Developments in Plant Breeding 11

H.T. Buck · J.E. Nisi · N. Salomón
Editors

Wheat Production in Stressed Environments

Springer

WHEAT PRODUCTION IN STRESSED ENVIRONMENTS

Developments in Plant Breeding

VOLUME 12

The titles published in this series are listed at the end of this volume.

Wheat Production in Stressed Environments

Proceedings of the 7th International Wheat Conference, 27 November–2 December 2005, Mar del Plata, Argentina

Edited by

H. T. BUCK
Buck Semillas S.A., La Dulce, Argentina

J. E. NISI
National Institute for Agricultural Technology and Animal Husbandry, Marcos Juarez, Argentina

and

N. SALOMÓN
National Southern University, Buenos Aires, Argentina

Springer

A C.I.P. Catalogue record for this book is available from the Library of Congress.

ISBN 978-1-4020-5496-9 (HB)
ISBN 978-1-4020-5497-6 (e-book)

Published by Springer,
P.O. Box 17, 3300 AA Dordrecht, The Netherlands.

www.springer.com

Printed on acid-free paper

Cover picture:
Bread wheat field in the Argentine pampas

TABLE OF CONTENTS

Crop and Natural Resource Management

Breeding for Resistance to Abiotic Stress

Physiology of Wheat Production

Biotechnology and Cytogenetics

Conservation and Management of Genetic Resources

Closing Remarks

PREFACE

Wheat researchers have made unique contributions and excellent progress to the production increase over the past several decades, mainly in the less developed countries; however, there are many challenges that still lie ahead to make food more accessible than ever before in a sustainable manner and to meet the needs of a global growing population. Numerous biotic and abiotic stresses affect wheat in major production areas and its future growth will most likely come from marginal environments where such stresses play even more important role. Developing countries are becoming increasingly urbanized. As urban populations grow, productive land disappears and this implies the need for more intensive cropping to keep pace. Water utilization for agriculture is also facing more competition from uses in urban areas. Focused efforts to improve wheat water-use efficiency are crucial to ensure sustainability of food production in water-constrained regions.

Current crop management systems such as reduced or zero tillage, stubble retention and precision agriculture are vital to satisfy the increasing needs of food and maintain at the same time the sustainability of natural resources. The widespread adoption of conservation farming techniques requires the introduction of changes to wheat varieties in disease resistance, particularly stubble-born diseases.

Global climate change will impact on agriculture due to temperature, precipitation and length of growing season alterations, and might also modify the impact of pests, diseases and weeds, increasing the risk of crop failure in certain wheat producing areas. Stresses represent a challenge for wheat researchers and impact the world food production; stabilization of production in all environments remains an important aim in the future. We believe that the future challenge to wheat production will find solutions much faster today than in the past: today the wheat community is more united to understand and handle problems in a collaborative way.

The program of the Seventh International Wheat Conference (7 IWC) held at Mar del Plata, Argentina, between November 27 and December 2, 2005, included, besides two opening lectures, oral and poster presentations grouped in seven sessions according to main topics. Each session was opened by two keynote talks delivered by invited speakers. A guided half day tour to Balcarce Experimental Station of the National Institute of Technology for Agriculture and Animal Husbandry located 60 Km W from Mar del Plata showed participants the Station wheat breeding program as well as wheat fields on different crop rotations and under several agricultural

systems. The 7 IWC Conference was preceded by a workshop on International Wheat Improvement at the CGIAR Centers and Global Initiatives on Rust sponsored by the US AID, the USDA-ARS and the National Wheat Improvement Committee. In addition, the Conference was an excellent opportunity for an open workshop organized by the International Wheat Genome Sequencing Consortium.

Feeding the world does not only mean addressing the need for total energy requirements but also the need of micronutrients. Wheat biofortification may be one way to reach this goal. In the post-Conference mini-symposium HarvestPlus: Breeding for Public Health, current researches and achievements of CGIAR HarvestPlus Challenge Program on this topic were presented. The Conference was distinguished by the presence of Dr. Norman Borlaug, 1970 Nobel Peace Prize Laureate, who was in charge of the Dinner Conference, and Dr. Evangelina Villegas, 2000 World Food Prize Laureate.

These Proceedings compile the 7 IWC oral presentations and hopefully will be useful to wheat researchers, breeders, agronomists and students.

We are indebted to Dr. S. Rajaram, Integrated Gene Management Director at ICARDA, and Chair of the International Scientific Committee of the Conference, for his guide in the outline of the scientific program and his overall support. We extend our appreciation to each of the International and Local Organizing Committees members and to the Session Chairs. Special thanks are due to the scientists who collaborated with valuable suggestions to improve this publication: P. Abbate, Z. Bedö, D. Calderini, F. García, S. Germán, M. M. Kohli, D. Lafiandra, S. Nagarajan, J. Rogers, P. Ruckenbauer, N. Saulescu, M.L. Seghezzo, E. Souza and R. Trethowan. We thank M. Pérez for the technical edition of the manuscripts and would like to express our special gratitude to Nelly Salomón for her permanent dedication, and our tribute, on behalf of the Local Organizing Committee, to Enrique Suárez, who joined us in this endeavour up to May 2005.

H. Buck and J. Nisi
Co-chairs of the Local Organizing Committee

INTERNATIONAL AND LOCAL COMMITTEES

INTERNATIONAL SCIENTIFIC COMMITTEE

Chairman

S. Rajaram

Members

R. Appels
S. Baenziger
Z. Bedö
L. Besopalova
A. Bonjean
H-J. Braun
G. R. Cunha
R. DePauw
W. Erskine
M. R. Jalal Kamali
Z. Kertesz
M. Keser
A. Kurishbayev
C. Le Roux
N. Litvinenko
S. Nagarajan
J. Nisi
P. Ruckenbauer
N. Saulescu
R. Sears
J. Snape

Local Organizing Committee

Co-Chairs

H. Buck
J. Nisi

Members

J. Annone
M. Cuniberti
M. Formica
M. García
L. González
M. Helguera
O. Klein
M. Kohli
N. Machado
R. Miranda
O. Rubiolo
N. Salomón
E. Satorre
E. Suárez†
G. Tranquilli

BENEFACTORS OF THE CONFERENCE

The Secretariat of Agriculture, Animal Husbandry, Fisheries and Food (SAGPyA)
National Institute of Technology for Agriculture and Animal Husbandry (INTA)

CONFERENCE SPONSORS

Financial support from the following organizations is gratefully acknowledged

DIAMOD SPONSORS

International Maize and Wheat Improvement Center (CIMMYT)
International Center for Agricultural Research in Dry Areas (ICARDA)

GOLD SPONSORS

BASF Argentina
Buck Semillas S.A.
Nidera S.A.

SILVER SPONSORS

Criadero Klein S.A.
Dow AgroSciences Argentina S.A.
MegaSeed
Relmo S.A.

BRONZE SPONSORS

Asociación Cooperativas Argentinas, Coop. Ltda.
Bayer CropScience
Bioceres S.A.
Buenos Aires Grain Exchange
Buenos Aires Grain Exchange Arbitration Chamber

Federation of Country Elevators Associations
Intertek – Caleb Brett
Profertil S.A.
Syngenta Agro S.A.
Wintersteiger - Seedmech Latinamerica

SPONSORS

Universidad Empresarial Siglo 21
Facultad de Ciencias Agropecuarias – Universidad Nacional de Córdoba


7th International
Wheat Conference
Nov 27 - Dec 2, 2005
Mar del Plata - Argentina

THE GLOBAL NEED FOR A SUSTAINABLE AGRICULTURAL MODEL

Its adoption and some of the benefits derived from the process with special reference to the Argentinean case

R.A. PEIRETTI

CAAPAS President (American Confederation of farmers Organizations for a Sustainable Agriculture) www.caapas.org

AAPRESID Director (Argentinean No Till farmers Association) www.aapresid.org.ar

E-mail: sdrob@idi.com.ar

THE CULTIVATION OF PLANTS AND THE FEEDING OF MANKIND. THE RELATIONSHIP BETWEEN THE RURAL AND URBAN SECTOR

Since the beginning of agriculture, ten thousand years ago, the cultivation of plants were basically managed by doing some kind of soil tillage. Even the strategy was useful to accompany an ever increasing human demand for agricultural products, the cost in terms of natural resources degradation — as soil erosion, fresh water depletion and other undesirable impacts — were too high and at the present time they should be consider as no longer affordable by humanity. A new conciliatory formula and agricultural approach is absolutely needed to be able to simultaneously keep and even enlarge the actual agricultural output and at the same time keep the capacity to do it in the future. In other words the achievement of a higher level of productivity and profit obtained with a higher level of sustainability must be seen as the new goal and as an urgent need.

In the present world and in direct relationship with the degree of socioeconomic development achieved by a society, an ever-growing disconnection between the urban and rural populations can be noticed worldwide. Frequently, this disconnection generates a state of ignorance (and even confusion) about the fundamental value and key role that the rural sector fulfils within societies and within our present civilization. This state of things delays — and even hinders — the possibilities for

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 1–7.

the global agricultural sector to speed up the process oriented to the achievement of the needed levels of productivity with sustainability and profit. However, reaching these levels proves indispensable to satisfy the ever-increasing demand of quality and quantity of food and other products and services derived form agriculture. At this point we should also take into account the fact that nowadays directly or indirectly it's from the cultivation of plants that mankind obtains around ninety percent of the food and that up to the present, with only a few exceptions of limited relative magnitude such as hydroponics (the cultivation of plants in an aquatic medium and under controlled conditions), the agro-productive processes are carried out on soils and environments reasonably apt for agriculture. Therefore, we should strive for society to reach an adequate level of information so it can appreciate the substantial role that the rural sector plays — by means of its agro-productive system — when it comes to using the soils and other natural resources as the basis to satisfy the feeding demands of mankind as a whole.

THE PROBLEM OF HUNGER AND THE ENLARGEMENT OF TOTAL PRODUCTION AND THE NEED TO ACHIEVE HIGH PRODUCTIVITY WITH SUSTAINABILITY

As part of the world agricultural production system we should have clear in mind that the problem of hunger is not only linked to total world food production if not also related to issues like the deficiencies in distribution and access to the productions as well as to technological, cultural, social, political, ideological, religious, economic, structural and even war-related constraints. Also, and even we are aware that most of these restrictions and problems extend beyond our reach and possibilities as producers, we clearly understand that without an increase in productivity and in the total world food production output, mankind will probably have very little chances to enlarge its cut so we have to keep our efforts oriented to permanently increase the capacity to enlarge the total world food output or the size of the world food cake.

Up to the present, there are only two basic mechanisms known to enlarge the total agricultural production; namely: the expansion of the agricultural area by converting ecosystems into agro-ecosystems and/or the achievement of a higher level of productivity on those already converted. During the past a combination of both mechanisms were utilized to accompany the increase of the food demand. While looking at the future and been aware of the progressive scarcity of the resources needed to carry out the agricultural process (particularly the natural resources), the permanent maximization of productivity within a more sustainable frame should be considered as a must and should be prioritized over the alternative of expanding the agricultural areas to increase total production as the basic mechanisms. If we do so we may be able to reduce the rate of conversion of less disturbed ecosystems into agro-ecosystems. Also, to be able to maximize productivity in a sustainable manner, the wise use of all the capacities and knowledge humanity has developed up to the present and will continue to develop in the future appears us a must. The capacities and knowledge needed to achieve this goal, span from those of pragmatic origin —

and even ancestral ones very much related to the observation and experience as producers in close contact with our environment and everyday agricultural process reality-, to those related to the most revolutionary and state of the art advancements of science and derived technology. None of them should be dismissed, but science — and not ideologies — should be the reference base in every case. History shows us that it's no other but this the pattern that mankind has adopted in the past to progress in other fields such as human health and the development and offering of all kinds of goods and services included the agricultural ones.

THE CAAPAS EXPERIENCE AND THE EMERGING OF A NEW AGRICULTURAL PARADIGM

Conventional agriculture, based on tillage (mainly plough) of soils, was the agricultural paradigm that humanity applied for nearly ten thousand years since the advent of agriculture. However, and even admitting that it help to feed humanity in the past, the utilization of this system in many cases generated an staggering level of degradation of the natural agro-ecosystem components as soil, water and others. Just as an example of such undesirable phenomenon we can consider that in many situations we were losing over ten tons of soil per each ton of grain produced. Clearly it represents a cost that mankind can not any longer afford in the present and will not be able to afford in the future. Therefore, we feel that this model (based on plough and tillage), upon which still nowadays most of the world's agricultural system is based, must be abandoned for good. The no till system, among others based on main principles as the absence of tillage and the covering of the topsoil by stubble, allows us to access a reasonable, sustainable — and even restoring — use of the basic agro-ecosystems components such as soils, water, biodiversity, etc.

Other technological tools such as the use of biotechnology, fertilizers and agrochemicals, the application of the most modern concepts regarding the handling of agro-ecosystems such as soil nutrition rather than crop fertilization, nutrient recycling, integrated pest management (weed, insects, diseases), the utilization of thresholds for economic damage of pests, the concept of rotation as general premise (and not just as variation of crops); constitutes only some examples of the pillar principles of the realistic building ground of the modern agriculture that we heavily promote and utilize within CAAPAS.

Considering the experience accumulated during the last twenty five years across many different agro-ecosystem conditions, we can state that wheat can be perfectly and successfully grown under No Till. Furthermore, along with other winter and summer grasses and legumes (and other broadleaf crops), play an important role in widening the rotational pattern needed to further improve the system functioning.

The fully utilization of them comprises a paradigmatic change absolutely needed to be able to enlarge the chances to satisfy the needs of a human population that doesn't cease to expand both in size and in economic capacity that allows to improve the diets both in quality and quantity further enhancing the need to enlarge total production.

The objective measurements carried out in the over 50 millions hectares under the No Till belonging to the countries members of CAAPAS (and over the around 90 millions hectares worldwide) show a significant increase in productivity and in a highly efficient control — and even reparation — of the erosion and degradation of our soils and water.

The above mentioned, together with the possibility of retaining atmospheric carbon (as organic substance of the soil) it's what makes our eco-systems become more healthy, what increases the biodiversity contained in them and what makes them become more resilient and at the same time more reactive, i.e., they deliver more output for each unit of applied input. (di Castri 2002) These achievements constitute only some of the indisputable proves of the value that this new paradigm can provide to make further advancements in the provision of food and in the relationship with our soils, our fellow men and the environment in general. The new agro-productive proposal of CAAPAS, is not based in a new hypothesis or in something that can theoretically happen, but in our daily reality as farmers under No Till, which, with the introduction of adaptations to the particular conditions of each agro-ecosystem, results applicable across the great variety of agro-ecosystem conditions that we can find across the world.

THE CASE OF ARGENTINA. NO TILL AND THE MOSHPA (MODERN SUSTAINABLE HIGH PRODUCTIVITY AGRICULTURAL) MODEL DEVELOPMENT AND ADOPTION

The Argentinean farmers were aiming, and found in No Till and the MOSHPA model principles, a valid (realistic and applicable) way to improve productivity and profit but at the same time to counteract the evident soil erosion (water and wind born) and general agro-ecosystem degradation process, that was becoming more and more evident and worrisome during the last third of the past century. (Casas, Roberto R. 1997). The first No Till extensive Argentinean trials started on the seventies; however, it was not until after the end of the eighties, and beginning of the nineties, that the adoption process boomed. The evolution of the adoption process can be seen on next Fig. 1. From a couple of hundred thousand hectares in 1990; the adoption jumped to more than sixteen millions hectares that account for around 65 % of the Argentinean grain cropped area.

The full utilization of new technical developments and approaches were strongly supporting and pillaring the Argentinean No Till adoption process. An evolved and more specific use of agro-chemicals to control weeds, diseases and insects as well as a further development and utilization of integrated weed, disease and insect management principles. Also, new rotational strategies; the development, selection and utilization of superior genotypes created by conventional breeding as well as by biotechnology; and the development of a new generation of specially adapted No Till drillers and planters able to properly operate under a soil covered condition and no tilled condition; can be considered as some of the most relevant factors that allowed the practical implementation and evolution of No Till.

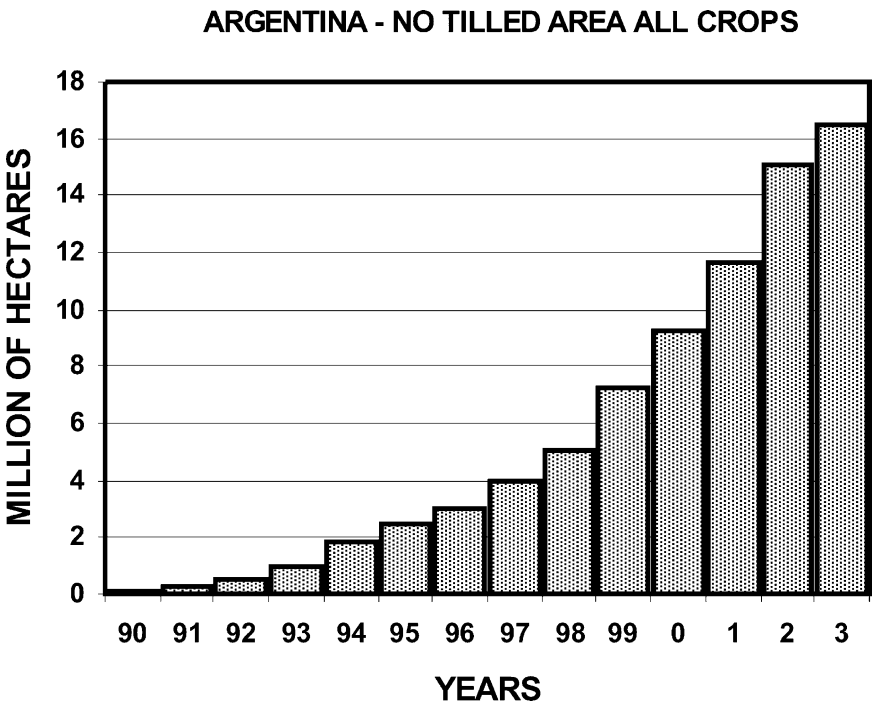


Figure 1. Evolution of the No Till adoption in Argentina

Also, a systemic approach and a clear proactive farmer attitude lead to the improvement of several agronomic and organizational strategies that allowed to quickly scaling up the process. Besides this, the strong domestic and international interactions among farmers promoted by CAAPAS (American Confederation of Organizations for a Sustainable Agriculture) and by AAPRESID (Argentinean No Till Farmers Association), represented a valid mechanism to keep improving, adjusting, evolving, and scaling up, the No Till and MOSHPA model principles adoption in America all and in other parts of the world. (Peiretti, Roberto A, 2003).

EVOLUTION OF THE ARGENTINEAN GRAIN PRODUCTIVITY AND TOTAL PRODUCTION

After 25 years of No Till experience in certain cases, it appears that the longer the period under no till the healthier and more productive the agro- ecosystem becomes. Erosion and soil deterioration symptoms almost disappeared and instead of them, clear evidences of a soil that increases its fertility or ability to produce can be detected.

During the nineties, all these phenomena together with an economic environment that strongly stimulates investment along all the Argentinean farming chain, yielded a significant increase of the total production in a sustainable manner. Focusing on

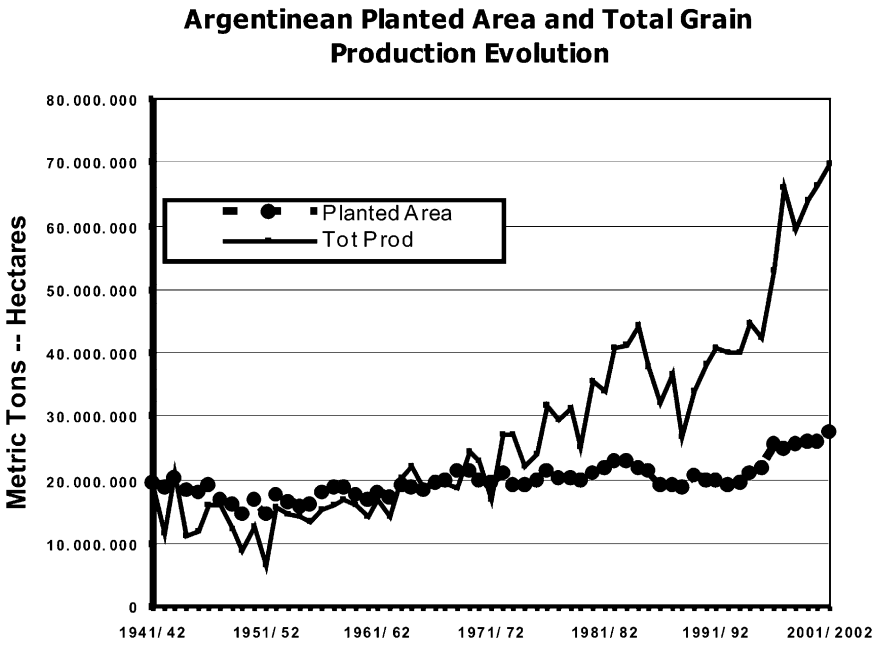


Figure 2. Evolution of Argentinean Grain Production

next graphic that includes the area cropped and the evolution of total Argentinean grain production between 1941/42 and 2001/02. In Fig. 2, we can see how much the total production grew especially during the last fifteen years in coincidence with cropping/farming system transformation.

Even part of the total production growth may be explained by an enlargement of the total area cropped on the country as well as from a variation of the area share among different crops, the biggest part of the explanation can be found in a relevant productivity increase. The productivity increase (paired with a significant reduction in production costs of all type), allowed a profitability and competitiveness improvement, but now obtained within a sustainable frame. (Peiretti, Roberto A., 2001).

The more evolved Argentinean cropping and farming system, yielded significant benefits that even with different intensity, reached all the farm size; from those large scale market oriented ones to the small scale mostly subsistence ones.

CONCLUSIONS

A few years ago, Dr. Norman Borlaug, the agronomist's father of the Green Revolution and Novel Peace Price Laureate 1970, stated: One of the most serious challenges that humanity will be faced with during XXI century, will be to develop the capacity to produce enough food for humanity but conserving the environment at the same time.

The new agro-productive model that we promote at CAAPAS is based on science — but not in ideologies — and also on humanitarian and realistic feeling. This model promotes the abandoning of tillage and the criteria of exploitation and plundering of resources to enter a new phase in which agriculture — based now on No Till — will develop efficiently, with high productivity and stability but also with sustainability and improvement of all the resources involved in the process. Without a doubt, the thorough understanding of these issues by the international community will help the world farming system to speed up the transformation of the farming system and agricultural paradigm toward a more evolved stage. If so, we will be increasing in a magnitude similar to our success, the generation of many benefits that, apart from benefiting the producers will spread simultaneously to the whole society and mankind as well.

REFERENCES

Casas RR (1997) Causas y Evidencias de la Degradación de los Suelos en la Región Pampeana. In: Solbrig OT, Veinesman L (eds) Hacia una agricultura productiva y sostenible en la pampa, Orientación Gráfica Editora. Buenos Aires, pp 99–129

di Castri F (2002) Globalización y Biodiversidad. (ed) Universidad de Chile. Universidad de Chile. Santiago. Reprint en AAPRESID 2005. Paraguay 777. 8° Piso. 2000. Rosario. Rep. Argentina

Peiretti RA (2001) Direct seed cropping in Argentina: economic, agronomic, and sustainability benefits. In: Solbrig OT, Paarlberg R, di Castri F (eds) Globalization and the Rural Environment, Massachusetts. Harvard University Press, Cambridge. ISBN 067400531-7 Section II, Chapter 9, pp 179–200

Peiretti RA (2003) The CAAPAS actions and the development of the MOSHPA. In: Proceedings of the II World Congress on Conservation Agriculture. Printed by Federacao Brasileira do Plantio Directo Na Phala. Rua 7 de Setembro, 800 – Sala 301 A – CEP 84350-210 – Ponta Grossa – Paraná – Brasil. vol I, pp 127–128

THE ECONOMICS OF WHEAT
Research challenges from field to fork

J. DIXON

Director, Impacts Targeting and Assessment, International Maize and Wheat Improvement Center (CIMMYT), Apdo. Postal 6-641, 06600 Mexico DF, Mexico
E-mail: j.dixon@cgiar.org

Abstract: During the two generations leading up to the turn of the century the global population grew by 90% whilst food production expanded by 115%. As with other food crops, wheat productivity rose steadily during the past 40 years through the availability of better varieties, inputs, markets and management. As a result of the growth in supply – wheat is the most widely internally-traded cereal- producer prices have fallen by approximately 40% during the past two generations. Notwithstanding the increase in per capita food production, around 800 million people are hungry and around 1.2 billion people live below the international consumption poverty line of US $ 1 per capita per day. Wheat is grown on a significant scale in 70 countries and for many poor households wheat is a significant production or consumption item. Nevertheless, global food security is quite fragile, particularly when looking towards the middle of the century: because of projected needs for human, animal and industrial uses, global wheat production is expected to increase from nearly 600 million tons to around 760 million tons in 2020, with limited expansion of sown area

The estimates of rates of returns on past breeding and agronomic research are very high, partly because of the wide adaptability of many new wheat cultivars. The paper distinguishes returns to productivity and maintenance research, as well as socio-economic and policy research. In the search for means to accelerate the achievement of the MDGs, the effectiveness of targeting research to marginal areas and marginal farmers becomes an important question

Wheat, therefore, is crucial to current and future global food security – the question is: can the achievements of the past be continued in the coming decades? The global demand for wheat is projected to grow modestly at 1.2% p.a. for food and 0.8% p.a. for feed. Greater growth may be experienced in certain end-uses including flour, pasta and bakery products; consequently, quality attributes are assuming greater importance. Many developing countries have implemented trade reforms but annual producer subsidies in OECD countries amount to about $17 billion. There has also been significant tariff

The author is responsible for the views expressed in this paper which do not necessarily represent the policies or priorities of CIMMYT.

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 9–22

escalation in flour, pasta, bakery products, so trade tends to occur within trading blocks such as EU and NAFTA. There is strong evidence of fast growth in value added along wheat value chains including retailing

Keywords: wheat economic , markets, trade, research impacts

INTRODUCTION

During the two generations leading up to the turn of the century the global population grew by 90%. Although food production expanded by 115% during this period, chronic hunger still afflicts more than 800 million people and some 1200 million people subsist on US $ 1 or less per day. The global community recently re-affirmed the importance of the reduction of hunger and poverty during the UN Millennium Summit. Adequate nutrition and rural livelihoods, which depend upon efficiency and value added along the chain from "field to fork", i.e. from production to consumption, underpin poverty reduction and rural economic development. Moreover, discussion of the link between food security and political stability is growing in the popular press and academic literature (see, for example, Falcon and Naylor 2005). Although the argument that poverty reduction is an international public good is contested, there is little doubt that most of the knowledge generated through international research system is an International Public Good (IPG) (Ryan 2005). The road to household and global food security faces major challenges, not least because of the substantial projected increases in food and feed requirements and the pressure on available land and water resources (Runge et al. 2003).

Wheat, along with maize and rice, underpin the world food supply, providing 44% of total edible dry matter and 40% of food crop energy consumed in developing countries. Bread wheat, which accounts for 90% of total wheat production, is grown on a substantial scale in more than 70 countries on 5 continents (Lantican et al. 2005). Durum wheat accounts for the remaining 10% of wheat production, of which more than half is found in North Africa and West Asia. Wheat is produced in a wide range of climatic environments, production environments and farming systems (Dixon et al. 2001). Of the total harvested bread and durum wheat area in 2005 of approximately 226 million ha (FAO 2006), about half is located in developing countries; and about one-third is found in countries where average farm household income is less than US $ 1 per capita per day.

As with other food crops, wheat science (including breeding, pathology, agronomy and economics) has contributed to a steady increase in wheat productivity and improved livelihoods of producers during the past 40 years through, inter alia, the availability of better varieties, more effective pest and disease control, better production practices and improved household management. In developing countries modern varieties were sown on 83% of irrigated and high rainfall wheat land by

the late 1970s and on practically all high potential crop land by 1990 and with substantial increases in productivity (Byerlee and Moya 1993).

Looking into the future, global wheat requirements are expected to increase from around the current 600 million tons to approximately 760 million tons by 2020 (CIMMYT 2004). Thus, wheat not only has a key role to play in current food security, but also in future global food security and poverty reduction. It is argued that the Green Revolution approach that created the enormous success in past decades is not the only, and indeed not necessarily the best, way forward in the coming decades: wheat scientists are now confronted with many new research challenges in relation to the sustainable intensification of production and value addition at all stages from field to fork as well as a new set of opportunities including the Gene Revolution (Pingali and Raney 2005).

The second section of the paper considers changes in wheat markets and trade. The third section summarizes evidence related to research impacts. These two analyses provide the basis for a discussion of drivers of change and plausible futures of wheat in the fourth section. The conclusions on ways forward appear in the fifth section.

WHEAT MARKETS AND TRADE

Notwithstanding the general decline in agricultural exports from developing countries, from more than 50% of international merchandise trade in 1960 to less than 20% now (FAO 2003), wheat is the most traded food grain in international markets. During the past few decades developing countries have gradually become major net importers (to the degree that wheat now accounts for 43% of food imports to developing countries). On the other hand, 81% of wheat is produced and utilized within the same country, and a large proportion within the same community or household. The increase in per capita supply and productivity has led to the well-known steady decline in international wheat prices – by some 40% since 1960 – while input prices are steady or rising. Such a situation can lead to increased domestic cost of production, favours the importation of wheat and may have discouraged the adoption of new cultivars.

Even though international market prices have declined, wheat production is protected in many countries. Consequently, producer prices differ markedly between countries, ranging from more than US $ 1000 per ton in Japan and more than US $ 500 per ton in Iran, Korea and Nigeria to less than US $ 200 per ton in a majority of countries during 2001 (Aksoy and Beghin 2003). The full implications of such high prices for sustainable production and for wheat science are not clear. Such high and stable producer prices provide incentives for the intensification of production, including the adoption of new cultivars, albeit at the cost to Government of the producer subsidies or the cost to consumers, notably the poor, of high food prices.

While urbanization increases demand, the marked shift from cereal-based diets to energy-dense diets with more vegetable oil, meat and sugar diminishes the relative importance of wheat in the diets of many middle and upper income countries

(FAO 2003). Moreover, as consumer incomes rise, processed foods account for a higher proportion of food budgets with a corresponding decrease in wheat's share of food budget expenditure. Nevertheless, some Middle Eastern and North African middle income countries have maintained high per capita levels of wheat consumption, for instance, 208 kg per capita per annum in Tunisia.

Another key trend in consumer preferences is the growing demand for wheat quality attributes of different types. The preferred quality attributes and the volume demanded depend very much on the market. For example, the traditional low-volume niche market in Syria for the traditional wheat product "friki" stands in marked contrast to the enormous market for wheat for the Chinese noodle industry. In both Turkey and Kansas, grain "whiteness" is of growing importance. Northern Kazakhstan has traditionally exported high quality wheat to blend with softer wheats from Russia for bread making. Two aspects are of primary concern to researchers: first, market differentiation of wheat of different qualities, and the implications for market access and price premia; and, second, the feasibility of breeding for the particular quality attributes demanded in each market.

Two further changes could have far reaching effects on the wheat industry. First, there is growing concentration of agricultural input suppliers, and also of agricultural product markets. One aspect of product market concentration is the growing strength of supermarkets, setting standards for quality, reliability, timeliness; and global purchasing policies. So far, supermarkets have had more impact on the marketing of fresh produce than that of food grains, but their emphasis on reliable product quality can also be expected to have an impact on the wheat industry. There is strong evidence of fast growth in value added along value chains and in retailing.

Second, many developing countries have implemented trade reforms whereas producer subsidies in most OECD countries (and some developing countries) still amount to about $17 billion per annum. There has also been significant tariff escalation in flour, pasta and bakery products, with the consequence that trade tends to occur within trading blocks such as EU and NAFTA (Aksoy and Beghin 2003). It is estimated that full trade liberalization would lead to an increase in international markets prices of the order of 10–15%; and global annual welfare gains of approximately $ 165 billion, three-quarters of which would accrue to developed countries, including Argentina and the CIS countries.

WHEAT RESEARCH IMPACTS

Of the wide variety of social sciences research on wheat, the most widely recognized may well be ex post impact assessments of wheat breeding of which many were published by the CIMMYT economists from the 1980s until the present day. Heisey et al. (2002) provide an historical sketch for economists of international wheat breeding. During the late 1950s and 1960s, wheat research in Mexico and South Asia, focused on semi-dwarf cultivars for irrigated areas, led to major increases in food production of the South Asian Green Revolution. By the late 1960s the scope had broadened to disease prone, higher rainfall environments, and shuttles were

established with Brazil in the 1970s and China in the 1980s. During the 1980s a winter wheat breeding program was established in Turkey which now covers 50 countries. As the geographic scope expanded, the selection for pests (e.g. Russian wheat aphid) and diseases (e.g. Septoria spp. Fusarium spp. karnal bunt and rusts) gained more attention. Although breeding for acid soils was a goal of the Brazilian shuttle, it was during the 1990s that the emphasis on biotechnology and abiotic stress, notably drought, really gained in importance internationally, along with attention to synthetic wheat, hybrid wheat, grain nutritional and economic quality and adaptation to conservation agriculture. During the past decade a shuttle has been established with Northern Kazakhstan to serve the high latitude wheat growing areas of Central Asia.

The enormous contribution of wheat breeding during past decades – from 1960 to 2000 modern varieties spread over approximately 95 per cent of global wheat area (Lantican et al. 2005) and average yields approximately doubled – was underpinned by widespread sharing of genetically improved materials (Evenson and Gollin 2003a). As an example of the partnership mode of operation of one actor in the global wheat improvement innovation system, CIMMYT invests in the exchange of germplasm and knowledge with regional and national partners (including NARS and sister CG Centers). Through these networks more than 1000 lines of improved wheat are distributed annually (Reynolds and Borlaug 2006a). The research productivity of NARS increased rapidly since the 1960s as a result of national and international capacity building (see Cooksey and Arelland 2006 for a discussion of CIMMYT training). The number of varieties released annually by NARS doubled to more than 100 varieties by the early 1990s (Lantican et al. 2005) of which more than 80% were products of international collaboration (Byerlee and Moya 1993). As a consequence of many years of collaboration between CIMMYT and NARS, by 2002 some 64% of world wheat area was sown to seed containing CIMMYT germplasm (Lantican et al. 2005). Wheat productivity in developing countries – where food security was most critical – grew at 3.4% p.a. during 1966–77 and 5.1% p.a. during 1977–85 (Pingali 1999) which was associated with the steady decline in the international price of wheat noted above.

Historically, the estimated returns on past wheat breeding and agronomic research have been very high – many times the usual returns to agribusiness investment – partly because of the wide adaptability of CIMMYT improved wheat lines and the associated nationally released varieties which many observers attribute to shuttle breeding. In a meta-analysis of more than 1700 estimates of rates of return to agricultural research, Alston et al. (2000) report average rates of return to wheat research of approximately 50%. The benefits of international collaborative wheat improvement to developing countries are substantial (Evenson and Gollin 2003b); for example, the annual economic benefit to international spring wheat research is estimated to be US $ 2500 million (Lantican et al. 2005). Taking into account contributions from other disciplines and sectors, Evenson and Gollin (2003b) estimates that 1% of the 3% p.a. increase in food production in Asia between 1961 and 1981 was attributable to improved cultivars of wheat and rice – the contributions of increased

fertilizer use and the expansion of irrigation were also substantial. In relation to wheat itself, Bell et al. (1995) estimate that 28% of the average annual increase in wheat grain yield in Yaqui Valley Mexico can be attributed to improvements in germplasm.

Turning from productivity enhancement to maintenance research, a study of the value of durable multigenic resistance to leaf rust in developing countries estimated a net present value of US $ 5.36 billion and a benefit cost ratio of 27:1 (Marasas et al. 2004). More recently, a scoping study of the impacts of Fusarium Head Blight also suggests high pay offs of return to maintenance research (la Rovere et al. 2006). The importance of maintenance research to the wheat industry is widely recognized: maintenance research usually generates high returns and will be increasingly required in order to defend past yield gains. Biotic threats can be sudden and unexpected, as in the case of the latest strain of stem rust (Ug99) which threatens enormous losses if and when it spreads from East Africa to South Asia (Hodson et al. 2005).

International wheat breeders were often criticized for supposedly contributing to a decline in genetic diversity of wheat. Contrary to popular belief, the genetic diversity of CIMMYT lines has been increasing during the past quarter century. Quite apart from molecular studies cited by Reynolds and Borlaug (2006a), the number of released spring wheat varieties increased during the 1990s, and the pedigrees of nationally released varieties with CIMMYT ancestry contained, on average, 45 landraces, compared with 19 landraces in the case of released varieties without CIMMYT ancestry (Smale 1997, Smale et al. 2002).

The contribution of crop management research can be seen in the increased productivity and sustainability of wheat-based Conservation Agriculture (CA) farming systems. For example, planting on permanent beds, a variant of CA, has been adopted rapidly by farmers in Mexico, China and the Indo-Gangetic Plain (Sayre and Hobbs 2004, Laxmi et al. 2005). The research for development behind this success was led by the Rice-Wheat Consortium which has received international recognition for its science-based and farmer-centred development of innovation systems (Seth et al. 2003). In the most mature rainfed wheat based CA systems, for example in southern Brazil and Paraguay, tillage has been eliminated. Reduced tillage in the form of chemical fallows is spreading rapidly in North Kazakhstan, and over time it is expected that rainfed CA systems in Asia and Africa will trend towards zero tillage, reduced costs, increased profit and reduced risk (Dixon 2003a).

For decades CIMMYT has been a source of frontier methodologies in economics and path breaking studies on returns to wheat research (e.g. Byerlee and Moya 1993; Byerlee and Traxler 1995; Maredia and Byerlee 1999; Lantican et al. 2005). There is natural synergy with IFPRI on many research activities including the spatial distribution and characterization of wheat environments. One key contribution of wheat socio-economists to such partnerships has been the detailed knowledge of the wheat industry, including wheat farming systems, crop diversity, cereal value chains, germplasm impact pathways and impacts on farm households. Nevertheless,

a range of methodological challenges persist for wheat impact studies, including the identification of suitable metrics for livelihoods and poverty reduction, the specification of counterfactuals, and the treatment of attribution (see Alston et al. 1995).

Impacts from wheat research have not been uniformly distributed across farming systems. The benefits of the Green Revolution were initially registered in the well-watered farming areas of the developing world where the new semi-dwarf varieties responded well to good moisture and increased fertilizer use. The relative effectiveness of targeting research to agro-ecologically and socio-economically marginal areas – and both aspects of marginality need to be considered – is an important question for poverty reduction. The rates of adoption of the early semi-dwarf cultivars were significantly higher in more productive environments: the cultivars performed less well in the marginal areas and farmers tended to be more risk adverse, and consequently they maintained their traditional cultivars (Byerlee 1994). Recent studies of the performance of new wheat lines in agro-ecologically marginal environments indicate substantial yield progress (of the order of 2–3% per annum) in both semi-arid and heat-stressed environments between 1979 and 1995 (Trethowan et al. 2002). Although the genetic basis of drought tolerance in wheat is complex (Reynolds and Borlaug 2006b), substantial genetic progress in drought tolerance has been made, and now many drier environments report significant adoption and improvements in productivity over time (Trethowan et al. 2002; Lantican et al. 2002; Evenson and Gollin 2003b).

The great progress with wheat described above required a strong enabling policy environment; and there is more than a little truth in the claims that poor seed and produce marketing systems and other support institutions hinder the adoption of modern cultivars. For this reason, social scientists have invested considerable effort in the analysis and promotion of institutional and policy options to improve input and grain marketing systems.

THE FUTURE OF WHEAT-BASED FARMING AND VALUE-ADDED SYSTEMS

Five global drivers of change in agricultural systems, including wheat production systems, value chains and consumption patterns, can be identified (see Dixon et al. 2001). The global agricultural research system has generated improved germplasm and technologies which underpinned the past growth of the wheat industry; and the continued supply of improved lines and new technologies is essential for future improvement in wheat production growth, without which global food security and the achievement of the Millennium Development Goals might be threatened. At the international level, policies and institutions shape international wheat markets and the work of the global agricultural research system. At the national and local levels, policies and institutions are key influencers of domestic markets, the incentives for the adoption of technologies and the share of benefits along value chains. As technologies become more knowledge-intensive and production more responsive to

markets, the importance of information and education are increasing. It is now recognized that farmers' resource sharing and group marketing can influence adoption and productivity, and that cooperation along value chains can increase efficiency and competitiveness.

Taking into consideration the current circumstances and drivers of change for the wheat industry during the coming decades, the wheat industry faces a number of challenges. Given declining farm gate prices and increasing input prices, can the competitiveness of wheat be maintained? Given the changes in wheat industry structure, can wheat markets and value chains be strengthened to improve value added and provide a premium to farmers to adopt quality wheat varieties? Given that farmers ultimately decide on whether to adopt new varieties and how to grow them, how can their views best be communicated to breeders? Given the prevalence of poverty in wheat growing areas, can wheat contribute significantly to poverty reduction, directly through wheat crop or indirectly by fostering diversification? Given the biotic stresses which threaten production, can the international wheat breeding community organize rapid response mechanisms to breed and distribute resistant germplasm? Given the burgeoning requirements for cereals for food and feed during the coming decades, can wheat continue to contribute a major share to global food security? Given the variation across eco-regions, impact pathways and livelihoods, what is the best way to identify priority traits for breeding?

Following the substantial technology-based growth in wheat supply, international wheat prices have fallen by approximately 40% since the 1960s. This fall in prices has been passed on to producers in many countries (some exceptions were noted in the previous section). Moreover, with the rise in oil prices the costs of production inputs, especially fuel and N-fertilizer, have increased. The cost-price squeeze is reducing returns to land, labour and capital relative to those of some other crops; therefore, wheat is being replaced by maize in the U.S.A. China and India. In relation to the increased N-fertilizer price, breeders could choose to focus on traits related to nutrient use efficiency. Another response of farmers and scientists to the cost price squeeze is the adoption and refinement of conservation agriculture practices which not only improve soil management and save water, but also reduce fuel consumption for tillage and other production costs (Lee 2005). Following its widespread adoption in Brazil, Paraguay and Argentina during past decades, conservation agriculture practices have spread rapidly during the past few years in the Indo-Gangetic Plain in India and Pakistan (Dixon 2003a).

An alternative research response to the cost price squeeze would be to focus on the structure, function and efficiency of the value chains that connect wheat producers to consumers (from the "field" to the "fork"). Value chains are the series of transactions necessary for the conversion of agricultural resources and inputs to farm produce, for its processing and marketing to the final consumer, involving a process of adding value at every stage. Thus, value chains connect consumers with producers, signal consumers preferences and willingness to pay for new quality attributes, and link inter-independent businesses together in "competitive cooperation". Well organized and coordinated chains underpin the competitive advantage of industries.

Prevailing wheat value chains are being transformed by urbanization, diet transitions, growing concentration of large scale agricultural input suppliers, agricultural marketing, wholesaling and retailing in developing countries (see, for example, Reardon and Berdegue 2002). Around half of consumers now live in cities and a large proportion are shifting from cereal-based diets to energy-dense diets containing more oils, fats and animal protein, and characterized by a higher proportion of processed food. The "supermarket tsunami", characterized by extremely rapid growth in supermarket sales, is rolling across developing regions including Asia, Africa and Latin America. The growing concentration of supermarket ownership enables global purchasing strategies and the setting of commercial standards for farm produce. Although the marketing of fresh produce is transformed first, cereal chains are also affected. There is an increasing demand for food quality, including wheat flour quality, which is manifested in premia for quality attributes in some markets and a growth in food standards and Good Agricultural Practices (GAPs). The diversity of products made from whole grain and milled wheat flour is not always recognized. The quality specifications for these products vary significantly across countries, and sometimes hinder access to export markets: for example, in Kazakhstan wheat quality specifications are based on gluten rather than the internationally recognized standard of protein content. Sometimes increases in yield potential have been associated with a reduction in grain quality. For example, the extensive use of semi-dwarf germplasm in Mexican wheat breeding between 1950 and 1985 boosted grain yield but initially reduced grain protein levels (Ortiz-Monasterio et al. 1997). Subsequently, however, further gains in grain yield were achieved while maintaining grain protein quality. Farmers' demand for high quality wheat varieties depends on, inter alia, the payment of price premia for quality attributes. Whilst price premia for various quality characteristics are evident in wheat wholesale and retail markets, the extent to which these premia are passed back by millers and traders to farmers varies from country to country.

Farmers' specific needs and preferences with regard to the characteristics of wheat varieties play an important role in the adoption of new varieties. The principal mechanisms for incorporating farmers' priorities in the selection and release of new varieties have tended to focus on "the greatest needs of the greatest number" which has led to high rates of return assessed at national or global levels of aggregation but not necessarily to much poverty reduction in marginal wheat growing areas. In relation to the minority of wheat producers that still grow landraces and for the majority with options for varietal replacement, secondary characteristics of new wheat varieties assume increasing importance. In some localities wheat scientists have pioneered participatory methods for varietal development with farmers, referred to as Participatory Varietal Selection (PVS). Following their demonstrated success in South Asia (Witcombe et al. 2006), it would be useful to extend PVS to other regions and to marginal groups of farmers who have been by-passed by mainstream wheat research and development efforts.

In relation to the contribution of wheat to global food security, the demand for wheat for human consumption in developing countries during the period leading

up to 2020 is expected to grow at 1.6% per annum, and for feed at 2.6% per annum. The global demand for wheat is projected to grow modestly at 1.2% p.a. for food and 0.8% p.a. for feed. Greater growth may be experienced in certain end-uses including flour, pasta and bakery products; consequently, quality attributes are assuming greater importance. Given the estimated demand and available resources and technologies, average yields are projected to increase during the coming 25 years from 2.6 tons ha^{-1} to 3.5 tons ha^{-1} (FAO 2003).

The relationship between intensification of wheat and the diversification to other crops and livestock is a critical determinant of poverty reduction among smallholders. Anecdotal evidence suggests many cases where the increase and stabilization of food crop yields releases resources, notably land, water, labour and cash, for diversification to cash crops and livestock. Some high potential areas with good market access, such as western parts of the Indo-Gangetic Plain, have witnessed rapid diversification away from both rice and wheat (Chand 2005). There is need for better data on the benefits and costs of cereal-cash crop rotations, and on development pathways characterized by the intensification of cereals and diversification to non-cereal crop and livestock enterprises.

High returns to maintenance research were noted above. The most recent biotic threat to global wheat production is Ug99, a virulent race of stem rust which has emerged from Uganda and has been confirmed at widely distributed testing locations in Kenya and Ethiopia. Yield losses of up to 71% have been recorded under experimental conditions in Kenya, and there is clear risk that Ug99 could move across the Red Sea to the Arabian Peninsula and through the Middle East to South Asia, with the potential to cause massive losses in wheat production and livelihoods where an estimated 1 billion people depend on wheat. The vast majority of current germplasm grown in the potential risk zone is susceptible to Ug99. Production loss scenarios range from 8.5 to 59.6 million tons, equivalent in value to US $ 1.1–8.3 billion (Hodson et al. 2005). Actual impacts would depend on the magnitude of indirect impacts (e.g. increased world wheat prices paid by consumers and importing countries) and offsetting adjustments to livelihood patterns by farmers (e.g. additional off-farm work). Given the magnitude of potential impacts, there is a clear need for more detailed spatial modeling of the likely spread and impacts of Ug99, as well as assessments of farmers' livelihood options. Such analysis would complement the international effort currently underway for the screening and selection of Ug99 resistant lines in Kenya.

The improvement of priority setting and targeting offers the means to increase payoffs to wheat breeding and crop management research. CIMMYT scientists created a resource Allocation Tool (RAT) for the comparing investments in crop improvement by the two commodities, maize and wheat, and geo-political regions. In a more refined spatial approach, global wheat mega-environments have been delineated which distinguish abiotic and biotic stress combinations for different wheat types (Trethowan et al. 2005). The growing recognition of the importance of socio-economic factors as determinants of adoption and productivity of new agricultural technologies (e.g. Lee 2005) suggests the inclusion of socio-economic

factors in such characterization. As spatial data availability and analytical power grow, GIS offers a viable platform to combine bio-physical and socio-economic information for priority setting, such as in the FAO-World Bank farming systems classification of Dixon et al. (2001). Using such a framework at global or national levels, an agricultural R & D can be systematically targeted to specific regions and farmer groups (see Dixon 2003b). Such an approach would benefit from systematic assembly and meta-analysis of farm and village level data on farm production practices, productivity, constraints and livelihoods.

Overall, there is a substantial social science research agenda to support wheat crop and industry improvement. Social sciences can provide the systematic data and knowledge for priority setting and targeting ("the big picture"), for assessing consumer demand for different types of wheat in different markets, for appraising value chains, for documenting and analyzing impact pathways and spillovers, for analyzing crop diversity, for conducting impact assessments of past (and possible future) research, and for providing advice on the impacts of alternative policies.

CONCLUSIONS

Historically, wheat production has made a major contribution to global food security for millennia. Given the steady increases in wheat productivity during the past 40 years underpinned by better varieties, inputs, markets and management, wheat has continued to play a major role in global food security. As a consequence, returns to past research to enhance the productivity of wheat have been high, as have the returns to maintenance research to defend those gains.

However, global food security remains quite fragile and thus the continued improvement of wheat germplasm, crop management and markets is required. Global wheat production is expected to increase from around 600 million tons to around 760 million tons by 2020, principally through intensification in existing wheat areas – and thus will continue to underpin global food security. As a consequence of the technology-based growth in wheat productivity and supply, producer prices have fallen by approximately 40% during the past two generations. Nevertheless, there is strong evidence of fast growth in value added along value chains including retailing; and of increasing demand for better grain quality.

Challenges for wheat scientists (including economists) include increasing productivity, especially for agro-climatically and socio-economically marginal areas, and defending past gains against pests and diseases, of which rust may well be the most important. In addition, the wheat industry faces a variety of new challenges which suggest that re-consideration of the wheat research portfolio may be in order. It is necessary to enhance competitiveness in production, taking into account complex farming systems, alternative uses of resources and structural transformation of value chains. Many producers are on a cost-price treadmill – high oil prices are driving up fuel and fertilizer costs and cereal prices are low and, at least until recently, declining. Profitability can be increased by breeding for water and nutrient use efficiency, as well as the promotion of cost-reducing conservation agriculture.

With increasing incomes and westernization of diets, consumers' preferences are shifting towards various quality characteristics of wheat products, in the context of the rapidly growing range of alternative foods. Thus, consideration should be given to strengthening wheat value chains to pass back price premia for wheat quality to farmers, and elevating the priority for grain quality in wheat breeding programs. Improved priority setting and targeting, underpinned by site-specific crop production, constraints and livelihoods meta-databases, offer the potential to increase payoffs to wheat improvement. Taken together, there is a substantial and important agenda for social science research.

ACKNOWLEDGEMENTS

The comments of Drs. Richard Trethowan, Erika Meng and Jon Hellin are gratefully acknowledged, as well as the assistance of Drs Pedro Aquino and Mina Lantican and Mr Frederico Carrion in the assembly and analysis of background data.

REFERENCES

Aksoy MA, Beghin MC (2003) Global Agricultural Trade and Developing Countries. World Bank, Washington

Alston JM, Norton GW, Pardey PG (1995) Science under Scarcity: principles and Practice for Agricultural Research Evaluation and Priority Setting. Cornell University Press in cooperation with the International Service for National Agricultural Research, Ithaca and London

Alston JM, Marra MC, Pardey PG, Wyatt TJ (2000) A meta analysis of rates of return to agricultural R&D: Ex pede Herculem? Research Report, vol 113. IFPRI, Washington, DC

Bell MA, Fischer RA, Byerlee D, Sayre K (1995) Genetic and agronomic contributions to yield gains: a case study for wheat. Field Crops Res 44:55–56

Byerlee D (1994) Modern Varieties, Productivity, and Sustainability: Recent Experience and Emerging Challenges. CIMMYT, Mexico, DF

Byerlee D, Moya P (1993) Impacts of International Wheat Breeding Research in the Developing World 1969–1990..: CIMMYT, Mexico, DF

Byerlee D, Traxler G (1995) National and international wheat improvement research in the post Green Revolution period: Evolution and impacts. Am J Agr Econ 77(2):268–78

Chand R (2005) The Rice-Wheat farming System, Punjab, India. Draft manuscript. FAO, Rome

CIMMYT (2004) Global Trends Influencing CIMMYT's Future. Prepared by the Global Trends Task Force in Support of Strategic Planning at CIMMYT. CIMMYT, Mexico, DF

Cooksey LJ, Arellano E (2006) CIMMYT Training: Activities & Impact. CIMMYT, Mexico, DF

Dixon J (2003a) Economics of Conservation Agriculture: farm profitability, risks and secondary benefits from the farmers' perspective, Keynote paper, Second World Congress of Conservation Agriculture (August 2003), Foz de Iguacu, Brazil

Dixon J (2003b) Targeting Agricultural Services to Different Farming Systems for Sustainable Agriculture and Rural Development in Iran, National Agricultural Development Workshop (December 2003), Tehran, Iran

Dixon J, Gulliver A, with Gibbon D (2001) Farming systems and poverty. Improving farmers' livelihoods in a changing world. FAO/World Bank, Rome/Washington DC

Evenson RE, Gollin D (2003a) Assessing the impact of the Green Revolution: 1960 to 2000. Science 300:758–761

Evenson RE, Gollin D (eds) (2003b) Crop Variety Improvement and Its Effect on Productivity: The impact of international agricultural research. CABI, Wallingford

Falcon W, Naylor RL (2005). Rethinking Food Security for the 21st Century. Am J Agr Econ 87(5): 113–1127

FAO (2003) World Agriculture to 2015/2030: an FAO perspective. FAO and Earthscan, Rome, Italy

FAO (2006) http://faostat.fao.org/faostat/accessed June, 2006

Heisey PW, Lantican M, Dubin HJ (2002) Impacts of international wheat breeding research in developing countries, 1967–97. CIMMYT, Mexico, DF, pp 3–14

Hodson DP, Singh RP, Dixon JM (2005) An initial assessment of the potential impact of stem rust (race UG99) on wheat producing regions of Africa and Asia using GIS. Poster presentation, 7th International Wheat Conference, Nov 27–Dec. 2, 2005, Mar del Plata, Argentina, p 142

Lantican MA, Pingali PL, Rajaram S (2002) Are marginal wheat environments catching up? In: Ekboir J (ed) CIMMYT 2000–2001 World Wheat Overview and Outlook: Developing No-Till Packages for Small Scale Farmers. CIMMYT, Mexico, DF, pp 39–44

Lantican MA, Dubin MJ, Morris ML (2005) Impacts of International Wheat Breeding Research in the Developing World, 1988–2002. CIMMYT, Mexico, DF

Laxmi V, Erenstein O, Gupta RK (2005) Assessing the impact of NRM research: The case of zero tillage in India's rice-wheat systems. SPIA NRM Case Study presentation to CGIAR AGM (December 2005), Marrakech, Morocco

La Rovere R, Hodson D, He Z, Mezzalama M, Ban T, Payne T, Trethowan R, Crouch J, Lewis J, Sayre K, Braun H-J, Nicol J, Capettini F, Smale M, Dixon J (2006) Early warning on scab epidemics: A scoping study. Draft research Report: CIMMYT

Lee DR (2005) Agricultural Sustainability and Technology Adoption: Issues and Policies for Developing Countries. Am J Agr Econ 87(5):1325–1334

Marasas CN, Smale M, Singh RP (2004) The economic impact in developing countries of leaf rust resistance breeding in CIMMYT-related spring bread wheat. Economics Program Paper 04–01. CIMMYT, Mexico, DF

Maredia MK, Byerlee D (eds) (1999) The Global Wheat Improvement System: Prospects for Enhancing Efficiency in the Presence of Spillovers. Research Report 5. CIMMYT, Mexico, DF

Ortiz-Monasterio JI, Sayre KD, Rajaram S, McMahon M (1997) Genetic progress in wheat yield and nitrogen use efficiency under four nitrogen rates. Crop Sci 37:898–904

Pingali PL (ed) (1999) CIMMYT 1998–99. World Wheat Facts and Trends. Global Wheat Research in a Changing World. Challenges and Achievements. CIMMYT, Mexico, DF

Pingali P, Raney T (2005) From the Green Revolution to the Gene Revolution: How Will the Poor Fare? ESA Working Paper 05–09, Agricultural and Development Economics Division, FAO, Rome, Italy

Reardon T, Berdegue JA (2002) The Rapid Rise of Supermarkets in Latin America: Challenges and Opportunities for Development. Development Policy Review 20(4):371–388

Reynolds MP, Borlaug NE (2006a) Impacts of breeding on international collaborative wheat improvement. J Agr Sci 144: 3–17

Reynolds MP, Borlaug NE (2006b) Applying innovations and new technologies for international collaborative wheat improvement. J Agr Sci 144:95–100

Runge CF, Senauer B, Pardey PG, Rosegrant MW (2003) Ending Hunger in Our Lifetime: Food Security and Globalization. John Hopkins University Press, Washington

Ryan J (2005) International Public Goods and the CGIAR Niche in the R for D Continuum: Operationalising Concepts. Discussion paper, CGIAR Annual General Meeting, Morocco

Sayre KD, Hobbs PR (2004) The raised bed system of cultivation for irrigated production conditions. In: Lal R, Hobbs PR, Uphoff N, Hansen DO (eds) Sustainable Agriculture and the International Rice-Wheat System. Marcel Decker, New York, pp 337–355

Seth A, Fischer K, Anderson J, Jha D (2003) The Rice-Wheat Consortium: An Institutional Innovation in International Agricultural Research on the Rice-Wheat Cropping Systems of the Indo-Gangetic Plains (IGP). New Delhi, The Review Panel Report. RWC

Smale M (1997) The Green Revolution and wheat genetic diversity: some unfounded assumptions. World Dev 25:1257–1269

Smale M, Reynolds MP, Warburton M, Skovmand B, Trethowan R, Singh RP, Ortiz-Monasterio I, Crossa J (2002) Dimensions of diversity in modern spring wheat in developing countries from 1965. Crop Sci 42:1766–79

Trethowan RM, van Ginkel M, Rajaram S (2002) Progress in breeding for yield and adaptation in global drought affected environments. Crop Sci 42:1441–1446

Trethowan RM, Hodson D, Braun H-J, Pfeiffer WH, van Ginkel M (2005) Wheat Breeding Environments. In Lantican MA, Dubin MJ, Morris ML. Impacts of International Wheat Breeding Research in the Developing World, 1988–2002. CIMMYT, Mexico, DF, Chapter 2

Witcombe JR, Gyawalli S, Sunwar S (2005) Participatory Plant Breeding is Better Described as Highly Client-Oriented Plant Breeding. II Optional Farmer Collaboration in the Segregating Generations. Exp Agr 42:79–90

THE FUSARIUM HEAD BLIGHT PATHOSYSTEM

Status and knowledge of its components

P. NICHOLSON*, N. GOSMAN, R. DRAEGER, M. THOMSETT, E. CHANDLER AND A. STEED

John Innes Centre, Norwich Research Park, Colney, Norwich NR4 7 UH

**E-mail: paul.nicholson@bbsrc.ac.uk*

Keywords: wheat, fusarium head blight

INTRODUCTION

Fusarium Head Blight (FHB), or scab, is a fungal disease affecting many small-grain cereals, but is of most importance on wheat and barley (Nelson et al. 1981). FHB reduces kernel weight and consequently grain yield. The germination rate and seedling vigour are reduced when the seeds are infected. The fungus digests proteins and starch and the use of infected kernels generates technical problems because enzymes and yeast growth are inhibited by by-products of the fungus that prevent bread production (Becthel et al. 1985, Saric et al. 1997). Metabolites produced by *Fusarium* species are also the cause of 'gushing' of beer. However, of primary concern, is the contamination of grain with mycotoxins produced by several of the species associated with FHB. Several of these compounds have been shown to be harmful to human and animal health.

Yield losses caused by FHB may reach 50–60% (Miller and Trenholm 1994, Windels 2000). In Paraguay, weather conditions in 1972–1975 favoured *Fusarium* and *Septoria* epidemics that together accounted for losses up to 70%. In the USA, yield losses and damage caused by FHB was estimated to be about $3 billion in the 90's and $220 million in the Canada (Windels 2000). Damages were also reported in China: FHB may affect up to 7 millions ha and 2.5 million tonnes of grain may be lost in epidemic years. In Minnesota, the disease has had an impact on cropping patterns, between 1992 and 1998 the amount of land planted to wheat decreased of 31% (Windels 2000).

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 23-36

CAUSAL AGENTS

Fusarium head blight (FHB) of wheat and barley is caused by several fungal species that cause similar symptoms. Although a large number of *Fusarium* species have been isolated from blighted wheat, relatively few are considered to be of overall significance (Parry et al. 1995). *Fusarium graminearum* is the major pathogen worldwide, while *F. culmorum* tends to predominate in maritime regions such as the UK. Recently, *Fusarium graminearum* has been recognised to consist of a number of lineages or groupings (O'Donnell et al. 1999, Carter et al. 2000, Carter et al. 2002). This culminated in the description of eight species within the *F. graminearum* clade. Isolates formerly described as lineage 7 retain the name *F. graminearum*. Particular lineages/groups appear to be associated with geographic regions and mycotoxin chemotypes (Nicholson et al. unpublished). *Fusarium avenaceum* and *F. poae* are also frequently associated with FHB, particularly in Northern Europe. In addition to the true *Fusarium* species, two varieties of *Microdochium nivale* (var. *nivale* and var. *majus*) also cause FHB and are particularly prevalent where cooler, wetter conditions prevail. These too, have recently been given species status. The relative contribution of each of the above species in causing disease will depend upon a range of variables, possibly the most important of which is environment.

FUNGAL TOXINS

The mycotoxins of primary concern with respect to FHB are the trichothecenes. Many *Fusarium* species produce trichothecene mycotoxins, including *F. culmorum, F. graminearum* (teleomorph: *G. zeae*), *F. poae, F. crookwellense, F. sporotrichioides* and *F. sambucinum* (teleomorph: *G. pulicaris*) (Desjardins and Hohn 1997).

Trichothecenes are sesquiterpene epoxides (Desjardins et al. 1993), and are low molecular weight, biologically active, fungal secondary metabolites (Freeman and Morrison 1948). Trichothecenes are potent inhibitors of protein synthesis in eukaryotic cells, and therefore active in a wide range of plant and animal hosts (Rotter et al. 1996). They interfere with the peptidyl transferase activity of ribosomes throughout the translational process (Cundcliffe and Davies 1977). Trichothecenes have been classified into 4 types, two of which are produced by *Fusarium* species; Type-A trichothecenes are characterised by a functional group other than a ketone at position C-8, Type-B trichothecenes have a ketone at that position (Gutleb et al. 2002).

The most common trichothecene in blighted grain is deoxynivalenol (DON), produced by *Fusarium graminearum* and *F. culmorum*. This compound often occurs along with acetylated forms such as 3-acetyldeoxynivalenol and 15-acetyldeoxynivalenol. A second, closely related trichothecene, produced by certain isolates of these species is nivalenol (NIV). This compound is believed to be more toxic than DON or its acetylated derivatives and hence is of importance with respect to food safety (Ryu et al. 1988). Other trichothecenes commonly found in infected wheat grain are diacetoxyscirpenol, T-2 and HT-2, the former

being produced by *Fusarium poae* and other species (Logrieco et al. 2002). *Fusarium avenaceum* is not known to produce trichothecenes but does produce other mycotoxins including moniliformin and enniatins (Golinski et al. 1996, Herrmann et al. 1996). Another mycotoxin, produced mainly by *F. culmorum, F. graminearum* and *F. cerealis* is zearalenone (ZEN) which commonly co-occurs with trichothecenes (Placinta et al. 1999). In contrast, neither variety of *Microdochium nivale* is known to produce mycotoxins.

INFECTION

Infection of the wheat head by macroconidia of *Fusarium* species such as *F. graminearum* is optimal in warm and wet weather: 100% humidity and 25 °C but other species have lower temperature optima. The spread of the disease can occur by various animal vectors, rain splash of conidia or by air-borne ascospores, produced on the base of the plant or crop debris (Champeil et al. 2004). Infection of wheat plants can occur at anytime between emergence and maturity, but wheat is most susceptible during flowering (Pugh et al. 1933). Two compounds, choline and betaine, present in anthers have been shown to stimulate growth of *F. graminearum* in the stage immediately post-germination (Strange and Smith 1978). Other studies have confirmed the absence of an effect at other growth stages (Engle et al. 2004). It appears that these compounds are limiting for initial growth of the fungus and their raised levels during anthesis aids infection. Physical emasculation of wheat flowers significantly reduced disease, demonstrating the enhanced susceptibility due to the anthers (Strange and Smith 1971). A similar effect of anthers has been shown in the interaction between *F. graminearum* and *Arabidopsis thaliana* (Urban et al. 2002).

Initial symptoms of FHB are small brown water-soaked marks on the glumes that expand to affect the whole spikelet. Under humid conditions the affected spikelet can become covered with white or pink mycelium and the disease spreads to other spikelets. The most severe symptoms are a result of fungal growth into the central axis of the ear and into the rachis. Then the fungus prevents translocation of water and nutrients. The ear takes on the appearance of premature ripening, and later, can become darkened because of the growth of saprophytic moulds.

A number of studies have provided insight into the infection and colonisation process. Where anthers are retained in florets, they can be covered by dense fungal growth (Kang and Buchenauer 2000) and pollen grains can be invaded and destroyed by *F. graminearum* (Ribichich et al. 2000). Where anthers are not retained within florets the route of infection of appears generally to be via the inner surface of glumes, lemma or palea although ovary tissue is also susceptible to infection (Kang and Buchenauer 2000). Dense hyphal networks developed on inner surfaces, in contrast to the sparse growth on outer surfaces (Kang and Buchenauer 2000). Direct penetration of these tissues was observed for *F. culmorum* (Kang and Buchenauer 2000) but reports for *F. graminearum* showed that penetration was predominantly through stomata (Ribichich et al. 2000, Pritsch et al. 2000). Fungal spread within tissues was shown to be both intra- and intercellular although hyphal growth at

the infection front was predominantly inter-cellular (Kang and Buchenauer 2000). Pritsch et al. (2000) concluded that chlorenchyma tissue beneath stomata on the abaxial surface of glumes was a target for early infection by *F. graminearum* and Ribichich et al. (2000) also found this tissue to be particularly damaged. Fungal growth from all infected tissues extended down to the rachis node and then on into the rachis. Kang and Buchenauer (2000) observed growth in rachis tissue above the node to be confined to intercellular spaces whereas below the node dense hyphal growth occurred. This difference may be associated with observations that fungal growth from infected spikelets tends to progress towards the base of the head. The development of *Fusarium*-tagged with reporter genes such as green fluorescent protein (GFP) permits real-time non-destructive study of host plant interaction but, to date, reports of the use of such isolates is limited (Skadsen and Hohn 2004).

TOXIN PRODUCTION *IN PLANTA*

Immunocytochemical localisation studies by Kang and Buchenauer (1999) showed that toxin accumulation in *F. culmorum* inoculated wheat heads had a close relationship with symptom appearance and fungal colonisation. Toxins were detected as early as 36 hours after inoculation in the cell wall and cells of the epidermis prior to invasion by the pathogen (Kang and Buchenauer, 2002a). Toxin was detected in xylem and phloem tissues above the point of inoculation while the fungus was absent. They concluded that mycotoxins can be translocated upwards through the xylem, and phloem sieve tubes, and downwards through the phloem sieve tubes (Kang and Buchenauer 1999).

The *TRI*5 gene encodes the enzyme trichodiene synthase and catalyses the first step of the trichothecene biosynthetic pathway (Desjardins et al. 1993), making it ideal for use in gene expression studies. Doohan et al. (1999) developed a reverse transcription PCR (RT-PCR) assay for the quantification of the *TRI*5 gene and observed differential expression of the *Tri*5 in grain and chaff tissues. These studies have been greatly extended and, while synthesis of toxin continues in chaff tissues throughout maturation, synthesis in the grain ceases soon after infection (Draeger et al. unpublished).

RESISTANCE TO FHB

Differences in susceptibility to FHB have been observed in wheat varieties and wild relatives in a number of studies (Snijders 1990a, Saur 1991). Four sources of FHB resistance tend to predominate in wheat breeding programmes, winter wheats from Eastern Europe (Snijders 1990a) and spring wheats from China, Japan and Brazil (Miedaner 1997). Resistance to head blight in wheat is generally thought to be non-specific and thus protect against all species of *Fusarium* and *Microdochium* (van Eeuwijk et al. 1995). However, recent studies indicate that interactions may be more complex (Nicholson et al. unpublished). Resistance is quantitative in nature and high levels of resistance are probably the result of several or many genes

(Snijders 1990b). The inheritance of the resistance can be described by a dominance-additive effect model, in which the additive effect is the essential factor (Buerstmayr et al. 1999). Some morphological characteristics such as head anatomy/positioning, or stature (short statured genotypes have a long grain filling and more disease than genotypes that are tall and have a rapid grain filling) influence susceptibility but they are of minor significance compared with physiological resistance (Rudd et al. 2001).

Schroeder and Christensen (1963) described 2 types of physiological resistance. Type I resistance is resistance to initial infection. It may be passive involving morphological characteristics of the wheat head. Alternatively type 1 resistance may be active and include defence reactions such as the activation of enzymes degrading the fungal cell wall or pathogenesis-related (PR) proteins. Type II resistance is generally considered to be prevention of movement of the pathogen from one infected spikelet to another via the rachis. The mechanisms involved in type II resistance are thought to be active but again may be due to morphological characteristics. Type II resistance is estimated by observing the spread of symptoms within the head after inoculation of individual spikelets. In contrast, type I resistance is assessed by spraying heads with conidia. This method, however, is complicated because, in the absence of significant type 2 resistance the pathogen will spread within the head making such assays estimates of the combined effect of type 1 and type 2 resistance. Two additional mechanisms of resistance have been proposed in relation to the effect of DON and these are also frequently termed 'types'. Type III resistance is the capacity to degrade deoxynivalenol (Miller et al. 1985) while type IV resistance is conferred by insensitivity to DON. Evidence for the existence of such mechanisms has been obtained for the Brazilian variety Frontana, (Miller and Arnison 1986, Miller and Ewen 1997) but confirmation of these results has yet to be obtained. Type V resistance is a resistance to kernel infection which results in differences in yield despite similar levels of symptoms. Type VI resistance is tolerance of yield, whereby yield is maintained despite the visual appearance of disease symptoms.

Among the diverse sources of resistance examined, only three chromosomes 1A, 6A and 1D have not been reported to possess loci for resistance (Zhou et al. 2002). However, it is generally recognised that Sumai 3 possesses one of the highest levels of resistance available in adapted germplasm and this has led to it being one of the best studied of resistance sources. The integration of phenotypic data with genetic maps (generated using molecular markers) combined with computer software to identify quantitative trait loci (QTL) has led to a significant advance in the dissection of polygenic traits such as FHB resistance. Several groups have identified a region on the short arm of chromosome 3B as carrying a QTL of major effect (Waldron et al. 1999, Buerstmayr et al. 2002, Anderson et al. 2001). Two additional loci (5A and 6B) have also been linked to FHB resistance of Sumai 3 in a number of studies (Waldron et al. 1999, Anderson et al. 2001, Buerstmayr et al. 2002). These loci, however, do not appear to be retained in all derivatives of Sumai 3, such as CM-82036 (Buerstmayr et al. 2002).

A single locus is unlikely to provide sufficient resistance to FHB to protect the crop under high disease pressure. For this reason it is highly desirable to identify loci on different chromosomes to facilitate combining them to enhance FHB resistance. Combining different types of resistance within a single variety should minimize the risk that the resistance will be overcome by changes in the pathogen(s) in addition to increasing the level of FHB resistance. For example, chromosome 4A of *Triticum macha* has previously been shown to possess resistance to FHB (Grausgruber et al. 1998, Mentewab et al. 2000). More recent study has shown that this resistance is of type I and also reduces DON accumulation in grain (Steed et al. 2005). Combining the 3BS resistance of Sumai 3 with that on 4A of *T. macha* may provide a means of combining type I and type II resistance. The number and combination of genes/QTL required to achieve durable, high level resistance to FHB in wheat remains to be determined. Whatever the source or genetic basis for FHB resistance it is essential that reduced symptoms are accompanied by reduced levels of trichothecene mycotoxins accumulating in the grain.

HOST RESPONSES

Inducible plant responses to attack by pathogenic micro-organisms are complex and many are believed to have a role in defence. These include, generation of reactive oxygen species, production of phytoalexins, cell wall modification (callose, glycine or hydroxyproline-rich proteins); and, production of pathogenesis-related (PR) proteins. The β-1,3 glucanases and chitinases (PR2 and PR3 proteins respectively) hydrolyze the chitin and β-glucan components of fungal walls.

Kang and Buchenauer (2002a) showed that the accumulation of β-1,3 glucanases and chitinases increased significantly more in wheat lemma, ovary and rachis tissues of the resistant variety Arina than in those of the susceptible variety Agent in response to infection by *F. culmorum*. They also observed thick-layered appositions and large papillae in the resistant host tissue, and detected β-1, 3-glucan (callose) in these structures. Similarly, Li et al. (2001), observed that the expression of genes for β-1,3-glucanases and acidic chitinases was induced in earlier in the FHB resistant genotype (Sumai-3) than the susceptible (Wheaton) variety following infection by *F. graminearum*. In a separate study, Anand et al. (2003) showed that transgenic wheat expressing both a β-1,3-glucanase gene and a chitinase gene have a delayed infection in greenhouse conditions but not in field conditions. More recently, a gene encoding acidic chitinase was found to be upregulated in the FHB resistant variety Ning7840 following inoculation with *F. graminearum* (Kong et al. 2005).

Expression of PR genes was studied in heads of FHB resistant (Sumai-3) and susceptible (Wheaton) varieties in response to *F. graminearum* (Pritsch et al. 2000). The expression of PR1, PR2 (β-1,3-glucanase), PR3 (chitinase), PR4 (wheatwin), PR5 (thaumatin-like protein) and peroxidase were measured by northern-blot. Expression of PR genes was detected 6 hpi with similar levels of PR1, PR2, PR3, and PR5 transcripts for the two varieties. Expression of PR4, however, was earlier and higher in Sumai-3 than in Wheaton. They concluded that the induction of the

defence gene expression was correlated to *F. graminearum* resistance. A subsequent study confirmed these results and demonstrated the systemic expression of these PR genes in response to infection by *F. graminearum* (Pritsch et al. 2001).

Kruger et al. (2002) generated 3,546 non-redundant sequences from a cDNA library prepared from Sumai-3 ears inoculated with *F. graminearum* to determine the array of genes expressed during the *F. graminearum* infection process in wheat. They found genes encoding PR-1, β-1,3-glucanase, chitinase and thaumatin-like proteins in multiple copies suggesting they were highly expressed in early infection by *F. graminearum*, which is in agreement with findings by Pritsch et al. (2000). They concluded that some of the sequences observed could encode proteins that act in the establishment of various plant-fungal interactions.

Other antifungal compounds have also been implicated in resistance to FHB. Polyphenol oxidase (PPO) and a peroxidase (POX) (PR9 protein) activities were shown to increase in response to infection of wheat heads by *F. graminearum*. However, only the activity of PPO was significantly higher in the resistant varieties (Wangshuibai and Sumai-3) than in the susceptible (Falat and Golestan) (Mohammadi and Kazemi 2002). Thus PPO may play a defensive role in FHB resistance in Sumai-3 and Wangshuibai.

Lignification and the deposition of phenolic compounds can prevent penetration by creating a mechanical barrier through modification of the cell walls, rendering the cells more resistant to cell wall degrading enzymes (Siranidou et al. 2002). Lignification may also reduce diffusion of toxin into host tissue and limit the flow of nutrients from host to fungus (Siranidou et al. 2002). Siranidou et al. (2002) showed that lignin deposition in cell walls adjacent to *Fusarium* increased in the FHB resistant variety Frontana but not in Agent (susceptible). The authors concluded that phenolic compounds have a role in the resistance of Frontana to *F. culmorum*. Other cell wall modifications have been associated with resistance to FHB. Accumulation of cell wall-bound thionins and hydroxyproline-rich glycoproteins (HRGP) increased significantly in the FHB resistant variety Arina but not in Agent in *F. culmorum* infected wheat heads (Kang and Buchenauer 2003).

Kang and Buchenauer (2002b) and Wanjiru et al. (2002) showed that cellulose, xylan and pectin are reduced in the wall of wheat cells in contact with invading *Fusarium* hyphae. Thus cellulases, xylanases and pectinases may be important for pathogenicity. Xylanase inhibitors such as TAXI (Igawa et al. 2004) and XIP (Juge et al. 2004) are hypothesised to have a role in preventing cell wall destruction, but to date, study of these proteins in relation to FHB has been limited.

ROLE OF MYCOTOXINS IN FHB

Genetic analysis of the role of trichothecenes in the virulence of *F. graminearum* on wheat and maize was summarised by Proctor et al. (2002). *TRI5* encodes trichodiene synthase which catalyses the first committed reaction in trichothecene biosynthesis. Mutants of *F. graminearum* with an inactivated *TRI5* gene cannot produce DON or any biosynthetic trichothecene intermediates (Proctor et al. 1995, Desjardins

et al. 1996). In the field trichothecene non-producing *TRI5*-disrupted mutants caused less disease than the trichothecene-producing progenitor strains (Desjardins et al. 1996). Similar results have been observed for maize (Harris et al. 1999). However, other studies involving different *TRI5* mutants showed that DON, while a virulence factor for infection of wheat did not contribute towards virulence on barley or maize (Maier et al. 2004).

Similar host-toxin interactions have been found for toxins produced by other *Fusarium* species. Enniatin non-producing strains of *F. avenaceum* were produced by disruption of the *ESYN1* gene encoding the multifunctional enniatin synthetase enzyme (Herrmann et al. 1996). Enniatin non-producing strains induced significantly less necrosis on potato tuber than the progenitor strains and it was concluded that enniatin production contributed to the virulence against potato tuber tissue (Herrmann et al. 1996). However, no such differences were observed for infection of seedlings of wheat or rye by these isolates (Nicholson et al. unpublished). Thus *Fusarium* toxins may not be entirely non-specific. There may be more complex interactions between toxins and different host species.

FHB AND RESISTANCE TO DON

Resistance to the effects of DON may limit the ability of the fungus to infect the grain and lead to reduced levels of mycotoxin or alternatively, other forms of resistance may prevent the growth of the fungus or restrict its ability to produce mycotoxin, again resulting in reduced levels of mycotoxin in grain. Highly resistant varieties tend to restrict accumulation of DON but few studies have demonstrated that disease and DON reduction is due to the action of the same gene(s). Work by Bai et al. (2001) showed that DON production is not necessary for initial infection and colonisation of inoculated spikelets by *F. graminearum*. However, their data indicated that DON production plays a significant role in the spread of FHB within the head. Thus DON may be important in respect to type II resistance but not for type I.

The putative site of action of DON is thought to be the 60S ribosomal protein L3 (*RPL3*) (Harris and Gleddie 2001). A Recent study has introduced a modified version of the *RPL3* gene into tobacco plants and found that tissues and cells from the transgenic plants with the modified gene had a significantly increased growth rate and ability to differentiate than those expressing the unmodified gene when grown in the presence of DON (Harris and Gleddie 2001). The modification of the target site for trichothecenes in host plants has allowed protein synthesis to continue in the presence of trichothecenes and so provides a possible mechanism for plant resistance to *F. graminearum* infection (Harris and Gleddie 2001). However, only very limited allelic diversity has been identified for the six (Group 4 and 5) *RPL3* genes in wheat and none has been associated with FHB resistance to date (Adam et al; Nicholson et al. unpublished). Alternative DON-related mechanisms of FHB resistance may exist.

Recently Lemmens et al. (2004) demonstrated that the type II resistance associated with the *Qfhs.nsdu-3BS* QTL of Sumai-3 confers resistance to DON. They also showed that DON was converted to DON-3-glucoside and concluded that this QTL either encodes a glucosyl-transferase or regulates expression of such an enzyme. In separate studies of the winter wheat variety WEK0609 an association between tolerance to DON (measured in a seed germination assay) and the *Qfhs.nsdu-3BS* QTL was also demonstrated. A similar relationship was also found for a second QTL conferring type II FHB resistance, derived from Aurora (Gosman et al. unpublished). In these studies we showed that a third QTL conferring type II FHB resistance was not associated with DON tolerance in germination assays. Thus, while type II resistance may be associated with tolerance to DON this may not always be the case.

Thus evidence indicates a role of DON in spread of the fungus within the wheat head and also for DON tolerance providing a potential mechanism to provide type II resistance. We have carried out a number of experiments involving DON-producing and non DON-producing isolates or FHB species. In all cases, DON production confers the ability to spread within the wheat head. In contrast NIV-producing isolates are severely compromised in their ability to spread within the head. Non trichothecene-producing species such as *M. nivale* appear to be unable to spread from one spikelet to another. This finding has implications for the accepted non-specific nature of FHB resistance as there may be limited or no requirement for type II resistance against non DON-producing species or isolates.

PATHOGEN-PATHOGEN INTERACTIONS AND FUNGICIDES

Tebuconazole and metconazole have both been found to be among the most effective fungicides available against FHB and DON accumulation in grain (Matthies and Buchenauer 2000, Simpson et al. 2001, Nicholson et al. 2003). Recent work has highlighted the significance of FHB being a disease complex, involving both toxigenic *Fusarium* species and non-toxigenic species, such as *M. nivale*. Simpson et al. (2001) demonstrated the complex interaction between the fungicide, disease, colonisation by particular species and mycotoxin accumulation. While fungicides such as tebuconazole and metconazole have good activity against *Fusarium* species others, such as azoxystrobin, have greater efficacy against *M. nivale*. Where *F. culmorum* and/or *F. graminearum* are present along with *M. nivale* application of azoxystrobin can result in a decrease in disease but a higher level of DON in grain (Simpson et al. 2001, Nicholson et al. 2003). *In vitro* studies indicate that azoxystrobin is not causing an increase in biosynthesis of DON (Covarelli et al. 2004) but rather that *M. nivale* and *F. culmorum* are antagonistic, with *M. nivale* reducing both growth and DON accumulation by *F. culmorum* (Simpson et al. 2004). It is hypothesised that fungicides such as azoxystrobin preferentially inhibit *M. nivale* allowing the *Fusarium* species to colonise the host tissues to a greater extent.

Even where the most effective fungicides are applied, control of disease and mycotoxin accumulation may not be achieved. The effectiveness of fungicides

such as tebuconazole decreases as the interval between treatment and inoculation increases (Matthies and Buchenauer 2000). Reduction of FHB following fungicide treatment has been reported to be greater on moderately susceptible, rather than highly susceptible varieties (Matthies and Buchenauer 2000). Thus a combination of resistant varieties and effective fungicides, appropriately applied, are likely to be required to provide sufficient protection against this disease and its associated mycotoxins where disease pressure is high.

CONCLUSIONS

While the availability of new research tools is enabling detailed study of the interaction between wheat and *Fusarium* species and between the pathogen species themselves, much remains unclear and we still await the cloning of the first gene conferring resistance to FHB.

ACKNOWLEDGEMENTS

This research was supported by a DEFRA-LINK project (No. LK0932), a European Union funded project (QLRT-2001-02044) and a BBSRC CASE studentship with Pioneer Hi-Bred International Inc. The Authors would like to thank our numerous collaborators from other labs for their invaluable input.

REFERENCES

Anderson JA, Stack RW, Liu S, Waldron BL, Fjeld AD, Coyne C, Moreno-Sevilla B, Fetch JM, Song QJ, Cregan PB, Frohberg RC (2001) DNA markers for Fusarium head blight resistance QTLs its two wheat populations. Theor Appl Genet 102:1164–1168

Anand A, Zhou T, Trick HN, Gill BS, Bockus WW, Muthukrishnan S (2003) Greenhouse and field testing of transgenic wheat plants stably expressing genes for Thaumatin-Like Protein, Chitinase and Glucanase against Fusarium Graminearum. J Exp Bot 54:1101–1111

Bai GH, Desjardins A, Plattner R (2001) Deoxynivalenol-nonproducing Fusarium graminearum causes initial infection but does not cause disease spread in wheat spikes. Mycopathologia 153:91–98

Bechtel DB, Kaleiken LA, Gaines RL, Seitz LM (1985) The effects of Fusarium graminearum infection on wheat kernels. Cereal Chem 62:191–197

Buerstmayr H, Lemmens M, Fedak G, Ruckenbauer P (1999). Back-cross reciprocal monosomic analysis of Fusarium head blight resistance in wheat (Triticum aestivum L.). Theor Appl Genet 98:76–85

Buerstmayr H, Lemmens M, Hartl L, Doldi L, Steiner B, Stierschneider M, Ruckenbauer P (2002). Molecular mapping of QTLs for Fusarium head blight resistance in spring wheat. I. Resistance to fungal spread (type II resistance). Theor Appl Genet 104(1):84–91

Carter JP, Rezanoor HN, Desjardins AE, Nicholson P (2000) Variation in Fusarium graminearum isolates from Nepal associated with their host of origin. Plant Pathol 49(4):452–460

Carter JP, Rezanoor HN, Holden D, Desjardins AE, Plattner RD, Nicholson P (2002) Variation in pathogenicity associated with the genetic diversity of Fusarium graminearum. Eur J Plant Pathol 108(6):573–583

Champeil A, Dore T, Fourbet JF (2004) Fusarium head blight: epidemiological origin of the effects of cultural practices on head blight attacks and the production of Mycotoxins by Fusarium in wheat grains. Plant Sci 166:1389–1415

Covarelli L, Turner AS, Nicholson P (2004) Repression of deoxynivalenol accumulation and expression of Tri genes in Fusarium culmorum by fungicides in vitro Plant Pathol 53:22–28

Cundcliffe E, Davies JE (1977) Inhibition of initiation, elongation and termination of eukaryotic protein synthesis by trichothecene fungal toxins. Antimicrob Agents Chemother 11:491–499

Desjardins AE, Hohn TM (1997) Mycotoxins in plant pathogenesis. Mol Plant Microbe Interact 10: 147–152

Desjardins, AE, Hohn TM, McCormick SP (1993). Trichothecene biosynthesis in Fusarium species: chemistry, genetics, and significance. Microbiol Mol Biol Rev 57:593–604

Desjardins AE, Proctor RH, Bai GH, McCormick SP, Shaner G, Buechley G, Hohn TM (1996) Reduced virulence of trichothecene-nonproducing mutants of Gibberella zeae in wheat field tests. Mol Plant Microbe Interact 9(9):775–781

Doohan FM, Weston G, Rezanoor HN, Parry DW, Nicholson P (1999) Development and use of a reverse transcription-PCR assay to study expression of Tri5 by Fusarium species in vitro and in planta. Appl Environ Microbiol 65:3850–3854

Engle JS, Lipps PE, Graham TL, Boehm MJ (2004) Effects of choline, betaine and wheat floral extracts on growth of Fusarium graminearum. Plant Dis 88:175–180

Freeman GG, Morrison RI (1948) Trichothecene: An antifungal metabolic product of Trichothecium roseum. Link. Nature 167:30

Golinski P, Kostecki M, Lasocka, I, Wisniewska H, Chelkowski J, Kaczmarek Z (1996) Moniliformin accumulation and other effects of Fusarium avenaceum (Fr) Sacc on kernels of winter wheat cultivars. J Phytopathol 144(9–10):495–499

Grausgruber H, Lemmens M, Burstmayr H, Ruckenbauer P (1998) Chromosomal Location of Fusarium head blight resistance and in vitro toxin tolerance in wheat using the Hobbit 'sib' (Triticum macha) chromosome substitution lines. J Genet Breed 53:173–180.

Gutleb AC, Morrison E, Murk AJ (2002) Cytotoxicity assays for mycotoxins produced by Fusarium strains: a review. Environ Toxicol Pharmacol 11:309–320

Harris LJ, Gleddie SC (2001) A modified Rpl3 gene from rice confers tolerance of the Fusarium graminearum mycotoxin deoxynivalenol to transgenic tobacco. Pysiol Mol Plant Pathol 58(4):173–181

Harris LJ, Desjardins AE, Plattner RD, Nicholson P, Butler G, Young JC, Weston G, Proctor RH, Hohn TM (1999) Possible role of trichothecene mycotoxins in virulence of Fusarium graminearum on maize. Plant Dis 83(10):954–960

Herrmann M, Zocher R, Haese A (1996) Enniatin production by Fusarium strains and its effect on potato tuber tissue. Appl Environ Microbiol 62(2):393–398

Igawa T, Ochiai-Fukuda T, Takahashi-Ando N, Ohsato S, Shibata T, Yamaguchi I, Kimura M (2004) New TAXI-type xylanase inhibitor genes are inducible by pathogens and wounding in hexaploid wheat. Plant Cell Physiol 45:1347–1360

Juge N, Payan F, Williamson G (2004) XIP-I, a Xylanase Inhibitor Protein from wheat: a novel protein function. Biochim Biophys Acta Proteins Proteomics 1696:203–211

Kang Z, Buchenauer H (1999) Immunocytochemical localization of Fusarium toxins in infected wheat spikes by Fusarium culmorum. Pysiol Mol Plant Pathol 55:275–288

Kang Z, Buchenauer H (2000) Ultrastructural and immunocytochemical investigation of pathogen development and host responses in resistant and susceptible wheat spikes infected by Fusarium culmorum. Pysiol Mol Plant Pathol 57:255–268

Kang Z, Buchenauer H (2002a) Immunocytochemical localization of β-1,3-glucanase and chitinase in Fusarium culmorum-infected wheat spikes. Pysiol Mol Plant Pathol 60:141–153.

Kang Z, Buchenauer H (2002b) Studies on the infection process of Fusarium culmorum in wheat spikes: degradation of host cell wall components and localization of trichothecene toxins in infected tissue. Eur J Plant Pathol 108:653–660

Kang Z, Buchenauer H (2003) Immunocytochemical localization of cell wall-bound thionins and hydroxyproline-rich glycoproteins in Fusarium culmorum-infected wheat spikes. J Phytopathology 151:120–129

Kong L, Anderson JM, Ohm HW (2005) Induction of wheat defense and stress-related genes in response to Fusarium graminearum. Genome 48:29–40

Kruger WM, Pritsch C, Chao S, Meuhlbauer GJ (2002) Functional and comparative bioinformatic analysis of expressed genes from wheat spikes infected with Fusarium graminearum. Mol Plant Microbe Interact 15:445–455

Lemmens M, Scholz U, Berthiller F, Koutnik A, Dall'Asta C, Schuhmacher R, Adam G, Mesterhazy A, Krska R, Beurstmayr H, Ruchenbauer P (2004) The role of resistance to deoxynivalenol in the complex Fusarium head blight resistance complex in wheat. In: Canty SM, Boring T, Wardwell J, Ward RW (eds) Proceedings of the 2nd International Symposium on Fusarium Head Blight; incorporating the 8th European Fusarium Seminar, 11–15 December, Orlando, FL, USA. Michigan State University, East Lansing, MI, USA, vol 1, p 88

Li WL, Faris JD, Muthukrishnan S, Liu DJ, Chen PD, Gill BS (2001) Isolation and characterization of novel CDNA clones of acidic chitinases and Beta-1,3-glucanases from wheat spikes infected by Fusarium Graminearum. Theor Appl Genet 102:353–362

Logrieco A, Mule G, Moretti A, Bottalico A (2002) Toxigenic Fusarium species and mycotoxins associated with maize ear rot in Europe. Eur J Plant Pathol 108(7):597–609

Maier FJ, Miedaner T, Salomon S, Hadeler B, Felk A, Henning S, Lemmens M, Shafer W (2004) The trichothecenes are major virulence factors of Fusarium graminearum to wheat, but dispensible for infection of maize and barley. In: Canty SM, Boring T, Wardwell J, Ward RW (eds) Proceedings of the 2nd International Symposium on Fusarium Head Blight; incorporating the 8th European Fusarium Seminar, 11–15 December, Orlando, FL, USA. Michigan State University, East Lansing, MI, USA, p 474

Matthies A, Buchenauer H (2000) Effect of tebuconazole (Folicur (R)) and prochloraz (Sportak (R)) treatments on Fusarium head scab development, yield and deoxynivalenol (DON) content in grains of wheat following artificial inoculation with Fusarium culmorum. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz-Journal of Plant Diseases and Protection, 107(1):33–52

Mentewab A, Rezanoor HN, Gosman N, Worland AJ, Nicholson P (2000) Chromosomal location of Fusarium head blight resistance genes and analysis of the relationship between resistance to head blight and brown foot rot. Plant Breed 119(1):15–20

Miedaner T (1997) Breeding wheat and rye for resistance to Fusarium diseases. Plant Breed 116:201–220

Miller JD, Arnison PG (1986) Degradation of deoxynivalenol by suspension-cultures of the fusarium head blight resistant wheat cultivar Frontana. Can J Plant Pathol 8(2):147–150

Miller JD, Ewen MA (1997) Toxic effects of deoxynivalenol on ribosomes and tissues of the spring wheat cultivars Frontana and Casavant. Nat Toxins 5:234–237

Miller JD, Trenholm HL (1994) Mycotoxins in grains: compounds other than aflatoxin. Eagan Press, St Paul, Minnesota.

Miller JD, Young JC, Sampson DR (1985) Deoxynivalenol and Fusarium head blight resistance in spring cereals. J Phytopathol 113:359–367

Mohammadi M, Kazemi H (2002) Changes in Peroxidase and Polyphenol Oxidase activities in susceptible and resistant wheat heads inoculated with Fusarium graminearum and induced resistance. Plant Sci 162:491–498

Nelson PE (1981) Fusarium: disease, biology and taxonomy. USA Pensylvania University Press 1981

Nicholson P, Turner JA, Jenkinson P, Jennings P, Stonehouse J, Nuttall M, Dring D, Weston G, Thomsett M (2003) Maximising control with fungicides of Fusarium ear blight (FEB) in order to reduce toxin contamination of wheat. Home-Grown Cereals Authority Report No. 299e. London, Home-Grown Cereals Authority

O'Donnell K, Kistler HC, Tacke BK, Casper HH (1999) Gene genealogies reveal global phylogeographic structures and reproductive isolation among lineages of Fusarium graminearum the fungus causing wheat scab. Proc Natl Acad Sci USA 97:7905–7910

Parry DW, Jenkinson P, MCleod L (1995) Fusarium ear blight (scab) in small-grain cereals – a review. Plant Pathol 44(2):207–238

Placinta CM, D'Mello JPF, Macdonald AMC (1999) A review of worldwide contamination of cereal grains and animal feed with Fusarium mycotoxins. Animal Feed Sci Technol 78(1–2):21–37

Pritsch C, Muehlbauer GJ, Bushnell WR, Somers DA, Vance CP (2000) Fungal development and induction of defense response genes during early infection of wheat spikes by Fusarium graminearum. Mol Plant Microbe Interact 13:159–169

Pritsch C, Vance CP, Bushnell WR, Somers DA, Hohn TM, Muehlbauer GJ (2001) Systemic expression of defense response genes in wheat spikes as a response to Fusarium graminearum infection. Pysiol Mol Plant Pathol 58:1–12

Proctor RH, Hohn TM, McCormick SP (1995) Reduced virulence of gibberella-zeae caused by disruption of a trichothecene toxin biosynthetic gene. Mol Plant Microbe Interact 8(4):593–601

Proctor RH, Desjardins AE, McCormick SP, Plattner RD, Alexander NJ, Brown DW (2002) Genetic analysis of the role of trichothecene and fumonisin mycotoxins in the virulence of Fusarium. Eur J Plant Pathol 108(7):691–698

Pugh GW, Johann H, Dickson JG (1933) Factors affecting infection of wheat heads by Gibberella saubinetii. J Agric Res 46:771–797

Ribichich KF, Lopez SE, Vegetti AC (2000) Histopathological spikelet changes produced by Fusarium graminearum in susceptible and resistant wheat cultivars. Plant Dis 84:794–802

Rotter BA, Prelusky DB (1996) Toxicology of deoxynivalenol (vomitoxin). J Toxicol and Environ Health 48:1–34

Rudd JC, Horsley RD, McKendry AL, Elias EM (2001) Host plant resistance genes for Fusarium head blight: sources, mechanisms, and utility in conventional breeding systems. Crop Sci 41:620–627

Ryu JC, Ohtsubo K, Izumiyama N, Nakamura K, Tanaka T, Yamamura H, Ueno Y (1988) The acute and chronic toxicities of nivalenol in mice. Fund Appl Toxicol 11(1):38–47

Saric M, Skrinjar M, Dimic G, Filipovic N, Rasic J (1997) Changes in hygienic and technological wheat quality caused by mould infection. Acta Alimentaria 26:255–269

Saur L (1991) Sources of resistance to head blight caused by Fusarium culmorum in bread wheat and related species. Agronomie 11(7):535–541

Schroeder HW, Christensen JJ (1963) Factors affecting the resistance of wheat to scab caused by Gibberella zeae. Phytopathology 53:831–838

Simpson DR, Weston GE, Turner JA, Jennings P, Nicholson P (2001) Differential control of head blight pathogens of wheat by fungicides and consequences for mycotoxin contamination of grain. Eur J Plant Pathol 107(4):421–431

Simpson DR, Thomsett MA, Nicholson P (2004) Competitive interactions between Microdochium nivale var. majus, M-nivale var. nivale and Fusarium culmorum in planta and in vitro. Environ Microbiol 6:79–87

Siranidou E, Kang Z, Buchenauer H (2002) Studies on symptom development, phenolic compounds and morphological defence responses in wheat cultivars differing in resistance to Fusarium head blight. J Phytopathology 150:200–208

Skadsen RW, Hohn TM (2004) Use of Fusarium graminearum transformed with gfp to follow infection patterns in barely and Arabidopsis. Pysiol Mol Plant Pathol 64:45–54

Snijders CHA (1990a) Genetic variation for resistance to Fusarium head blight in bread wheat. Euphytica 50(2):171–179

Snijders CHA (1990b) The inheritance of resistance to head blight caused by Fusarium culmorum in winter-wheat. Euphytica 50(1):11–18

Steed A, Chandler E, Thomsett M, Gosman N, Faure S, Nicholson P (2005) Identification of Type 1 resistance to Fusarium head blight controlled by a major gene located on chromosome 4A of Triticum macha. Theor Appl Genet 111:521–529

Strange RN, Smith H (1971) Fungal growth stimulant in antherswhich predisposes wheat to attack by Fusarium graminearum. Physiol Plant Pathol 1:141–150

Strange RN, Smith H (1978) Effects of choline, betaine and wheat germ extract on growth of cereal pathogens. Trans Roy Mycol Soc 70:193–199

Urban M, Daniels S, Mott E, Hammond-Kosack K (2002) Arabidopsis is susceptible to the cereal ear blight fungal pathogens Fusarium graminearum and Fusarium culmorum. Plant J 32:961–973

van Eeuwijk FA, Mesterhazy A, Kling CI, Ruckenbauer P, Saur L, Burstmayr H, Lemmens M, Keizer LCP, Maurin N, Snijders CHA (1995) Assessing non-specificity of resistance in wheat to head

blight caused by inoculation with European strains of Fusarium culmorum, F. graminearum and F. nivale using a multiplicative model for interaction. Theor Appl Genet 90: 221–228

Waldron BL, Moreno-Sevilla B, Anderson JA, Stack RW, Frohberg RC (1999) RFLP mapping of QTL for fusarium head blight resistance in wheat. Crop Sci 39(3):805–811

Wang YZ, Miller JD (1988) Effects of Fusarium-graminearum metabolites on wheat tissue in relation to Fusarium head blightresistance. J Phytopathol 122(2):118–125

Wanjiru MW, Zhensheng K, Buchenauer H (2002) Importance of cell wall degrading enzymes produced by Fusarium graminearum during infection of wheat heads. Eur J Plant Pathol 108:803–810

Windels CE (2000) Economic and social impacts of Fusarium head blight: changing farms and rural communities in the Northern Great Plains. Phytopathology 90:17–21

Zhou WC, Kolb FL, Bai GH, Shaner G, Domier LL (2002) Genetic analysis of scab resistance QTL in wheat with microsatellite and AFLP markers. Genome 45(4):719–727

THE STATUS OF RESISTANCE TO BACTERIAL DISEASES OF WHEAT

H. MARAITE[1,*], C. BRAGARD[1] AND E. DUVEILLER[2]

[1] *Unité de Phytopathologie, Université catholique de Louvain, Croix du Sud 2/3, B-1348 Louvain-la-Neuve, Belgium*

[2] *CIMMYT South Asia Regional office, P.O. Box 5186, Kathmandu, Nepal*

[*] *E-mail: maraite@fymy.ucl.ac.be*

Abstract: Bacterial leaf streak and black chaff caused by *Xanthomonas translucens* pv. *undulosa* (XTU), basal glume rot caused by *Pseudomonas syringae* pv. *atrofaciens* (PSA) and bacterial leaf blight caused by *P. syringae* pv. *syringae* (PSS) are the most frequently reported bacterial diseases on wheat. Eight other bacteria have been described on wheat but have not shown severe outbreaks during the last years. Seed contamination by XTU, PSA or PSS has been demonstrated. It results from spike infection but in some cases also from contamination by bacteria released from diseased leaves of highly susceptible genotypes. Several pathovars are distinguished among *X. translucens* strains isolated from small grains on base of differences in host range. Transmission electron microscopy reveals in the compatible interaction, characterized by water-soaked lesions on the leaf, an abundant bacterial multiplication and the formation in intercellular spaces of a matrix with fibrillar material; while in incompatible reactions only limited bacterial multiplication and a restricted amount of matrix or fibrillar material is noticed. XTU designates strains pathogenic to several graminaceous hosts. XTU mutants affected in their capacity to infect some hosts can be obtained through transposon mutagenesis. DNA fingerprinting of XTU isolates reveals various genotypes. Nevertheless, in pathogenicity tests only quantitative differences in virulence are noticed, except for a few South American isolates. Field screening under natural or artificial infection has been effective in identifying wheat genotypes with high level of partial resistance. Crosses have revealed the occurrence of several resistance genes as well as both additive and dominant genetic control for partial resistance. The ranking of the genotypes appears stable over time, although differences in inoculum level and environmental conditions induce variations in actual disease severity. Inoculation at the seedling stage under controlled conditions and rating of the water-soaking reaction is not a reliable means for screening wheat for XTU resistance

The discrimination of PSA and PSS is difficult because some PSS strains also show the capacity to induce basal glume rot. Both bacteria appear to have an asymptomatic epiphytic growth in the phyllosphere of wheat, other annual or perennial *Gramineae* and possibly unrelated hosts. Symptoms develop on susceptible cultivars after periods of very humid, cool weather and wind-driven rain. Quantitative difference in virulence among strains and occurrence of races are reported. PSA and PSS are

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 37–49.

producing syringomycine and other phytotoxic and surface tension active compounds. The differences in virulence require careful selection of strains for resistance screening of germplasm under controlled conditions. A range of inoculum concentrations has been used to identify genotypes with partial resistance among winter and spring wheat cultivars of various origins. *Aegilops* genotypes show a high level of resistance

Keywords: wheat, bacterial diseases, genetic resistance

INTRODUCTION

As most crops, wheat is affected by a range of bacterial diseases that interfere in optimal leaf development, decrease yield, and put restriction on international grain trade. Nevertheless, overall relatively little attention is devoted by the farmer to the yearly control of bacterial diseases on wheat during the cropping season, compared to rusts, or Helminthosporium and Septoria leaf blights. This is due to the fact that the symptoms are sometimes confused with those of physiological or stress-induced damage. Also, the epidemic development requires the conjunction of specific climatic conditions and is thus sporadic from one to another year or across countries and continents. Differences in cultivar susceptibility or in degree of seed contamination induce further variation. Finally, management through pesticide protection of established crops is difficult and presently not considered as a practical option. Thus the main control strategies through cultivar resistance and production of disease-free seed are upstream of farmer's operation.

During this presentation we shall review data on the importance of the various bacterial diseases, and for the most important, the ecology of the diseases, pathogenic variation of the causal agent as well as sources and mechanisms of resistance in order to draw prospects of improved genetic management. The cornerstone for this review has been the book 'The Bacterial Diseases of Wheat: Concepts and Methods of Disease Management' (Duveiller et al. 1997).

MINOR BACTERIAL DISEASES OF WHEAT

Not less than 14 bacterial taxa belonging to 6 genera have been reported causing diseases on wheat (Table 1).

White blotch symptoms associated with *Bacillus megaterium* pv. *cerealis* has been reported from North Dakota (Hosford 1982) and Australia (Brennan and Murray 1998). Nevertheless, the bacteria is considered as an epiphytic colonizer of wheat leaves which leads to its isolation from spots of possibly physiological origin rather than induced by it. Some *B. megaterium* pv. *cerealis* strains have shown high potential in biocontrol of crucifer black rot (Luna et al. 2002).

Clavibacter michiganensis* subsp. *tessellarius causes small yellow undefined lesions distributed over the whole leaf. The symptoms can be confused with hypersensitive reaction to rust or physiological disorder. Although generally considered as

Table 1. Bacterial diseases reported on wheat

Causal agent	Disease	Distribution
Bacillus megaterium pv. *cerealis*	white blotch, tan streak	Australia, USA
Clavibacter michiganensis subsp. *tessellarius*	mosaic	Australia, Canada, USA,
Erwinia rhapontici	Pink seed	Australia, Europe, Israel, North America
Pseudomonas cichorii	Stem & head melanosis	Canada
Pseudomonas fuscovaginae	brown sheath rot	Brazil, Mexico, Nepal
Pseudomonas syringae pv. *atrofaciens*	basal glume rot	Africa, Australia, Europe, Syria, North America, New Zealand
Pseudomonas syringae pv. *japonica*	black node, stripe blight	Japan
Pseudomonas syringae pv. *syringae*	leaf blight	worldwide
Rathayibacter iranicus	gumming	Iran, Turkey
Rathayibacter tritici	tundu, yellow ear, rot	worldwide
Xanthomonas translucens pv. *undulosa* and related pv. *cerealis*, pv. *translucens*, pv. *secalis*	leaf streak, leaf stripe, black chaff	worldwide

of minor importance, significant losses due to bacterial mosaic have been reported (McBeath 1993). *C. michiganensis* subsp. *tessellarius* is seed-borne. The wide range of host response to the disease in spring wheat genotypes suggests that genetic improvement of this trait is feasible.

Pink seed caused by *Erwinia rhapontici* is occasionally observed on bread and durum wheat in the Northern hemisphere and Australia (Diekmann and Putter 1995). It has been recently reported from common bean (Huang et al. 2002), confirming the wide host range of this opportunistic pathogen. The light pink appearance is due to a diffusible pigment produced by the bacteria in the testa. The symptom is similar to the colouration of some fungicide seed dressings or *Fusarium* infection. Pink seed do not germinate well due to interference in the Fe^{++} uptake by the root of the proferrosamine released by the bacteria.

Pseudomonas cichorii is also a widespread bacteria with a broad host range. Spectacular head and stem melanosis have been observed in Canada on the wheat cultivar Park. The disease is favoured by the combination of high humidity and high temperature after anthesis as well as on copper deficient soils (Piening and MacPherson 1985).

P. fuscovaginae is an important opportunistic pathogen of rice when low temperatures and high humidity occur at the booting-heading stage. Under these conditions it has also been spotted on other cereals including wheat. It causes on the flag leaf sheath irregular angular blackish brown necrotic lesions bordered by a

purple-black water-soaked area. This significantly affects spike exertion and leads to partial sterility and seed contamination (Duveiller and Maraite 1990). Differences in genotype susceptibility have been observed in Nepal with Annapurna-1, Annapurna-2, Annapurna-3 and WK685, but not RR21, showing high level of infection (Anonymous 1995).

Rathayibacter iranicus was considered since 1961 as restricted to Iran (Scharif 1961). However, during recent analysis it was also revealed on wheat seeds from Central Anatolia in Turkey. Since gumming can be a serious disease, new research is undertaken on the identification and discrimination of this bacteria from closely related species such as *R. toxicus* producing a deadly animal toxin (Anonymous 2003).

Spike blight is a disease complex that includes *R. tritici* and the nematode *Anguina tritici*. It is characterized by a bright yellow gum on the leaf surface, by deformed culms, aborted spikes and seed galls (ear cockle) in some varieties (Paruthi and Bhatti 1985). The larvae and galls of *A. tritici* are vectors of the bacterium and are involved in seed-transmission. The disease has been reported on five continents but has become rare in most wheat growing areas.

DISEASES CAUSED BY *PSEUDOMONAS SYRINGAE* PATHOVARS

Taxonomy, Symptoms and Importance

Strains belonging to the heterogeneous *P. syringae* group have been associated with a wide range of diseases on herbaceous as well as arborescent plants. Up to 57 pathovars have been described but the taxonomy of this group is still discussed. On wheat, *P. syringae* pv. *atrofaciens* (PSA) is associated with basal glume rot. This disease is characterized by dull, brownish black area at the lower third of the glumes, more conspicuous at the inner surface of the glumes than at the outside. The bacterial colonization of the glumes and of the rachis spreads to the grain where it causes brown to charcoal black discolouration at the germ end (Toben 1989). This symptom can be confused with black point or infection by *Bipolaris sorokiniana*. PSA has also been isolated from dark brown or black longitudinal streaks on the upper part of the stem just above the node. However, this symptom as well as dark streaks on the entire glume, may be due to melanism induced on some cultivars by abiotic stress.

In Japan, *P. syringae* pv. *japonica* (PSJ) has been associated with dark brown lesions on the nodes and stripes on the internodes of wheat and barley (Fukuda et al. 1990). Cautiousness is thus required for identification of the cause of dark brown spots on stems and glumes.

PSJ has been considered by Young (1992) as a synonym of *P. syringae* pv. *syringae* (PSS), a pathovar associated with an extraordinary broad range of diseases on different plants. Leaf symptoms incited by PSS on wheat are generally referred to as bacterial leaf blight or leaf necrosis. Initial symptoms are observed at booting or early heading in the form of tiny, inconspicuous water-soaked spots which expand and often

coalesce into large, greyish-green, desiccated areas on the upper leaves. These areas become necrotic and bleach to a light tan or white colour (Otta 1974, Sellam and Wilcoxson 1976). PSS was also isolated from similar lesions on younger plants.

PSA and PSS cannot be differentiated by colony morphology nor by physiological, serological and basic genetic features. Evaluation of the symptoms induced on cereals and other plants allows some characterization. Nevertheless, definite discrimination between PSA and PSS remains difficult because some PSS strains also induce basal glume rot on spikes (von Kietzell 1995).

Basal glume rot leads to poor grain filling and is considered as a major pathogen of cereals in Russia (Matveeva et al. 2003). Considerable research has also been devoted to PSA in Ukraine (Pasichnyk 2000) and Bulgaria (Vassilev et al. 1995). In Germany, the economic losses were considered to be low (Toben et al. 1991). In Belgium, PSA produced conspicuous symptoms sporadically only on cv. Fidel. It is also observed on durum wheat (Alexandrova et al. 1995), barley and oats.

Leaf blight caused by PSS occurs worldwide. Locally foliage destruction may exceed 50%, but yield losses have not been thoroughly assessed (Diekmann and Putter 1995).

Ecology

PSA and PSS are commonly found on wheat and barley seeds. Both have also important epiphytic phases not only on cereals (Shane and Baumer 1987) but also on various annual or perennial weeds showing no symptoms (Taghavi and Keshavarz 2003). Disease outbreaks only occur under specific environmental conditions. Glume blotch is favoured by periods of extraordinary humid, cool weather. Leaf blight has been reported in years of abundant moisture and wind-driven rain or overhead irrigation. It is also noticed when, while still wet after a heavy rainfall, wheat leaves experience rapid heat loss due to radiance.

Pathogenic Variation and Pathogenicity

Evaluation of the virulence on 13 wheat and barley cultivars of strains isolated in Russia from barley, rye and wheat revealed clustering of the barley and rye strains into two separate clusters while strains from wheat were found in both clusters (Matveeva et al. 2003). A wide range of quantitative difference in virulence has been demonstrated among 1120 PSA strains pathogenic to wheat in Bulgaria. Furthermore, winter bread wheat cvs Sadovo 1 and Trakia were used to differentiate 4 races (Vasilev et al. 1988). Difference of reaction on cv. Star also suggested the occurrence of races among PSA strains in Germany (Toben et al. 1991). This has implications in testing wheat genotypes for resistance.

PSA and PSS are producing syringomycine and other phytotoxic and surface tension active compounds (Bultreys and Gheysen 1999). They may facilitate penetration of the epiphytic bacteria into the plant tissues and through pore formation in the membranes increase availability of nutrients and lead to the plant cell death.

Recently, PSS has been found to secrete syringolins, a family of closely related peptide derivatives that activates defence related genes in the non-host plant rice and induces resistance towards the rice blast fungus. The function of the syringolins in the interaction with the host plant during bacterial pathogenesis is presently unknown. On wheat, syringolin A has been shown to induce hypersensitive death of cells colonized by powdery mildew and, thus, to re-programme a compatible interaction into an incompatible one by counteracting the suppression of the host defence reaction imposed by the pathogen on the colonized cells (Waspi et al. 2001, Amrein et al. 2004). Epiphytic populations of PSS may thus interfere in other pathosystems.

Resistance

Differences in bacterial basal glume rot and leaf blight severity are observed amongst wheat cultivars in field trials. However, systematic evaluation of genotype resistance under field conditions is difficult because of the specific climatic requirements and changes in susceptibility during growth. At flowering, the spikes are resistant to PSA but thereafter susceptibility increases steadily until the dough stage (Toben 1989). There are only a few reports on large scale screening for resistance to PSA in the field with artificial inoculation. Most of the publications deal thus with resistance screening in growth chambers or greenhouses after inoculation of plants at various growth stages. The quantitative and qualitative differences in virulence require careful selection of strains, of plant stage, as well as of the environmental conditions. Zaharieva and Vassilev (1995) have evaluated the resistance of various wheat and *Aegilops* genotypes to PSA at booting stage in a greenhouse by injecting a bacterial suspension into the boot; while Vassilev et al. (1995) used pricking or injecting plantlets at the third leaf stage with a range of inoculum concentrations. A higher level of resistance was observed in the *Aegilops* genotypes than in wheat cultivars. These results confirmed previous reports by Kotlyarov (1991) who furthermore observed resistance to Russian PSA strains in the wheat cultivars Erythrospermum 19-16, Sadovo 1 and Katya. Resistance was also identified among other Russian winter wheat and spring wheat cultivars as well as in Cooperacion Calquin, Punjab 96, Prointa Superior, Prointa Imperial, Lhanaa, Bedhao and Rhabour-15 (Matveeva et al. 2003). The wheat cultivars Len, Marchal, Nowesta, Red River 68, Bounty 208, Bonanza and Alex were found resistant to PSS (Kotlyarov 1991). At our knowledge, no systematic breeding for resistance of wheat to PSA or PSS is undertaken.

DISEASES CAUSED BY *XANTHOMONAS TRANSLUCENS* PATHOVARS

Taxonomy, Symptoms and Importance

Bacterial leaf streak or leaf stripe on wheat is caused by *X. translucens*. Early symptoms are characterized by translucent water-soaked stripes producing abundant

honey-like exudates under humid conditions. If undisturbed, the exudates harden into yellowish granules studding the surface of the lesion and are easily detachable. When there is dew, drizzle or guttation water the exudates droplets coalesce to form conspicuous milky drops that may spread over the leaf surface and dry as thin, greyish, almost transparent flakes. Streaks coalesce into irregular light brown blotches. Symptoms often develop in the middle of the leaf where dew remains longer in the morning. Infection of culms, rachis, glumes and awns induces black chaff characterized by a greasy appearance or alternating bands of diseased or healthy areas on the awns. This can help in distinguishing XTU infection from pseudo-black chaff or brown melanosis induced by high temperature combined with high humidity on some genotypes with the Sr2 gene for stem rust resistance.

The species *X. translucens* encompasses different pathovars, all pathogenic to *Gramineae*. Each of these pathovars has a narrow host range, sometimes overlapping. Wheat is mainly affected by *X. translucens* pv. *undulosa* (XTU) and occasionally by *X. translucens* pv. *cerealis*. These pathovars as well as *X. translucens* pv. *hordei*, a synonym of *X. translucens* pv. *translucens*, affect barley as well. Pathovars thus defined through their host range, are correlated to molecular markers revealed by restrictions fragment length polymorphism using either 16+23 S rDNA or avrBS3 probes and by amplified fragment length polymorphism (Bragard et al. 1995, Bragard et al. 1997). These studies have demonstrated that strains of various genotypes are gathered within XTU.

XTU has a worldwide distribution but it remains a constraint for international germplasm exchange (Duveiller et al. 1997). Little quantitative information is available on losses caused by XTU because of the difficulty to obtain plots with different disease levels for a given cultivar. Yield losses as high as 40% have been reported from Idaho, USA. By comparing the yield of single tillers showing various levels of diseased flag leaf area in a temperate high rainfall environment in Mexico, we calculated that losses <5% could be expected when the percentage of flag leaf area damaged at early milk-dough stage was <10%, with losses up to 20% yield anticipated if 50% of the flag leaf was diseased (Duveiller and Maraite 1993). The disease tends to appear more frequently in breeding stations than farmer's fields, as confirmed in recent surveys in Kazakhstan (Unpublished data).

Ecology

Seed contamination by XTU has been demonstrated. It results from spike infection, but in some cases also from contamination by bacteria released from diseased leaves of highly susceptible genotypes (Tubajika et al. 1998). Seed is considered as the most important source of primary inoculum, despite a very low transmission rate. Due to its broad host range, XTU can also survive on some perennial grasses. Epiphytic populations of the pathogen have been detected on some monocotyledons and even on non-host dicotyledons near wheat fields.

Epiphytic populations on wheat leaves were found to decrease after heavy rainfall, which suggests that XTU are present on the surface before they actually penetrate

the parenchyma. Pathogen population size on asymptomatic leaves was found to be highly predictive of subsequent bacterial leaf streak severity (Stromberg et al. 1999). Bacterial antagonists can reduce disease severity by reducing the population size of the pathogen prior to disease onset (Stromberg et al. 2004). Bacteria are though to enter into the leaves and awns through the stomata and micro-injuries covered by a film of water. Irrigation sometimes favours bacterial leaf streak development (Carmona et al. 1997). XTU tolerates a wide range of temperatures and grows best when temperatures are above 26 °C. Pathogen multiplication in leaf tissue is thus directly dependent on temperature, and dry air conditions do not limit disease progress. As a consequence, XTU infections prevail in warm wheat growing areas where rainfall is limited but dew is enough to favour penetration of the parenchyma.

Pathogenic Variation and Pathogenicity

As for other pathovars within the xanthomonads, XTU mutants affected in their capacity to infect specific hosts can be obtained through transposon mutagenesis (Waney et al. 1991). By this strategy, both host-specific virulence and general virulence determinants have been identified. For a successful interaction between xanthomonads and plants, a type three secretion system (TTSS) is needed (Minsavage et al. 1990, Buttner and Bonas 2002). Xanthomonads mutants lacking a functional TTSS are non pathogenic when associated with the host plant, because they are unable to grow within the intercellular spaces. Bacterial growth in these spaces is indeed dependent on resources such as nutrient and water made available from within host cells. TTSS is thus viewed as the essential channel for translocation of specific proteins or effector proteins within the plant cell, hence liberating its content (Alizadeh et al. 1997, Hotson et al. 2003). Amongst well-known effectors, avirulence proteins (avr) are the most commons. In the interaction between xanthomonads and their host plant, a striking evidence is the number of different effectors that have been pointed out, emphasizing the complexity of the interaction. Typing of several XTU strains by hybridisation using an avrBS3 homologous probe from *X. axonopodis* pv. *manihotis* revealed the presence of multiple copies of the gene, according to the strains. Hence, as observed for *Xanthomonas oryzae* pv. *oryzae*, it is probable that avirulence genes contribute differently and specifically to pathogen aggressiveness (Bai et al. 2000). This could explain the quantitative differences in virulence observed with most XTU strains.

Indeed, the assessment of the pathogenicity of 36 XTU strains from Africa, America and Asia revealed that most strains were pathogenic, but with quantitative variations from moderate to high aggressiveness. Only a few South American strains were less aggressive on wheat and barley. Interestingly, such strains show only a single and faint hybridisation band with the avrBS3 probe, and their reaction on small grain cereals might be compared to a hypersensitive reaction. Transmission electron microscopy revealed in the compatible interaction, characterized by water-soaked lesions on the leaf, an abundant bacterial multiplication and the formation in the intercellular spaces of a matrix with fibrillar material; while in incompatible reaction induced by the less aggressive South American strains only limited bacterial

multiplication and a restricted amount of matrix or fibrillar material was noticed (Bragard et al. unpublished data).

On base of the inoculation of 19 wheat cultivars with 81 *X. translucens* isolates from North and South America, Milus and Chalkley (1994) found a highly non-significant cultivar x strain interaction and no evidence for races among the strains that best represented strains capable of causing bacterial streak on wheat in the field.

For *X. translucens* pv. *graminis*, a pathovar closely related to XTU, Kölliker et al. (2005) have observed the presence of three groups with low, medium and high pathogenicity. Using three different cultivars, a significant interaction between cultivars and isolates was demonstrated, indicating an at least partial race-specific resistance.

RFLP analyses of genomic DNA using probes including hrp genes from *X. campestris* revealed a very high diversity amongst *X. translucens* strains from cereals and grasses in Iran compared to other origins (Alizadeh et al. 1997), suggesting that the centre of origin of this pathogen could be in this area. RFLP analysis with different probes appeared useful in tracing the dispersion of particular strains through contaminated seed.

Resistance

Immunity to bacterial leaf streak does not occur in wheat. Disease may develop even in seemingly resistant parents, if inoculum pressure is sufficiently strong and time for disease development is allowed. This indicates that resistance is partial or incomplete. However, relative resistance was consistently confirmed over years and appears to be relatively stable. Wheat materials such as Trigo Br 10, Igapo, Buck Ombu, Batuira, Juriti and Thornbird have also shown indications of presence of partial resistance. Resistance to XTU was furthermore detected in cultivars Cacatu, Ibiara, Pirata (Mehta 1996) and Terral 101 (Tillman et al. 1996a). Somaclonal variation has not induced variation in XTU resistance (Mehta and Angra 2000). Reaction ranks of greenhouse-grown cultivars after infiltration with a bacterial suspension are significantly and positively related to ranks of cultivars evaluated for bacterial leaf streak in the field, although on base of the percentage of water-soaking under controlled conditions some field-susceptible cultivars would be kept and some field-resistant wheat would be discarded (Milus and Mirlohi 1994; Milus et al. 1996). For 428 accessions from the USDA National Small Grains Collection no positive correlation was found between the water-soaking reaction to XTU after inoculation in growth chamber and bacterial streak severity developing in field trials during 2 years. Field screening was effective in identifying resistance, but reaction of genotypes was confounded by relative maturity and genotype x environment interactions (Tillman et al. 1996b).

Very little research on the genetics of resistance to bacterial diseases in wheat has been undertaken. A study on the inheritance of bacterial leaf streak resistance was conducted in Mexico under artificial disease pressure in the field using a XTU strain and involved a full combination of crosses from five parents differing in resistance: Pavon and Mochis, (resistant); Angostura (intermediate), and Alondra and

Turaco, (susceptible) (Duveiller et al 1993). The methodological approach required complete coherence and consistency among all cross combinations in regard to the postulation of genes involved and of the genotypic constitution of the parents. Also, data were obtained at two dates during the same epiphytotic confirming the stability and reliability of observations. This study showed resistance to bacterial leaf streak or black chaff in the five wheat genotypes to be conditioned by five genes. No evidence of cytoplasmic effect was found from the comparison between reciprocal F1 crosses. The results suggested that the several genes controlling bacterial leaf streak differed in strength of expression of resistance. One of the two strongest genes, *Bls1*, is present in all three superior parents, Pavon, Mochis and Angostura. These genes might be related to different mechanisms of resistance. It is likely that an accumulation of diverse genes, associated with one or more mechanisms of resistance, confer increased levels of resistance in the field. None of the five parents contained the full set of five resistance genes identified, suggesting that cultivars more resistant than Pavon and Mochis can be found. A Chinese cultivar, Nanjing 8331, appeared to be much more resistant than Pavon and Mochis under similar conditions, although nothing can be inferred about the presence of any of the genes identified.

Black chaff severity and bacterial streak severity in field trials is not correlated. Since yield loss is related to bacterial streak but not to black chaff, wheat should be evaluated for resistance to bacterial streak rather than black chaff (Tillman et al 1996a).

CONCLUSIONS

The minor bacterial diseases have not shown severe outbreaks during the last years. Nevertheless, because of their sporadic epidemic development under particular environmental conditions on some genotypes and because of their seed-borne nature, some of these bacteria are searched for in seed testing. Their detection may hamper grain trade. Disease problems considered unimportant today may become economically significant in the future as a result of evolving cropping conditions or changes in prevalent cultivars.

High level of resistance to PSA and PSS has been identified in some wheat and especially *Aegilops* genotypes by inoculation under controlled conditions. The relation with field resistance is not well documented. Because of the widespread occurrence of PSA, its wide host range, the differences in aggressiveness and the occurrence of races, resistance observed in one area could not apply for another one.

The progress in the understanding of the molecular interactions between xanthomonads and their host plants highlights the complexity of the mechanisms leading to the establishment of a successful interaction, but also the need to use for resistance screening well-characterized strains covering the range of pathogenicity genes already discovered within XTU. Seen the overall absence of cultivar x strain interactions, cultivars rated as resistant in fields under severe disease pressure are likely to remain resistant.

The accumulation of resistant sources in breeding programs, combined with sound crop management practices and adequate seed-health procedures has certainly

contributed to reducing the effect of bacterial leaf streak epiphytotics in recent years. Overall progresses in incorporating further genetic resistance to bacterial leaf streak in breeding programs are limited because epidemics are sporadic. Although, marker assisted selections could possibly contribute to faster resistance improvement, results so far have been unsatisfactory (Feingold et al. 1992).

Identification of hotspots for the various bacterial diseases and international agreement on germplasm testing at these locations could warrant a faster evaluation of genotype resistance and minimize the risk of release of susceptible cultivars.

A better understanding of the plant factors affecting epiphytic growth of XTU, PSA or PSS as well as of possible antagonists in the phyllosphere, may offer new opportunities for genetic management of the bacterial diseases of wheat.

ACKNOWLEDGEMENTS

This research was funded by the Belgian Administration for Development Cooperation and is a collaborative research between CIMMYT and Unité de Phytopathologie, Faculté d'ingénierie biologique, agronomique et environnementale, Université catholique de Louvain.

REFERENCES

Alexandrova M, Zaccardelli M, Stefani E, Bazzi C (1995) Testing of Pseudomonas syringae pv. atrofaciens and Xanthomonas campestris pathovars on cereals in Italy. EPPO Bulletin 25:437–448

Alizadeh A, Arlat M, Sarafi A, Boucher CA, Barrault G (1997) Restriction fragment length polymorphism analyses of Iranian strains of Xanthomonas campestris from cereals and grasses. Plant Dis 81:31–35

Amrein H, Makart S, Granado J, Shakya R, Schneider-Pokorny J, Dudler R (2004) Functional analysis of genes involved in the synthesis of syringolin A by Pseudomonas syringae pv. syringae B3001 D-R. Mol Plant Microbe Interact 17:90–97

Anonymous (1995) Occurrence of sheath rot, a new disease on wheat. Lumle Newsletter, Lumle Agricultural Research Centre, Pokkara, Nepal 1:3

Anonymous (2003) ARS/USDA. Foreign Disease-Weed Science: 2003 Annual Report. http://www.ars.usda.gov/research/projects/projects.htm?ACCN_NO=406860&showpars=true&fy=2003

Bai JF, Choi SH, Ponciano G, Leung H, Leach JE (2000) Xanthomonas oryzae pv. oryzae avirulence genes contribute differently and specifically to pathogen aggressiveness. Mol Plant Microbe Interact 13:1322–1329

Bragard C, Verdier V, Maraite H (1995) Genetic diversity among Xanthomonas campestris strains pathogenic for small grains. Appl Environ Microbiol 61:1020–1026

Bragard C, Singer E, Alizadeh A, Vauterin L, Maraite H, Swings J (1997) Xanthomonas translucens from small grains: diversity and phytopathological relevance. Phytopathology 87:1111–1117

Brennan JP, Murray GM (1998) Economic importance of wheat diseases in Australia. NSW Agriculture GRDC, Australia

Bultreys A, Gheysen I (1999) Biological and molecular detection of toxic lipodepsipeptide-producing Pseudomonas syringae strains and PCR identification in plants. Appl Environ Microbiol 65:1904–1909

Buttner D, Bonas U (2002) Getting across – bacterial type III effector proteins on their way to the plant cell. EMBO Journal 21:5313–5322

Carmona MA, Irigoyen ED, Pioli R (1997) Presencia de Xanthomonas campestris pv. undulosa agente causal del rayado bacteriano en cultivos de trigo bajo riego. Revista de la Facultad de Agronomia (Universidad de Buenos Aires) 16:211–212

Diekmann M, Putter CAJ (1995) FAO/IPGRI Technical Guidelines for the Safe Movement of Germplasm. No. 14. Small Grain Temperate Cereals. Food and Agriculture Organization of the United Nations, Rome/International Plant Genetic Resources Institute, Rome

Duveiller E (1994) Bacterial leaf streak or black chaff of cereals. EPPO Bulletin 24:135–157

Duveiller E, Maraite H (1990) Bacterial sheath rot of wheat caused by Pseudomonas fuscovaginae in the highlands of Mexico. Plant Dis 74:932–935

Duveiller E, Maraite H (1993) Study of yield loss due to Xanthomonas campestris pv. undulosa in wheat under high rainfall conditions. J Plant Dis Prot 100:453–459

Duveiller E, van Ginkel M, Thijssen M (1993) Genetic analysis of resistance to bacterial leaf streak caused by Xanthomonas campestris pv. undulosa in bread wheat. Euphytica 66:35–43

Duveiller E, Fucikovsky L, Rudolph K (1997) The bacterial diseases of wheat: concepts and methods of disease management. CIMMYT, Mexico

Feingold SE, Hoisington DA, Duveiller E, van Ginkel M (1992) Bacterial leaf streak of wheat: RFLP mapping of resistance. Agronomy Abstracts, ASA-CSSA-SSSA 1992 Annual Meetings, November 1–6, Minneapolis, MN, USA, p 96

Fukuda T, Azegami K, Tabel H (1990) Histological studies on bacterial black node of barley and wheat caused by Pseudomonas syringae pv. japonica. Annals of the Phytopathological Society of Japan 52:252–256

Hosford RM Jr (1982) White blotch incited in wheat by Bacillus megaterium pv. cerealis. Phytopathology 72:1453–1459

Hotson A, Chosed R, Shu H, Orth K, Mudgett MB (2003) Xanthomonas type III effector XopD targets SUMO-conjugated proteins in planta. Mol Microbiol 50:377–389

Huang HC, Erickson RS, Yanke LJ, Mundel H-H (2002) First report of pink seed of common bean caused by Erwinia rhapontici. Plant Dis 86:921

Kölliker R, Kraehenbuehl R, Boller B, Widmer F (2005) Genetic diversity and pathogenicity of the grass pathogen Xanthomonas translucens pv. graminis. Syst Appl Microbiol 29(2):109–119

Kotlyarov VV (1991) Methods of evaluating resistance to bacterial diseases in wheat and triticale. Selektsia I Semenovodstvo (Moskva) 3:31–32

Luna CL, Mariano RLR, Souto-Maior AM (2002) Production of biocontrol agent for crucifer black rot disease. Braz J Chem Eng 19:133–140

Matveeva IEV, Pekhtereva ESH, Polityko VA, Ignatov AN, Nikolaeva EV, Schaad NW (2003) Distribution and virulence of Pseudomonas syringae pv. atrofaciens, causal agent of basal glume rot, in Russia. In: Iacobellis NS, Collmer A, Hutcheson SW, Mansfield JW, Morris CE, Schaad NW, Stead DE, Surico G, Ullrich MS (eds) Presentations from the 6th International Conference on Pseudomonas syringae pathovars and related pathogens, Maratea, Italy, September 15–19, Kluwer Academic Publishers, Dordrecht, Netherlands, pp 97–105

McBeath JH (1983) Other bacterial diseases. In: Mathur SB, Cunfer BM (eds) Seed-borne diseases and seed health testing of wheat. Jordbrugsforlaget, Frederiksberg, Denmark, pp 137–146

Mehta YR (1996) Resistencia de cultivares de trigo a Xanthomonas campestris pv. undulosa. Summa Phytopathologica 22:200–204

Mehta YR, Angra DC (2000) Somaclonal variation for disease resistance in wheat and production of dihaploids through wheat x maize hybrids. Genet Mol Biol 23:617–622

Milus E, Chalkley DB (1994) Virulence of Xanthomonas campestris pv. translucens on selected wheat cultivars. Plant Dis 78:612–615

Milus E, Mirlohi AF (1994) Use of disease reactions to identify resistance in wheat to bacterial streak. Plant Dis 78:157–161

Milus E, Duveiller E, Kirkpatrick TL, Chalkley DB (1996) Relationship between disease reaction and bacterial streak severity in wheat. Plant Dis 80:726–730

Minsavage GV, Dahlbeck D, Whalen MC, Kearny B, Bonas U, Staskawicz BJ, Stall RE (1990) Gene-for-gene relationships specifying disease resistance in Xanthomonas campestris pv. vesicatoria–pepper interactions. Mol Plant Microbe Interact 3:41–47

Otta JD (1974) Pseudomonas syringae incites a leaf necrosis on spring and winter wheats in South Dakota. Plant Dis Reporter 58:1061–1064

Paruthi IS, Bhatti DS (1985) Estimation of loss in yield and incidence of Anguina triticina on wheat in Haryana (India). International Nematology Network Newsletter 2:13–16

Pasichnyk LA (2000) Antigenic properties of bacteria of pathovars of Pseudomonas syringae affecting cereals. Mikrobiologichnii Zhurnal 65:18–22.

Piening LJ, MacPherson DJ (1985) Stem melanosis, a disease of spring wheat caused by Pseudomonas cichorii. Canadian Journal of Plant Pathology 7:168–172

Scharif G (1961) Corynebacterium iranicum sp. nov. on wheat (Triticum vulgare L.) in Iran and a comparative study of it with C. tritici and C. rathayi. Entomologie et phytopathologie appliquées 19:1–24

Sellam MA, Wilcoxson RD (1976) Bacterial blight of wheat in Minnesota. Plant Dis Reporter 60:242–245

Shane WW, Baumer JS (1987) Population dynamic of Pseudomonas syringae pv. syringae on spring wheat. Phytopathology 77:1399–1405

Stromberg KD, Kinkel LL, Leonard KL (1999) Relationship between phyllosphere population size of Xanthomonas translucens pv. translucens and bacterial leaf streak severity on wheat seedlings. Phytopathology 89:131–135

Stromberg KD, Kinkel LL, Leonard KL (2004) Quantifying the effect of bacterial antagonists on the relationship between phyllosphere population sizes of Xanthomonas translucens pv. translucens and subsequent bacterial leaf streak severity on wheat seedlings. Biol Contr 29:58–65

Taghavi SM, Keshavarz K (2003) Identification of the causal agent of bacterial wheat blight in Fars and Kohgiluyeh & Boyrahmad provinces and the reaction of certain wheat cultivars to them. J Sci Tech Agr Nat Res 6:171–180

Tillman BL, Harrison SA, Russin JS, Clark CA (1996a) Relationships between bacterial streak and black chaff symptoms in winter wheat. Crop Sci 36:74–78

Tillman BL, Harrison SA, Clark CA, Milus EA, Russin JS (1996b) Evaluation of bread wheat germplasm for resistance to bacterial streak. Crop Sci 36:1063–1068

Toben H (1989) Untersuchungen über die basale Spelzenfäule an Weizen und Gerste, hervorgerufen durch Pseudomonas syringae pv. atrofaciens ((McCull.) Young, Dye, Wilkie). Diploma thesis. Universität Göttingen, Institut für Pflanzenpathologie und Pflanzenschutz, Germany

Toben H, Mavridis A, Rudolph K (1991) Zum Vorkommen der basalen Spelzenfäule an Weizen und Gerste, hervorgerufen durch Pseudomonas syringae pv. atrofaciens, in West-Deutschland. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz 98:225–235

Tubajika KM, Tillman BL, Russin JS, Clark CA, Harrison SA (1998) Relationship between flag leaf symptoms caused by Xanthomonas translucens pv. translucens and subsequent seed transmission in wheat. Plant Dis 82:1341–1344

Vassilev V, Kolev K, Zaharieva M, Sevov V (1995) Resistance of Aegilops, maize and wheat genotypes to Pseudomonas syringae pathovars atrofaciens and syringae. Agronomie 15:25–29

Vasilev VI, Schnaider YJ, Ilyuhina MK (1988) Virulent properties of the phytopathogenic bacteria from the genus Pseudomonas Migula causing cereal crop bacteriosis. Genetika I Selektsiya 21:308–313

von Kietzell J. (1995) Untersuchungen zur Epidemiologie und Spezifität von Pseudomonas syringae pv. atrofaciens ((McCulloch), Dye, Young, Wilkie), dem Erreger der basalen Spelzenfaüle des Getreides. Dissertation, Universität Göttingen, Institut für Pflanzenpathologie und Pflanzenschutz, Germany

Waney VR, Kingsley MT, Gabriel DW (1991) Xanthomonas campestris pv. translucens genes determining host specific virulence and general virulence on cereals identified by Tn5-gusA insertion mutagenesis. Mol Plant Microbe Interact 4:623–627

Waspi U, Schweizer P, Dudler R (2001) Syringolin reprograms wheat to undergo hypersensitive cell death in compatible interaction with powdery mildew. Plant Cell 13:153–161

Young JM (1992) Pseudomonas syringae pv. japonica (Mukoo 1955) Dye et al. 1980 is a junior synonym of P. syringae pv. syringae van Hall 1902. Lett Appl Microbiol 15:129–130

Zaharieva M, Vassilev V (1995) Study of adult wheat and Aegilops resistance to Pseudomonas syringae pathovar atrofaciens. Agronomie 15:21–24

SPREAD OF A HIGHLY VIRULENT RACE OF *PUCCINIA GRAMINIS TRITICI* IN EASTERN AFRICA

Challenges and opportunities

R.P. SINGH[1,*], M.G. KINYUA[2], R. WANYERA[2], P. NJAU[2], Y. JIN[3] AND J. HUERTA-ESPINO[4]

[1]*CIMMYT, Apdo. Postal 6-641, 06600, Mexico DF*
[2]*KARI-NPBRC, P.O. Njoro, Kenya*
[3]*USDA-ARS CDL, Univ. of Minnesota, St. Paul, MN, USA*
[4]*INIFAP-CEVAMEX, Apdo. Postal 10, 56230, Chapingo, Mexico*
[*]*E-mail: r.singh@cgiar.org*

Abstract: Stem or black rust, caused by *Puccinia graminis*, has historically caused severe losses to wheat (*Triticum aestivum*) production worldwide. Its control for over 30 years through the use of genetic resistance in semidwarf cultivars is a remarkable success story. However, this situation also has led to decline in research and breeding for resistance in many countries. In 1999, high susceptibility of CIMMYT germplasm was noted in Uganda and an increase in stem rust incidence and severity was seen in Kenya. The causal race, commonly known as Ug99 and designated as TTKS based on the North American nomenclature, carries virulence for several genes commonly present in wheat germplasm including gene *Sr31* located in the 1BL.1RS wheat-rye translocation known to be present in several leading spring and winter wheat cultivars and germplasm worldwide. Race Ug99 is now widespread in wheat growing areas of Kenya and Ethiopia and has caused susceptibility of many popular cultivars. This race is expected to migrate further to northern Africa through Arabian Peninsula and then to Middle East, West Asia and eventually to South Asia as recently happened with yellow rust. Severe losses are likely to occur because several major cultivars in the migration path are susceptible to this race. The challenge is to identify/develop suitable resistant cultivars in a relatively short time and implement appropriate strategies to replace the susceptible cultivars before rust migrates out of Eastern Africa. Although several alien genes will provide resistance to this race, the long-term strategy should focus on rebuilding the *Sr2*-complex (combination of slow rusting gene *Sr2* with other unknown additive genes of similar nature) to achieve long-term durability once again. A Global Rust Initiative has been launched to monitor the further migration of this race, facilitate field testing in Kenya or Ethiopia of wheat cultivars and germplasm developed by wheat breeding programs worldwide, understand the genetic basis of resistance especially the durable

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 51–57

type, and carry out targeted breeding to incorporate diverse resistance genes into key cultivars and germplasm

Keywords: wheat, stem rust, virulences

INTRODUCTION

Stem or black rust, caused by *Puccinia graminis tritici (Pgt)*, is known historically for causing severe losses to wheat (*Triticum aestivum* and *T. turgidum*) production. However, it has been controlled effectively through the use of genetic resistance in cultivars associated with green revolution during the 1960s and 1970s. Over 80% of the developing countries spring wheat area is currently sown to cultivars either derived directly from CIMMYT-germplasm or CIMMYT germplasm used as parent. For more than 30 years, a major proportion of the CIMMYT wheat germplasm and germplasm developed by other breeding programs has remained resistant to stem rust. Resistance gene *Sr31*, located on rye translocation 1B.1R contributed to high level of resistance in several wheat cultivars developed worldwide. Consequently stem rust disease is often not considered important and in many countries wheat breeding is currently done in the absence of stem rust and research in the last two decades also declined substantially.

Detection in 1999 of *Pgt* race Ug99 in Uganda with broad virulence including the virulence for *Sr31* and its migration to Kenya and Ethiopia has been recognized as a highly significant event and led to the launch on Global Rust Initiative on September 9, 2005.

Present paper describes the avirulence/virulence phenotype of race Ug99, sources of resistance that can be used and discusses the breeding strategy that should be implemented.

MATERIALS AND METHODS

Stem rust urediniospores samples were collected in Kenya from infected wheat plants, multiplied on a susceptible cultivar and then sent to USDA-ARS Cereal Disease Laboratory for characterization on differentials. Standard greenhouse practices were used for growing seedlings, inoculation, incubation, etc. and infection type scoring. The original *Sr31*-virulent stem rust race from Uganda (Pretorius et al. 2000) was also included in the test for comparison.

Field screenings of Kenyan cultivars, CIMMYT's International Nurseries and introduced germplasm from various countries were carried out during 2004 and/or 2005 crop seasons at KARI's Wheat Research Station at Njoro, Kenya. Plot sizes consisted of two rows 2 m length. Both years heavy stem rust epidemic developed from natural infections. Germplasm was scored according to modified Cobb Scale when the most susceptible lines in nurseries displayed about 80% severity. Field

observations were made on differentials and various CIMMYT's International Nurseries at Ethiopian Agricultural Research Organization's (EARO) Research Station at Kulumsa in Ethiopia.

RESULTS AND DISCUSSION

The Race, Effective Resistance Genes and Likely Migration Path

When tested on wheat seedlings with many of the named resistance genes, the Ug99 race from Uganda and all collections from Kenya fit the designation TTKS based on the naming system of North American rust workers (Roelfs and Martens 1988). The effective resistance genes in seedling tests are *Sr7a, 13, 22, 23, 24, 25, 26, 27, 28, 29, 32, 33, 35, 36, 39, 40, 44* and *Tmp*; and the ineffective resistance genes are *Sr5, 6, 7b, 8a, 8b, 9a, 9b, 9d, 9e, 9g, 11, 12, 15, 17, 21, 30, 31* and *38* (Wanyera et al. 2005). These results and observations made in field nurseries in Kenya and Ethiopia indicate that Ug99 race is now widely spread in Eastern African highlands. Genes that gave effective levels of resistance in field nursery at Njoro (tested as single gene lines) include: *Sr22, 24, 25, 26, 27, 29, 32, 33, 35, 36, 39, 40, 44* and *Tmp*. Under high disease pressure in field conditions *Sr7a, Sr13* and *Sr23* are not expected to provide enough resistance. Among the effective genes, *Sr22, 24, 26, 29, 36* and *Tmp* may have some immediate value; the other genes located in alien chromosome segments are either in need of further shortening the length of the alien segment, or have lacked durability. CIMMYT triticales, which often carry *Sr27* or *SrSatu* were highly resistant in the field at Njoro. While *Sr36* confers resistance to Ug99, another race in the region caused an epidemic on cultivar Enkoy, which carries *Sr36*, in 1994 and subsequent years.

Recent evidence has indicated that an *Yr9*-virulent *P. striiformis* race first evolved in Eastern Africa and then migrated to South Asia through Arabian Peninsula, North Africa, Middle East and West Asia in about 10 years and caused severe epidemics in its migration path (Singh et al. 2004). Several important cultivars grown in these areas are susceptible to Ug99 race and could suffer severe losses if Ug99 follows a migration pattern similar to that of *P. striiformis*. Predominant wind patterns indicate all possibilities for its migration to the above mentioned regions. A human error can expedite the migration or even introduce it to unexpected areas as has happened to leaf rust and stripe rust in recent years.

Sources of Resistance in Wheat Germplasm

About 10% of the current wheat germplasm tested in Kenya during 2005 showed high to acceptable levels (up to 15% severity) of resistance. A large portion of the highly resistant germplasm from South America, Australia and CIMMYT appears to possess *Sr24*. This gene is located on the *Agropyron elongatum* translocation on chromosome 3DL where the leaf rust resistance gene *Lr24* is present. There are three distinct *Sr24* carrying translocations: the original one linked to a gene for

red grain color, the shorter segment with white grain, and a third segment where a very small segment has been retranslocated onto chromosome 1BS. In all three segments both *Sr24* and *Lr24* are present together. Therefore, selection for *Lr24* with avirulent leaf rust isolates can be used as an indirect selection strategy. This gene would look like an attractive candidate for future breeding efforts; however it must be used in combination with other effective resistance genes because virulence to *Sr24* is already known in South Africa and India.

The *Sr25* gene is also located on *A. elongatum* translocation together with leaf rust resistance gene *Lr19* on chromosome 7DL. A white floured mutant of the translocation, developed by D. R. Knott, was transferred into some Australian and CIMMYT wheat backgrounds. *Sr25* conferred high level of resistance only in some genetic backgrounds, especially when the slow rusting adult plant resistance gene *Sr2* was also present, e.g. lines 'Super Seri#1' (yellow flour) and 'Wheatear' (white flour). Virulence to *Sr25* is not known.

Gene *Sr26* of *A. elongatum* origin, translocated to chromosome 6AL, has been used successfully in Australia and remains effective despite its large scale deployment in 1980s. It is not known to be present in cultivars from other countries and the translocation used initially may have yield penalty. Shorter translocations were developed by I. Dundas in Australia and could be better sources.

Gene *Sr27* of rye origin has not been used in wheat improvement. Its deployment in triticale in Australia resulted in a rapid selection for virulence. Strategically this gene should be left for triticale improvement in areas where virulence is not known. Although Ug99 is avirulent on genes *Sr28* and *Sr36*, numerous virulent races are known to occur worldwide. Genes *Sr29, 32, 33, 35, 37, 39, 40* and *44* have not been tested widely for their effectiveness to other races and also not used in breeding. Gene *SrTmp* from 'Triumph' is present in some US wheat cultivars and can be used in breeding. Moreover, an additional unnamed resistance gene located in rye chromosome translocation 1A.1R and different from *Sr31*, is present in some US winter wheats, such as TAM200, and can also be used (Jin and Singh 2006). This translocation is present in a CIMMYT spring wheat line TAM200/Tui. Both TAM200 and TAM200/Tui also possess *Sr24* gene, hence could be a better parent to transfer two effective resistance genes simultaneously. Certain hexaploid synthetic wheat derived advanced lines and some lines where certain Chinese cultivars such as Shanghai#7 are parents also have shown high level of resistance. However, the genetic basis of resistance is not known.

Durable Resistance and Hope Derived Sr2-Complex

Durable stem rust resistance of some older US, Australian and CIMMYT spring wheats is believed to be due to the deployment of *Sr2* in conjunction with other unknown minor, additive genes. McFadden transferred Gene *Sr2* to hexaploid wheat in 1920s from tetraploid emmer wheat cultivar 'Yaroslav'. The slow rusting gene *Sr2* confers only moderate levels of resistance when present alone. Its presence can be detected through its complete linkage with pseudo-black chaff phenotype. On wheat lines that displayed pseudo-black chaff, we observed varying degrees of

disease with a maximum severity reaching about 60% compared to 100% severity for highly susceptible materials and reaction varying from MR to S (moderately resistant to susceptible) on the same internodes clearly indicated that *Sr2* did confer at least some resistance. However, the level of resistance conferred by *Sr2* alone under high disease pressure in Kenya was not sufficient. *Sr2* was detected in several highly resistant old, tall Kenyan cultivars, including Kenya Plume (Singh and McIntosh 1986), and CIMMYT-derived cultivars Sonalika (a cultivar associated with the Green Revolution in South Asia) and Pavon 76. Both Sonalika and Pavon 76 were resistant during 2004 and 2005 with maximum disease score of 15MR. Because Pavon 76 is susceptible as seedlings with race Ug99, its resistance as speculated earlier (Rajaram et al. 1988) is based on the multiple additive genes where *Sr2* is an important component. US wheat cultivar Chris, which is not known to carry *Sr2* but possesses *Sr7a* (Singh and McIntosh 1987) also displayed high level of resistance and hence its adult plant resistance may involve interaction of moderately effective gene *Sr7a* and other unknown adult plant resistance genes. High level of resistance in Selkirk may involve interactions of moderately effective genes *Sr2* and *Sr23* (linked to leaf rust resistance gene *Lr16*) and perhaps additional unknown adult plant resistance genes. These observations although need validation through genetic analyses indicate that complex resistance to stem rust present in some tall as well as some semidwarf cultivars developed in the 1960s and 1970s continue to remain effective.

Breeding Strategies for Resistance to Pgt Race Ug99

The fastest way to correct the susceptibility of important wheat cultivars and the best new germplasm is to systematically incorporate diverse sources of resistance into them through limited or repeated backcrossing. Race-specific resistance genes that will confer resistance to Ug99 and other important races were described earlier. Because most of these genes are of alien origin, co-segregating molecular markers for several of them are already available (Mago et al. 2005, Prins et al. 2001) and can aid selection. Where the alien stem rust resistance genes are linked to leaf rust resistance genes, screening for leaf rust in seedlings or adult plants can also be practiced.

The best strategy to use race-specific resistance genes is to use them in combinations. Molecular markers will provide a powerful tool to identify plants that carry combinations of resistance genes. However, it may be difficult to combine more than two translocations in a single genotype as their negative impact on yield and quality can be quite large. To transfer two or more effective resistance genes into an adapted cultivar the better crossing strategy would be to first cross the resistance sources and then cross F1 plants with the adapted cultivar. If molecular markers are to be applied then apply them on the top-cross plants and select plants that have desirable agronomic features and carry multiple resistance genes. Because such plants are expected to be in low frequency, it is desirable to maintain large family size of approximately 400, which can be obtained by emasculating and pollinating 20 spikes. Further backcross on selected plants will help restore the characteristics of the recurrent parent.

It is advisable to restore the durable resistance in current cultivars and recent wheat germplasm. At present little is known on the genes involved in durable resistance, however some earlier work done by Knott (1982) and knowledge on durable resistance to leaf and yellow rusts (Singh et al. 2004) indicate that such resistance will involve multiple minor genes with additive effects. Accumulating such complex resistance in the absence of disease pressure caused by Ug99 race and lack of molecular markers will not be an easy task. Molecular markers linked to the slow rusting resistance gene *Sr2* are known and can be used in selection; however this gene can also be identified in the field from its linkage with pseudo-black chaff phenotype. *Sr2* is present in over half of the current CIMMYT's spring wheat germplasm including some of the most high-yielding recent genotypes that have high level of resistance to leaf and yellow rusts and good industrial quality. Our strategy is to transfer the adult-plant resistance from Pavon 76, and a few other wheats identified so far, in a range of important CIMMYT wheat germplasm by using the "single-backcross, selected bulk" breeding approach (Singh et al. 2004). In this strategy the resistance sources are crossed with the adapted high yielding wheats and then a single backcross is made with the recurrent parent to obtain about 500BC_1 seeds. BC_1 plants will be selected for desired agronomic features and resistance to leaf and yellow rusts and will be harvested as bulk. Large F2 populations of about 2500 plants will be grown and plants will be selected in Mexico for agronomic traits and resistance to other diseases and harvested as bulk. A similar selection will be practiced in the F3 generation to obtain F4 populations. At this stage we will try to select for adult plant resistance by growing dense sown F4-Bulk populations in Kenya or Ethiopia, under high stem rust pressure created by inoculating with Ug99 race. Populations will be bulk harvested and plumper grains selected to grow F5 generation in Mexico. Because stem rust affects grain filling, we expect that plants with insufficient resistance will have shriveled grains. Moreover, by F4 generation enough homozygosity is achieved for the selection of additive resistance genes. Individual plants with desired agronomic features and resistance to other diseases will be selected in the F5 generation and those with good grain characteristics will be grown in F6 as hill plots in Kenya or Ethiopia as well as small plots in Mexico for final selection. Finally the resistant F6 plots will be harvested for conducting yield trials in the following crop season. Same methodology is also proposed to transfer resistance from old, tall Kenyan cultivars into adapted semidwarf wheats. The proposed approach is expected to rebuild the durable resistance in modern wheat germplasm.

Genetic analyses will be necessary to understand the number and type of resistance genes involved in sources contributing the adult plant resistance. Genomic locations of additive resistance genes will be determined through molecular mapping.

The Global Rust Initiative (GRI)

GRI was launched on 9th September 2005 in Nairobi, Kenya to raise the awareness from the risk posed by Ug99 race. The recommendations from the expert panel

report (CIMMYT 2005) to implement a global strategy were also endorsed. Efforts are currently underway to generate human and financial resources to implement a global control strategy. The recommendations relevant to wheat improvement community include: tracking the migration of Ug99 and its further evolution, establishment of a warning system, identification of resistance in global wheat germplasm by testing in Kenya and Ethiopia, implementation of a breeding strategy to incorporate diverse genetic resistance into key germplasm before the race migrates to those areas, and enhancement of human resources and structural capacities.

Testing in Kenya and Ethiopia of a limited number of germplasm from CIMMYT, ICARDA and various countries was already implemented. This activity should increase during 2006 provided new funding becomes available. Germplasm identified to be resistant is being multiplied in Mexico for international distribution in 2006. However, information on resistant entries in various international nurseries either being grown currently in the Southern hemisphere or being planted in rest of the areas can be obtained from the first author and will be distributed widely to enable selection and crossing. The success of GRI lies in a timely replacement of stem rust susceptible cultivars with resistant cultivars with equal or better yield potential and other characteristics. This will require concerted and integrated efforts from scientists working in a range of disciplines.

REFERENCES

Jin Y, Singh RP (2006) Resistance in US wheat to recent eastern African isolates of Puccinia graminis f. sp. tritici with virulence to resistance gene Sr31. Plant Dis. Submitted

Knott DR (1982) Multigenic inheritance of stem rust resistance in wheat. Crop Sci 22:393–399

Mago R, Bariana HS, Dundas IS, Speilmeyer W, Lawrence GJ, Pryor AJ, Ellis JG (2005) Development of PCR markers for the selection of wheat stem rust resistance genes Sr24 and Sr26 in diverse wheat germplasm. Theor Appl Genet 111:496–504

Pretorius ZA, Singh RP, Wagoire WW, Payne TS (2000) Detection of virulence to wheat stem rust resistance gene Sr31 in Puccinia graminis f. sp. tritici in Uganda. Plant Dis 84:203

Prins R, Groenewald JZ, Marias GF, Snape JW, Koebner RMD (2001) AFLP and STS tagging of Lr19, a gene conferring resistance to leaf rust in wheat. Theor Appl Genet 103:618–624

Rajaram S, Singh RP, Torres E (1988) Current CIMMYT approaches in breeding wheat for rust resistance. In: Simmonds NW, Rajaram S (eds) Breeding Strategies for resistance to the Rust of Wheat. CIMMYT, Mexico, DF, pp 101–118

Roelfs AP, Martens JW (1988) An international system of nomenclature for Puccinia graminis f. sp. tritici. Phytopathology 78:526–533

Singh RP, McIntosh RA (1986) Genetics of resistance to Puccinia graminis tritici and Puccinia recondita tritici in Kenya plume wheat. Euphytica 35:245:256

Singh RP, McIntosh RA (1987) Genetics of resistance to Puccinia graminis tritici in 'Chris' and 'W3746' wheats. Theor Appl Genet 73:846–855

Singh RP, William HM, Huerta-Espino J, Rosewarne G (2004) Wheat Rust in Asia: Meeting the Challenges with Old and New Technologies. New directions for a diverse planet: Proceedings of the 4th International Crop Science Congress, 26 Sep-1 Oct 2004, Brisbane, Australia, ISBN 1 920842 20 9. www.cropscience.org.au

Wanyera R, Kinyua MG, Jin Y, Singh R (2005) The spread of stem rust caused by Puccinia graminis f. sp. tritici, with virulence on Sr31 in wheat in Eastern Africa. Plant Dis. 89 (in press)

INHERITANCE OF ADULT PLANT RESISTANCE GENES AND ASSOCIATED MARKERS FROM A DURABLE RESISTANT CULTIVAR TO LEAF RUST

L. INGALA[1,*], H. SAIONE[1], M. HELGUERA[2], M. NISI[2] AND F. SACCO[1]

[1]*Instituto de Genética EA Favret, CICVyA, INTA, CC25 1712 Castelar, Bs.As, Argentina*
[2]*EE INTA Marcos Juárez, Córdoba, Argentina*
[*]*E-mail: lingala@cnia.inta.gov.ar*

Abstract: Leaf rust, incited by the biotrophic fungus *Puccinia triticina*, is one of the most important diseases of wheat worldwide, causing in Argentina annual yield losses of about 5–10%. The use of resistance genes, particularly from varieties that show durable resistance, may be an outstanding contribution for controlling this disease. In addition, the development of linked DNA molecular markers can assist the introduction, and selection of genes to be efficiently used in plant breeding programs. In wheat, a number of varieties showed durable resistance, including some old South American varieties as Sinvalocho MA and Buck Manantial among others. The objective of the present work was to identify leaf rust resistance genes, both at seedling and adult stage, their mode of inheritance and the search of associated DNA markers, in the wheat cultivar Sinvalocho MA

The identification of resistance genes was carried out by using families of an F3 segregating population from the cross Sinvalocho MA and the susceptible line Gama 6, tested with different races of *P. triticina*. The searching for molecular markers (RAPDs, Microsatélites and AFLPs) was done by Bulk Segregant analysis. Two adult expressed resistance genes were determined (temporary named SV1 and SV2) and one for specific seedling resistance (*Lr3*). For SV1 gene two AFLPs, one RAPD and four associated Microsatellites were identified and located on chromosome 2DS. For SV2 gene two AFLPs and two associated Microsatellites were found, located on chromosome 3BS, for which no adult resistance gene was previously reported. The *Lr3* gene for seedling resistance was previously mapped on distal 6BL linked to several AFLPs. For genetic mapping an F9 population of 93 recombinant inbred lines (RILs) was used, allowing to encompass an interval of 13.7 cM and 11.9 cM, that included SV1 and SV2 genes, respectively. The closest marker to SV1 gene was established at 1.7 cM and for SV2 gene at 0 cM for two molecular markers. Durable resistance in Sinvalocho MA cultivar would be explained by the combination of adult resistance genes and specific genes expressed at seedling stage

Keywords: wheat, leaf rust, durable resistance, molecular markers

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 59–63

INTRODUCTION

Leaf rust, incited by the biotrophic fungus *Puccinia triticina*, is one of the most important diseases of wheat worldwide, causing in Argentina annual yield losses of about 5–10%. A large amount of genetic variation for pathogenicity is commonly observed in rust populations as well as the speed at which these populations adapt to resistance genes in wheat cultivars, making difficult to achieve a genetic control of the disease. Usually, most of new commercial varieties are resistant to rust populations. However, these varieties carrying different resistant gene combinations frequently become susceptible when are widely grown over the years, because of the occurrence and selection of new virulent strains. In spite of this situation some wheat varieties remain resistant for a long time. This kind of resistance was operationally defined as "durable" by Johnson (1981). Some old South American varieties as La Prevision 13, Pergamino Gaboto, Sinvalocho MA and Buck Manantial, among others, showed durable resistance (Favret et al. 1983, Dyck 1989). The study of the genetic basis of durable resistance, and the use of resistance genes coming from varieties showing this feature, may be an outstanding contribution for controlling this disease. In addition, the development of linked DNA molecular markers can assist the introduction, and selection of genes to be efficiently used in plant breeding programs.

The objective of the present work was to identify leaf rust resistance genes, both at seedling and adult stage, their mode of inheritance and the search of associated DNA markers, in the wheat cultivar Sinvalocho MA.

MATERIALS AND METHODS

The identification of resistance genes was carried out by using an F3 segregating population of 93 individuals from the cross Sinvalocho MA and the susceptible line Gama6. F3 family progeny tests, both at seedling and flag leaf stages were carried out using local races of *P. triticina*. To detect adult plant resistant genes, races to which Sinvalocho MA behaved as susceptible at seedling stage and resistant to adult stage (flag leaf) were used. An average of 10–15 plants per family was tested.

The searching for associated molecular markers (RAPDs, Microsatélites and AFLPs) was done by Bulk Segregant Analysis (BSA), by using polymorphic markers tested on 10 resistant and 10 susceptible F3 families for each identified resistance gene (Caetano Anolles and Gresshoff 1997, Michelmore et al. 1991). A total of 100 decamers were used for RAPDs, 75 primers for microsatellites and 109 primers combinations for AFLPs.

By using Chinese Spring nulli-tetrasomic and ditelosomic lines, chromosome arm location for adult plant resistance genes was determined.

For genetic mapping an F9 population of 93 recombinant inbred lines (RILs) from the cross Sinvalocho MA x Gama6 was used. Linkage analysis and distances were estimated using Mapmaker version 3.0 (Lander et al. 1987).

RESULTS AND DISCUSSION

Ninty three F3 families from the cross Sinvalocho x Gama6 were tested at flag leaf stage using *P. triticina* races F0-5 and 99-28. These 2 races allowed the detection of 2 independent resistance genes for adult expression. Race C2-12 allowed to detect a gene for seedling resistance. The three genes fitted monogenic and independent mendelian segregations (Tables 1, 2 and 3). The two adult expressed resistance genes determined, were temporary named SV1 and SV2, and the one for specific seedling resistance corresponds to *Lr3* gene.

For SV1 gene two AFLPs, one RAPD and four associated Microsatellites were identified and located on chromosome 2DS. For SV2 gene two AFLPs

Table 1. Ninety three F3 families from the cross Sinvalocho X Gama6 were tested at flag leaf stage using *P. triticina* races F0-5 and 99-28. The p value (independence test) between SV1 and SV2 genes was 0.5–0.7

		Race F05 $p(1:2:1) = 0.90-0.95$			
		Resistant	Heterozygous	Susceptible	
Race 99−28	Resistant	6	9	8	23
$p(1:2:1) =$	Heterozygous	14	24	11	49
0.80−0.90	Susceptible	5	12	4	21
		25	45	23	93

Table 2. Ninety three F3 families from the cross Sinvalocho X Gama6 were tested at flag leaf and seedling stage using *P.triticina* races F0-5 and C2-12 respectively. The p value (independence test) between SV2 y Lr3 genes was 0.30–0.50

		Race F05 $p(1:2:1) = 0.90-0.95$			
		Resistant	Heterozygous	Susceptible	
Race C2-12	Resistant	10	9	5	24
$p(1:2:1) =$	Heterozygous	11	28	12	51
0.30−0.50	Susceptible	4	8	6	18
		25	45	23	93

Table 3. Ninety three F3 families from the cross Sinvalocho X Gama6 were tested at flag leaf and seedling stage using *P. triticina* races 99-28 and C2-12 respectively. The p value (independence test) between SV1 y Lr3 genes was 0.30–0.50

		Race 99−28 $p(1:2:1) = 0.80-0.90$			
		Resistant	Heterozygous	Susceptible	
Race C2-12	Resistant	7	11	6	24
$p(1:2:1) =$	Heterozygous	11	31	9	51
0.30−0.50	Susceptible	5	7	6	18
		23	49	21	93

and two associated Microsatellites were found, located on chromosome 3BS. On chromosome 2DS was reported the location of two alleles of adult plant resistance gene *Lr22* (McIntosh et al. 1995). SV1 gene found in Sinvalocho could be an allele of *Lr22b*, but no allelism test was carried out yet. On the other hand, gene SV2 mapped on chromosome 3BS, on which no adult resistance gene was previously reported.

For genetic mapping an F9 population of 93 recombinant inbred lines (RILs) was used, allowing to encompass an interval of 13.7 cM and 11.9 cM, that included SV1 and SV2 genes, respectively. The *Lr3* gene for seedling resistance was previously mapped on distal 6BL linked to several AFLPs (Dieguez et al. 2005). The closest marker to SV1 gene was established at 1.7 cM and for SV2 gene at 0 cM.

The three genes identified in this variety were mapped at chromosome arm level and linked molecular markers were found, which would facilitate the potential use in plant breeding. Molecular marker assisted back crosses could be the fastest way to introgress genes into commercial varieties.

The durable resistance observed in Sinvalocho MA wheat may be explained by the combination of adult resistance genes and specific genes expressed at seedling stage (Sawhney et al. 1989, Saione et al. 1993).

ACKNOWLEDGEMENTS

This work has been supported by grants from CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas), SECYT (Secretaría de Ciencia y Técnica) and from INTA (Instituto Nacional de Tecnología Agropecuaria), Argentina. We wish to thank to Lic. Lida Pimper and Lic. Emiliano Altieri.

REFERENCES

Caetano-Arnolles G, Gresshoff PM (eds) (1997) DNA markers: protocols, applications and overviews. Wiley-VCH

Dieguez MJ, Altieri E, Ingala LR, Perera E, Sacco F, Naranjo T (2005) Physical and genetic mapping of amplified fragment length polymorphisms and the leaf rust resistance *Lr3* gene on chromosome 6BL of wheat. Theor Appl Genet DOI 10.1007/s00122–005–0122 (on line)

Dyck PL (1989) The inheritance of leaf rust resistance in wheat cultivars Kenyon and Buck Manantial. Can J Plant Sci 69:1113–1117

Favret EA, Saione HA, Franzone PM (1983) New approaches in breeding for disease resistance. Cereal Breeding and Production Symp, Argentina. Oregon State University, Special Report, p 718

Johnson R (1981) Durable resistance: definition of, genetic control, and attainment in plant breeding. Phytopathology 71:567–568

Lander E, Green P, Abrahamson J, Barlow A, Daley M, Lincoln S, Newburg L (1987) MAPMAKER: an integrative computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

McIntosh RA, Wellings CR, Park RF (eds) (1995) Wheat rusts: an atlas of resistance genes.. Kluwer Academic publishers

Michelmore RW, Paran I, Kesseli V (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci USA, 88:9828–9832

Saione HA, Favret EA, Franzone PM, Sacco F (1993) Host genetic analysis by using Puccinia recondita tritici induced mutants for increased virulence. J Phytopathology 138:225–232

Sawhney RN, Nayar SK, Sharma JB, Bedi R (1989) Mechanism of durable resistance: a new approach. Theor Appl Genet 78:229–232

CHARACTERIZATION OF GENES FOR DURABLE RESISTANCE TO LEAF RUST AND YELLOW RUST IN CIMMYT SPRING WHEATS

H.M. WILLIAM[1,3,*], R.P. SINGH[1], J. HUERTA-ESPINO[2] AND G. ROSEWARNE[1]

[1] *CIMMYT, Apdo-Postal 6-641, Mexico DF 06600, Mexico*
[2] *Campo Experimental Valle de Mexico-INIFAP, Apdo-Postal 10, 56230, Chapingo, Edo. De Mexico, Mexico*
[3] *Australian Molecular Plant Breeding Cooperative Research Centre (MPBCRC)*
[*] *E-mail: M.William@cgiar.org*

Abstract: Leaf and stripe rusts, caused by *Puccinia triticina* and *P. striiformis*, respectively, are globally important fungal diseases that cause significant yield losses in wheat. The objectives of our study were to characterize genetic loci associated with resistance to leaf and yellow rusts by using molecular markers in multiple populations derived from a common susceptible cultivar Avocet crossed with several improved CIMMYT spring wheats. Results obtained from two crosses are presented. Using bulked segregant analysis and partial linkage mapping with AFLPs, SSRs and RFLPs, we identified six independent loci that contributed to adult plant resistance (APR) to the two rust diseases in Avocet x Pavon76 population. The loci identified on chromosomes 1BL, 4BL and 6AL influenced resistance to both yellow and leaf rust commonly whereas two additional loci, on chromosomes 3BS and 6BL, had effects on yellow rust only. In Avocet x Parula population, four independent loci were identified. The loci on chromosomes 1BL and 7DS had effects commonly for the two rust diseases, whereas the locus on chromosome 7B had effects only on leaf rust and the locus on chromosome 3BS had effects only on yellow rust. A single chromosome recombinant line population was used to map the *Lr46/Yr29* locus as a simply inherited Mendelian trait

Keywords: wheat, leaf rust, stripe rust, durable resistance

INTRODUCTION

Among the biotic stresses that pose challenges to the global wheat production, diseases such as leaf (brown) rust, caused by *Puccinia triticina* Eriks and stripe rust, caused by *Puccinia striiformis* Westend, respectively, are considered important

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 65-70

diseases where heavy yield losses can occur when susceptible cultivars are used in areas with potential for high inoculum pressure. Although a large number of genes that confer race specific resistance to these rust diseases have been identified and characterized in hexaploid wheat, a majority of them have already been overcome by the emergence of new pathotypes (McIntosh et al. 1995, 2003). CIMMYT's wheat germplasm enhancement efforts have emphasized the use of durable rust resistance for the past several decades and have developed and deployed a number of cultivars with adequate levels of slow rusting resistance that are widely used in many developing country wheat growing areas (van Ginkel and Rajaram 1993). The known slow rusting genes that have remained effective since their deployment include *Lr34/Yr18* and *Lr46/Yr29*. Cultivars containing *Lr34/Yr18* have been deployed for over 50 years and Pavon76 *with Lr46/Yr29* has been effective since its release in 1976.

The aims of this study were: (1) characterize and establish genome locations of loci that confer adult plant resistance to leaf and yellow rust and (2) identify closely linked markers for possible applications in wheat improvement efforts.

MATERIALS AND METHODS

Two recombinant inbred line populations derived from crosses between the susceptible cultivar Avocet S and the resistant cultivars Pavon76 (146 F_6 lines) and Parula (139 F_6 lines), and a set of 184 F_5 families of a single chromosome recombinant line (SCRL) population involving chromosome 1B of Pavon76 were used. The procedures used in field evaluations of leaf rust and yellow rust resistance is described in William et al. (2003). Visual estimates of percent leaf area infected were recorded for both diseases. Readings were taken when the susceptible parent displayed severity of between 80 and 100% on flag leaves. A second reading was taken approximately 12 to 15 days after the first evaluation. The single chromosome recombinant line population (Singh et al. 1998) derived from the cross between Lalbahadur x Lalbahadur (Pavon 1B) was used to confirm the monogenic inheritance of *Lr46*. The SCRL population was evaluated for leaf rust resistance in order to classify the homozygous resistant, homozygous susceptible and segregating families, according to the procedures described in William et al. (2003).

Three sets of field data were collected for leaf rust and yellow rust in the two mapping populations of Avocet x Pavon76 and Avocet x Parula. Resistant bulks were made by combining equal quantities of DNA of 8–10 entries with good levels of resistance consistently across different data sets. Similarly, susceptible bulks were made by combining 10–12 entries with most susceptible rust scores. Bulked segregant analysis (Michelmore et al. 1991) and partial linkage mapping were used. The parents and bulks were screened with 96 *Pst*1/*Mse*1 AFLP primer combinations and once polymorphic primer combinations that distinguished the bulks were identified, they were used to screen the entire population. Furthermore, a random set of wheat microsatellites, RFLPs and STS markers identified from published wheat linkage maps (Sommers et al. 2004, Graingenes

website http://wheat.pw.usda.gov/GG2/index.html) were used to assign genomic locations of the identified loci as well as to expand the linkage groups associated with significant effects on the two rust diseases. Procedures involved in molecular characterization are according to Hoisington et al. (1994) and William et al. (2003).

Linkage analysis was conducted using MAPMAKER 3.0. Linkage group establishment and marker order assignments were done using a threshold LOD value of 3.0 and a recombination frequency value of 0.4 theta. Simple linear regression was used to calculate the coefficient of determination (R^2); a measure of the proportion of the total phenotypic variation explained by each marker. Computer software such as Q-gene (Nelson 1997) and composite interval mapping (CIM) developed at CIMMYT (Jiang and Zeng 1995) were used in interval mapping. The final readings of the leaf rust and yellow rust infection severities were utilized in all analysis of marker trait associations and interval mapping.

RESULTS AND DISCUSSION

Yellow rust and leaf rust severities of the two mapping populations displayed a continuous pattern of distribution between the severities of the two parents. Bulked segregant analysis with 96 AFLP primer combinations identified several polymorphic primer combinations between the two parents and the bulks in the two populations. Entire populations were used for genotyping with the identified, polymorphic primer combinations. Some primer combinations identified clear presence and absence of amplified fragments between the two bulks whereas others identified clear intensity differences between the two bulks under repeated conditions. A total of 27 AFLP primer combinations were genotyped across Avocet x Pavon76 population, whereas, 15 primer combinations were genotyped across the Avocet x Parula population. The genotypic data for AFLPs were combined with SSR, STS and RFLP genotypic data. A total of 233 markers were genotyped across the entire population of Avocet x Pavon76 and a total of 181 markers were genotyped on Avocet x Parula population. The putative loci identified by single factor analysis of variance were further analyzed by simple interval mapping (SIM). The QTL analysis of the three years of data for leaf and yellow rust were conducted individually using Q-gene (Nelson 1997) and the CIM program (Jiang and Zeng 1995). When slight shifts of the QTL peaks during different years were observed during interval mapping analysis, the peak identified with the joint analysis was considered to be the common peak associated with that QTL using the CIM program.

In Avocet x Pavon76 population, a total of six linkage groups were identified with effects on both yellow and leaf rust commonly or on only one of the two diseases. Three loci located on chromosomes 1BL, 4BL and 6AL had effects commonly on the two rust diseases. The locus identified on chromosome 3BS and 6BL had effects only on yellow rust, whereas there was a locus, identified by AFLPs that had minor effects on leaf rust. The LOD scores, additive effects and the percentage phenotypic variation explained by each marker as well as the total phenotypic variation explained jointly by all markers (R^2%) for each of the three years are

Table 1. Loci associated with slow rusting resistance in Avocet x Pavon76 population

Yellow rust chromosome	Closest marker	Year 1			Year 2			Year 3			Joint	Effect
		LOD	Additive effect	R2 (%)	LOD	Additive effect	R2 (%)	LOD	Additive effect	R2 (%)	LOD	
1 BL	gwm259	13.02	17.9	33.0	16.79	17.4	39.9	14.25	13.5	36.5	17.1	Pavon76
3BS	pAATmCAC	1.40	6.0	4.3	1.05	4.6	3.3	1.89	5.1	5.9	2.03	Pavon76
4BL	gwm495	2.60	8.5	8.0	2.76	7.7	8.4	4.35	8.0	13.1	4.51	Avocet
6AL	gwm617	2.63	9.7	7.90	2.82	8.9	8.4	1.91	6.2	5.8	3.09	Avocet
6BL	pAGGmCGA	6.54	13.1	18.60	2.98	8	9	4.86	8.5	14.0	7.64	Pavon76
Total R^2(%)				53.30			52.3			55.6		
Leaf Rust												
1BL	gwm259	19.9	20.1	49.7	33.7	31.4	61.6	21.64	25.7	50.9	33.9	Pavon76
4BL	gwm495	2.05	8.8	6.1	0.81	6.2	2.9	1.60	8.2	5.5	2.80	Avocet
6AL	gwm617	2.02	8.0	4.4	1.34	8.6	2.9	0.93	6.8	2.4	2.35	Avocet
Unknown	pACGmCAG	2.22	8.4	7.1	1.29	8.1	3.3	1.43	8.1	5.0	2.38	Pavon76
Total R^2(%)				55.20			63.7			53.7		

Table 2. Loci associated with slow rusting resistance for leaf rust and yellow rust in Avocet X Parula population. Three year average % reduction in disease severity between the two marker classes

Chromosome	Closest marker	Leaf rust	Stripe rust	Named genes
1BL	*gwm259*	15	16	*Lr46, Yr29*
7DS	*gwm295*	56	46	*Lr34, Yr18*
7B	PCR105	29	–	–
3BS	*Sun2*	–	12	*Yr30, Sr2*

indicated in Table 1. The markers associated with chromosome 1BL are identifying the genes *Lr46/Yr29*, whereas the markers associated with 3BS identify a locus that has minor effects of yellow rust, identified by *Yr30* which is in the same region as the durable stem rust resistance gene *Sr2*.

Similar observations were made in the loci identified in Avocet x Parula population. Markers identified to be associated with genes *Lr46/Yr29* in Avocet x Pavon76 also identified a locus with common effects on both leaf and yellow rust in Avocet x Parula population. Moreover, we have validated the markers identified by Suenaga et al. (2003) associated with genes *Lr34/Yr18* in Avocet x Parula population. In addition to these two loci, we have identified two additional loci, one on chromosome 3BS, associated with *Yr30* that had minor effects on yellow

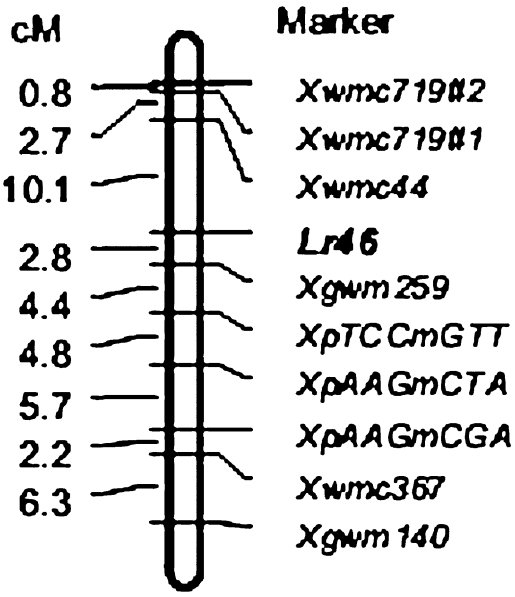


Figure 1. Linkage map of chromosome 1B of the single chromosome recombinant line population of Lalbahadur x Lalbahadur (Pavon1B)

rust and another locus on chromosome 7B with significant effects on leaf rust only. The loci identified in Avocet x Parula population are in Table 2.

We have also used the SCRL population of Lalbahadur x Lalbahadur (Pavon1B) to map the gene *Lr46* as a single Mendelian trait. Markers already known to be associated with *Lr46Yr29* in Avocet x Pavon76 were used in developing the linkage group of the SCRL population. The linkage map developed using 184 F_5 families is app. 40cM in length and is in Fig. 1. The linkage group has five microsatellite markers as well as three AFLP markers. The microsatellite markers flanking the genes *Lr49* could possibly be used in marker assisted selection efforts aimed at manipulating the durable leaf rust as well as yellow rust genes *Lr46/Yr29* in breeding material.

REFERENCES

Hoisington D, Khairallah M, Gonzalez de Leon D (1994) Laboratory protocols: CIMMYT applied molecular genetics laboratory. 2nd edn. Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT), Mexico DF, Mexico

Jiang C, Zeng ZB (1995) Multiple-trait analysis of genetic mapping for quantitative trait loci. Genetics 140:1111–1127

McIntosh RA, Wellings CR, Park RF (1995) Wheat Rusts: An Atlas of Resistance Genes. CSIRO Publications, Melbourne, Australia, p 200

McIntosh RA, Yamazaki Y, Devos KM, Dubkowsky J, Rogers WJ, Appels R (2003) Catalogue of gene symbols for wheat. Proceedings of the 10th International Wheat Genetetics Symposium, Paestum, Italy

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci USA 88:9828–9832

Nelson JC (1997) Q-GENE: Software for marker-based genomic analysis and breeding. Mol Breed 3:231–245

Singh RP, Mujeeb-Kazi A, Huerta-Espino J (1998) Lr46: a gene conferring slow-rusting resistance to leaf rust in wheat. Phytopathology 88:890–894

Sommers DJ, Isaac P, Edwards K (2004) A high density microsatellite consensus map for bread wheat (Triticum aestivum L). Theor Appl Genet 109:1105–1114

Suenaga K, Singh RP, Huerta-Espino J, William HM (2003) Microsatellite markers for genes Lr34/yr18 and other quantitative trait loci for leaf rust and stripe rust resistance in bread wheat. Phytopathology 93:881–889

van Ginkel M, Rajaram S (1993) Breeding for durable disease resistance in wheat: an international perspective. In: Jacobs T, Parlevliet JE (eds) Durability of Disease Resistance. Kluwer Academic Press, Dordrecht, The Netherlands, pp 259–272

William HM, Singh RP, Huerta-Espino J, Ortiz-Islas S, Hoisington D (2003) Molecular marker mapping of leaf rust resistance gene Lr46 and its association with stripe rust resistance gene Yr29 in wheat. Phytopathology 93:153–159

EPIDEMIOLOGY OF *PUCCINIA TRITICINA* IN GANGETIC PLAIN AND PLANNED CONTAINMENT OF CROP LOSSES

S. NAGARAJAN[1,*] AND M.S. SAHARAN[2]

[1]*Chairman, Protection of Plant Varieties and Farmers' Rights. Department of Agriculture and Cooperation, Ministry of Agriculture, NASC Complex, Todapur, New Delhi 110012.*
[2]*Directorate of Wheat Research, Kunjpura Road, Karnal, Haryana, India.*
[*]*E-mail: snagarajan@flashmail.com.*

Abstract: Leaf or brown rust of wheat incited by *Puccinia triticina* Eriks. is the most important one. The pathogen spreads across national boundaries, virulences get mixed and the disease severity level reached does affect the crop productivity. The Gangetic Plain (GP) being warmer sowing is done in November and wheat is harvested by mid April. The 18 million hectares under wheat in this area is predominantly irrigated and well fertilized having a cultivar mosaic of at least 30 or more genotypes differing for their leaf rust resistance. The primary inoculum source is the Cental Himalaya (CH) of Central Nepal and here the CIMMYT sponsored activities have promoted varietal diversification. Shift in sowing time and cultivation of several varieties differing in resgene combination over eastern GP has delayed the process of establishment of the primary foci of leaf rust here before January end. Insufficient inoculum multiplication then curtails the spread of urediospores westward beyond Delhi. Build up of disease severity levels to epidemic proportions gets delayed by at least two weeks. As a consequence of this temporal delay, wheat gets harvested before the severity could inflict crop losses. Occasional inter-zone exchange of inoculum before the crop harvest facilitates the injection of new virulences

Keywords: leaf rust, epidemiology, resistance genes, Gangetic Plains

INTRODUCTION

India, the major wheat grain producer in South Asia (SA), is both climatically and agriculturally interwoven with the adjoining countries. Nearly two billion people live in the South Asian nations of Bangladesh, Bhutan, India, Nepal and Pakistan. In this vast stretch of land, despite several similarities visible shift in geographic features and flora are observed as one traverses a distance of 400 km or moves up an elevation of 2000 m altitude. The SA also has largest undernourished cattle

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 71–76.

population and the wheat straw meets the fodder demand during summer months. Wheat (*Triticum* spp.) is an important life sustaining food grain crop of this area. In this tract bread wheat or *T. aestivum* L. emend. Fiori & Paol. is the most important followed by macaroni wheat or *T. durum* Desf and occasionally fields of *T. monococcum* L. have also been spotted.

Establishment of Initial Foci of *Puccinia Triticina (Ptr)* in Northern India

In the Gangetic Plain (GP) wheat sowing is done in November and the crop is harvested by the end of March/mid April. The 18 million hectares under wheat in the GP is predominantly irrigated and adequately fertilized. In the Himalayas, the alternate host namely, *Thalictrum* spp., is not functional. The pathogen survives in the higher Himalayan valleys as urediospore around the year on the normal season wheat or self sown wheat or on the off-season crop. Inoculum of Ptr available from the self sown plants or grasses spread to the foot hills of Nepal, Bihar and east Uttar Pradesh by early January, enabled by the mid low level wind circulation pattern. The urediospores land on crop surface (Metha 1952) and the frequent dew condensation ensures infection and establishment of primary foci. Such foci occur in the GP to a distance of about two hundred and seventy kilometers from the foot hills of central Nepal. By mid January, several widely distributed foci occur all over the eastern GP. These foci serve as the multiplication ground for inoculum build up and its subsequent spread over the whole of the GP.

By the end of January on vulnerable varieties leaf rust severity reaches 10S as per Cobbs scale. The winter precipitation that occurs in the GP during January to April is as a consequence of weather systems called the "Western Disturbances" (Nagarajan and Joshi 1978, Pisharoty and Desay 1956). Under the spell of this, the urediospores of P*re* originating the multiple initial foci get churned, transported and deposited all over the western parts of the GP (west Uttar Pradesh, Haryana, Punjab and northern Rajasthan). The weather system spreads the inoculum all over the eastern GP and provides a congenial weather for severe disease development (Joshi et al. 1977). The terminal disease severity in the next 6–8 weeks time reaches a level to inflict severe crop loss (Joshi 1980). A massive build up of Ptr over the eastern parts of the GP favors the retreat of inoculum gradually back to the Himalayan valleys in Nepal during April where still vulnerable standing crop of wheat is available. Thus the virulences selected in the GP retreat to the Himalayan valley to survive and evolve. Thus these matching virulences ensure a disease recurrence in the GP during next season and accelerate the process of varietal breakdown.

Survival of *Ptr* during the Desiccating Summer Months of May to July

The north – western Himalayas are relatively cooler than central Nepal parts of the mountain range. This is reflected by the fact that centres of leaf rust infection start very close to the foot hills in western GP mainly due to the "Katabatic winds" that inject the inoculum from the hills into the plains all along the river openings. Due

to this along the foot hills of Uttarachal, Himachal Prasedh, Punjab and Jammu region of J&K foci of Ptr appear by early January on vulnerable varieties invariably at the mouth of the river. By early March ambient temperature increases activating these foci to expand and spread and together with the massive incoming inoculum from eastern GP result in severe leaf epidemic development. By mid April the terminal leaf severity score often exceeds 60S to 80S on susceptible wheat and grain production over the vast 9.0 million ha, can get educed by 15–20 per cent. Since the western GP produce around 35 million tons of grain or half of India's wheat production and is a major surplus grain contributor any fall in total production has several ramifications.

Cross Border Flow of *Ptr* Inoculum Between Nepal to India and then Between India and Pakistan

During severe Ptr years in the adjacent Punjab of Pakistan also, leaf rust epidemic occurs as the weather on both sides of Punjab is comparable. But the primary inoculum source of Ptr in Pakistan Punjab is from the North West Frontier Province (NWFP). Naturally the virulence spectrum of Pakistan is not identical to that of India's NWPZ. Not withstanding this, Pakistan also gets *Ptr* inoculum from Afghanistan side. Prior to harvest, during mid late April, there occurs a free flow of inoculum from Pakistan side to adjacent areas of western GP. Such very late admixtures of urediospores enable the spread of new *Ptr* virulence to the western GP and to the Himalayan Valleys. Thereafter, the urediospores of Ptr retreat to the interior valleys of the Himalayas and survive there on the spring sown wheat crop. Again next year during December, Ptr virulence gets blown from the interior Himalaya to outer Himalayan foot hills and to the plains of western GP to initiate fresh foci of leaf rust along the foot hills (Joshi et al. 1977). After the harvest of wheat in the western GP, the inoculum gradually retreats to the interior valleys of Himalayas (Nagarajan and Joshi 1985) to over-summer, survive, evolve on the summer sown wheat crop and in this process there occurs a gradual shift in virulence pattern resulting in 'Boom and Burst' cycle.

Spread of *Ptr* from Himalaya (Nepal) to India and Bangladesh

Bangladesh almost covers the delta of two great rivers namely, Ganges and the Brahmaputra. The Ptr does occur in Bangladesh by January as the country has nearly a million hectares under wheat in the northern parts close to the Indian state of Bengal. Leaf rust pathogen does not survive during the summer and heavy downpour monsoon and arrives each year from the Himalaya and from the Indian parts of the eastern GP. The virulence spectrum is similar to the eastern GP and since the varietal spectrum in Nepal, India and Bangladesh are no more identical variety based gene deployment has happened curtailing the disease severity in all these countries by creating a temporal and special hurdle.

Epidemiological Summing

The nature and recurrence of leaf rust over the GP has distinct epidemiological zones namely, Zone 1: central Nepal/western Himalaya/Hindu Kush region where the pathogen survives around the year and supplies the primary inoculum to the Gangetic plain and Punjab each year. This undulating steep terrain provides various niches for the pathogen to survive, evolve and spread. The Zone 2 covers a narrow strip of fifty kilometers or so from the foot hills of Jammu to Pantnagar and then balloons up to Banarus and narrowing down to the Darjeeling foot hills. The eastern part of the GP is warm, foggy and misty and is the area where primary foci develop and huge increase of inoculum (urediospores) happens. The Zone 3 is the remaining part of the western GP, the cool well irrigated highly productive wheat continuum (Fig. 1). This clear zonation offers enormous opportunity for gene/varietal deployment to contain the *Ptr* epidemic in the GP (Nagarajan et al. 1980). It is visualized that by releasing wheat varieties with different *Lr* gene

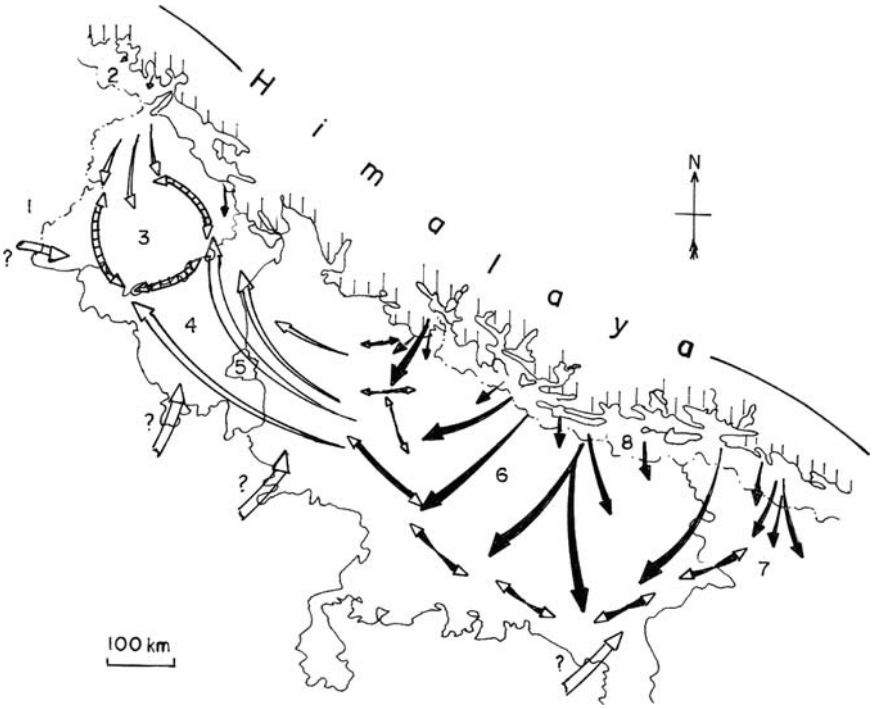


Figure 1. Geophytopathology map on the spread of P.triticina urediospores from the over wintering Himalayan source to the Indus plain. Filed 'Black arrow' shows spread of pathogen till January end, white arrow is spread during February and dotted denotes spread during March. Arrow ending with? denotes stray intrusion during April just prior to harvest. Shaded Himalaya means elevations of 2000m. The plains are just 50m above mean sea level

combinations in Zone 1 than in Zone 2, Ptr build-up can be mitigated. Then handling zone-3 will become easy due to cut down and delay of initial doses of Ptr inoculum reaching the eastern GP. This strategy was actually implemented and the occurrence of epidemic for two decades was efficiently avoided.

Gene Deployment was Planned and Executed

For containing the crop losses inflicted by the leaf rust epidemics it is necessary to understand the pathogen spectrum, its distribution, dynamics and the leaf rust resistance genes present in the recommended wheat cultivars. The virulence analysis procedure was modified from differential based race identification to (near) isogenic line/lines with known gene(s) based virulence analysis procedure (Nagarajan et al. 1983). The virulence nomenclature itself indicated the virulence genes present in the isolate. By choosing the appropriate virulences and challenging each one of them on the tester line host-pathogen reaction matrix was generated and following the gene matching techniques (Nagarajan et al. 1987) speculating the possible *Lr* gene combinations in the new wheat varieties became possible. Following this procedure several of the pipeline wheat varieties queuing to get released for cultivation in the Himalayan region and the GP were evaluated for the leaf rust resistance genes they carry. Thereby, wheat varieties with different leaf rust gene(s) combination were recommended for cultivation and the varietal mosaic thus created effectively delayed the development of leaf rust epidemic (Nagarajan et al. 1980). And for the last two decades it has been successfully followed as a national varietal strategy to contain leaf rust.

Support from Search for New Resistance Genes

Using marker aided selection several *Lr* gene combinations have been made in good varietal background and the materials generated serve as genetic stock and facilitate breeding for leaf rust resistance (Cherukuri et al. 2005, Gupta et al. 2005, Prabhu et al. 2004). Efforts were focused to identify and characterize new *Lr* genes, to keep the gene deployment strategy functional. Saini et al. (2002) reported two new genes *Lr 48* and *Lr 49* and are available for plant breeders. At Directorate of Wheat Research, Karnal, the presence of rust resistance genes *Lr 10, Lr 19, Lr 24, Lr 26* and *Lr 37* in AVT entries can now be detected through molecular markers. Also STS marker based detection of *Lr 19* in Thatcher NIL (Tc*Lr19) and Inia66//CMH81A575, *Lr 24* in Arkan, Blue Boy II, Agent and CI17907 has been accomplished (Anonymous, 2005). This integrated effort was facilitated by supportive fund and help from ACIAR, CIMMYT and Indo-Swiss program apart from regular Indian grants from the DBT and ICAR. This international hook-up enabled the national program achieve the set target in an effective manner (Nayar 1989).

REFERENCES

Anonymous (2005) Marker assisted selection for rust resistance. In: Jag S, Mishra B (eds) Project Director Report. Directorate of Wheat Research, Karnal, 132 001, Haryana, India, pp 14–15

Cherukuri DP, Gupta SK, Charpe A, Koul S, Prabhu KV, Singh RB, Rizwanul Haq QM (2005) Molecular mapping of Aegilops speltoides derived leaf rust resistance gene Lr 28 in wheat. Euphytica 143:19–26

Gupta SK, Charpe A, Koul S, Prabhu KV, Rizwanul Haq QM (2005) Development and validation of molecular markers linked to an Aegilops umbellulatae- derived leaf rust resistance gene, Lr 9 for marker-assisted selection in bread wheat. Genome 48:823–830

Joshi LM (1980) The disease surveillance system in India. In: Govindu HC, Veeresh GK, Walker PT, Jenkyn JF (eds) Assessment of Crop Losses due to Pests and Diseases. Univ Agric Sci, Hebbal, Bangalore, UAS Tech Ser N° 3 pp 211–217

Joshi LM, Nagarajan S, Srivastava KD (1977) Epidemiology of brown and yellow rusts of wheat in India. I. Place and time of appearance and spread. Phytopath Z 90:116–22

Mehta KC (1952) Further studies on cereal rusts in India. Part II. Sci. Monogr. N°18, Indian Council of Agricultural Research, New Delhi, India

Nagarajan S, Joshi LM (1978) Epidemiology of brown and yellow rusts of wheat over north India. II. Associated meteorological conditions. Plant Dis Rep 62:186–188

Nagarajan S, Joshi LM (1985) Epidemiology in the Indian sub continent. In: Roelfs AP, Bushnell WR (eds) The Cereal Rusts, Diseases, Distribution, Epidemiology, and control, Vol. II. Academic Press, New York, pp 371–399

Nagarajan S, Joshi LM, Srivastava KD, Singh DV (1980) Epidemiology of brown and yellow rusts of wheat in north India. IV. Disease management recommendations. Cereal Rusts Bull 7:15–20

Nagarajan S, Nayar SK, Bahadur P (1983) The proposed Brown Rust of Wheat (Puccinia recondita f. sp. tritici) Virulence Monitoring System. Curr Sci 52:413–416

Nagarajan S, Nayar SK, Bahadur P, Bhardwaj SC (1987) Evaluation of some Indian wheats for Yr, Lr, and Sr genes by matching technique and genetic uniformity observed. Cereal Rusts Bull 15:53–64

Nayar SK (1989) Genetics and Breeding for Rust Resistance in Wheat. ACIAR-ICAR Collaborative Research Report, Plant Breeding Institute, University of Sydney, Australia, p 50

Pisharoty PR, Desai BN (1956) Western disturbances and Indian weather. Indian J Meteorol Geophys 7:333–338

Prabhu KV, Gupta SK, Charpe A, Koul S (2004) SCAR marker tagged to the alien leaf rust resistance gene Lr 19 uniquely marking the Agropyron elongatum gene Lr 24 in wheat: a revision. Plant Breed 123:417–420

Saini RG, Kaur M, Singh B, Sharma S, Nanda GS, Nayar SK, Gupta AK, Nagarajan S (2002) Gene Lr 48 and Lr 49 for hypersensitive adult plant leaf rust resistance in wheat (Triticum aestivum L). Euphytica 124:365–370

STRIPE RUST RESISTANCE IN CHINESE BREAD WHEAT CULTIVARS AND LINES

X.C. XIA[1,*], Z.F. LI[1], G.Q. LI[1,2], Z.H. HE[1,3,*], AND R.P. SINGH[4],

[1]*Institute of Crop Science/National Wheat Improvement Center, Chinese Academy of Agricultural Sciences (CAAS), Zhongguancun South Street 12, Beijing 100081, China*
[2]*Faculty of Agronomy, Xinjiang Agricultural University, Nanchang Road 42, Urumqi 830052, Xinjiang, China*
[3]*CIMMYT China Office, c/o CAAS, Zhongguancun South Street 12, Beijing 100081, China*
[4]*International Maize and Wheat Improvement Center (CIMMYT), Apdo. Postal 6–641, 06600, Mexico D.F., Mexico*
[*]*E-mail: xiaxianchun@caas.net.cn or zhhe@public3.bta.net.cn*

Abstract: Identification of stripe rust resistance genes is extremely important for developing new resistant cultivars to control the disease. A total of 98 Chinese wheat cultivars and advanced lines were inoculated with 26 isolates of *Puccinia striiformis* f. sp. *tritici* (PST) for postulation of stripe rust resistance genes at seedling stage. The results indicated that 42 cultivars and lines possess the resistance gene *Yr9*, either independently or in combination with other resistance genes. Nineteen accessions carry *Yr24* or *Yr26*. Seven entries showed resistant to the 26 isolates tested, whereas, six cultivars and lines were susceptible to all of them. It was indicated in the test that *Yr10, Yr15, Yr24*, and *Yr26* were effective resistance genes against Chinese PST isolates, whereas *Yr1* and *Yr6* were susceptible to them. SSR analysis indicates that *YrCH42* in Chinese wheat cultivar Chuanmai 42 is closely linked to *Xgwm498* and *Xwms273*, with genetic distances of 1.6 cM and 2.7 cM, respectively. *YrCH42*, *Yr24* and *Yr26* are probably common genes according to their reaction patterns to the 26 isolates tested, as well as their chromosomal location and origins. In addition, a new stripe rust resistance gene on chromosome 7BL was detected in the Chinese wheat line Zhou 8425B, which is closely linked to *Xcfa2040* and *Xbarc32*, with genetic distances of 1.4 cM and 4.8 cM, respectively

Keywords: common wheat (*Triticum aestivum* L.), stripe rust, *Puccinia striiformis* f. sp. *tritici*, gene-for-gene specificity, SSR marker

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 77–82

INTRODUCTION

Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici* (PST), is a major disease of common wheat (*Triticum aestivum* L.) in cool and moist production regions (Singh et al. 2000). In terms of area affected by stripe rust, China is the largest epidemic region in the world (Stubbs 1988). Stripe rust is most destructive to autumn-sown wheat in northwest and southwest China when susceptible cultivars are grown and the weather is favorable for the disease (Wan et al. 2004). Destructive epidemics of stripe rust in China occurred in 1950, 1964, 1990, and 2002, which caused yield losses of 6.0, 3.2, 1.8 and 1.3 million tonnes, respectively (Li and Zeng 2000; Wan et al. 2004). The most recent country-wide epidemic in 2002 was caused by a new Chinese PST isolate designated CYR32 (Wan et al. 2004).

The use of resistant cultivars is the most economical and environmentally sound method to reduce damage caused by stripe rust. Molecular markers are useful tools for pyramiding of different resistance genes and developing multi-line cultivars targeting for durable resistance to the disease. The objectives of this study are to investigate probable resistance genes conferring resistance in Chinese wheat cultivars and advanced lines, and identify stripe rust resistance genes in Zhou 8425B and Chuanmai 42 using molecular markers.

MATERIALS AND METHODS

Wheat Germplasm and PST Isolates

A total of 98 Chinese wheat cultivars and advanced lines were employed for the test of seedling resistance to 26 PST isolates in the greenhouse. These accessions are available at the National Wheat Improvement Center of China upon request. The F_1 offspring, 611 F_2 plants and 97 F_3 lines derived from the cross of Zhou 8425B/Chinese Spring, and 787 F_2 plants from Chuanmai 42/Taichuang 29 were included in the genetic analysis. Three cultivars, Maris Huntsman, Heines VII and Clement, with different resistance genes were kindly provided by Dr. Y. C. Niu, Institute of Plant Protection, Chinese Academy of Agricultural Sciences (CAAS). The near-isogenic lines Yr6/6*Avocet S, Yr15/6*Avocet S, Yr24/3*Avocet S and Yr26/3*Avocet S were kindly provided by Dr. C. R. Wellings, Plant Breeding Institute, University of Sydney, Australia. The 26 PST isolates used for seedling test were collected from China and other countries (Niu et al. 2000).

Seedling Test

Seeds were planted in flowerpots with a diameter of 10 cm and 10 cm in height, with seven plants in each pot. Seedlings were inoculated with PST isolates when the first leaf was fully expanded. After inoculation, the seedlings were placed in a dew chamber at 90 and 100% of relative humidity for 24 hr, and then transferred to a greenhouse maintained with 14 hr light/10 hr dark photoperiod at 12–17 °C. Infection types (IT) were scored 15–16 days after inoculation when the susceptible check, Mingxian169, was fully infected.

Infection type was scored using a 0–4 scale (Bariana and McIntosh 1993), in detail, with 0 for no visible uredia, 0; for small chlorotic flecks without sporulation, $0;^{+}$ for large chlorotic areas without sporulation, 1 for chlorosis and necrosis associated with extremely limited uredial development, 1^{+} for chlorosis and necrosis associated with limited uredial development, 2 for chlorosis and necrosis with little intermediate sporulation, 2^{+} for chlorosis and necrosis among abundant intermediate sporulation, 3^{-} for chlorosis and necrosis among increased uredial development, 3 for chlorosis with increased uredial development, 3^{+} for occasionally necrosis with abundant sporulation, and 4 for abundant sporulation without chlorosis. The plants with an IT of $0–2^{+}$ were considered as resistant and those with an IT of $3^{-}–4$ as susceptible.

SSR Analysis

Genomic DNA was extracted using the CTAB protocol (Sharp et al. 1988). The resistant and susceptible bulks, composed of equal amounts of DNA from 20 resistant and 20 susceptible F_2 plants, respectively, were used for bulk segregant analysis (BSA) (Michelmore et al. 1991).

In total, 819 pairs of wheat SSR primers were surveyed with two parents and the resistant and susceptible bulks, in which, 240 GWM (Gatersleben wheat microsatellite) primer sequences were described by Röder et al. (1998) and Pestsova et al. (2000), 560 WMC primer sequences developed by the Wheat Microsatellite Consortium (WMC), a private effort coordinated by Dr. P. Isaac (IDnagenetics, Norwich, UK), 10 BARC markers on chromosome 1B were from Song et al. (2002), and nine CFA and CFD markers on chromosome 1B from Dr. P. Spurdille (INRA). These SSR primers were available at http://www.graingenes.org.

PCR reaction was performed in a PTC200 Peltier Thermal Cycler in a volume of 20 μl containing 1.0U of Taq DNA polymerase, 2 μl of 10×buffer 50 mmol of KCl, 10 mmol of Tris-Cl, 1.5 mmol of $MgCl_2$, pH 8.3), 200 μmol of each of dNTPs, 6 pmol of each of primers and 50–100 ng of template DNA. The PCR conditions were as follows: denaturation at 94 °C for 4 min, followed by 35 cycles of 94 °C for 1 min, 50–61 °C (depending on primers) for 1 min, 72 °C for 1 min, and a final extension for 10 min at 72 °C. PCR products were mixed with 4 μl of the formamide loading buffer (98% formamide, 10 mM EDTA, 0.25% bromophenol blue, 0.25% xylene cynol, pH 8.0) and heated at 94 °C for 5 min. Five to 7 μl of each sample was loaded on 6% denaturing polyacrylamide gels and run at 80 W for approximately 1.5 hr, and then resolved by the silver staining method as described by Bassam et al. (1991).

Statistical Analysis and Genetic Mapping

Chi-squared (χ^2) test was used to evaluate the goodness of fit for the observed and expected ratios of segregation in the F_2 and F_3 populations. Linkage analysis was conducted with Mapmaker 3.0b (Lincoln et al. 1992). The Kosambi (1944) function was employed to calculate the map distance in Mapmaker 3.0b, and a LOD score

of 3.0 was used as a threshold for declaration of linkage. The genetic map was drawn with the software Mapdraw V2.1 (Liu et al. 2003).

RESULTS AND DISCUSSION

Seedling Resistance Genes in Chinese Wheat Cultivars and Advanced Lines

The seedling test of 98 Chinese wheat cultivars and advanced lines with 26 PST isolates indicated that *Yr2*, *Yr3a*, *Yr4a*, *Yr6*, *Yr7*, *Yr9*, *Yr26*, *Yr27*, *YrSel*, and *YrSD*, either independently or in certain combinations, were detected in 72 genotypes, while known resistance genes were not identified in the other 26 accessions. The resistance genes *Yr9* and *Yr26* were found in 42 and 19 accessions, respectively. *Yr3a* and *Yr4a* were detected in two entries, and four cultivars may contain *Yr6*. Three cultivars were postulated to possess *YrSD* and one carried *Yr27+YrSel*, and one cultivar may possess *Yr7*.

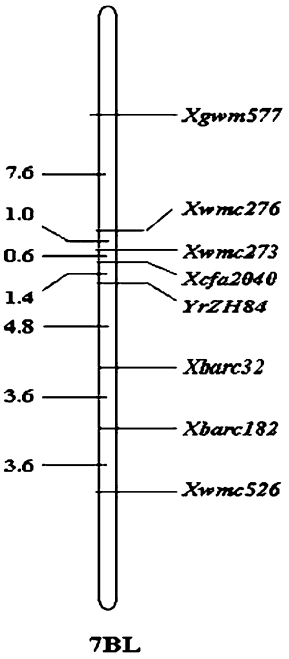


Figure 1. Linkage map of stripe rust resistance gene *YrZH84* and seven SSR markers on chromosome 7BL. Locus names and corresponding locations on the genetic map are indicated on the right side. Map distances (Kosambi) in centi-Morgans, are shown on the left side

Mapping of Stripe Rust Resistance Gene in Zhou 8425B

Bulk segregant analysis detected seven polymorphic markers on chromosome 7BL, which were used for genotyping $F_2$and F_3 populations. Linkage analysis indicated that Zhou 8425B carries a single dominant resistance gene, temporarily designated *YrZH84*, closely linked to SSR markers *Xcfa2040-7B* and *Xbarc32-7B* with genetic distances of 1.4 cM and 4.8 cM, respectively (Fig. 1). In a seedling test with 26 PST isolates, the reaction patterns of *YrZH84* were different from those of lines carrying *Yr2* and *Yr6*. It was concluded that *YrZH84* is a new stripe rust resistance gene based on its chromosomal location and reaction patterns to the 26 PST isolates tested.

Mapping of Stripe Rust Resistance Gene in Chuanmai 42

Bulk segregant analysis detected nine polymorphic markers, which were subsequently used for genotyping the F_2 population. Results indicate that the stripe rust resistance of Chuanmai 42 is conferred by a single dominant gene, temporarily designated *YrCH42*, and close to the centromere of chromosome 1B and resided in a region flanked by nine SSR markers *Xwmc626*, *Xgwm273*, *Xgwm11*, *Xgwm18*,

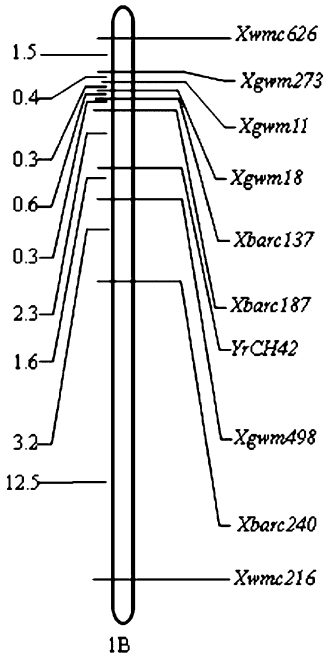


Figure 2. Linkage map of stripe rust resistance gene *YrZH42* and nine SSR markers on chromosome 1B. Locus names and corresponding locations on the genetic map are indicated on the right side. Map distances (Kosambi) in centi-Morgans, are shown on the left side

Xbarc137, *Xbarc187*, *Xgwm498*, *Xbarc240* and *Xwmc216*. The resistance gene was closely linked to *Xgwm498* and *Xbarc187* with genetic distances of 1.6cM and 2.3cM, respectively (Fig. 2). In the seedling test with 26 PST isolates, *YrCH42*, *Yr24* and *Yr26* showed common reaction patterns to the isolates tested, indicating that they are probably common resistance genes in considering their common origin and common chromosomal location.

ACKNOWLEDGEMENTS

We are grateful to Dr. Colin Wellings, Plant Breeding Institute, University of Sydney, Australia, for providing a set of isogenic lines with different stripe rust resistance genes. This project was funded by National 863 program (2003AA207090)and National Natural Science Foundation of China (30471083 and 30220140636).

REFERENCES

Bariana HS, McIntosh RA (1993) Cytogenetic studies in wheat XV. Location of rust resistance genes in VPM1 and their genetic linkage with other disease resistance genes in chromosome 2A. Genome 36:476–482

Bassam BJ, Caetano-Anolles G, Gresshoff PM (1991) Fast and sensitive silver staining of DNA in polyacrylamide gels. Annu Biochem 196:80–83

Kosambi DD (1944) The estimation of map distances from recombination values. Annu Eugen 12: 172–175

Li ZQ, Zeng SM (eds) (2000) Wheat Rusts in China. China Agriculture Press, Beijing

Lincoln S, Daly M, Lander E (1992) Constructing genetic maps with Mapmaker/EXP3.0. Whitehead Institute Techn Rep, 3rd edn. Whitehead Institute, Cambridge, Masachussetts, USA

Liu RH, Meng JL (2003) MapDraw: a Microsoft Excel macro for drawing genetic linkage maps based on given genetic linkage data. Hereditas (Beijing) 25(3):317–321

Michelmore RI, Paran R, Kesseli RV (1991) Identification of markers linked to disease resistance genes by bulked segregant analysis. Proc Natl Acad Sci USA 88:9828–9832

Niu YC, Qiao Q, Wu LR (2000) Postulation of resistance genes to stripe rust in commercial wheat cultivars from Henan, Shandong, and Anhui provinces. Acta Phtopathologica Sinica 30(2):122–128

Pestsova E, Ganal MW, Röder MS (2000) Isolation and mapping of microsatellite markers specific for the D genome of bread wheat. Genome 43:689–697

Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier M-H, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007–2023

Sharp PJ, Kreis M, Shewry PR, Gale MD (1988) Location of β-amylase sequence in wheat and its relatives. Theor Appl Genet 75:286–290

Singh RP, Nelson JC, Sorrells ME (2000) Mapping *Yr28* and other genes for resistance to stripe rust in wheat. Crop Sci 40:1148–1155

Stubbs RW (1988) Pathogenicity analysis of yellow (stripe) rust of wheat and its significance in a global context. In: Simmonds NW, Rajaram S (eds) Breeding Strategies for Resistance to the Rusts of Wheat. CIMMYT, Mexico DF, Mexico, pp 23–28

Wan AM, Zhao ZH, Chen XM, He ZH, Jin SL, Jia QZ, Yao G, Yang JX, Wang BT, Li GB, Bi YQ, Yuan ZY (2004) Wheat stripe rust epidemic and virulence of *Puccinia striiformis* f. sp. *tritici*. Plant Dis 88:896–904

INTROGRESSION OF LEAF RUST AND STRIPE RUST RESISTANCE GENES FROM *AEGILOPS UMBELLULATA* TO HEXAPLOID WHEAT THROUGH INDUCED HOMOEOLOGOUS PAIRING

P. CHHUNEJA[1,*], S. KAUR[1], R.K. GOEL[1], M. AGHAEE-SARBARZEH AND H.S. DHALIWAL[2]

[1]*Dept. Plant Breeding, Genetics and Biotechnology, Punjab Agricultural University, Ludhiana 141 004, India*

[2]*Indian Institute of Technology, Roorkee, India*

[*]*E-mail: pchhuneja@rediffmail.com*

Abstract: First alien leaf rust resistance gene *Lr9* was transferred from *Ae. umbellulata* into hexaploid wheat in the year 1956 through irradiation induced translocation. A number of other genes were subsequently transferred from non-progenitor and progenitor *Aegilops* species and exploited commercially. However, the appearance of new virulences necessitates the search of novel sources of rust resistance. An *Ae. umbellulata* acc. 3732 was found to be resistant to several wheat diseases such as leaf rust, stripe rust, Karnal bunt, powdery mildew and cereal cyst nematode. An amphiploid (AABBUU) synthesized from the cross of *Ae. umbellulata* acc. 3732 and *T. durum* cv. WH890 was crossed to CS PhI for inducing homoeologous pairing between *Ae. umbellulata* and wheat chromosomes. The F1 (AABBDU) was crossed to a susceptible hexaploid wheat cv. WL711 and introgression lines carrying resistance to leaf and stripe rust were selected in backcross progenies. The BC2F1 plants with leaf and stripe rust resistance genes were selfed, and homozygous lines with least linkage drag were selected and screened against five leaf rust and three stripe rust pathotypes. The introgression lines were resistant to all the leaf rust pathotypes and stripe rust pathotypes. Transfer of *Lr9* was ruled out by screening the introgression line with 77-7, virulent on *Lr9*. For studying the inheritance of transferred rust resistance genes, an F_2 population was generated by crossing the introgression line with the recipient parent WL711. Screening of F_2 population at the seedling stage against leaf rust pathotype 77-5 revealed that a single dominant gene governs the resistance at seedling stage. The F_2 plants screened at the seedling stage were grown in the field and screened at adult plant stage against a mixture of pathotypes under artificial epiphytotic conditions. All plants that were resistant at the seedling stage maintained resistance at adult plant stage. Out of a total of 48 susceptible seedlings, 28 showed resistance at adult plant stage indicating the presence of adult plant resistance (APR) gene as well. The population segregated for two genes for leaf rust, one seedling and one APR, with a $\chi 2$ (15:1) of 12.5. Thus from *Ae. umbellulata*, two novel leaf rust resistance genes(one seedling resistance and one APR) and stripe

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 83–90

rust resistance has been transferred to hexaploid wheat. The introgression lines were analysed with SSR markers for identifying the introgressed regions. The introgressions were detected for chromosomes of homoeologous group 2, 4 and 5. Bulk segregant analysis is in progress to identify the SSR markers segregating with resistance

Keywords: leaf rust resistance, stripe rust resistance, *Aegilops umbellulata*, homeologous pairing

INTRODUCTION

Leaf rust (*Puccinia triticina*) and yellow rust (*Puccinia striformis*) are the two major rust diseases of wheat. Leaf rust or brown rust caused by the fungal pathogen *Puccinia triticina* is one of the most common diseases affecting wheat production worldwide. The impact of leaf rust on yield reduction in wheat ranges from 10 percent under moderate conditions to 65 percent under intense epidemics (Saari and Prescott 1985). An effective, economical and ecologically safe method to control leaf and stripe rust epidemics is the cultivation of resistant cultivars. More than 50 leaf rust resistance genes have been designated (Knott 1989, McIntosh et al. 1995) and of these 46 genes have been mapped to specific chromosomes. Out of these 50 genes, 20 are of alien origin having been introgressed into the wheat genome from wild relatives like *Agropyron* and *Aegilops* spp. (Kolmer 1996). However, a number of genes transferred from related species of wheat viz. *Lr9*, *Lr19*, *Lr24*, *Lr26*, *Yr 9* and *Pm8* etc. and exploited commercially, have been overcome by the emergence of virulent pathotypes, therefore, necessitating the search for new sources of resistance. Less closely related *Aegilops* species especially non-progenitor C, U and M genome species are excellent sources of resistance to various wheat diseases (Dhaliwal et al. 1991, 1993, Harjit-Singh et al. 2000).

However, only a few genes for resistance to diseases and other traits transferred from non progenitor species have been commercially exploited due to substantial amount of undesirable genetic information (linkage drag) associated with useful genes and yield reduction (Jiang et al. 1994). Chen et al. (1994) transferred the dominant homoeologous pairing inducer Ph^1 gene from *Aegilops speltoides* high pairing line to hexaploid wheat cultivar Chinese Spring (CS). This gene suppresses the effect of the Ph^1 locus and induces homoeologous pairing between wheat and alien chromosomes in F_1 hybrids (Chen et al. 1994, Aghaee-Sarbarzeh et al. 2000). Leaf and stripe rust resistance genes has been transferred from *Ae. ovata* chromosome 5M to wheat chromosome 5D through induction of homoeologous pairing with CS(Ph^1) (Aghaee-Sarbarzeh et al. 2002).

Evaluation of different accessions of wild *Triticum* and *Aegilops* species maintained at the Punjab Agricultural University, Ludhiana, Punjab (India) has led to the identification of a number of novel sources of resistance to leaf rust, stripe rust, Karnal bunt, powdery mildew and cereal cyst nematode (Dhaliwal et al. 1993, Gill et al. 1995, Dhaliwal and Harjit-Singh 1997, Harjit-Singh et al. 1998, Harjit-Singh and Dhaliwal 2000). *Aegilops umbellulata*, a diploid species with UU genome, was

found to be highly resistant to different wheat diseases such as leaf rust, stripe rust, Karnal bunt, powdery mildew and cereal cyst nematode. An accession of *Ae. umbellulata* with multiple disease resistance was selected and leaf and stripe rust resistance has been transferred from this accession to cultivated wheat through induction of homoeologous pairing using CS(Ph^I). In the present communication, the transfer of leaf rust and stripe rust resistance from *Ae. umbellulata* through induction of homoeologous pairing and molecular characterization of introgression lines using SSR markers is being reported.

MATERIAL AND METHODS

An amphiploid was synthesized between *T. durum* cv. WH890 and *Ae. umbellulata* accession 3732 and crossed with *T. aestivum* cv Chinese spring carrying Ph^I gene of *Ae. speltoides* to induce homoeologous pairing. The F_1 plants from this cross were selfed and also crossed to a rust susceptible *T. aestivum* cv. WL711 having non-necrotic gene WL711(NN). All BC_1 plants were screened at the seedling stage for leaf and stripe rust. The resistant plants were backcrossed to WL711(NN) to recover the recurrent genotype. Homozygous BC_1F_4 and BC_2F_4 introgression lines with leaf and stripe rust resistance transferred from *Ae. umbellulata* have been selected.

Leaf and Stripe Rust

The rust resistant genes were followed in the segregating generations by screening against leaf rust pathotypes 77-5 and 104-2 and stripe rust pathotype 46S119 at the seedling stage. The resistant plants were transplanted in the field and terminal disease severity recorded for leaf and stripe rust. The homozygous leaf and/or stripe rust resistant introgression lines were tested with five most virulent and prevalent pathotypes of leaf rust 12-2, 77-2, 77-5, 77-7, and 104-2, and three pathotypes of stripe rust 46S119, 46S102 and 46S103 at the seedling stage and under artificial epiphytotic conditions in the rust screening nursery in the field. Seedling reaction was recorded in parents and introgression lines using the standard inoculation procedure (Nayar et al. 1997). Infection type was recorded 14 days after inoculations following the modified 0; -4 scale of Stakman et al. (1962). The disease severity under field conditions was recorded as percentage of leaf area covered by rust following modified Cobb's scale (Peterson et al. 1948).

Molecular Studies

SSR markers were used to detect alien transfer, in the homozygous wheat-*umbellulata* introgression lines. The PCR was performed as described by Röder et al. (1998) with some modifications. PCR products were resolved by electrophoresis in 2.5% agarose gels. Gels were visualized by staining with ethidium bromide using UVP Gel Documentation System.

RESULTS AND DISCUSSION

Leaf and Stripe Rust Screening

All the selected ILs showed resistance to the four test leaf rust pathotypes at the seedling stage and remained free from leaf rust in the rust screening nursery at PAU, Ludhiana (Table 1) and off season nursery at Wellington, Neelgiri Hills, India (data not given). A leaf rust resistance gene transferred from U genome of *Ae. umbellulata* (Sears 1956) is present on chromosome 6BL as a compensating transfer. To establish that the leaf rust resistance gene present in these introgression lines is different from *Lr*9, some of the lines were tested with *Lr*9 virulence 77-7. Three of the selected ILs 329-1, 333-4 & 367-4, however, showed susceptible infection type to 77-7 at the seedling stage indicating that these ILs might be carrying *Lr* 9. All the introgression lines except 329-1, 333-4, 367-4, 380-3 were found to be resistant to stripe rust at the seedling stage and under field conditions (Fig. 1).

Inheritance Studies

One of the ILs 403-1 was crossed with recurrent parent WL711 and leaf and stripe rust resistant F_1 plants were selfed to develop an F_2 for studying the inheritance of leaf rust resistance in this IL. The F_2 was screened at the seedling stage against leaf rust pathotype 77-5 and all the plants were then transplanted in the field and studied for leaf rust reaction at the adult plant stage. The population segregated for single gene for leaf rust at the seedling stage with a χ^2 of 3.5 and for two genes for leaf rust at the adult plant stage with a χ^2 (15:1) of 12.5. However, out of 48 susceptible plants at the seedling stage, 28 plants were observed to be resistant at the adult plant stage indicating that an APR gene for leaf rust is present in addition to the seedling resistance gene. The progeny of another introgression line 351-1 was also found to be segregating for one seedling and one APR gene for leaf rust resistance (Table 2).

SSR Analysis

A total of 100 SSR markers were amplified in the parents and 19 primers were found to be polymorphic. 13 primers did not amplify in *umbellulata* and hence were not used for genotyping of introgression lines. The analysis of the wheat-*Ae. umbellulata* introgression lines using Xwmc and Xgwm SSR markers led to the identification of introgressed segments of *umbellulata* chromatin in homoeologous group 2 in different introgression lines (Fig. 2). The 2AL/2DL mapped SSR marker Xwmc181 detected the introgression in the progenies of 315-5, 351-1, 388-5 and 403-1. The Xwmc181 was amplified in the two leaf and stripe rust resistant and one susceptible progeny derived from IL 351-1. The introgression was detected in the progeny carrying seedling resistance gene for leaf rust (lane 16) and wheat type allele was observed in the progeny with APR gene and in the susceptible progeny. The five leaf and stripe rust resistant and one susceptible F_3 progeny of IL 403-1

Table 1. Leaf and stripe rust reaction of the selected introgression lines from the cross *T. durum* c. WH890-*Ae. umbellulata* acc. PAU#3732/CS (*PhI*)//WL711(NN)

S. No.	IL ID	Generation	Leaf rust-seedling					Stripe rust-seedling			Rust reaction-Field	
			12-2	77-2	77-5	77-7	104-2	46S119	46S102	46S103	Leaf	Stripe
1	WL711		3	3	3	3	3	3	3	3	60S	80S
2	*Ae. umbellulata*		;	;	;	;	;	0;	;	;	0	0
3	CS (Ph^I)		33+	3	3	3	3	3	3	3	20S	20S
4	WH890		33+	3	3	-	3	3	3	3	10S	10S
5	*T. durum-Ae. umbellulata* amphiploid		-	-	-	-	-	-	-	-	40S	40S
6	315-5	F_7	;	-	;	;-	;	3	;	-	0	0
7	329-1	F_7	-	-	0;	3+	;	3	-	-	0	80S
8	333-4		-	1	;	3+	1	3	-	-	0	40S
9	339-4	BC_1F_7	-	-	;1	1	1	;	;	0;	0	0
10	351-1	BC_1F_6	-	-	3	-	3	-	-	-	0	5MR
11	351-5	BC_1F_6	;1	0 ;	;1	-	1	0 ;	;	0;	0	0
12	353-2	BC_1F_6	-	-	;	-	;	;	-	-	0	0
13	354-4	BC_1F_6	-	-	;	-	;	;	;	;	0	0
14	357-1	BC_1F_6	;1	;	;1	-	;1	;	;	;	0	0
15	359-1	BC_1F_6	-	-	;	;	;	;	;	;	0	0
16	367-4	BC_2F_5	-	-	0 ;	33+	;	3	-	-	0	40S
17	380-3	BC_3F_4	;	0 ;	0 ;	-	;	3	3	3	0	40S
18	388-5	BC_1F_6	;1	;1	;1	;1	;1	0;	-	-	0	0
19	393-4	BC_2F_5	;1	0 ;	;1	;	;1-	;	;	-	0	0
20	403-1	BC_2F_5	-	-	3	2	-	0;	-	-	0	0

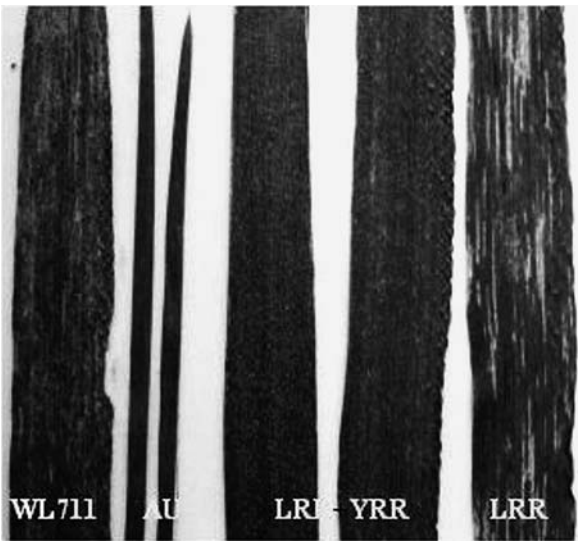


Figure 1. Rust reaction of wheat-*Ae umbellulata* introgression lines under artificial epiphytotic conditions in the field

Table 2. Segregation for leaf rust resistance in the F_2 developed from the cross of IL403-1 with recipient parent WL711

	Resistant plants	Susceptible plants	Segregation ratio	χ^2
Seedling	104	48	3:1	3.5
Adult plant	132	20	15:1	12.5

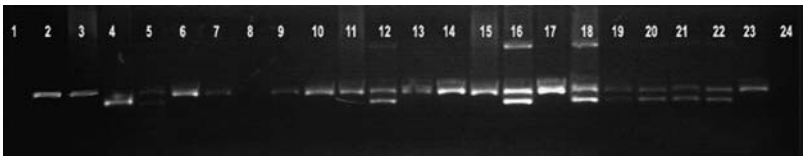


Figure 2. PCR amplification profile of SSR marker Xwmc181 in the homozygous resistant progenies of some selected leaf and/or Stripe rust resistant Introgression lines developed from the cross *T. durum* cv. WH890-*Ae. Umbellulata* acc. PAU#3738/CS (*Ph*)//WL711(NN) and segregating F_3 progenies of 351-1 and 403-1. Lane 1. *T. durum* WH890; Lane 2. WL711; Lane 3. CS(*Ph*); Lane 4. *Ae. Umbellulata* Lane 5-14. Progeny of 315-5, 353-2, 357-1, water, 367-4, 380-3, 393-4, 388-5, 403-1 and 403-1 (repeated); Lane 15-17. 351-1(R), 351-1(R), 351-1(S); Lane 18-22 resistant F_3 progenies of 403-1; Lane 26. susceptible F_3 progeny of 403-1

were also analysed with Wmc181. The introgression was detected in all the resistant progenies and only wheat type allele was amplified in susceptible progeny. The amplification of wheat as well as *umbellulata* specific alleles of the 2A/2D mapped Xwmc181 in resistant introgression lines and progenies indicates the presence of introgressed segment from 2U of *Ae. umbellulata* on the either chromosome 2A or 2D as a compensating transfer. The work to define the introgressed regions and to tag the genes using F_2 population is in progress.

Results indicated the precise transfer of one seedling and one APR gene for leaf rust and one seedling resistance gene for stripe rust from *Ae. umbellulata* acc. 3732 to hexaploid wheat cultivar WL711 and the introgression of *Ae. umbellulata* chromatin, carrying these genes, has been detected on wheat homoeologous group 2.

The leaf and stripe rust resistance genes transferred from *Ae. umbellulata* are novel genes which can be exploited for broadening the genetic base of cultivated wheats for rust resistance. The identification of the closely linked molecular markers may lead to marker-assisted mobilization of these genes in elite wheat backgrounds.

ACKNOWLEDGEMENTS

The financial assistance of USDA-ARS under Project IN-ARS-842, and Grant Number FG-In-792 to carry out the research work is gratefully acknowledged. The rust inoculum was provided by Director, Regional Research Station, Directorate of Wheat Research, Flowerdale, Shimla, India.

REFERENCES

Aghaee-Sarbarzeh M, Harjit-Singh, Dhaliwal HS (2000) Ph1 gene derived from Ae. speltoides induce homeologous pairing in wide crosses of Triticum aestivum. J Hered 91:417–421

Aghaee-Sarbarzeh M, Ferrahi M, Singh S, Singh H, Friebe B, Gill BS, Dhaliwal HS (2002) PhI–induced transfer of leaf and stripe rust-resistance genes from Aegilops geniculata and Ae. triuncialis to bread wheat. Euphytica 127:377–382

Chen PD, Tesujimoto H, Gill BS (1994) Transfer of PhI gene promoting homoeologous pairing from Triticum speltoides into common wheat and their utilization in alien genetic introgression. Theor Appl Genet 88:97–101

Dhaliwal HS, Singh H (1997) Breeding for resistance to bunts and smuts: Indian Scenario. In: Malik VS, Mathre DE (eds) Bunts and smuts of wheat. North Am Plant Protection Organ, Ottawa, USA, pp 327–347

Dhaliwal HS, Singh H, Gupta S, Bagga PS, Gill KS, Singh S (1991) Evaluation of Aegilops and wild Triticum species for resistance to leaf rust (Puccinia recondita f. sp. tritici) of wheat. Int J Trop Agric 9:118–22

Dhaliwal HS, Singh H, Gill KS, Randhawa HS (1993) Evaluation and cataloguing of wheat germplasm for disease resistance and quality. In: Damania AB (ed), Biodiversity and Wheat improvement. John Wiley & Sons NY, Icarda, Syria, and Sayce Publishing Co, UK, pp 123–140

Harjit-Singh, Dhaliwal HS (2000) Intraspecific genetic diversity for resistance to wheats in wild Triticum and Aegilops species. Wheat Inf Serv 90:21–30

Harjit-Singh, Grewal TS, Dhaliwal HS, Pannu PPS, Bagga PS (1998) Sources of leaf rust and stripe rust resistance in wild relatives of wheat. Crop Improv 25(1):26–33

Harjit-Singh, Tsujimoto H, Sakhuja PK, Singh T, Dhaliwal HS (2000) Transfer of resistance to wheat pathogens from Ae. triuncialis into bread wheat. Wheat Inf Ser 91:5–10

Gill KS, Dhaliwal HS, Harjit-Singh (1995) Cataloguing and pre-breeding of wheat genetic resources. Project In ARS-639, Terminal Report

Jiang J, Friebe B, Gill BS (1994) Recent advances in alien gene transfer in wheat. Euphytica 73:199–212

Knott DR (1989) The Wheat rusts: Breeding for resistance. Springer-Verlag Publ. Berlin Germany

Kolmer JA (1996) Genetics of resistance to leaf rust. Ann Rev Phytopathology 34:435–55

McIntosh RA, Wellings CR, Park RF (1995) Wheat Rusts: An Atlas of Resistance Genes. CSIRO, East Melbourne, Victoria 3002, Australia

Nayar SK, Prashar M, Bhardwaj SC (1997) Manual of current techniques in wheat rusts. Research Bull. No.2, 32pp Regional Station, Flowerdale, Shimla 171002, India

Peterson RF, Campbell AB, Hannah AE (1948) A diagrammatic scale for rust intensity on leaves and stems of cereals. Can J Res 26:496–500

Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier M, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007–2023

Sears ER (1956) The transfer of leaf rust resistance from Aegilops umbellulata to wheat. Brookhaven Symp Biol. 9:1–22

Saari EE, Prescott JM (1985) World distribution in relation to economy losses. In: Roelfs AP, Bushnell WR (eds) Cereal rusts, vol II. Academic press, Orlando, Florida, USA, pp 259–298

Stakman EC, Stewart DH, Loegering WQ (1962) Identification of physiologic races of Puccinia graminis var. tritici. USDA Agri. Res. Serv. No. E617(Rev.) p 53

ENHANCEMENT OF FUSARIUM HEAD BLIGHT RESISTANCE IN BREAD WHEAT AND DURUM BY MEANS OF WIDE CROSSES

G. FEDAK[1,*], W. CAO[2], A. XUE, M. SAVARD, J. CLARKE[2] AND D.J. SOMERS[3]

[1]*Eastern Cereals & Cereals & Oilseeds Research Centre, Bldg 50, Central Experimental Farm 960 Carling Avenue, Ottawa, Ontario. K1A 0C6*
[2]*Agriculture & Agri-Food Canada, P.O. Box 1030, Swift Current SK. S9H 3X2*
[3]*Cereal Research Centre, 195 Dafoe Road, Winnipeg, Manitoba. R3T 2M9*
[*]*E-mail: fedakga@agr.gc.ca*

Abstract: Fusarium head blight resistance from wild relatives is being introgressed into bread wheat and durum wheat. The resistance from *Triticum monococcum*, *T. timopheevi* and *Aegilops speltoides* has now been stably incorporated into bread wheat. Transfer of resistance from other species is progressing

Keywords: wheat, fusarium head blight, genetic resistance, wide crosses

INTRODUCTION

Fusarium head blight has become a serious disease of cereals in virtually all temperate regions of the world. The inoculum seems to be sufficiently widely distributed that the occurrence of rainfall during flowering is almost certain to cause an epidemic. The disease manifests itself by yield reduction through kernel shrivelling and secondly by deposits of a vomitoxin in the grain. Under our conditions the causal organism is *Fusarium graminearum* Schwabe [teleomorph: *Gibberella zea* Schev. (Petch)] and the vomitoxin produced is deoxynivalenol (DON). The economic losses in Canada due to FHB infection have been substantial over the years. Cultural and chemical controls of the disease are subject to precise timing, subject to environmental factors and in general are not overly dependable and in any case do not give complete control. Genetic resistance seems to be the most obvious alternative to controlling FHB.

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 91–95.

In hexaploid wheats, resistance has been reported in accessions originating in China, Japan and Brazil. Of particular interest are the cultivars Sumai3, Nyu Bay and Frontana respectively. These have been widely used successfully in breeding programs worldwide. It was suggested some time ago that Sumai 3 and Frontana had different FHB resistance genes (Van Ginkel et al. 1996) and this has since been proven to be true with numerous reports describing the resistance QTL in Sumai3 to be located on chromosome 3BS (Anderson et al. 2001, Buerstmayr et al. 2002) with the main QTL in Frontana being located on chromosome 3AS (Steiner et al. 2004). More recently, reports of the FHB-QTL in winter wheat have appeared (Gervais et al. 2003). It appears the QTL profile in the winter wheats in some cases is different than that in spring wheats. In our experience, cultivars such as Sumai3, Frontana and Nyu Bay under growing conditions in an epiphytotic nursery will become infected so that up to 20% of florets will show symptoms and threshed grain samples will contain up to 5.0 ppm of DON. In view of these observations, there is a need to enhance the FHB resistance level of the original introductions. This approach has met with some success, as lines with stable resistance have been isolated from the progeny of Wuhan X Nyu Bay crosses (Somers et al. 2003).

Another approach would be the isolation of resistance from alien species and introgress these into wheat to enhance existing wheat-derived resistance. Early large-scale screening efforts of wild species were successful. (Wan et al. 1997, Fedak et al. 1997). This was followed by reports of integration of the alien resistance into wheat (Chen et al. 1993, Fedak et al. 2003) and most recently by screening of various wheat-alien aneuploid stocks (Oliver et al. 2005). This report will describe our efforts at transferring into bread wheat, FHB resistance from *Triticum* and *Aegilops* species and early results of transfer of alien resistance into durum wheat.

MATERIALS AND METHODS

Several hundred accessions of numerous species of *Triticum* and *Aegilops* were initially screened to identify resistant lines. This report will only deal with the species from which resistance was introgressed into wheat. The critical lines of *T. timopheevi* were found in a collection provided by Gina Brown-Guidera (Brown-Guidera et al. 1996) of USDA, ARS, at Manhattan Kansas. Critical lines of *T. monococcum* were obtained from Dr. Maxine Trottet of INRA, Le Rhen, Cedex France and the critical *Aegilops speltoides* accessions were obtained from Dr. Maria Zaharieva of INRA Centre de Montpellier in France. The screening of introductions of all species was conducted in greenhouses and mist chambers. Spikes were inoculated (point and spray) at 50% anthesis with 50,000 spores/ml. suspension of *F. graminearum*. Plants with inoculated spikes were misted for 48 hours and symptoms scored 21 days later. Inoculation was repeated on plants showing minimal symptoms.

Resistant accessions of *T. monococcum* and *Ae. speltoides* were crossed onto cultivar Superb and hybrid embryos cultured on B_5 medium. The hybrid with *T. monococcum* was backcrossed to the cultivars Fukuhokomugi and advanced to BC_2 F_4 whereas three backcrosses to Superb were required to restore fertility of the hybrid involving *Ae. speltoides*. The *T. timopheevi* accession was crossed to the experimental line Crocus (carrying crossability loci *kr1*, *kr2*, *kr3* in a Domain background), backcrossed once and then 1300 BC, seeds were advanced to F_9 by SSD. The derived lines were seeded as one meter double rows in an FHB nursery, inoculated twice with corn spawn and irrigated twice a day. Symptoms were scored at 50% anthesis. Incidence and severity scores were assigned visually whereas FDK was determined on threshed samples. Samples of seed were ground and DON content determined as described previously. (Sinha and Savard 1996).

RESULTS AND DISCUSSION

The incidence, severity, FHB index, FDK values and DON levels for the interspecific derivatives and check cultivars are shown in Table 1. The fact that the check varieties performed much as expected indicated that a high level of infection occurred in the 2004 epiphytotic nursery. The susceptible cultivar Roblin was

Table 1. Fusarium head blight symptoms in progenies of interspecific crosses with bread wheat (2004)

Sources of resistance	Generation	Incidence	Severity (%)	FHB index	*FDK (%)	**DON content (ppm)
Ae. speltoides						
Line 1	BC3F4	30	25	7,5	11,7	8,5
2		15	10	1,5	7,0	3,6
3		10	10	1	6,3	4,3
4		15	10	1,5	14,7	5,3
5		15	10	1,5	6,7	3,2
6		15	10	1,5	10,0	2,9
7		10	10	1	7,0	4,8
T. monococcum						
Line 1	BC2F4	10	5	0,5	15,3	8,9
T. timopheevi						
TC 67	F9-SSD	19,0	11,3	2,1	13,9	5,7
Checks						
Sumai 3		10	5	0,5	9,0	5,5
Nyu Bay		33	16	5,3	13,2	3,4
Fukuhokomugi		15	5	0,8	50,0	7,7
Roblin		80	80	16,0	90,0	35,0
AC Barrie		45	10	4,5	20,3	16,3

* Fusarium damaged kernels.

** Deoxynivalenol (trichothecene vomitoxin) in parts per million

highest in all parameters scored; AC Barrie, considered to be somewhat resistant was intermediate in all parameters scored. Among the three resistant check cultivars, Fukuho gave a low FHB index but a very high percentage of FDK and somewhat high level of DON. Sumai3, as expected gave the lowest FHB index and FDK and relatively low DON content. Nyu Bay gave the lowest DON content of the checks and relatively low FDK. Of the derived lines, the *T. timopheevi* derivative gave slightly higher values for FDK and DON than the *T. monococcum* derivative and both were slightly higher than the checks. The seven *Ae. speltoides* derivatives gave a range of FDK values from 6.3 to 14.7 and DON values from 2.9 ppm to 8.5 ie spanning the values of the checks. The DON levels from plots grown in 2005 were relatively similar to those shown in Table 1. The overall levels of DON were lower in 2005 than 2004 but relative relationships were similar. For example the DON levels in Roblin were 9.1 ppm, while in Sumai3 and Nyu Bay they were 0.3 and 0.4 ppm respectively.

The DON values in the *T. monococcum* derivatives ranged from 0.1 to 1.5 ppm and the range in levels in the *Ae. speltoides* derivatives was 0.3 to 2.0 ppm. Thus following two years of testing it appears that the FHB resistance of the interspecific derivatives is stable. In addition, molecular marker studies are underway to tag the resistance QTL; to determine if the QTL differ from those carried by resistant wheat accessions; and if so, to pyramid these resistance QTL with those already known in wheat.

FHB resistance has also been found in accessions of *T. miguschovae* (AG genome) and *Lophopyrum elongatum*. In the case of the former, a high level of resistance was detected among BC_2 segregants and these are being advanced to homozygosity. The *L. elongatum* derivatives are also being advanced to homozygosity.

In the case of durum wheat, it was at one time considered that the main acreage occurring in the drier brown soil zones would not be threatened by FHB. However it has been shown that the FHB incidence is moving westward with increasing severity from its focal point in the Red River valley of Manitoba and is into the durum-growing areas. A screening of durum accessions has revealed no good sources of resistance. In terms of related wild species, levels of resistance have been found in accessions of *T. dicoccoides*. In our screening efforts, we have found some level of resistance in *T. carthlicum*, *Hordeum chilense* (via *Tritordeum*) and *L. elongatum*. Homozygous lines have now been derived from crosses of durum with *Tritordeum* that showed some level of resistance in field trials in 2005. Hybrid materials from other combinations are being advanced to homozygosity.

The data in Table 1 suggests that useable FHB resistance has now been isolated from several wild species. It will be interesting to determine the numbers of QTL controlling the resistance in the interspecific derivatives. In virtually all QTL studies in hexaploid wheat published to date, FHB resistance was found to be controlled by 1–2 major QTL plus several minor QTL. Some epistatic interactions have been implied in a few studies. If the resistance in the derived lines listed in Table 1 is controlled by several QTL, then markers will be essential to pyramid this resistance

to augment the resistance currently found in hexaploid wheat. Our approach at pyramiding will involve the addition of the alien resistance to lines having resistance combined from several bread wheat accessions.

REFERENCES

Anderson JA, Stack RW, Liu S, Waldron BL, Field AD, Coyne C, Moreno-Sevilla B, Mitchell Fetch J, Song QJ, Cregan PB, Frohberg RC (2001) DNA markers for Fusarium head blight resistance QTL in two wheat populations. Theor Appl Genet 102:1164–1168

Brown-Guidera GL, Cox TS, Gill BS, Hatchett JH, Becker WW, Leath S, Peterson CJ, Thomas JB, Zeven P (1996) Evaluation of a collection of wild timopheevi wheat for resistance to disease and arthropod pests. Plant Dis 80:928–933

Buerstmayr H, Lemmens M, Hartl L, Doldi B, Steiner B, Stierschneider M, Ruckenbauer P (2002) Molecular mapping of QTLs for Fusarium head blight resistance in spring wheat. I. Resistance to fungal spread (Type II resistance). Theor Appl Genet 104:84–91

Chen, PD, Wang ZT, Wang SL, Huang L, Wang YZ, Liu DJ (1993) Transfer of scab resistance from Elmus giganteus into common wheat. In: Li ZS, Xin XY (eds) Proc 8th Inter Wheat Genetics Symposium. China Agricultural Scientch Press, Bejing, 1:153–157

Fedak G, Armstrong KC, Sinha RC, Gilbert J, Procunier JD, Miller JD, Pandeya R (1997) Wide crosses to improve Fusarium head blight resistance in wheat. Proceedings of the 5th European Fusarium Seminar, Szeged Hungary, 29 August–5 September. Cereal Res Comm 25:651–654

Fedak G, Cao W, Han F (2003) Germplasm enhancement for FHB resistance in spring wheat through alien introgression. Third Canadian Workshop on Fusarium Head Blight, Winnipeg, Manitoba, December 09–12, p 132

Gervais L, Dedryver F, Morlais J-Y, Bodusseau V, Negre S, Bilous M, Groos C, Trottet M (2003) Mapping of quantitative trait loci for field resistance to Fusarium head blight in a European winter wheat. Theor Appl Genet 106:961–970

Oliver RE, Cai X, Wu SS, Chen X, Stack R (2005) Wheat-alien species derivatives: a novel source of resistance to Fusarium head blight in wheat. Crop Sci 45:1353–1360

Sinha RC, Savard ME (1996) Comparison of immunoassay and gas chromatography methods for detection of the mycotoxin deoxynivalenol in grain samples. Can J Plant Path 18:233–236

Somers DJ, Fedak G, Savard M (2003) Molecular mapping of novel genes controlling Fusarium head blight resistance and deoxynivalenol accumulation in spring wheat. Genome 46:555–564

Steiner B, Lemmens M, Grieser M, Scholz U, Schondelmaier J, Buerstmayr H (2004) Molecular mapping of resistance to Fusarium head blight in the spring wheat cultivar Frontana. Theor Appl Genet 109:215–224

Van Ginkel M, Van Der Schaar W, Yang Z (1996) Inheritance of resistance to scab in two wheat cultivars from Brazil and China. Plant Dis 80:863–867

Wan YF, Yen C, Yang JL, Lui DC (1977) The diversity of resources resistant to scab in Triticeae (Poaceae). Wheat Information Service 84:7–12

LEAF RUST RESISTANCE GENE *LR34* IS INVOLVED IN POWDERY MILDEW RESISTANCE OF CIMMYT BREAD WHEAT LINE SAAR

M. LILLEMO[1,*], R.P. SINGH[2], J. HUERTA-ESPINO[3], X.M. CHEN[4], Z.H. HE[5] AND J.K.M. BROWN[6]

[1] *Dept. of Plant and Environmental Sciences, Norwegian University of Life Sciences, P.O. Box 5003, N-1432 Ås, Norway*
[2] *CIMMYT, Apdo. Postal 6–641, 06600, México, D.F., México*
[3] *Campo Experimental Valle de Mexico-INIFAP, Apdo. Postal 10, 56230, Chapingo, Edo. de Mexico, Mexico*
[4] *Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Zhongguancun South Street 12, 100081, Beijing, P.R. China*
[5] *CIMMYT, c/o Chinese Academy of Agricultural Sciences, Zhongguancun South Street 12, Beijing 100081, P.R. China*
[6] *Dept. of Disease and Stress Biology, John Innes Centre, Colney, Norwich NR4 7UH, UK*
[*] *E-mail: morten.lillemo@umb.no*

Abstract: The CIMMYT bread wheat line Saar has a high level of adult plant resistance to leaf rust (LR, caused by *Puccinia triticina*) and stripe rust (YR, caused by *P. striiformis* f. sp. *tritici*) based on *Lr34/Yr18* in combination with additional minor resistance genes. Because Saar also has good partial resistance to powdery mildew (PM, caused by *Blumeria graminis* f. sp. *tritici*), experiments were set up to test whether *Lr34* could be involved in its resistance to PM. A population of 113 recombinant inbred F_6 lines from a cross between Saar and Avocet-*YrA* was tested for all three diseases. Correlations among the disease data for LR, YR and PM were strong and highly significant, suggesting that the cross segregated for at least one common genetic factor that affected the resistance to all three diseases. Strong correlations with leaf tip necrosis (LTN), a phenotypic marker for *Lr34*, indicated that this gene was indeed involved in the PM resistance of Saar. Disease testing of near-isogenic lines for *Lr34* and *Lr46* in the genetic background of Avocet-*YrA* and *YrLrPrl1* in the background of Lalbahadur showed that all three genes were associated with significantly reduced levels of LR, YR and PM compared to their susceptible genetic backgrounds. It is concluded that resistance to both rust and PM is not only confined to *Lr34*, but could be a general phenomenon of LTN-associated resistance genes, including *Lr46* and *YrLrPrl1*

Keywords: wheat, leaf rust, powdery mildew, genetic resistance

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 97–102

INTRODUCTION

Powdery mildew (PM), caused by *Blumeria graminis* (DC) E.O. Speer f. sp. *tritici* EM. Machal, is an important disease of bread wheat (*Triticum aestivum* L.), especially under intensive production systems in maritime and temperate areas of the world. Breeding for resistance is considered an economic and environmentally friendly way of keeping this disease under control. However, owing to the lack of naturally occurring epidemics of PM in Mexico, where CIMMYT has its main breeding operations, most bread wheats from CIMMYT have never been selected directly for resistance.

An important breeding goal has been to achieve good levels of race-non-specific resistance to leaf rust (LR, caused by *Puccinia triticina* Eriks.) and stripe rust (YR, caused by *P. striiformis* Westend.f. sp. *tritici*) based on genes that confer adult plant resistance without any hypersensitive reactions associated with race-specific resistance. One key source of resistance has been the LR resistance gene *Lr34*, which is either tightly linked to or pleiotropic with the YR resistance gene *Yr18* (Singh 1992a). The resistance based on *Lr34/Yr18* has, when combined with a few additional minor genes, resulted in high levels of non-specific resistance, and is believed to be durable since it has remained effective for a long period of time in widely grown wheat cultivars (Singh et al. 2000). Leaf tip necrosis (LTN), a phenotype consistently associated with *Lr34/Yr18*, is widely used as a phenotypic marker for the gene (Singh 1992b).

One product of the rust resistance breeding program at CIMMYT is the bread wheat line Saar, which has good levels of APR to LR and YR based on *Lr34/Yr18* in combination with other minor genes (Navabi et al. 2003, 2004). Despite never having been exposed to powdery mildew during its development, the line has exhibited high levels of partial resistance to PM in field trials conducted in Europe, Asia and South America, and genetic analysis has shown that at least three genes are involved in its resistance to powdery mildew (Lillemo et al. 2005).

The present study was set up: (i) to test whether *Lr34/Yr18* is involved in the PM resistance of Saar, and (ii) to investigate whether other LTN-associated APR genes to LR and YR could cause similar effects.

MATERIALS AND METHODS

Plant Material

A population of 113 random inbred F_6 lines was developed from a cross between the CIMMYT bread wheat line Saar (selection history CG25-099Y-099M-4Y-2M-3Y-0B) and Avocet-*YrA*, which is highly susceptible to leaf rust, stripe rust and powdery mildew. $F_{5:6}$ seed of the population was kindly provided by A. Navabi, the University of Alberta, Edmonton, and F_6 lines were derived as one randomly selected head from each F_5 family. Near-isogenic lines (NILs) for *Lr34/Yr18* and *Lr46/Yr29* in the background of Avocet-*YrA* and *YrLrPrl1* in the background of the susceptible Indian variety Lalbahadur were developed in Australia (*Lr34/Yr18*) by C. Wellings and Mexico (*Lr46/Yr29* and *YrLrPrl1*) by the second and third authors.

Disease Testing

The Avocet-*YrA* x Saar population was tested for LR resistance at CIANO, CIMMYT's Research Station near Cd. Obregon in North-Western Mexico during the 2004–2005 cropping season, and for YR in Toluca in the Mexican highlands during the 2005 cropping season. The plot size at both locations was 2 rows of 1 m length with two reps. Artificial disease epidemics were created by inoculating the spreader rows at appropriate time with selected races of the respective pathogens with which the seedlings of the two parents were susceptible. Disease severity on flag leaves was scored according to the modified Cobb Scale at the time when Avocet-*YrA* had just reached maximum severity. In addition, LTN was scored at CIANO. PM assessment was done at two locations in Norway: Hamar and Ås. The lines were planted in small hillplots with two replications following a randomized complete block (RCB) design and the percentage of leaf area covered with PM was assessed on the penultimate leaf at 4–7 days intervals in July 2005 based on a modified Cobb Scale, commencing at the time of heading (GS 50–59) and ending when Avocet-*YrA* had reached maximum severity (around GS 69–71). Powdery mildew was also assessed in Beijing, China, on 2 m rows with 100 grains and 30 cm between the rows planted in three replications following a RCB design and scored on 24th of May 2005 and 3rd of June 2005 based on a 0–9 scale.

The NILs were tested for LR and YR in Mexico and PM in Norway at the same locations and following the same procedure as for the population. In addition, they were tested for PM resistance in Bawburgh, Norfolk, England during the 2005 cropping season. The lines were planted in small hillplots with four reps in an RCB design. The percentage of the area of the upper four leaves covered with PM was scored twice in June and July 2005 using the NIAB scale.

RESULTS AND DISCUSSION

Correlation coefficients for selected disease variables of the Avocet-*YrA* x Saar population are shown in Table 1. Strong and highly significant correlations between the scores for powdery mildew and the two rust diseases indicated that one or more common genetic factors were responsible for resistance to all three diseases. This common gene is most likely *Lr34*, because of the high correlation of LTN with all three diseases (Table 1). The effects of *Lr34* on LR, YR and PM are given in Table 2, and show that, at the two locations in Norway, PM levels on progeny lines with *Lr34* were reduced to half compared to those without this gene. The effect was less dramatic than for LR, but in the same order of magnitude as for YR. It is also evident that the resistant parent Saar possesses other minor resistance genes that are specific to each of the three diseases.

NILs for *Lr34* and two other adult plant resistance genes associated with LTN (*Lr46* and *YrLrPrl1*) were tested for resistance to LR, YR and PM to verify the effects of *Lr34* on PM resistance and to test whether other LTN-associated APR genes could cause similar effects. All three genes reduced the levels of PM

Table 1. Correlation coefficients among mean disease resistance parameters for 113 lines in the Avocet-*YrA* x Saar population. All correlations were highly significant ($P < 0.0001$)

Parameter[1]	LR	YR	PM Hamar	PM Ås	PM Beijing
LTN	−0.849	−0.640	−0.674	−0.713	−0.575
LR		0.762	0.764	0.797	0.547
YR			0.718	0.768	0.552
PM Hamar				0.903	0.695
PM Ås					0.672

[1] LTN = Leaf Tip Necrosis, CIANO, Mexico (absence/presence, 0–1), LR = Leaf Rust, CIANO, Mexico (0–100), YR = Stripe Rust, Toluca, Mexico (0–100), PM Hamar = Powdery Mildew, last assessment date, Hamar, Norway (0–100). PM Ås = Powdery Mildew, last assessment date, Ås, Norway (0–100) and PM Beijing = Powdery Mildew, Beijing, China (0–9).

significantly at the two locations in Norway, compared to the susceptible genetic backgrounds without these resistance genes (Table 3). Consistent with the rust data from Mexico, *Lr34* and *YrLrPrl1* were more effective against PM than *Lr46*. All three genes resulted in reduced PM levels at the UK site. Although disease levels were much lower than in Norway, NILs with each of the three genes had much lower PM levels than their recurrent parents. Powdery mildew progress curves for the NILs at Ås, Norway, (Fig. 1) indicate that all three resistance genes were effective both in delaying the onset of PM infection and in slowing down its development.

It can not be ruled out that the PM resistance associated with *Lr34* could be caused by a gene closely linked to *Lr34*, but we did not observe any recombination between LTN and PM resistance. This is consistent with the work of Spielmeyer et al. (2005) who found complete cosegregation between *Lr34* and resistance to PM in a population of 110 F_6 lines from the cross Thatcher x RL6058. The fact that two other LTN-associated adult plant resistance genes to LR and YR gave similar effects on PM rather suggests that *Lr34* confers a general type of disease resistance in which the premature leaf tip senescence causing the characteristic LTN might be involved. Nevertheless, genes like *Lr34, Lr46* and *YrLrPrl1* are highly valuable

Table 2. Parental means and effects of *Lr34* on disease severity, based on the phenotypic marker leaf tip necrosis (LTN). Data for 106 lines in the Avocet-*YrA* x Saar population with unambiguous scores for LTN. All differences were highly significant ($P < 0.0001$)

		LR	YR	PM Hamar	PM Ås	PM Beijing
Saar		1	7.5	6.3	2.5	2.26
Avocet-*YrA*		100	97.5	83.0	90.0	6.42
Lr34	n = 61	6.9	32.2	30.2	27.6	3.42
lr34	n = 45	71.2	62.1	62.6	66.7	4.64

Table 3. Mean disease severities of near-isogenic lines for *Lr34* and *Lr46* in the Avocet-*YrA* background and *YrLrPrl1* in the background of Lalbahadur. The leaf rust and stripe rust scores are given together with their corresponding infection types. The letters behind powdery mildew scores indicate significant differences at level $\alpha = 0.05$ based on the Tukey method

	LR	YR	PM Hamar	PM Ås	PM Bawburgh
Avocet-*YrA*	100 S	100 S	80.0b	85.0c	9.3a
Avocet-*YrA* + *Lr34*	10 MSS	50 MS	45.0a	35.0a	4.5a
Avocet-*YrA* + *Lr46*	30 MSS	60 MS	50.0a	55.0b	5.5a
Lalbahadur	100 S	40 MR-MS	85.0b	90.0c	15.8b
Lalbahadur + *YrLrPrl1*	20 MSS	15 MR	50.0a	40.0a	7.0a

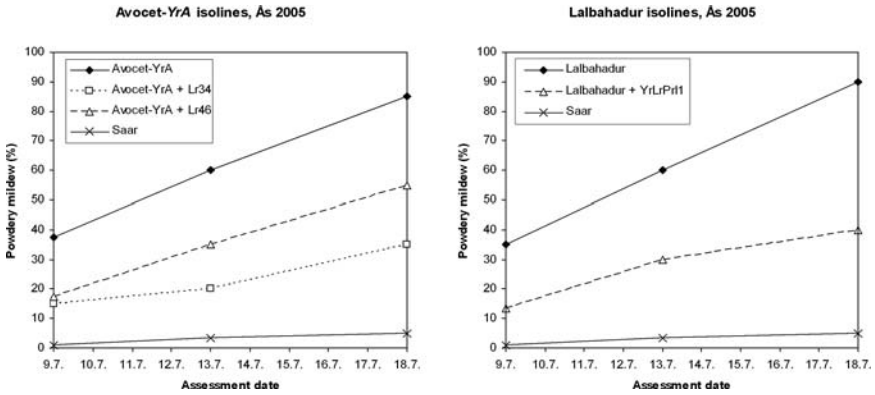


Figure 1. Disease progress curves for powdery mildew on near-isogenic lines planted in Ås, Norway. % leaf area of powdery mildew was scored on penultimate leaves. Data for Saar is included for comparison

as sources of disease resistance since they allow for simultaneous selection of resistance to LR, YR and PM. Further characterization of the mechanisms involved in their resistance is underway.

ACKNOWLEDGEMENTS

Part of the work presented in this paper was supported by grants from the Research Council of Norway and GRDC, Australia. The authors are thankful to A. Navabi for providing the seed of the Avocet-*YrA* x Saar population that was used to produce the F_6 lines used in the study.

REFERENCES

Lillemo M, Skinnes H, Singh RP, van Ginkel M (2005) Genetic analysis of partial resistance to powdery mildew in bread wheat line Saar. Plant Dis (accepted)

Navabi A, Singh RP, Tewari JP, Briggs KG (2003) Genetic analysis of adult-plant resistance to leaf rust in five spring wheat genotypes. Plant Dis 87:1522–1529

Navabi A, Singh RP, Tewari JP, Briggs KG (2004) Inheritance of high levels of adult-plant resistance to stripe rust in five spring wheat genotypes. Crop Sci 44:1156–1162

Singh RP (1992a) Genetic association of leaf rust resistance gene *Lr34* with adult plant resistance to stripe rust in bread wheat. Phytopathology 82:835–838

Singh RP (1992b) Association between gene *Lr34* for leaf rust resistance and leaf tip necrosis in wheat. Crop Sci 32:874–878

Singh RP, Huerta-Espino J, Rajaram S (2000) Achieving near-immunity to leaf rust and stripe rust in wheat by combining slow rusting resistance genes. Acta Phytopathol Entomol Hung 35:133–139

Spielmeyer W, McIntosh RA, Kolmer J, Lagudah ES (2005) Powdery mildew resistance and *Lr34/Yr18* genes for durable resistance to leaf and stripe rust cosegregate at a locus on the short arm of chromosome 7D of wheat. Theor. Appl. Genet. 111:731–735

STRATEGIES OF THE EUROPEAN INITIATIVE FOR RESISTANCE BREEDING AGAINST FUSARIUM HEAD BLIGHT

P. RUCKENBAUER, H. BUERSTMAYR AND M. LEMMENS

Department of Applied Plant Science and Plant Biotechnology, Institute of Agronomy and Plant Breeding, BOKU-University of Natural Resources and Applied Life Sciences, Gregor Mendel Str. 33, A-1180 Vienna, Austria
E-mail: peter.ruckenbauer@boku.ac.at

Abstract: Wheat is Europe's most important cereal crop, cultivated on over 16 Million ha, yielding over 88 million metric tons per year. The wheat quality and consumer safety is threatened by Fusarium Head Blight (FHB) caused by i.e. *Fusarium culmorum, F. graminearum and F. avenaceum*. Most of the wheat varieties grown in the EU today are susceptible to this disease. Crop management and chemical measures to prevent the disease and associated mycotoxin contamination are either not available or not feasible. The development of resistant varieties is the most reliable and environmentally sound means to combat this disease

In the presented paper, firstly an overview about initiatives on European, national and regional levels to tackle the disease are described involving a wide range of expertise from public and private research institutions as well as breeding and food processing companies

In the second part and in more details, the results about two European-wide projects are presented

1. From the EU-funded project "FUCOMYR" where research institutes and breeding companies from 6 European countries are cooperating together, results about the efficiency of conventional selection for resistant lines, artificial infection in mist irrigated fields and under controlled condition are demonstrated. This enabled the team of participants to detect 3 new DNA markers for Type II resistance by QTL mapping of segregating populations. Three genomic regions were found to be significantly associated with FHB resistance: the most-prominent effect was detected on the short arm of chromosome 3B, explaining up to 60% of the phenotypic variance for type II resistance. A further QTL was located on chromosome 5A and a third one on 1B. The QTL regions on 3B and 5A were tagged with flanking SSR markers, the 1B QTL was found associated with the high-molecular-weight glutenin locus. These results indicate that FHB resistance is under control of a few major QTL's operating together with an unknown number of minor genes
2. In the EU-Concerted Action "MYCOTOCHAIN", partners from breeding research institutions, breeding companies, milling industries and food processors from five European countries were aiming to synchronise their activities and efforts towards

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 103–107.

mycotoxin control in cereal and cereal products for food and feed. The results of the proposed recommendations for mycotoxin contamination thresholds for the whole production chain from wheat breeding strategies until the final consumer products for European and possibly worldwide acceptance are demonstrated and discussed

Keywords: wheat, fusarium head blight, breeding for resistance

INTRODUCTION

Due to the agricultural policy of the EU which favours the cultivation of cereals and corn and the narrow crop rotations in the last two decades – where wheat and corn have been grown alternatively – increased the incidence of Fusarium Head Blight (FHB). Since all *Fusarium ssp*. that infect cereals are facultative saprophytes and survive on crop debris and direct drilling or minimal cultivation techniques are used, the risk of infection from contaminated debris is high. Crop management and chemical control to prevent the disease and associated mycotoxin contamination have strong limitations. Wheat grains produced by intensive farming, low input sustainable farming and organic farming are all infected by FHB. To date, the cultivation of resistant cultivars seems to be the best option to reduce the threat of mycotoxin contamination of cereal food and feed. Unfortunately till now nearly all European wheat varieties are susceptible to the disease while many exotic sources have high level of FHB resistance. Resistance to FHB in wheat is of quantitative, multigenic nature. However, it is a tedious, expensive and time consuming task for plant breeders to develop FHB tolerant or resistant cultivars adapted to European conditions.

Current Research and Breeding Activities against FHB in Wheat in Europe

In Europe, several research groups are involved in breeding for tolerance or resistance against this pathogen of which the team in Hungary, United Kingdom, Ireland, Germany and Austria are the most active. Mesterhazy (1983, 1995, 2002) at Szeged/Hungary is a pioneer cereal breeder involved with FHB since 1980's. Their team focussed on the establishment of broad crossing populations between Hungarian, Eastern European and Asian FHB resistant varieties such as Sumay 3 and Nobeoka Bozu. From thousands of advanced lines screened under artificial inoculation plants that had no disease (FHB) symptoms were selected and in the next generation plants with no Fusarium Diseased Kernels (FDK) were advanced further. With this 2-step selection procedure and with the use of various inoculi with different aggressiveness, but simultaneously also under different times of inoculation treatments during flowering, a number of varieties for the hard and dry condition of Hungary and the neighbouring countries could be released.

Nicholson et al. (2003) investigated the molecular-genetic base of the host-pathogen relationship of *Fusarium ssp*. Differential expression of pathogenesis related protein has been observed between resistant and susceptible varieties accompanied by a reduced accumulation of deoyxynivalenol (DON) content. Use of DNA–microarrays for the pathogens and a number of wheat hosts revealed the signalling process between host and pathogen and opened up new opportunities to study tolerance or resistance.

Browne and Cook (2004) developed detached wheat leaf assays to detect components of partial disease resistance (PDR) after infecting it with *Microdochium nivale*. From their experiments it was evident that incubation period is a major component of PDR of FHB resistance and there is considerable genetic variation for this trait. The extended inoculation period can serve as a useful selection criterion in breeding for FHB.

Rabenstein et al. (2004) developed and utilized methods for quantification disease severity of FHB in grains of breeding material. A fast, economical and reliable tool is essential for further resistance evaluation and selection. Immunological methods appear to be particularly suitable for such an approach. Polyclonal antisera prepared to antigens from *Fusarium ssp*. were tested in immunological detection systems. The antiserum assessments resulted in one antiserum that was appropriate for the detection of Fusarium – exoantigens (ExAg) in cereal grains by an indirect ELISA-format – the plate trapped antigen (PTA-ELISA). The results show that an increase of FHB disease severity is associated with an increase in fungus colonisation expressed as higher amounts of ExAg and higher DON contents. To increase further the test sensitivity, monoclonal antibodies (MAbs) have been developed to *F. culmorum* surface washings.

Selected Results of Two European Joint Research Projects

1. In Austria, concerted effort to breed for wheat varieties tolerant to FHB started only in 1991. Because of the impressive progress made in a decade, a generously funded six nation European union network project was granted to Ruckenbauer et al. (2001) and we present here the most significant findings of the project "FUCOMYR".

After the detection of the major QTL for FHB resistance on the wheat chromosome 3BS for type II resistance (Anderson et al. 2001) and the second major QTL on chromosome 5A (Buerstmayr et al. 2002) for type I resistance, a number of experiments were carried out to establish the non-species specify of these QTL – markers (Lemmens et al. 2004). A wheat population of 96 doubled haploid (DH) lines from a cross between a moderate resistant CIMMYT – line "CM-82036" and a Fusarium – susceptible spring wheat variety from Germany "Remus" was investigated in detail for FHB-resistance. "CM-82036" carries two QTLs for resistance (*Qfhs.ndsu-3BS* and "*Qfhs.ifa-5A)*. All four QTL-classes resulting from all possible combinations of these QTLs were present in the nursery (24 lines in each class).

Resistance was assessed after spray inoculation with eight different fungal strains on separate plots. Disease incidence (Type I resistance) and disease severity (Type I and II) were assessed 4–5 times. These experiments were performed for two year on two locations (Tulln/Austria and Szeged/Hungary). Spread of the symptoms (number of diseased spikelets) was assessed four times after inoculation and prior to harvest.

The results showed that highly significant difference for FHB resistance exists between genotypes and QTL classes both after spray and point inoculation. It was concluded that genotypes with the QTL *Qfhs.ndsu-3BS* improved only Type II resistance (disease severity). Genotypes carrying the QTL *Qfhs.ifa-5A* improved mainly the type I resistance (disease incidence). In the ongoing commercial breeding programmes with this material, both the QTLs have demonstrated their excellence marker qualities in backcrossing programmes to improve the overall resistance of wheat genotypes adapted to Central and Eastern European conditions.

2. The EU-sponsored "Concerted Action": "Quality Control Measures in the Production and Processing Chain to Reduce Fusarium Mycotoxin Contamination of Food and Feed Grains" with the acronym "MYCOTOCHAIN" was executed January 1999 to June 2002 – involving twenty different research groups spread over Europe (Scholten et al. 2003). The aim was to collect the state of art of all possibilities in Europe to reduce Fusarium mycotoxin contaminations of food and feed grain. The partners from 8 EU-countries: Austria, Denmark, France, Germany, Italy, Sweden, The Netherlands and the United Kingdom belong to fundamental research organisations, commercial breeding companies, the trade, the milling industry and food and feed safety organisations. The document comprises of six chapters, all relevant to the goal to achieve mycotoxin-free cereals for the benefit of European consumers.

Important information on the status of susceptibility of all European wheat varieties and the results of several comparative field trials across the continent with or without artificial inoculations or mist-irrigation treatments are covered. Although some varieties were only slightly infected, all collected results showed clearly that **none** of them was completely resistant. The correlation between disease incidence and DON content of the grain samples was estimated at 0.80.

The various efforts of the public and private European wheat breeders indicated the slow progress by using exotic Asian wheat sources for resistance QTLs in crossing programmes. Recent marker-aided selection procedures clearly demonstrates that it can significantly facilitate the current breeding programmes, and in tackling the quantitative nature of disease resistance.

Adequate information has been generated on the FHB occurring after harvest storage, processing and decontamination of the pathogen. Humidity and temperature were important factors that influence fungal growth. All chemical, microbiological or physical detoxification treatments tried so far have been found to be inadequate to provide an acceptable level of freedom from the otherwise dangerous trichothecenes.

REFERENCES

Anderson JA, Stack RW, Liu S, Waldon BL, Fjeld AD, Coyne C, Moreno-Sevilla B, Mitchel Fetch J, Song OJ, Cregan PB, Frohberg RC (2001) DNA markers for Fusarium head blight resistance QTLs in two wheat populations. Theor Appl Genet 102:1164–1168

Browne RA, Cook BM (2004) Development and evaluation of an *in vitro* detached leaf assay for pre-screening resistance to Fusarium head blight in wheat. Eur J Plant Pathol 110:91–102

Buerstmayr H, Lemmens M, Hartl L, Doldi L, Stierschneider M, Ruckenbauer P (2002) Molecular mapping of QTLs for Fusarium head blight resistance in spring wheat. I. Resistance to fungal spread (type II resistance). Theor Appl Gene. 104:84–91

Lemmens M, Buerstmayr H, Scholz U, Mesterhazy A, Ruckenbauer P (2004) Non-species specifity of *QFhs.ndsu-3BS* and *QFhs.ifa-5A* in wheat. In: Canty SM, Boring T, Wardwell J, Ward RW (eds) Proceedings of the 2nd International Symposium on Fusarium Head Blight; incorporating the 8th European Fusarium Seminar, 11–15 Dec, Orlando, FL, USA. Michigan State University, East Lansing, MI, USA, vol 1, p 87

Mesterhazy A (1983) Breeding wheat for resistance to *Fusarium graminearum* and *Fusarium culmorum*. Z Pflanzenzuchtg 91:295–311

Mesterhazy A (1995) Types and components of resistance against Fusarium Head Blight of wheat. Plant Breed 114:377–386

Mesterhazy A (2002) Role of deoxynivalenol in agressivness of *F. graminearum* and *F. culmorum* and resistance to Fusarium Head Blight. Eur J Plant Pathol 108:675–684

Nicholson P, Chandler E, Draeger RC, Gosman NE, Simpson DR, Tamssett M, Wilson AH (2003) Molecular tools to study epidemiology of Fusarium Head Blight in cereals. Eur J Plant Pathol 109:691–703

Rabenstein F, Rohde S, Lind V, Heinze M, Unger O, Schachschneider R, Hamman T, Richter K, Miedaner T (2004) Immunological estimation of exoantigen content in small grain cereals for assessment of resistance to Fusarium Head Blight. In: Canty SM, Boring T, Wardwell J, Ward RW (eds) Proceedings of the 2nd International Symposium on Fusarium Head Blight; incorporating the 8th European Fusarium Seminar, 11–15 Dec, Orlando, FL, USA. Michigan State University, East Lansing, MI, USA, vol 1, p 153

Ruckenbauer P, Buerstmayr H, Lemmens M (2001) Present strategies in resistance breeding against scab (*Fusarium ssp.*). Euphytica 119:121–127

Scholten OE, Ruckenbauer P, Visconti A, van Osenbruggen WA, den Nijs APM (2003) A chain wide approach to reduce Fusarium mycotoxins. Commission of the European Community. FAIR-CT 98–4094 document, 84pp.

GENETIC ANALYSIS OF *SEPTORIA TRITICI* BLOTCH TO IMPROVE RESISTANCE IN EUROPEAN WHEAT BREEDING PROGRAMMES

L.S. ARRAIANO* AND J.K.M. BROWN

Disease and Stress Biology Department, John Innes Centre, Norwich Research Park, Colney Lane, Norwich, NR4 7UH, United Kingdom
**E-mail: lia.arraiano@bbsrc.ac.uk*

Abstract: In Europe, the need to understand and use host plant resistance to septoria tritici blotch (STB) has assumed new urgency with the widespread development of resistance to QoI (strobilurin and related) fungicides in the STB pathogen, *Mycosphaerella graminicola* (Fuckel) Schrot. We have investigated factors that contribute to reducing STB levels among wheat cultivars in field conditions, including resistance to specific isolates of *M. graminicola* and aspects of plant development and morphology that contribute to disease escape. This has allowed the identification of cultivars with isolate-non-specific, foliar resistance to STB which should be valuable in wheat breeding

Keywords: septoria tritici, resistance genes, partial resistance

INTRODUCTION

Septoria tritici leaf blotch has been the major disease of wheat in Britain and much of the rest of Europe for nearly two decades (Pillinger et al. 2004). Until recently, most popular varieties were susceptible, which means that septoria has been the principal target for foliar fungicides on wheat. The recent discovery of resistance to strobilurin (QoI) fungicides (Fraaije et al. 2005) has increased interest in breeding and growing cultivars resistant to STB as a cost-effective means of controlling the disease and wheat breeders throughout Europe consider it one of the major targets for resistance breeding. Until very recently however, almost nothing of practical value was known about the genetics of resistance to septoria, despite its importance on wheat world-wide. This limited the ability of the plant breeding industry to provide farmers with a reliable supply of septoria-resistant varieties. Brown et al. (2001)

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 109–112.

showed that there are several isolate-specific resistances present in European wheat cultivars but it is not known how frequent they are among germplasm in current use by breeders. For many years European wheat breeders believed they did not have access to adequate genetic variation in resistance to STB. This was partly because of a lack of knowledge about the genetics of resistance, particularly the distribution of resistance genes among different cultivars, which would indicate if different genes could be combined. In recent years twelve major genes for resistance to the fungal pathogen, *Mycosphaerella graminicola*, have been identified and mapped. Of these gene Stb6, which has been demonstrated to follow a gene-for-gene relationship, is widespread in sources of resistance to STB world-wide (Chartrain et al. 2004a).

There have been many reports of increased STB severity in earlier heading and shorter cultivars, in some of which the effects of heading date and height on STB were analysed genetically (Chartrain et al. 2004b, Simón et al. 2004, Arraiano et al. 2005). These studies varied greatly in the methods used for disease trialling and disease assessment. It has been proposed that resistance in a range of cultivars can only be assessed reliably if disease severity is measured at the same stage of plant development and not at the same moment in time (Arama et al. 1999). Disease escape mechanisms may also involve times of leaf emergence and the degree of overlap between successive leaves in a canopy and these may be significant factors in disease development in crop canopies (Royle 1994). The risk of disease progression is therefore dynamically complex and specific to each crop canopy (Lovell et al. 2004).

Breeding for durable resistance to STB therefore requires knowledge about which aspects of plant development and morphology are more significantly associated with disease escape and knowledge of the distribution of isolate-specific resistances within wheat germplasm. The objective of the work reported here was to identify such resistances in European wheat cultivars relevant to current breeding programmes in the UK and to investigate which of these genes might make useful contributions to reducing levels of STB in field situations as well as investigating other areas of the genome that might be linked to partial resistance against STB.

MATERIALS AND METHODS

A total of 226 wheat genotypes were studied, including cultivars grown in the UK and other lines relevant to UK breeding programmes, including progenitors of current cultivars. Eleven field trials were carried out at eight sites over three years, in which STB and escape factors were scored. All 226 lines were screened with single isolates to identify the presence of specific resistance genes using the detached leaf technique (Arraiano et al. 2001). A sub-set of 98 lines were scored with 121 microsatellite markers covering all 21 chromosomes. The markers used were developed by Wheat Microsatellite Consortium (WMC- Agrogene SA, Moissy Cramayel, France), the Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben (Röder et al. 1998), the John Innes Centre (Stephenson et al. 1998), the Beltsville Agricultural Research Centre, Maryland, USA and the Dupont Company,

Wilmington, USA. For this study Fluorescent PCR products were separated on an ABI 3700 capillary sequencer and their sizes measured using Applied Biosystem's GENESCAN and GENOTYPER fragment analysis software (Applied Biosystems, Foster City, Calif.).

RESULTS AND DISCUSSION

At least two specific resistance genes were associated with significant reduction of STB in the set of wheat genotypes. One of these was the most important predictor of resistance other than height to flag leaf. The development of a model which combines escape factors (heading date, plant height and leaf spacing) with specific resistances that are correlated with disease levels has enabled us to detect cultivars with high levels of foliar resistance to STB. These are likely to be useful as new sources of STB resistance for wheat breeding programmes in the UK and elsewhere in Europe. Other regions of the genome that may contain hitherto unknown resistance genes were identified by linkage to microsatellite markers in an association genetic analysis.

This research is providing breeders with knowledge about the potential value of genes for resistance to STB and about previously unidentified sources of resistance within the pool of wheat germplasm adapted to northern European conditions. This is expected to lead to improved selection of wheat varieties with resistance to STB and in due course, to a sustained, gradual improvement in the resistance of European wheat varieties to STB and thus to a reduced need for fungicides to control this disease.

ACKNOWLEDGEMENTS

This research was done in collaboration with Advanta Seeds UK, Cebeco Seed Innovations, Elsoms Seeds, Nickerson, SW Seed and Syngenta Seeds through the Sustainable Arable LINK Programme.

REFERENCES

Arama P, Parlevliet JE, van Silfhout CH (1999) Heading date and resistance to septoria tritici blotch in wheat not genetically associated. Euphytica 106:63–8

Arraiano LS, Brading PA, Brown JKM (2001) A detached seedling leaf technique to study resistance to *Mycosphaerella graminicola* (anamorph *Septoria tritici*) in wheat. Plant Pathol 50:339–46

Arraiano LS, Brading PA, Dedryver F, Brown JKM (2005). Resistance of wheat to septoria tritici blotch (*Mycosphaerella graminicola*) and associations with plant ideotype and the 1BL-1RS translocation. Plant Pathol, Doi: 10.1111/j.1365–3059.2005.01319.x

Brown JKM, Kema GHJ, Forrer HR, Verstappen ECP, Arraiano LS, Brading PA, Foster EM, Fried PM, Jenny E (2001) Resistance of wheat cultivars and breeding lines to septoria tritici blotch caused by isolates of *Mycosphaerella graminicola* in field trials. Plant Pathol 50:325–38

Chartrain L, Brading PA, Makepeace JC, Brown JKM (2004a) Sources of resistance to septoria leaf blotch and implications for wheat breeding. Plant Pathol 53:454–60

Chartrain L, Brading PA, Widdowson JP, Brown JKM (2004b) Partial resistance to septoria tritici blotch (*Mycosphaerella graminicola*) in wheat cultivars Arina and Riband. Phytopathology 94:497–504

Fraaije BA, Cools HJ, Fountaine J, Lovell DJ, Motteram J, West JS, Lucas JA (2005) Role of ascospores in further spread of QoI-resistant cytochrome b alleles (G143A) in field populations of *Mycosphaerella graminicola*. Phytopathology 95:933–941

Lovell DJ, Parker SR, Hunter T, Welham SJ, Nichols AR (2004) Position of inoculum in the canopy affects the risk of septoria tritici blotch epidemics in winter wheat. Plant Pathol 53:11–21

Pillinger C, Evans EJ, Whaley JM, Knight SM, Poole N (2004) Managing Early-drilled Winter Wheat: Seed Rates, Varieties and Disease Control. HGCA Project Report no. 348. Home-Grown Cereals Authority,, London

Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier MH, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007–23

Royle DJ (1994) Understanding and predicting epidemics: a commentary based on selected pathosystems. Plant Pathol 43:777–89

Simón MR, Worland AJ, Struik PC (2004) Influence of plant height and heading date on the expression of the resistance to septoria tritici blotch in near isogenic lines of wheat. Crop Sci 44:2078–85

Stephenson P, Bryan G, Kirby J, Collins A, Devos K, Busso C, Gale M (1998) Fifty new microsatellite loci for the wheat genetic map. Theor Appl Genet 97:946–9

INHERITANCE AND ALLELIC RELATIONSHIP OF RESISTANCE GENES TO SPOT BLOTCH OF WHEAT CAUSED BY *BIPOLARIS SOROKINIANA*

S. KUMAR[1,*], L.C. PRASAD[2], U. KUMAR[2], K. TYAGI[2], B. ARUN[2] AND A.K. JOSHI[2,3]

[1]*Department of Biotechnology, Sardar Vallabh Bhai Patel University of Agriculture and Technology, Meerut-250110, U.P., India*

[2]*Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi-221005, India*

[3]*Present Address: International Maize and Wheat Improvement Center (CIMMYT) Apdo. Postal 6-641, C.P. 06600, D.F. Mexico*

[*]*E-mail: sundeepksharma77@rediffmail.com*

Abstract: Three varieties viz., Longmai 10, Jinmai and Sanghai, known for their resistance to spot blotch were crossed with a susceptible parent Sonalika. Disease severities of F_1's were intermediate to parents and thus indicated no dominance. Around 150–200 progeny rows of three resistant × susceptible crosses were evaluated in the F_3, F_4 and F_5 generations. Spot blotch severity (%) for each progeny row was measured at three different growth stages viz. 69, 77 and 83 (Zadoks scale, 1974). Based on disease score, F_3, F_4 and F_5 progenies were grouped into three classes: homozygous resistant, homozygous susceptible and segregating. Number of genes was estimated following χ^2 and quantitative approach. Two parents showed two genes control while, Jinmai appeared to be under the control of three genes

Resistant x resistant crosses were made to confirm the allelic relationship of resistance genes. The F_3 progenies of all the crosses did not show susceptible plants. This proved that at least one gene was common among parents for resistance. However, the appearance of transgressive segregants was an indication of the non-allelic relationship. The study indicated the possibility of enhancement of resistance through gene pyramiding

Keywords: wheat, spot blotch, genetic resistance

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 113–118

INTRODUCTION

Spot blotch disease of wheat caused by *Bipolaris sorokiniana* (Sacc.) Shoem. (teleomorph *Cochliobolus sativus* (Ito&Kurib.)) is of increasing concern in South East Asia and Latin American countries (Sharma et al. 1997, Chand et al. 2003, Joshi et al. 2004a, b, Pandey et al. 2005). It is a disease of importance where warm humid conditions persist during wheat cropping season. The average yield losses due to leaf blight for South Asia and India have been estimated to be around 20% (Saari 1998).

One of the major constraints in breeding progress to enhance resistance against spot blotch is the lack of clear information about the inheritance of resistance for this disease. Inheritance studies on resistance to spot blotch are limited and nature of inheritance is still debatable. Further, allelic relationship of the resistant genes is also not known. In view of the importance of the spot blotch disease of wheat and lack of sufficient information about the inheritance of resistance, the present investigation was conducted to throw more light on the nature of resistance and their allelic relationship.

MATERIALS AND METHODS

Crosses were made between three resistant parents viz., Longmai 10, Jinmai and Sanghai with the susceptible cultivar cv Sonalika. All the generations (F_1 to F_5) were raised along with parents under artificial epiphytotic conditions in the field at Varanasi in the year 2000–01, 2001–02 and 2002–03. Spot blotch severity was assessed three times; at growth stages 69 (flowering complete), 77 (late milk) and 83 (early dough) (Zadoks et al. 1974) using spot blotch severity (%). For each line, the disease scores of all the plants, including the most susceptible and most resistant, were recorded. Areas under disease progress curve (AUDPC) (van der Plank, 1963) was estimated based on the plot disease severities at different growth stages (Joshi et al. 2002). Number of genes were estimated following the χ^2 analysis (Singh et al. 1993, Joshi and Chand 2002) as well as the quantitative approach using a modified formula of Wright (1968) (Singh et al. 1995, Joshi et al. 2004a).

The three resistant parents were crossed among themselves for test of allelism of resistance genes. Around 150–200 random single plants for each of the crosses were advanced to the F_3 generation following a pedigree method. Parents, F_1's, F_2's and F_3 lines were evaluated for spot blotch resistance under induced epiphytotic conditions during crop season 2001–02. In F_2 populations, individual plants were classified while for each F_3 line the range of severity (the most resistant to the most susceptible plant) was recorded using spot blotch severity (%) for each genotype at growth stage 83 (early dough) (Zadoks et al. 1974).

RESULTS

Performance of the Parents and F_1's

The disease severity (%) of resistant parents was less than 30 in both the years (2000–2001 and 2001–02) while it was around 90% in case of the susceptible parent (Sonalika). The AUDPC was less than 500 in resistant parents while, it was higher than 2000 in case of the susceptible parent (Sonalika) (data not shown). Compared to the parents, the spot blotch scores of the F_1's were intermediate indicating absence of dominance for genes governing resistance.

Performance in Segregating Generations

The χ^2 values in F_3, F_4 and F_5 generations revealed a goodness of fit for two genes in the two parents (Longmai 10 and Sanghai) while, it was three in case of Jinmai (Table 1). The F_3 line distributions in these crosses showed partially continuous to skewed variation indicating that resistance genes interacted in an additive manner (Fig. 1). The result of the modified formula of Wright (1968) (Singh et al. 1995), using severity (%) as well as AUDPC values, showed that the gene number were quite close to that obtained by χ^2 analysis (Table 2).

Table 1. Goodness of fit of ratios observed and hypothesized class frequencies for F_5 lines from three resistant × susceptible crosses

Cross	Generation	Sowing date	Observed ratio in F_3			Hypoth. ratio	χ^2 value	P value	Gene Number
			HRPT[a]	Seg[b]	HSPT[c]				
Longmai 10 × Sonalika	F_5	Ist	134	35	41	10.9375:2:3 .0625	3.57	>0.15	2
		IInd	140	32	38	10.9375:2:3 .0625	1.47	>0.48	2
Sanghai × Sonalika	F_5	Ist	120	27	33	10.9375:2:3 .0625	1.04	>0.60	2
		IInd	122	28	30	10.9375:2:3 .0625	1.93	>0.35	2
Jinmai × Sonalika	F_5	Ist	10	161	9	4:56:4	0.63	>0.70	3
		IInd	12	160	8	4:56:4	1.03	>0.60	3

[a] Homozygous for resistant parental type (homozygous for all resistance alleles).
[b] Segregating for disease levels higher than that of resistant parent but less than or equivalent to that of susceptible parent (homozygous for at least one resistant allele or heterozygous for at least one locus and homozygous for susceptibility alleles at other loci).
[c] Homozygous for susceptible parental type (homozygous, lacking all resistance alleles).

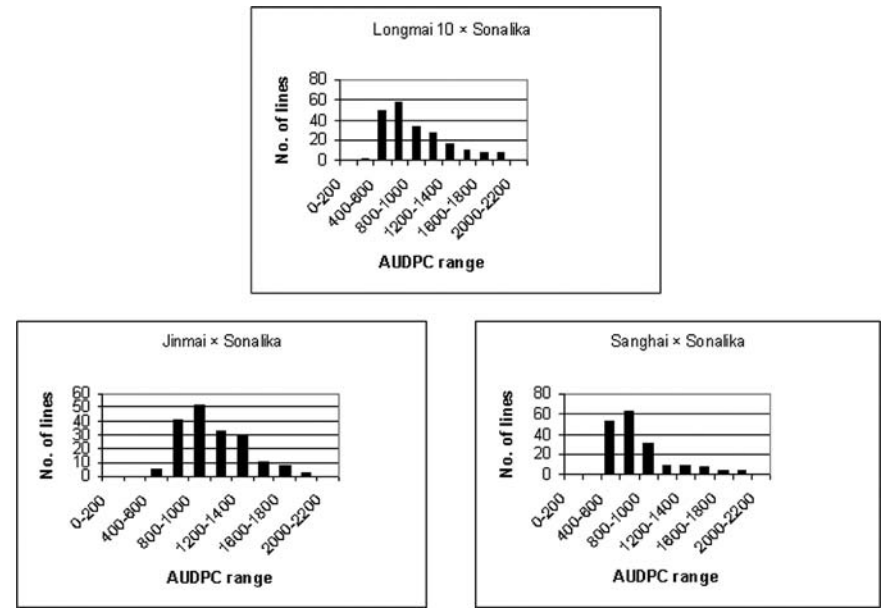


Figure 1. Distribution of AUDPC of F_3 lines of resistant × susceptible crosses for spot blotch severity

Table 2. Estimation of minimum number of effective genes segregating for spot blotch in the different crosses by using Wright's (1968) formula modified for F_3, F_4 and F_5 generation (Singh et al., 1995)

Cross	Generation	No. of Lines	% disease severity		AUDPC	
			Without heritability	With heritability	Without heritability	With heritability
Longmai	F_3	210	2.70^b	1.57^a	2.14^a	1.55^a
10 ×	F_4	210	2.32^a	1.54^b	2.46^b	1.59^b
Sonalika	F_5	210	2.28^a	1.62^b	3.23^a	2.16^b
Sanghai ×	F_3	180	2.39^a	1.52^b	2.48^a	1.72^b
Sonalika	F_4	180	2.82^a	1.90^b	2.52^a	1.53^b
	F_5	180	2.37^a	1.53^b	2.77^a	1.54^b
Jinmai ×	F_3	180	3.97^a	2.53^b	3.50^a	2.52^b
Sonalika	F_4	180	3.94^a	2.53^b	3.80^a	2.56^b
	F_5	180	4.26^a	2.55^b	4.15^a	2.53^b

TEST OF ALLELISM

All the F_3 lines obtained from intercrosses of resistant parents showed a common range of disease severity and completely lacked susceptible plants (Table 3). The absence of extreme types in the cross Jinmai × Sanghai provided strong evidence that both the resistance genes of Sanghai might be present in Jinmai.

Table 3. F_1 severity, F_2 severity range and classification of F_3 lines based on disease severity in the crosses of resistant parents tested with *Bipolaris sorokiniana*

Cross	F_1 severity	F_2 severity range	Number of F_3 lines with plants of greatest severity							Total F_3 lines
			T	10	15	20	25	30	35	
Longmai 10 × Jinmai	22.43	10–25	–	4	20	33	21	3	–	81
Longmai 10 × Sanghai	24.47	10–30	–	–	7	19	28	22	5	81
Ning 8201 × Jinmai	21.20	10–30	–	2	21	39	18	3	–	83
Ning 8201 × Sanghai	22.53	15–30	–	–	8	16	26	21	7	78
Jinmai × Sanghai	23.45	10–30	–	4	22	29	21	3	–	79

DISCUSSION

It appeared that around two genes were responsible for spot blotch resistance in two wheat lines Longmai 10 and Sanghai 4, while Jinmai carried three genes. In some of the Indian studies, one or two gene control has been reported (Srivastava et al. 1971, Srivastava 1982, Adlakha et al. 1984). However, these studies did not go beyond F_2 generation and utilized limited population size. Further, the genetic stocks used in these studies were less potent than those used in this study. The breeding progress using such parents did not prove satisfactory for a long time and disease became increasingly important in the Indian subcontinent (Saari 1998, Joshi and Chand 2002). Recently, Joshi et al. (2004b), using resistance sources viz., Mons/Ald, Acc. 8206 and Suzhoe 8, reported that around three genes having additive effect operated in controlling resistance to spot blotch. Joshi et al. (2004a) also reported that leaf tip necrosis (*Ltn*), which is under the control of a major gene, is associated with moderate resistance to spot blotch and can be used as a morphological marker to facilitate selection for resistance.

The heritability estimates for the three crosses were moderate with a range of 0.77 to 0.84. A few other reports (Sharma et al. 1997, Joshi et al. 2004b) also suggested moderate estimates of heritability with respect to spot blotch resistance. Joshi et al. (2004b) suggested that by creating effective artificial epiphytotic and recording observations taking care of the growth stage, one could reduce the effect of environment and thereby obtain fairly high estimates of heritability.

One of the important reasons of slow breeding progress in spot blotch resistance has been suggested to be the absence of suitable resistant parents and proper information about the inheritance of resistance (Joshi et al. 2004b). Immunity for spot blotch is also not known (van Ginkel and Rajaram 1998). However, substantial gains in resistance were obtained by selecting for low AUDPC lines in the segregating generations (Sharma et al. 1997, Joshi and Chand 2002). The presence

of only two non-additive genes observed in most of the crosses of this study imply that spot blotch resistance can be easily manipulated by following selection for low scoring genotypes at appropriate growth stages under high inoculum pressure even in early generations. In case of additive effect of three genes as obtained in case of Jinmai, enhancement in resistance can be realized by growing fairly large segregating populations and selecting for low scoring genotypes under high inoculum pressure. Even in this case, since genes are only a few and heritability is fairly high, effective selection could be applied in the early segregating generations (Joshi et al. 2004b). For further gains, strong selection pressure could be applied in advance generations when high homozygosity has been achieved (Singh et al. 1995, van Ginkel and Rajaram 1998, Joshi et al. 2004b).

REFERENCES

Adlakha KL, Wilcoxson RD, Raychaudhuri SP (1984) Resistance of wheat to leaf spot caused by *Bipolaris sorokiniana*. Pl Dis 68(4):320–321

Chand, R, Pandey SP, Singh HV, Kumar S, Joshi AK (2003) Variability and its probable cause in natural populations of spot blotch pathogen (*Bipolaris sorokiniana*) of wheat (*T. aestivum* L.) in India. J Pl Dis Pro 110(1):27–35

Joshi AK, Chand R (2002) Variation and inheritance of leaf angle, and its association with spot blotch (*Bipolaris sorokiniana*) severity in wheat. Euphytica 124:283–291

Joshi AK, Chand R, Arun B (2002) Relationship of plant height and days to maturity with resistance to spot blotch in wheat. Euphytica 123:221–228

Joshi, AK, Chand R, Kumar S, Singh RP (2004a) Leaf tip necrosis-a phenotypic marker for resistance to spot blotch disease caused by *Bipolaris sorokiniana* in wheat (*Triticum aestivum* L.). Crop Sci 44:792–796

Joshi AK, Kumar S, Chand R, Ortiz-Ferrara G (2004b) Inheritance of resistance to spot blotch pathogen caused by *Bipolaris sorokiniana* in spring wheat. Plant Breed 123:213–219

Pandey SP, Kumar S, Kumar U, Chand R, Joshi AK (2005) Sources of inoculum and reappearance of spot blotch of wheat in rice-wheat cropping system in eastern India. Eur J Plant Pathol 111:47–55

Saari EE (1998) Leaf blight diseases and associated soil borne fungal pathogens of wheat in north and south East Asia. In: Duveiller E, Dubin HJ, Reeves J, McNab A (eds.) Helminthosporium Blights of Wheat: Spot Blotch and Tan Spot. CIMMYT, Mexico D.F., pp 37–51

Sharma RC, Dubin HJ, Devkota RN, Bhatta MR (1997) Heritability estimates of field resistance to spot blotch in four spring wheat crosses. Plant Breed 116:64–68

Singh RP (1992) Genetic association of leaf rust resistance gene *Lr34* with adult plant resistance to stripe rust in bread wheat. Phytopathology 82:835–838

Singh RP, Ma Hong, Rajaram S (1995) Genetic analysis of resistance to scab in spring wheat cultivar Frontana. Plant Dis 79(3):238–240

Srivastava OP (1982) Genetics of resistance to leaf blights of wheat. Indian J Genet 42:140–141

Srivastava OP, Luthra JK, Narula PN (1971) Inheritance of seedling resistance to leaf blight of wheat. Indian J Genet Plant Breed 45:941–943

van Ginkel M, Rajaram S (1998) Breeding for resistance to spot blotch in wheat: Global perspective. In: Duveiller E, Dubin HJ, Reeves J, McNab A (eds.) Helminthosporium Blights of Wheat: Spot Blotch and Tan Spot. CIMMYT, Mexico D.F., pp 162–170

van der Plank JE (1963) Plant Diseases: Epidemics and Control. Academic Press, New York

Wright S (1968) Evolution and Genetics of Populations. Vol I. Genetics and Biometric Foundation. University of Chicago Press. Chicago

Zadoks JC, Chang TT, Konzak CF (1974) A decimal code the growth stages of cereals. Weed Res 14:415–421

RESISTANCE TO *MAGNAPORTHE GRISEA* AMONG BRAZILIAN WHEAT GENOTYPES

A.M. PRESTES[1,*], P.F. ARENDT[2], J.M.C. FERNANDES[1] AND P.L. SCHEEREN[1]

[1]*National Wheat Research Center of the Brazilian Agricultural Research Corporation (Embrapa) and CNPq Fellow. Caixa Postal 451, 99001-970 Passo Fundo, RS, Brazil*
[2]*Graduate student at the University of Passo Fundo. Passo Fundo, RS, Brazil*
[*]*E-mail: ariano@upf.br*

Abstract: *Magnaporthe grisea*, the causal organism of wheat head blast may induce important yield losses in wheat and barley crops in Brazil. Chemical control besides been expensive is not effective on highly susceptible varieties. Thus, the use of wheat genotypes with reasonable level of resistance associated with fungicides appears as the most effective means of control of the disease. The research carried out has the objective of evaluating the resistance of Brazilian wheat genotypes to wheat blast under controlled environment conditions. A great variability in reaction to head blast of wheat was observed among Brazilian varieties and advanced breeding lines tested. The range of head infection varied from 10 % to 86 % in average, but none among hundred varieties tested displayed high level of resistance to *M. grisea*. Nevertheless, some varieties and advanced breeding lines showed moderate resistance and eighteen of them displayed average infections lower than the control BR 18, a known moderately resistant variety under field condition

Keywords: wheat, wheat blast, *Magnaporthe grisea*, genetic resistance

INTRODUCTION

Magnaporthe grisea (Hebert) Barr [(anamorph *Pyricularia grisea* (Cook) Sacc.], the causal organism of blast, may induce yield losses over 50% whenever susceptible wheat cultivars are grown in Brazil. Besides wheat (*Triticum aestivum* L.), *M. grisea* also infects several species of grass weeds that are common in farmers' fields (Urashima et al. 1993). The disease was firstly reported on wheat in the State of Paraná, in 1985 (Igarashi et al. 1986). However, it may be possible that it was already present on wheat fields, since its symptoms in early stages could be inadvertently confused with Fusarium head blight. Soon after its first report, the

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 119–123.

disease became widely distributed over wheat growing areas of several Brazilian States (Igarashi, 1990; Picinini and Fernandes 1990; Prabhu et al. 1992, Goulart and Paiva 2000). Because fungicides are not effective and resistant cultivars are seldom reported, wheat blast became one of the major diseases of wheat in Brazil (Urashima et al. 2004). It produces lesions on all above–ground parts of wheat plants, but head infection is more common and more destructive. Nevertheless, typical blast lesions may occur shortly after wheat plant emergence, under favorable environment. The objective of the present study was to evaluate wheat genotypes for resistance to head blast, under controlled environment.

MATERIAL AND METHODS

The objective of the present work was to evaluate one hundred Brazilian wheat genotypes from the working collection of the National Wheat Research Center of the Brazilian Agricultural Research Corporation (Embrapa Trigo) for resistance to head blast, under controlled environment. Plants were grown up to head stage in 7 kg capacity pots distributed in the greenhouse as a completely randomized treatment experimental design. The pathogen was then inoculated in a spore density of $2.5 \times 10^5\ mL^{-1}$, onto plants at flowering stage in growth chamber with temperature adjusted to 24 °C under saturated moisture provided by spraying nozzles (De Vilbiss, model SGA 570) during 24 hours. Plants were then transferred to the greenhouse and maintained under adjusted temperature of 24 °C. Evaluation was done 10 days after inoculation by counting the number of infected spikelets per head to determine blast severity. The data was analyzed using the statistical SAS FASTCLUS (SAS 1998) cluster procedure, and the varieties were classified into three groups of blast severity.

RESULTS AND DISCUSSION

The groups of severity resulted from the cluster analyses were: group 1- wheat genotypes displaying mean blast severity $81.6\% \pm 10.8\%$; group 2- mean blast severity $49.4 \pm 9.4\%$; and group 3- mean blast severity $16.5 \pm 10.3\%$ (Table 1). There was a great variability in head blast severity among the Brazilian wheat commercial varieties and advanced breeding lines tested. Mean range of blast severity varied from 10% to 86%. No genotype completely resistant to *M. grisea* under the testing condition in controlled environment was found. Nevertheless, eighteen genotypes among commercial varieties and advanced breeding lines showed moderate resistance and displayed mean severity of head infection lower than the control BR 18, a known moderately resistant variety under field conditions. Those were BRS 120, BRS 49, BRS 220, IA 0310, IAPAR 53, LD 0221, LD 0324, LD 2004, LD 2010, LD 0320, PF 953239, PF 970177, PF 980503, PF 980571, PF 990692, PF 999245, IPF 758669, and IPF 75876. Other 56 wheat genotypes displaying head infection over 40% were considered susceptible. Moderate resistance has also been reported under field conditions (Goulart and Paiva 1992, 1993).

Table 1. Groups of wheat genotypes according to severity of head infection by *Magnaporthe grisea* under controlled environment, in 2004[1]

Blast severity group	Genotype	Severity (%)
	LD 0319	86.50
	IA 0314	83.59
	BRS 210	83.50
	IA 0214	78.38
	LD 0323	76.29
Group 1	IA 0212	72.56
(81.6% ± 10.8%)	IA 0210	70.27
	IWT 2005	69.62
	WT 00010	69.12
	IA 0311	68.61
	LD 2005	68.59
	IA 0304	68.53
	IA 0301	67.67
	IA 0305	66.83
	IA 0302	65.73
	IA 0203	64.86
	BRS Camboatá	63.89
	WT 01039	62.98
	LD 0321	58.54
	PF 973994	58.29
	LD 0317	53.65
	IAPAR 17	51.18
	LD 0322	61.28
	IA 0303	59.23
	LD 992	58.03
	IPR 87	57.76
	IA 0204	56.94
	IA 0215	55.92
	WT 00246	55.00
	IA 0307	54.35
	BRS 208	53.47
	IAPAR 60	53.33
	WT 99207	52.41
	WT 00007	51.91
	BRS 209	51.25
	BRS Guabiju	51.00
	BRS 179	50.00
	WT 01050	50.00
	PF 990695	49.41
Group 2	BR 35	47.56
(49.4% ± 9.4%)	BRS Timbaúva	47.33
	IA 0308	47.23
	PF 001024	47.18
	LD 2006	46.14
	LD 0220	45.88

(*Continued*)

Table 1. (*Continued*)

Blast severity group	Genotype	Severity (%)
	WT 00298	45.00
	IPR 90	43.59
	IPR 85	43.01
	PF 001069	41.53
	Nesser	40.44
	BRS 177	39.22
	BRS 193	38.44
	LD 2007	37.08
	IPF 79813	37.02
	PF 001104	40.78
	IPF 79812	39.82
	BRS Buriti	39.29
	IA 0315	38.89
	PF 001122	38.42
	PF 003113 A	37.56
	BRS Louro	35.93
	IA 0209	33.68
	IAPAR 78	31.16
	BRS Angico	30.44
	LD 991	29.47
	PF 001102	28.91
	IPR 110	28.75
	WT 01110	28.57
	IPR 109	28.41
	WT 00066	28.00
	WT 00249	25.26
Group 3	**BR 18 – MR Control**	**24.86**
(16.7% ± 10.4%)	IPF 75869	24.79
	LD 0324	23.81
	BRS 120	23.13
	BRS 220	22.06
	BRS 49	21.72
	LD 2010	20.93
	PF 953239	19.84
	PF 980503	19.85
	PF 990692	19.74
	PF 970177	19.34
	IAPAR 53	19.26
	PF 999245	16.67
	IPF 75876	16.67
	PF 980571	15.94
	LD 0221	12.19
	LD 0320	11.31
	LD 2004	10.00
	IA 0310	09.61

[1] "FASTCLUS procedure" (Cluster) – SAS 1998.

According to Urashima et al. (2004), no promising resistant cultivar has been identified, but BR 18 showed the best performance. However, they observed a high degree of genetic variation in the pathogen, what could explain the difficulty in identifying wheat genotypes with good resistance.

REFERENCES

Goulart ACP, Paiva FA (1992) Incidência da brusone (*Pyricularia oryzae*) em diferentes cultivares de trigo (*Triticum aestivum*) em condições de campo. Fitopatologia Brasileira 17:321–325

Goulart ACP, Paiva FA (1993) Response of wheat cultivars and breeding lines to blast (*Pyricularia grisea*) under field conditions. Annual Wheat Newsletter 9:109–112

Goulart ACP, Paiva FA (2000) Perda no rendimento de grãos de trigo causada por *Pyricularia grisea*, nos anos de 1991 e 1992, no Mato Grosso do Sul. Summa Phytopathologica 26:279–282

Igarashi S (1990) Update on wheat blast (*Pyricularia oryzae*) in Brazil. In: Saunders DA (ed) Proceedings of the International Conference- Wheat for nontraditional warm areas. CIMMYT, Mexico, pp 480–483

Igarashi S, Utimada CM, Igarashi LC, Kazuma AH, Lopes RS (1986) *Pyricularia sp*. em trigo. I. Ocorrência de *Pyricularia sp*. no Estado do Paraná. Fitopatologia Brasileira 11:351–352

Picinini EC, Fernandes JMC (1990) Ocorrência da brusone (*Pyricularia oryzae*) em lavouras comerciais de trigo (*Triticum aestivum*) no Estado do Rio Grande do Sul. Fitopatologia Brasileira 15:83–84

Prabhu AS, Filippi MC, Castro N (1992) Pathogenic variation among isolates of *Pyricularia grisea* affecting rice, wheat, and grass in Brazil. Tropical Pest Management 38:367–371

Urashima AS, Igarashi S, Kato H (1993) Host range, mating type and fertility of *Pyricularia grisea* from wheat in Brazil. Plant Dis 77:1211–1216

Urashima AS, Lavorent NA, Goulart ACP, Mehta YR (2004) Resistance spectra of wheat cultivars and virulence diversity of *Magnaporthe grisea* isolates in Brazil. Fitopatologia Brasileira 29:511–518

THE INTERNATIONAL BREEDING STRATEGY FOR THE INCORPORATION OF RESISTANCE IN BREAD WHEAT AGAINST THE SOIL BORNE PATHOGENS (DRYLAND ROOT ROT AND CYST AND LESION CEREAL NEMATODES) USING CONVENTIONAL AND MOLECULAR TOOLS

J.M. NICOL[1,*], N. BOLAT[2], A. BAGCI[3], R.T. TRETHOWAN[1], M. WILLIAM[1], H. HEKIMHAN[2], A.F. YILDIRIM[2], E. SAHIN[1], H. ELEKCIOGLU[4], H. TOKTAY[2], B. TUNALI[5], A. HEDE[1], S. TANER[2], H.J. BRAUN[1], M. VAN GINKEL[1], M. KESER[2], Z. ARISOY[2], A. YORGANCILAR[2], A. TULEK[2], D. ERDURMUS[2], O. BUYUK[2] AND M. AYDOGDU[2]

[1]*CIMMYT International (International Wheat and Maize Improvement Center), Turkey Regional Office and Mexico*
[2]*MARA (Turkish Ministry of Agriculture and Rural Affairs), Turkey*
[3]*Selcuk University, Sarayonu, Konya, Turkey*
[4]*Cukurova University, Adana Turkey*
[5]*Ondokuz Mayis University, Samsun, Turkey*
[*]*E-mail: j.nicol@cgiar.org*

Abstract: Soil borne pathogens (SBPs) including the Dryland Root Rots and Cereal Nematodes are causing economic yield loss in many parts of the world where cereals dominate the cropping system and sub-optimal growing conditions or cultural practices are common. One of the most effective control measures of these SBPs is the use of host resistance, whereby the inoculum level of these pathogens can be reduced to below economically damaging thresholds. CIMMYT International in collaboration with The Turkish Ministry of Agriculture and Rural Affairs have established an International field and laboratory screening program for identifying spring and winter wheat accessions with resistance to SBPs. Several screening protocols for assessing resistance to both cereal root rots and nematodes have been modified and optimized. Known resistance sources to SBPs from other regions of the world have been tested against Turkish isolates of SBPs and several of these have been shown to be effective in the region. In addition new sources of resistance with genetic variability have been identified against the prevalent SBPs. These diverse genes for resistance are being pyramided into both spring and winter bread wheat backgrounds using both conventional and molecular tools where feasible

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 125–137.

Keywords: resistance, wheat, dryland root rot, Fusarium, Bipolaris, Cereal Cyst Nematode, Root Lesion Nematode

INTRODUCTION

Soil Borne Pathogens (SBPs), including Dryland Cereal Root Rots and Cereal Nematodes are a major constraint to cereal production worldwide, particularly where cereals dominate rotations, and sub-optimal growing conditions and or cultural practices are common. Dryland root rots also commonly known as root, crown, or foot root rots include a complex of fungi with several species of Crown Root (CR) (*Fusarium* spp.) and Common Root Rot (CRR) (*Bipolaris sorokiniana* (syns. *Helminthosporium sativum, H. sorokiniana*, Teleomorph *Cochliobolus sativus* (Ito & Kurib.) Dresch.ex Dast.)). The two most reported *Fusarium* species are *F. pseudograminearum* (formerly *F. graminearum* Group 1, Teleomorph *Gibberella coronicola*) and *F. culmorum*. Furthermore two groups of microscopic nematodes are commonly found on wheat roots and include several species of the Cereal Cyst Nematode (CCN) *Heterodera* spp. and at least two important species of the Root Lesion Nematode (RLN) *Pratylenchus thornei* and *P. neglectus*.

Frequently two or more SBPs can occur in the soil at one time, making a disease complex and hence a holistic approach in management principally based primarily on resistance but where possible integrated with rotational options is required.

Yield loss caused by these SBPs has been reviewed and documented in many regions of the world including Europe, America and in particular the more marginal cereal production areas of West Asia, North Africa, Australia and Canada with losses reported between 3–50% (Diehl et al. 1983, Burgess et al. 2001, Singh et al. 2005, Nicol et al. 2001, Nicol et al. 2004a, McDonald and Nicol 2005). Recent yield losses studies in Turkey have confirmed that cereal root rots and cereal nematodes are associated with yield losses of 42 and 45% in commonly cultivated winter wheats (Nicol et al. 2005, Hekimham et al. 2004). Considering the similarity in WANA (West Asia and North Africa), parts of South America, South Africa and other parts of the world in relation to cropping patterns and climate, it is likely that soil borne pathogens could cause similar economic losses in these regions.

Resistance, which is defined as a reduction in the multiplication of the pathogen, is one of the best methods to control these diseases. Although these nematodes and fungi have been considered important for several decades in certain countries, little advancement in breeding has been made. This is due to the difficulties of screening for these pathogens under field and greenhouse conditions. Currently there are very few known effective sources of resistance against these pathogens available in commercially grown wheat varieties, and many of the identified resistant sources are found in unadapted germplasm which will require considerable breeding investment

to produce commercial varieties. Hence a precise laboratory/field breeding strategy has been established by Turkish and CIMMYT scientists in Turkey with CIMMYT Mexico to identify and incorporate new sources of resistance, particularly those identified in well adapted backgrounds.

MATERIALS AND METHODS

International Linkages

A strategy for screening, validating and disseminating germplasm has been developed linking CIMMYT Mexico and the International Winter Wheat Improvement Program (IWWIP) in Turkey which is a tripartite breeding effort between the Turkish Ministry of Agriculture and Rural Affairs, CIMMYT and sister center ICARDA. Spring wheats are developed in Mexico at CIMMYT headquarters and winter wheats in Turkey under the IWWIP program as indicated in Fig. 1.

Field Screening for Cereal Root Rots to Identify Resistance

Fig. 2 illustrates the clear strategy for germplasm screening, validation and subsequent incorporation into both spring and winter wheat breeding programs has been developed (Nicol et al. 2004b). Germplasm entering these nurseries is sourced from several breeding programs around the world including CIMMYT, IWWIP, Turkish national materials and a number of Advanced Research Institutes and National

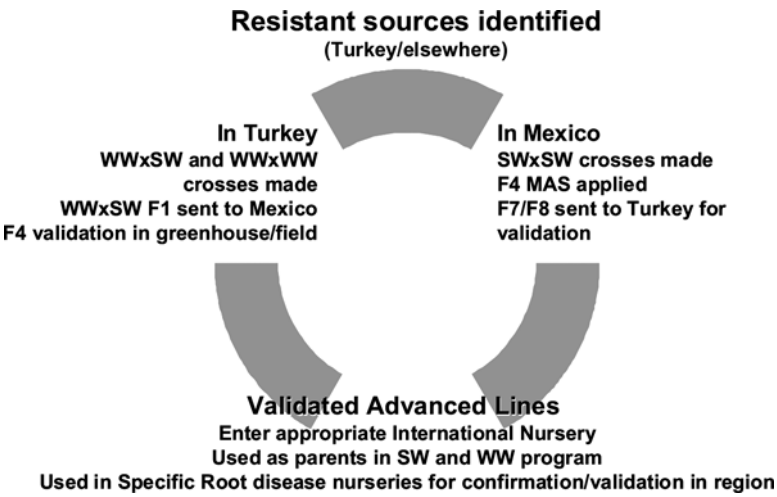


Figure 1. International breeding strategy to develop Dryland Root Rot Disease and Cereal Nematode Resistant Wheat Germplasm. (WW = winter wheat, SW = spring wheat, MAS = marker assisted selection)

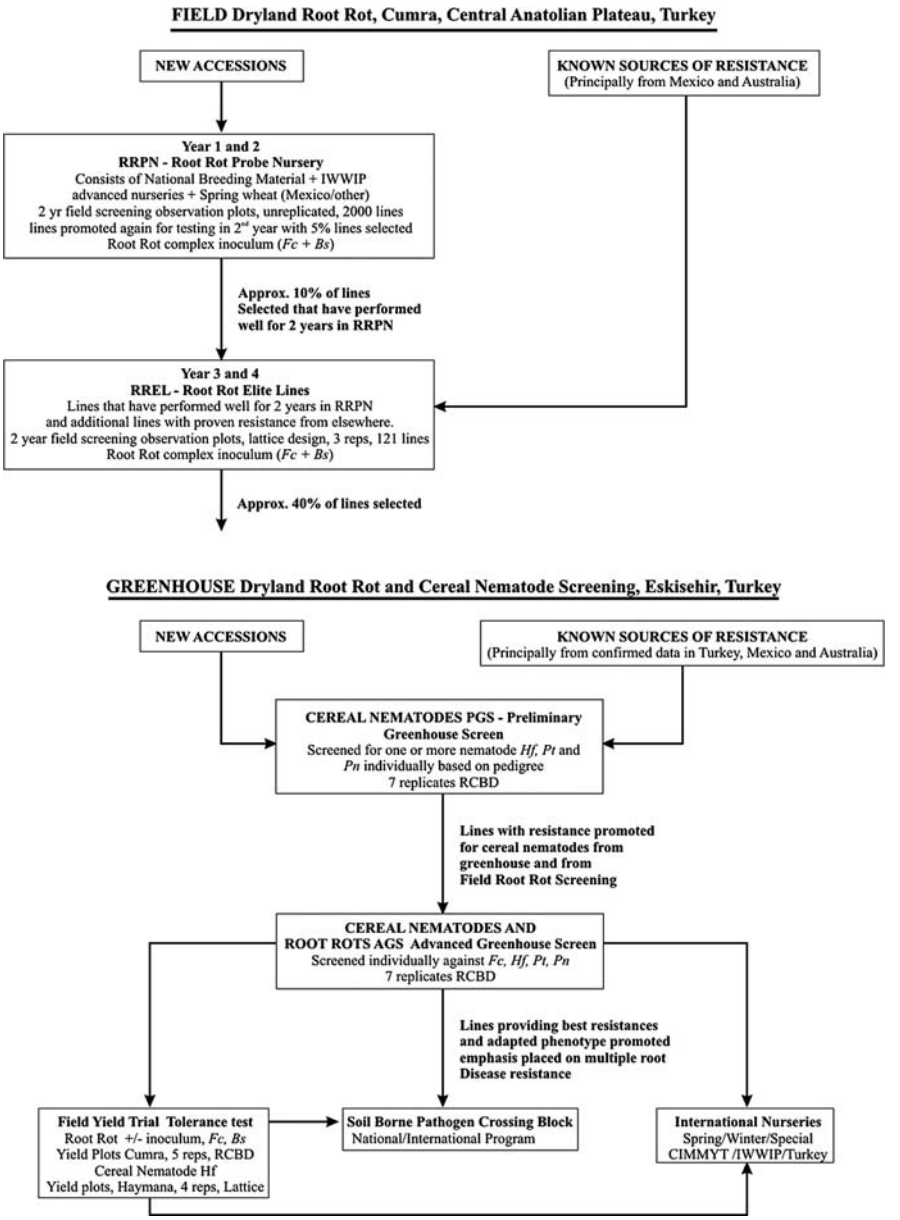


Figure 2. International screening program to identify resistance and tolerance in bread wheat against Soil Borne Pathogens, including cereal root rots ($Fc = F.\ culmorum,\ Bs = Bipolaris\ sorokiniana$) and Cereal Nematodes (Cereal Cyst $Hf = Heterodera\ filipjevi$, Root Lesion $Pt = Pratylenchus\ thornei,\ Pn = Pratylenchus\ neglectus$)

Programs. In all cases the observation plots are inoculated under field conditions in 1.5m pairwise plots, one with inoculum and the other without. The inoculated plots have monosporic conidial suspensions of Turkish isolates of *F. pseudograminearum* (F4), *F. culmorum* (F2) and *B. sorokinana*(B1) which are mixed at a concentration of 2×10^5 spores ml^{-1} to the seed before planting. The yield trial seed is similarly inoculated. Confirmation of the inoculation effectiveness is determined during the growing season by collected selected plants from inoculated and non-inoculated plots and extracting the root rotting pathogens from the root and crown sections.

The plants are scored twice, comparing with and without inoculation plots, side by side for the symptom development of these root pathogens (Nicol et al. 2004a). After heading on two occasions at ripening scoring white head development and (Zadoks growth stage 91–94) and at full maturity (Zadoks growth stage 99) scoring the growth reduction with inoculation. These two scores are used to rank the material for subsequent promotion.

Greenhouse Screening for Cereal Root Rots and Cereal Nematodes

Pre-germinated seeds are grown in open-ended electrical conduit tubes which are placed in large trays at 22 °C (±5 °C), 16 hour day 8 hour night. With RLN, a 70: 29: 1 (Sand: field soil: organic matter) soil mixture placed in 12.5cm × 3 cm diameter tubes whilst a 90: 10 (Sand: field soil) is used for CCN. RLN are reared on carrots and CCN is extracted by hatching cysts collected from a naturally infested field site in Haymana-Ankara, population *Hf1*. Each plant is inoculated at a rate of 100 juveniles for CCN or 400 nematodes (juveniles and adults) for RLN in a 1 ml aliquot of water per plant, immediately or 1 week after planting germinated grains, respectively (Yorgancilar, 2006). The same monosporic cultures of CR and CRR fungi used in field inoculation are assessed individually by pipetting a conidial suspension on the stem base of the seedlings medium one week after planting at 250μ (1×10^6 spore ml^{-1}) spore suspension is following a modified method of Mitter et al. (2006). Water is supplied in adequate amounts for plant growth using a capillary bottom up by capillary reaction.

All greenhouse tests use a randomized complete block design with seven replicates per genotype. For all pathogens a range of known check lines are included. After two months the lesions on the roots are scored for both CR and CRR on a qualitative scale of lesion development (Nicol et al. 2001). Similarly after two months the plants are harvested for nematodes. In the case of CCN the number of cysts per plant is counted under a stereomicroscope after washing the roots and collecting on a 250μm sieve. For RLN, the number of nematodes per plant is determined by extracting nematodes from the root system and surrounding soil and counting them microscopically. It should be noted that CCN comprises a complex group of several closely related species with varying pathotypes as reviewed by Nicol et al. (2004a), however presently only the Turkish population of *H. filipjevi Hf1* has been screened.

Field Tolerance Screening for Dryland Root and Cereal Cyst Nematode

The tolerance of the best resistant germplasm is assessed in Cumra for Dryland root rot using the same field protocol as with the observation plots, however with yield plots being used instead. In the near future tolerance against the Cereal Cyst Nematode will be screened under natural field populations near Ankara (Haymana) with and without the application of the nematicide Aldicarb (Temik® 15G).

Molecular Validation with Markers

Crosses segregating for sources of root disease resistance that have molecular tags are firstly screened in the F1 top cross or F2 generation. This is a process of allele enrichment as most of the available markers are dominant and heterozygotes cannot be identified. Once gene frequency has been influenced in this way, markers are not applied again until fixed lines have been developed. Presently thre molecular markers are routinely used in CIMMYT Mexico including *Cre1, Cre3* for CCN and rln1 for RLN – *P. neglectus*. New markers that have been developed for CR resistance from the sources 2–49 and Sunco are currently being tested and optimized. Those lines expressing the desired combination of resistance genes are then sent to Turkey for confirmation under field conditions.

Intergration of Resistance and the Development of Advanced Lines

Currently, around one third of all spring crosses made at CIMMYT in Mexico for the drier areas segregate for soil borne pathogen resistance. New advanced materials with improved root disease resistance have shown excellent yield performance even in environments where root diseases do not occur (Table 1). More than 300 targeted winter wheat crosses have been made since 2001 and in 2006 these will undergo validation process using this strategy. Since 2002 this spring wheat germplasm has been sent out with the International Semi Arid nurseries from CIMMYT Mexico and the frequency of these entries has continually increased from 3% in 2002 to 16% in 2005. In addition more than 20 special disease nurseries have been sent to collaborators working with these soil borne pathogens, particularly in West Asia and North Africa and also Advanced Research Institutions in Australia and America.

RESULTS AND DISCUSSION

As clearly illustrated in Table 1 more than 24 spring wheat lines from CIMMYT Mexico have been validated with resistance to one or more SBP. One quarter of these represent synthetic derivatives which have also provided excellent sources of other biotic and abiotic resistances. It is very reassuring to note that resistant sources identified from other countries such as Sunco and Silverstar provide the same resistant reaction in Turkey and Mexico, suggesting the pathogen complexity of some of the SBPs is relatively conserved. Within the winter wheat 16 sources

Table 1. Summary of the confirmed spring and winter wheat lines identified from the joint TURKEY/CIMMYT screening program with resistance to one or more Soil Borne Pathogen(s). Score of 1* indicates a higher level of resistance than the best known resistant check, 1 indicates resistance equivalent to the best known resistant check line, 2 level of moderate resistance not as high as best known check, but still effective. Partially resistant (PR) and Susceptible (S) check lines used for each soil borne pathogen. [1]refers to characterized single gene for resistance against different various pathotypes of the Cereal Cereal Nematode *Heterodera avenae* (see Nicol et al. 2003). [2]these sources also have known resistance against Fusarium Head Blight (*Fusarium graminearum*) from CIMMYT Mexico, AUS Australia, IWWIP International Winter Wheat Improvement Program, MX Mexico, SP Spain, TK Turkey

	[1]Characterised Cereal Cyst Nematode Gene	Greenhouse Cereal Cyst Nematode *Heterodera filipjevi*	Greenhouse Root Lesion Nematode *Praty-lenchus thornei*	Greenhouse Root Lesion Nematode *Praty-lenchus neglectus*	Greenhouse Crown Rot *Fusarium culmorum*	Field Screening Crown Rot & Common Root Rot *F. culmorum* & *Bipolaris sorokinana*	SW Spring Wheat WW Winter Wheat	CROSS NAME	IWWIP ACCESSION #	CIMMYT MEXICO CID	CIMMYT MEXICO SID	CIMMYT MEXICO SELECTION HISTORY	COUNTRY OF OCCUR-RENCE
1	–	–	1	–	1*	1*	SW	CROC_1/ AE.SQUARROSA (224)// OPATA	020615	72726	531	CMBW91Y00935S –80Y-11KBY -1KBY-010M -1Y-2M -0Y-0SY	MX
2	–	–	–	–	1*	1	SW	CROC_1/ AE.SQUARROSA (224)// OPATA	020616	72726	532	CMBW91Y00935S -80Y-11KBY -1KBY-010M -1Y-3M-0Y -0SY	MX
3	–	–	–	–	1*	1	SW	CROC_1/ AE.SQUARROSA (224)//OPATA	030825	72726	530	CMBW91Y00935S	MX

(Continued)

Table 1. (Continued)

	[1]Characterised Cereal Cyst Nematode Gene	Greenhouse Cereal Cyst Nematode *Heterodera filipjevi*	Greenhouse Root Lesion Nematode *Praty-lenchus thornei*	Greenhouse Root Lesion Nematode *Praty-lenchus neglectus*	Greenhouse Crown Rot *Fusarium culmorum*	Field Screening Crown Rot & Common Root Rot *F. culmorum* & *Bipolaris sorokinana*	SW Spring Wheat WW Winter Wheat	CROSS NAME	IWWIP ACCESSION #	CIMMYT MEXICO CID	CIMMYT MEXICO SID	CIMMYT MEXICO SELECTION HISTORY	COUNTRY OF OCCUR-RENCE
4	–	–	–	–	1	1	SW	SABUF/7/ ALTAR 84/ AE.SQUARROSA (224)//YACO/ 6/CROC_1/ AE.SQUARROSA (205)/5/ BR12*3/4/ IAS55*4/ CI14123/ 3/ IAS55*4/EG, AUS// IAS55*4/ALD	020632	167180	136	CASS94Y00045S	MX
5	–	–	–	–	2	1	SW	[2]YAV79// DACK/RABI /3/SNIPE/4/ AE.SQUARROSA (460)	020635	154092	7	CIGM88.1348 MX BW30157	MX
6	–	2	–	–	2	–	SW	[2]MAYOOR// TK SN1081/ AE.SQUARROSA (222)	031035	167144	420	MX	MX

7	–	–	–	–	1*	–	SW	[2]ALTAR 84/ AE.SQUARROSA (224)// YACO/6/ CROC_1/ AE.SQUARROSA (205)/5/ BR12*3/4/ IAS55*4/ CI14123/3/ IAS55*4/EG, AUS// IAS55*4/ALD	031037	152384	14	MX	MX
8	–	–	–	–	1*	–	SW	[2]CATBIRD	031031	21597	2279	MX	MX
9	*Cre3*	–	1*	2	–	–	SW	VP1620 (VF304/ TTAU.69.5–33// YANAC)	030901	–	–	–	AUS
10	*Cre1*	1	1*	–	–	–	SW	SILVERSTAR	031017	–	–	–	AUS
11	*Cre2*	1*	1*	1	–	–	SW	ID-2150	020626	–	–	–	SP
12	*Cre7*	1*	2	–	2	–	SW	T-2003	020628	–	–	–	SP
13	–	–	–	–	–	1	SW	302–5	020637	221383	0	–	AUS
14	–	1*	2		1	2	SW	SUNCO	020650	76058	0	–	AUS
15	*Cre8?*	–	–	–	1*	2	SW	SUNCO/ FRAME// PASTOR	–	394740	50	CMSS99M01589 T-040Y-0P0M-16 SY-010M-010 Y-5M	MX
16	–	2	–	–	1*	2	SW	SUNCO/ PASTOR	–	429528	44	CMSS98GH00077 S-0100M-040Y- 020M-040SY- 23M-0Y	MX
17	–	–	–	–	2	2	SW	SUNCO/2* PASTOR	030802	431763	51	CMSS99Y05530T– 10M-1Y-010M- 3SY-0B	MX
18	–	2	–	–	1*	1	SW	SUNCO/3/ URES/JUN// KAUZ/4/URES/ JUN//KAUZ	–	394737	15	CMSS99M01586T- 040Y-0P0M- 040SY-040M- 040SY-10M	MX

(Continued)

Table 1. (Continued)

	[1]Characterised Cereal Cyst Nematode Gene	Greenhouse Cereal Cyst Nematode *Heterodera filipjevi*	Greenhouse Root Lesion Nematode *Praty-lenchus thornei*	Greenhouse Root Lesion Nematode *Praty-lenchus neglectus*	Greenhouse Crown Rot *Fusarium culmorum*	Field Screening Crown Rot & Common Root Rot *F. culmorum* & *Bipolaris sorokinana*	SW Spring Wheat WW Winter Wheat	CROSS NAME	IWWIP ACCESSION #	CIMMYT MEXICO CID	CIMMYT MEXICO SID	CIMMYT MEXICO SELECTION HISTORY	COUNTRY OF OCCUR-RENCE
19	–	2	1*	–	–	1	SW	AUS GS50AT34/ SUNCO// CUNNINGHAM	030799	431762	31	CMSS99Y05529 T–12M-6Y-010 M-3SY-0B	MX
20	*Cre?*	2	2	–	–	–	SW	AUS4930 5.3/ Spear DH#44	030921	–	–	–	MX
21	*Cre?*	1	2	–	–	–	SW	AUS 4930.7/2* PASTOR	030857	431784	83	CMSS99Y05544 T–9M-10Y-020M-9Y	MX
22	–	–	1	–	1	2	SW	CANADIAN/2* PASTOR	–	394714	20	CMSS99M01563 M-040Y-0P0M-040SY-040M-040SY-15M	MX
23	–	–	–	–	2	1	SW	CANADIAN/ CUNNINGHAM// KENNEDY	–	394715	8	CMSS99M01564 T-040Y-0P0M-040SY-040M-040SY-3M	MX
24	–	–	–	–	1*	1	SW	CANADIAN/ CUNNINGHAM// KENNEDY		394718	21	CMSS99M01567 T-040Y-0P0M-040SY-040M-040SY-16M	MX
25	–	2	–	–	–	1*	WW	ALTAY 2000	010627	–	–	–	TK

26	–	–	–	–	1*	1	WW	BAGCI 2002	030845	–	–	–	TK
27	–	–	–	–	1*	1	WW	SONMEZ	950193	–	–	–	TK
28	–	–	–	–	1*	2	WW	KATEA-1	950590	–	–	–	TK
29	–	–		–	2	1*	WW	MVR27-82// LI7/LE2062	000406	–	–	–	TK
30	–		–	–	1*	1*	WW	TAM201/4/ BL/AU/3/ AGRI//HYS/ 7C/5/F134.71/ NAC	000240	–	–	–	TK
31	–	1*	1*	–	–	–	WW	BEZ/HAWK //ES14	030788	–	–	–	TK
32	–	–	–	–	1*	2	WW	SKP35/SAM2/ 4/55-1744/ D101//MAYA.S/ 3/MUS.S/ DRM.MAYA/ ALD.S	030791	–	–	–	TK
33	–	–	–	1*	–	1	WW	ES84-24/ DYNASTY	030793	–	–	–	TK
34	–	1*	–	–	–	1	WW	BILINMIYEN96.7	000064	–	–	–	IWWIP
35	–	–	–	–	1*	1*	WW	LOV41// LI7/LE2062	000161	–	–	–	IWWIP
36	–	–	–	1*	–	1*	WW	BILINMIYEN96.7	000330	–	–	–	IWWIP
37	–	–	–	–	1*	1	WW	JING411// PLK70/LIRA /3/GUN91	000393	–	–	–	IWWIP
38	–	–	–	–	1*	1	WW	F130L1.12/ ATTILA	980872	–	–	–	IWWIP
39	–	–	–	–	2	1*	WW	BURBOT-6	990857	–	–	–	IWWIP
40	–	–	–	–	1	1	WW	ZANDER-39	010221	–	–	–	IWWIP

have been identified, 4 of which are released Turkish cultivars and the others represent Turkish National and IWWIP sources of advanced highly adapted lines. Tolerance work in Mexico has revealed that several of the sources of confirmed spring resistance against specific soil borne pathogens are in significantly higher yielding backgrounds (from 10 up to 90%) that the parental source without disease pressure. These are now being validated under disease pressure in Turkey.

As mentioned CCN is much more complex than the other SBPs and will require more extensive studies to understand the regional complexity of the different species and their relative importance, however work with the other resistant sources for RLN and CR seem to be transferable between countries. Molecular tools have and as they become further developed will help aid this developing of advanced germplasm with greater efficiency.

Through integrated efforts of CIMMYT Mexico, the Turkish Ministry of Agricultural and Rural Affairs and CIMMYT Turkey substantial progress has been made in development of SBP disease resistant germplasm. We welcome collaboration with any other scientific groups.

ACKNOWLEDGEMENTS

The authors would like to thanks the Grains Research Development Corporation of Australia, the Turkish Ministry of Agriculture and Rural Affairs and CIMMYT Management for their financial and corporate support.

REFERENCES

Burgess LW, Backhouse D, Summerell BA, Swan LJ (2001) Crown rot of wheat. In: Summerell BA, Leslie JF, Backhouse D, Bryden WL, Burgess LW (eds) '*Fusarium*: Paul E Nelson Memorial Symposium'. The American Phytopathological Society, St Paul, Minnesota. pp 271–294

Diehl JA, Tinline RD, Kochhann RA (1983) Perdas em trigo causadas pela podridao comum de raizes no Rio Grande do Sul, 1979–1981. Fitopatologia Braseleira 8:507–511

Hekimhan H, Bagci A, Nicol J, Arisoy Z, Taner S, Sahin S (2004) Dryland Root Rot: a major threat to winter cereal production under sub-optimal growing conditions. Poster paper presented at the International Crop Science Congress – New directions for a diverse planet. Proceeding of the 4th International Crop Science Congress, 26th Sep–1st Oct 2004, Brisbane, Australia. http://www.cropscience.org.au/icsc2004/poster/3/7/2/1322_nicol.htm

Mitter V, Zhang MC, Liu C, Ghosh R, Ghosh M, Chakraborty S (2006) A high-throughput glasshouse bioassay to detect crown rot resistance in wheat germplasm. Plant Pathol 55:433–441

Nicol JM, Rivoal R, Trethowan RM, van Ginkel M, Mergoum M, Singh RP (2001) CIMMYT's approach to identify and use resistance to nematodes and soil-borne fungi, in developing superior wheat germplasm. In: Bedo Z, Lang L (eds) Wheat in a Global Environment. Proceedings of the 6th International Wheat Conference, 5–9th June 2000, Budapest, Hungary. Kluwer Academic Publishers, The Netherlands, pp 381–389

Nicol J, Rivoal R, Taylor S, Zaharieva M (2004a) Global Importance of Cyst (*Heterodera spp*.) and Lesion Nematodes (*Pratylenchus spp*.) on Cereals: yield loss, population dynamics, use of host resistance and integration of molecular tools. Nematology Monographs and Perspectives 2:233–251

Nicol JM, Bagci A, Hekimhan H, Bolat N, Braun HJ, Trethowan R (2004b) Strategy for the identification and breeding of resistance to dryland root rot complex for International spring and winter wheat breeding programs. Poster paper presented at International Crop Science Congress - New directions

for a diverse planet. Proceedings of the 4th International Crop Science Congress, 26th Sep–1st Oct 2004, Brisbane, Australia

Nicol JM, Bolat N, Yildirim AF, Yorgancilar A, Kaplan A, Braun HJ (2005) The Cereal Cyst Nematode is causing economic damage on rainfed wheat production systems of Turkey. Paper presented at Joint Meeting Annual Western Soil Fungus Conference and American Phytopathology Soceity, 28th June–1st July, Portland, USA

McDonald A, Nicol JM (2005) Nematode Parasites of Cereals 2005. In: Bridge J, Sikora R, Luc M (eds) Plant Parasitic Nematodes in Tropical and Subtropical Agricultural. CABI Publishing, Wallingford, Oxon, United Kingdom. Chap 5: pp 131–191

Singh RP, Huerta Espino J, Fuentes G, Duveiller E, Gilchrist L, Henry M, Nicol JM (2005) Resistance to Diseases, Chapter 10. In: Royo C, Nachit M, Di Fonzo N, Araus J, Pfeiffer WP, Slafer G (eds) In Durum Wheat Breeding: Current Approaches and Future Strategies.Food Product Press, Haworth Press Inc, New York, USA, ISBN 1–56022–967–5, pp 291–327

Yorgancilar A, Sahin E, Nicol JM, Bolat N, Yildirim AF (2006) Identification of multiple resistance against cereal cyst (*Heterodera filipjevi*) and lesion (*Pratylenchus thornei* & *P. neglectus*) nematodes for International bread wheat improvement. Poster presented at the 28th European Society of Nematologists Symposium, 5–9th June, Blagoevgrad, Bulgaria

GENETIC RESISTANCE TO GREENBUG IS EXPRESSED WITH HIGHER CONTENTS OF PROTEINS AND NON-STRUCTURAL CARBOHYDRATES IN WHEAT SUBSTITUTION LINES

A.M. CASTRO[1,2,*], A.A. CLÚA[1], D.O. GIMENEZ[1], E. TOCHO[1,2], M.S. TACALITI[1], M. COLLADO[1], A. WORLAND[†], R. BOTTINI[2,4] AND J.W. SNAPE[3]

[1]*Department of Biological Sciences, Faculty of Agronomy, National University of La Plata, CC31, 1900-La Plata, Argentina*
[2]*National Council of Scientific Research (CONICET)*
[3]*John Innes Centre, Colney, Norwich NR4 7UH, UK*
[4]*Faculty of Agricultural Science, National University of Cuyo, Almirante Brown 500, M5528AHB Chacras de Coria, Argentina*
[*]*E-mail: amcastro@isis.unlp.edu.ar*

Abstract: This paper studied the endogenous levels of reduced, non-reduced, total non-structural carbohydrates, soluble proteins and biomass in aerial and rooting structures of bread wheat, *Triticum aestivum* (2n = 6x = 42), in response to aphids, as a first step for understanding the cascade of transductional events that may account for antixenosis, antibiosis and tolerance to greenbug. Up to now, few studies have been made on the relationship between aphid resistance and these traits. A set of wheat intervarietal chromosome substitution lines, with "Chinese Spring" (CS, a greenbug susceptible line) as a recipient and a synthetic wheat (*Triticum dicoccum* x *T tauschii*, = [Syn]) as the donor, and both parents were used. Plants were cultivated in hydroponic solutions to the fully expanded 3rd leaf stage. Half of the plants of every genotype were infested 72 h with greenbugs, and the remaining uninfested plants were used as controls. Carbohydrate and protein contents and dry matter mass were determined for aerial and root tissues Lines 5A and 6A had lower aerial, root and, consequently, total dry weights in both control and infested plants. These lines have been previously reported to be antixenotic against greenbug and Russian Wheat Aphid (RWA), implying these lines carry genes for constitutive defences. Four substitution lines (1A, 1B, 7B and 7D) showed significant increases in protein content when infested, compared to their controls and to the CS susceptible parent. Considering that these substitution lines have been previously reported to reduce greenbug and RWA fertilities and longevities, the antibiotic

[†] Deceased

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 139–147.

resistance to greenbugs may be related to gene expression for enhanced protein levels. Most of the D genome substitution lines showed an increase of total root carbohydrates with the greatest increase in total root and aerial carbohydrates under infestation in the 1D and 6D substitution lines. Since these lines have been reported as being tolerant to greenbug, their highest carbohydrate contents probably protect them against biotic stress by enhancing growth. Greenbug resistance genes have been mapped only on the 1A, 6A, 7A and 7D chromosomes. Nonetheless, it was possible to identify other substitution lines that showed effects in the photosynthesis, the C and N metabolisms in the cascade of transductional signals that account for antixenosis, antibiosis and tolerance to greenbug in wheat

Keywords: greenbug, host plant resistance, non-structural carbohydrates – proteins, *Schizaphis graminum*, substitution lines, wheat

INTRODUCTION

Greenbug (*Schizaphis graminum* Rond) is one of the most economically important insect pests of wheat, It causes decreases in yield and even seedling death, depending on the intensity and the moment of infestation, In susceptible cultivars greenbug infestation diminishes total root biomass, and volume, thereby reducing the capacity for water and mineral uptake (Castro et al. 1988). After 4 days of infestation it leads to shorter leaves decreasing leaf area (Burton 1986, Castro et al. 1994) and reducing photosynthesis (Ryan et al. 1987). In susceptible genotypes of barley, oat and sorghum, greenbug inhibits differentiation of new leaf primordia and nodal roots after 48 h of infestation (see Castro et al. 2001). It has been claimed that these alterations in aerial and root growth were consequence of disturbances in nutrient uptake and transport, (Giménez et al. 1990), suggesting that greenbug induces systemic damage (Giménez et al. 1997). Accordingly, tolerant cultivars of barley and wheat showed no decrease in growth or production under aphid infestation (Castro et al. 1988, 2001). These previous results are consistent with those found in studies of plant responses to insect attack in the *Nicotiana attenuata-Manduca sexta* system (Hermsmeier et al. 2001). Changes in the metabolism of infested plants involved constitutive and induced defences, and these responses are based on very complex transcriptional regulation that include genes coding for both primary and secondary metabolites, wound- and jasmonate-elicited responses induced or repressed photosynthesis, carbon and nitrogen metabolisms (Kessler and Baldwin 2001, 2002).

The genetic control of the synthesis of reducing, non-reducing and total non-structural carbohydrates and soluble proteins in aerial and root tissues of wheat seedlings has been reported in a set of substitution lines (Clúa et al. 2002). The same set was used to study the chromosomal effects on the different mechanisms of greenbug resistance (Castro et al. 2001). These authors reported the Chinese Spring/Synthetic substitution lines that contributed the highest levels of antixenosis (5A and 6A), antibiosis (1A, 1B and homoeologous group 7 chromosome) and

tolerance (1D and 6D) to greenbug. However, to date aphid R genes in wheat have been located only on the 1A, 1D, 7D (McIntosh et al. 2003), 7A (Boyko et al. 2004) and 6A chromosomes (Castro et al. 2005). Since, the relationship between wheat chromosomes involved in the control of total biomass, contents of carbohydrates and proteins, and the plant responses to aphids has not been studied, the aim of this paper was to evaluate these plant traits as a first step towards the knowledge of the relationship between aphid resistance and these three metabolisms in the cascade of events that account for antixenosis, antibiosis and tolerance to greenbug. By restricting the variability to a single chromosome, it should be possible to isolate the effects of chromosomes carrying genes involved with photosynthesis, and with C and N metabolisms in the infested plants.

MATERIALS AND METHODS

Plant Material

A set of intervarietal chromosome substitution lines of wheat, *Triticum aestivum* L, (Law and Worland 1996) was used in the current research The lines 2A, 4A, 7A, 2B and 6B were not included because the molecular characterizations of these chromosomes were not correct "Chinese Spring" (CS) was the recipient variety into which chromosomes from a synthetic wheat (*T dicoccum* x *T tauschii* = Syn) were introduced. Two hundred seeds of each of the 16 substitution lines and of the parental lines were sown singly in 20 ml plastic vials perforated at the base, on a substrate of vermiculite. The vials were then placed in trays under natural conditions of light and temperature in a glasshouse, in La Plata, Argentina (34° 55′ SL, 57° 57′ WL). The trays were filled with nutrient solution (Hoagland and Arnon 1959) to enable a free supply of water and minerals and to maintain the volume constant along the experiments.

Source of Aphids

Greenbugs were collected from wheat plants in the vicinity of Tres Arroyos, Argentina (38° 20′ SL, 60° 15′ WL), and reared under controlled conditions. The clone aisolated from this population used in the trail was characterized as biotype C (Castro et al. 2004).

Assay Procedures

The responses of wheat plants to aphid infestation were evaluated when plants had reached the fully expanded 3rd leaf stage. Half of the plants of every genotype were transferred to another tray and infested during 72 h with 10 adult aphids per plant. The remaining uninfested plants were used as controls. Afterwards, aphids were removed, plants were harvested and divided into aerial and root tissues and the fresh weight of each portion was determined Four plants of every genotype

in each treatment were sampled together, representing one replicate. At least 20 replicates per genotype and treatment were analysed for protein (Bradford 1976) and carbohydrate contents (Cronin and Smith 1979). The remaining aerial and root biomass of the 18 genotypes was oven dried at 60°C until constant weight, and root (RDW) and aerial dry weights (ADW) were determined ANOVA was applied for all the parameters studied, and Duncan's test was used to determine significant differences between means (SAS 1998).

RESULTS AND DISCUSSION

There were few significant differences in RDW between control or infested plants of the parental varieties and the corresponding substitution lines. The infestation caused an increase in the RDW of both parental varieties and in most of the substitution lines. The 7B substitution line showed a significantly higher RDW than parental varieties either with or without infestation (Table 1) and the 5A and 6A substitution lines showed a significantly lower RDW compared to the parental controls and infested plants. The aerial dry weight (ADW) and the total dry weight (TDW) were reduced by greenbug feeding in most of the substitution lines, nonetheless, these differences were not significantly different, except for 5A and 6A substitution lines that showed significantly lower ADW and TDW

Table 1. Root (RDW), aerial (ADW), and total dry weights (TDW) of 16 wheat substitution lines and both parents (CS and Syn), subjected to aphid infestation and in uninfested control plants

Lines	RDW		ADW		TDW	
	Control	Infested	Control	Infested	Control	Infested
1A	508efgh	524defg	1254abcdefg	1314abcd	1762abcdefghi	1838abcde
3A	558bcdef	580bcde	1214bcdefgh	1224bcdefgh	1772abcdefgh	1804abcdefg
5A	**402ijk**	**434hij**	**960o**	**962°**	**1362mopq**	**1416mnop**
6A	**412ijk**	**458ghij**	**818op**	**824op**	**1230pqr**	**1272opqr**
1B	454ghij	486fghi	996 klmn	1040ijklmn	1450lmno	1526jklmn
3B	552bcdef	558bcdef	1218 bcdefgh	1134 fghijkl	1770abcdefgh	1692defghijk
4B	522defg	568bcdef	1282abcdef	1326abcd	1804abcdefgh	1894abc
5B	516defgh	536defg	1130fghijkl	1042 ijkllmn	1646efghijkl	1578hijklm
7B	**624ab**	**636a**	1200 cdefg	1220 bcdefgh	1824abcde	1904ab
1D	596abcd	624abc	960mno	994klmn	1556ijklmn	1618fghijklm
2D	500efgh	508efg	1010jklmn	960mno	1510jklmn	1487klmn
3D	592abcd	606abc	1106ghijmn	1006jklm	1698cdefghijk	1612fghijklm
4D	580bcde	634a	1226 bcdefgh	1194cdefghi	1806abcdefg	1848abcde
5D	525defg	562bcdef	1223 bcdefgh	1251 abcdefg	1748bcdefghi	1813abcdef
6D	572bcde	574bcde	1178defghi	1082hijklmn	1750abcdefghi	1656efghijkl
7D	550bcdef	616abc	1232 bcdefgh	1214 bcdefgh	1782abcdefgh	1830abcde
CS	512defgh	540cdef	1137fghijkl	1114ghijklm	1681deghijk	1665efghijkl
Syn	526defg	578bcde	1346abc	1336abcd	1872abcd	1914ab

Values in bold are significantly different ($P \geq 0.05$). Values within RDW, ADW or TDW column with a similar letter are not significantly different.

than CS either with or without infestation (Table 1). The rest of the substitution lines did not show differences from parental varieties or uninfested controls. The substitution lines that showed significantly lower ADW, RDW and TDW (5A, and 6A) compared to CS both with and without greenbug infestation, have been reported to contribute to antixenosis to greenbug and RWA infestation (Castro et al. 2001) and a new gene for greenbug antixenotic resistance has been mapped on the 6A (Castro et al. 2005). These chromosomes may carry genes for constitutive defences that affect photosynthesis yielding a poor plant biomass, which made these genotypes unattractive for aphid feeding, or induce repellency. Since antixenosis to aphids has been associated with secondary compounds, the lower plant weights could be the result of the costs devoted by these genotypes to constitutive synthesis of secondary plant metabolites (Martin et al. 2003), distracting resources from plant growth and production regardless of the presence of insects (Cipollini 2002). On the other hand, the 1A, 1D, and 7D substitution lines that are reported to carry aphid R genes (McIntosh et al. 2003), showed no lowering in plant biomass under greenbug infestation.

Protein content showed highly significant differences between the control and infested plants of the parental lines (Table 2). Protein contents significantly increased as a result of greenbug infestation in the 1A, 1B, 7B and 7D substitution lines compared to both parents and to their controls. The 7B line showed the highest value and another seven substitution lines (3A, 6A, 4B, 1D, 3D, 4D and 5D) showed a significantly lower protein content than their controls and CS under infestation. The remaining substitution lines did not show significant differences between control and infested plants The 1A, 1B, 7B and 7D substitution lines were previously reported to reduce greenbug and/or RWA fertilities and longevities (Castro et al. 2001). Increases in proteins have also been found in microbe infections (Martin et al. 2003) and after *Manduca sexta* attack due to ethylene, jasmonic acid and salicylic acid elicited a significant number of genes involved with N metabolism under the stress of *Manduca sexta* attack (Hui et al. 2003). Therefore the increases in protein content registered on 1A, 1B, 7B and 7D substitution lines could be then consequence of an enhanced protein synthesis necessary to support high enzymatic requirements due to the over-expression of genes in the downstream chain of events of plant defence that implies a greater metabolic activity under stress. The R genes carried by chromosome 7D (Castro et al. 2004) may interact with aphid elicitors to induce a cascade of transductional events involved with the higher protein synthesis that affect greenbug and RWA life cycles and fertilities (Castro et al. 2001). The remaining substitution lines (1A, 1B, 7B) have genes involved with N metabolism (McIntosh et al. 2003) that in the absence of aphid-specific R genes (located on Synthetic 7D), could induce proteins related with indirect defences (ie: defence proteins, peroxidases) that also affected aphid performance. Moreover, 121 candidate R-genes have been physically located with most mapping on the 1A, 1B and 7B chromosomes (Dilbirligi and Gill 2003, Dilbirligi et al. 2004). Further characterization is necessary to find out the relationship between these R-gene candidates, N metabolism and the antibiosis to greenbug.

Table 2. Protein content of aerial biomass, reduced and non reduced carbohydrates in the aerial and root biomass of 16 wheat substitution lines and both parents (CS and Syn), subjected to aphid infestation and in uninfested control plants

Lines	Protein		Aerial carbohydrates				Root Carbohydrates			
			Reduced		Non Reduced		Reduced		Non Reduced	
	Control	Infested	Control	Infested	Control	Infested	Control	Infested	Control	Infested
1A	2469f	**2966cd**	1275qrst	62BC	4636uv	3283z	987GH	1175CD	3942yz	3549B
3A	2218hi	983v	1241rstu	936xy	6471q	8666i	1329yz	1268zAB	4516w	5069t
5A	2376gh	2255hi	2561a	2243cd	9828d	6689pq	1367xy	772 K	4614uv	3199C
6A	2936cd	1605op	**1648kl**	**2326b**	7941kl	8029k	1296z	2045 i	3981y	2574D
1B	1573pq	**2791de**	2023gh	1217stu	6459q	5387t	1275zAB	1151CE	5207r	7253h
3B	1685no	1708no	1324opqr	675BC	2947B	2468D	1857k	2229g	3908z	6563lm
4B	1890lm	1539pqr	1863ij	1589lm	2652C	3145A	1778n	2084h	5822o	7989c
5B	1623op	2035jk	1402no	816zA	3627x	3074AB	1619opq	173 o	**6518m**	**9185b**
7B	1875l	**3531a**	1209tuv	104x	2208E	1495F	1079EF	614 L	2713C	2389E
1D	3008bc	2221hi	**2354ef**	**255a**	**6528q**	**11771b**	**1985 j**	**4114a**	5516q	7501ef
2D	1485qrs	1453rs	1118wx	731AB	3394z	3004B	879 J	2363e	4401x	7856cd
3D	1388st	542x	2089fg	1342nopq	9132f	7201no	1245B	2684c	5674p	7178i
4D	2092 jk	1741mn	1124vwx	1297pqrs	4322w	2518D	1559rs	2328f	5109st	7617e
5D	1946kl	1234tu	2001h	155m	9955c	6124r	**128**[a]	**2728b**	5636pq	7813d
6D	1554pqr	1473qr	**2142ef**	**2373b**	**8961g**	**9377e**	1782mn	2458d	**6747k**	**9607a**
7D	2085 jk	**3149b**	**1947hi**	**2132ef**	3483y	4782u	1388uw	1793 m	552q	7423fg
CS	1942kl	1978kl	1716jk	1417mn	6526q	5936rs	1403tu	1841 ll	5119rs	6212n
Syn	2921cd	1716no	1055x	653BC	3779x	3243z	934 I	918 I	2615CD	2058F

Values in bold are significantly different ($P \geq 0.05$). Values within a trait category with a similar letter are not significantly different.

The parental lines showed significant differences in the reducing carbohydrates (RC) of the aerial tissues, with a significantly lower value for Syn (Table 2). Only infested plants of the 6A, 1D, 6D and 7D substitution lines showed a significantly higher RC than their controls and also than CS. The contents of non reducing carbohydrates (NRC) showed significant differences between control and infested plants of both parental lines, CS showing the highest value (Table 2). The greatest values of NRC in the infested plants were recorded for the 6D and 1D substitution lines with the latter showing the highest significant increases, consequently these substitution lines also showed the highest total aerial carbohydrates. There are significant differences in reducing carbohydrates in roots between control and infested plants of both parents RC values were significantly increased in CS and in most of the substitution lines Infested plants of the 1D and 5D lines showed the highest contents compared to CS and to their controls. There were significantly greater differences in non-reducing carbohydrates of the roots in control and infested plants of both parental lines, and CS showed the highest values (Table 2). Infestation significantly increased the NRC in CS and in most of the lines, with the highest values for 5B and 6D The total carbohydrates resulted significantly higher in 1D and 6D. Greenbug infestation was reported to produce a reduction in carbohydrates either due to ingestion or to chloroplast membrane injury (Al-Mousawi et al. 1983). Carbohydrate contents play an important role in the osmoregulation and osmoprotection of winter cereals. The current results show that the contents of different categories of carbohydrates were significantly different in several substitution lines subjected to aphid infestation. Most of the B and D genome substitution lines showed a significant increase in root carbohydrates but only 1D and 6D showed the highest increases in both the root and aerial parts under infestation. These substitution lines have been previously reported as tolerant to greenbug and RWA infestation (Castro et al. 2001). Nonetheless, only chromosome 1D has been reported to carry aphid R genes (McIntosh et al. 2003) Possibly on chromosome 6D there are genes that cope with an increase in C metabolism. The metabolism of C is known to be one of the most reconfigurated under infestation (Ryan et al. 1987, Behle et al. 1994, Kessler and Baldwin 2002, Hui et al. 2003). The responses that could minimize the fitness consequences of insect attack represent the tolerance responses, a largely unstudied mechanism of defence (Cipollini 2002). In the current research, the 1D and 6D lines showed significant increases in total carbohydrates of aerial and root parts, which could be related to an improved performance for nutrient uptake under stress. Nevertheless further research should be performed in order to understand the complex regulation of the tolerance to greenbug.

ACKNOWLEDGEMENTS

The authors wish to thank Ms. Mariana Barragan, and Ms. Mirna Ricciardi for the technical assistance. CONICET and British Council funded the current research.

REFERENCES

Al-Mousawi AH, Richardson PE, Burton RL (1983) Ultrastructural studies of greenbug (*Hemiptera: Aphididae*) feeding damage to susceptible and resistant wheat cultivars. Ann Entomol Soc Am 76:964–971

Behle RW, Michels GJ, Gilstrap FE (1994) Greenbug (*Homoptera*: *Aphididae*) feeding effects on nonstructural carbohydrates in sorghum. Environ Entomol 23:965–970

Boyko E, Starkey S, Smith M (2004) Molecular genetic mapping of *Gby*, a new greenbug resistance gene in bread wheat. Theor Appl Genet 109:1230–1236

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Burton RL (1986) Effect of greenbug (*Homoptera: Aphididae*) damage on root and shoot biomass of wheat seedlings. J Econon Entomol 79:633–636

Castro AM, Rumi CP, Arriaga HO (1988) Influence of greenbug on root growth of resistant and susceptible barley genotypes. Environ Exp Botany 28:61–72

Castro AM, Martín LM, Martín A, Arriaga HO, Tobes N, Almaraz L (1994) Screening for greenbug resistance in *Hordeum chilense* Roem et Schult. Plant Breed 112:151–159

Castro AM, Ramos S, Vasicek A, Worland A, Giménez D, Clúa A, Suárez E (2001) Identification of wheat chromosomes involved with different types of resistance against greenbug (*Schizaphis graminum* Rond) and the Russian wheat aphid (*Diuraphis noxia* Mordvilko). Euphytica 118:321–330

Castro AM, Worland AJ, Vasicek A, Ellerbrook C, Giménez DO, Tocho E, Tacaliti MS, Clúa A, Snape JW (2004) Mapping quantitative trait loci for resistance against greenbug and Russian wheat aphid. Plant Breed 123:361–365

Castro AM, Vasicek A, Manifesto M, Giménez DO, Tacaliti MS, Dobrovolskaya R, Röder MS, Snape JW, Börner A (2005) Mapping antixenosis genes on chromosome 6A of wheat to greenbug and to a new biotype of Russian wheat aphid. Plant Breed 123:229–233

Cipollini D (2002) Does competition magnify the fitness costs of induced responses in *Arabidopsis thaliana*? A manipulative approach. Oecologia 131:514–520

Clúa A, Castro A, Giménez D, Tacaliti M, Worland JA (2002) Chromosome effects in the endogenous contents of non-structural carbohydrates and proteins in wheat (*Triticum aestivum*). Plant Breed 121:142–145

Cronin D, Smith S (1979) A simple and rapid procedure for the analysis of reducing total and individual sugars in potato. Potato Research 22:99–105

Dilbirligi M, Gill KS (2003) Identification and analysis of expressed resistance gene sequences in wheat. Plant Mol Biol 53:771–787

Dilbirligi M, Erayman M, Sandhu D, Sidhu D, Gill KS (2004) Identification of wheat chromosomal regions containing expressed resistance genes. Genetics 166:461–481

Giménez DO, Rumi CP, Brocchi G, Castro AM, Almaraz LB, Arriaga HO (1990) P transport shortage in susceptible barley plants under 3 hrs of greenbug (*Schizaphis graminum*). Ann Plant Resistance to Insects Newsl 16:74–75

Giménez D, Castro A, Rumi C, Brocchi G, Almaraz L, Arriaga H (1997) Greenbug systemic effect on phosphate influx in barley (*Hordeum vulgare* L). Environ Exp Botany 38:109–116

Hermsmeier D, Schittko U, Baldwin I (2001) Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host *Nicotiana attenuata*: I. Large-scale changes in the accumulation of growth- and defense-related plant mRNAs. Plant Physiol 125:687–700

Hoagland DR, Arnon D (1959) The water culture method for growing plants without soil. California Agriculture Experimental Station Circular 347:1–32

Hui D, Iqbal J, Lehmann K, Gase K, Saluz H, Baldwin I (2003) Molecular interactions between the specialist herbivore *Manduca sexta* and its natural host *Nicotiana attenuata*: V. Microarray analysis and further characterization of large-scale changes in herbivore-induced mRNAs. Plant Physiol 131:1877–1893

Kessler A, Baldwin I (2001) Defensive function of herbivore-induced plant volatile emissions in nature. Science 291:2141–2144

Kessler A, Baldwin I (2002) Plant responses to insect herbivory: the emerging molecular analysis. Annu Rev Plant Biol 53:299–328

Law CN, Worland AJ (1996) Intervarietal chromosome substitution lines in wheat-revisited. Euphytica 89:1–10

Martin G, Bogdanove A, Sessa G (2003) Understanding the functions of plant disease resistance proteins. Annu Rev Plant Biol 54:23–61

McIntosh RA, Yamazaki Y, Devos K, Dubcovsky J, Rogers J, Appels R (2003) Catalogue of gene Symbols for wheat http://wwwgrsnigacjp/wheat/komugi/genes/

Ryan JD, Johnson R, Eikenbary R, Dorschner J (1987) Drought/greenbug interactions: photosynthesis of greenbug resistant and susceptible wheat. Crop Sci 27:283–288

SAS Institute (1998) SAS/STAT guide for personal computers, version 6.03, carry NC

UTILIZATION AND PERFORMANCE IN WHEAT OF YELLOW DWARF VIRUS RESISTANCE TRANSFERRED FROM *THINOPYRUM INTERMEDIUM*

H. OHM[1,*] AND J. ANDERSON[2]

[1] *Purdue University, Department of Agronomy*
[2] *USDA-ARS West Lafayette, IN 47907-2054, USA*
[*] *E-mail: hohm@purdue.edu*

Abstract: Barley yellow dwarf virus (BYDV – previously PAV) and cereal yellow dwarf virus (CYDV – previously RPV) which cause the yellow dwarf (YD) disease are serious pathogens of the small cereal grain crops like barley, wheat, oat, and rye in many cereal-growing areas of the world. Highly effective resistance to yellow dwarf viruses was introgressed into wheat (*Triticum aestivum*) from *Thinopyrum intermedium*. A translocation line containing the *Th. intermedium*-derived *Bdv*3 locus for YD virus resistance was identified during inbreeding to adapted soft winter wheat. Two selections from this germplasm line P961341 (Ohm et al. 2005) were released as the cultivars INW0315 and INW0316. These lines have excellent grain yield and soft wheat milling and baking qualities. They also have resistance to an array of other diseases, including *Soil borne mosaic virus* and the fungal diseases: powdery mildew, stem rust, stripe rust and tan spot. Current research is in progress to shorten the 7E translocation segment yet retain the YD resistance that is on this segment

Keywords: wheat, disease resistance, barley yellow dwarf virus, *Thinopyrum intermedium*, virus resistance, translocation

INTRODUCTION

Viruses that cause the disease yellow dwarf (YD), including barley yellow dwarf virus (BYDV-PAV) and cereal yellow dwarf virus (CYDV-RPV, previously BYDV-RPV) are serious pathogens of the small cereal grain crops like barley, wheat, oat, and rye in many cereal-growing areas of the world. Although partial resistance and tolerance to YD have been identified in *Hordeum vulgare*, *Avena sativa* and *Secale cereale*, searching for resistance to YD in cultivated wheat, including *Triticum aestivum* and *T. durum*, has been largely unsuccessful. Highly effective resistance

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 149–152.

has been identified in certain species that are related to wheat, including intermediate wheatgrass (*Thinopyrum intermedium*) having the E1, E2 and ST genomes.

MATERIAL AND METHODS

An immature embryo that resulted after pollination of many emasculated florets of winter wheat (*Triticum aestivum*) cultivar 'Abe' with pollen from clones of *Thinopyrum intermedium* cultivar 'Oahe' was transferred to culture medium to initiate differentiation into a seedling, which was backcrossed to a wheat pollen parent. Two additional cycles of backcrossing to adapted winter wheat lines were performed along with phenotyping by ELISA for YD resistance and cytology to select for low chromosome number and resistance. After BC3 we identified a $2n = 43 F_2$ plant with YD resistance, from which we developed the $2n = 42$ 7D/(7E) YD resistant substitution line, P29 (Sharma et al. 1997) and a $2n = 44$, YD resistant addition line, P107. Resistance to both BYDV and CYDV was determined as low virus concentration by ELISA at 14 days after infestation by viruliferous *Rhopalosiphum padi*. Hybrid seeds from crossing P29 x adapted winter wheat and seeds of P107 were irradiated with γ rays from ^{60}Co using a 120-Gy radiation dose. In subsequent generations of inbreeding along with phenotyping using ELISA, and genotyping by presence/absence of wheat-specific DNA markers along chromosome 7D, Crasta et al. (2000) localized the resistance QTL to the distal end of chromosome arm 7EL, and identified the chromosome carrying YD resistance that was transferred from *Th. intermedium* into P29 (and P107) as 7E. The chromosome 7E of P29 is different from the *Th. intermedium* group 7 chromosome present in the YD-resistant wheat-*Thinopyrum* addition line, L1 (Brettell et al. 1988) at a number of RFLP loci (Sharma et al. 1995); the telomere-specific element pAW161 (Anderson et al. 1998, Francki et al. 2001); and L1 likely has chromosome 7St (Crasta et al. 2000). The YD resistance in L1 and derived translocations is named *Bdv*2 (Stoutjesdijk et al. 2001) whereas the YD resistance locus in P29 and P107 and subsequent translocations and cultivars has been named *Bdv*3.

RESULTS AND DISCUSSION

An M4 translocation plant with a low BYDV-PAV ELISA value was used as YD resistance donor parent in backcrossing to adapted soft winter wheat, followed by selection during inbreeding, resulting in the germplasm line, P961341, described in Ohm et al. (2005). Two selections from P961341 were released as the cultivars INW0315 and INW0316. The two cultivars are competitive with current wheat cultivars for grain yield in the absence of YDV infection, and are superior to YDV-susceptible wheat cultivars in the presence of the disease. They have acceptable soft wheat milling and baking qualities. These cultivars also have resistance from one or another wheat parent to *Soil borne mosaic virus* and have slow mildewing resistance to *Blumeria graminis* DC. E.O. Speer. INW0315 and INW0316 are highly resistant to Indiana isolates of *Puccinia graminis* Pers: Pers.f. sp. *tritici*

Eriks. & E. Henn. and *P. striiformis* Westend., but do not have the dominant diagnostic STS marker (Dubcovsky, pers. comm.) for the *Lr37-Yr17-Sr38* resistance block from the parent line Roazon. The other wheat parent lines are susceptible to *P. graminis* and *P. striiformis*. INW0315 and INW0316 also have resistance to *Phaeosphaeria nodorum* (E. Muller) Hedjaroude and *Pyrenophora tritici-repentis* (Died.) Dresch., although their wheat parent lines are moderately susceptible or susceptible to these diseases.

Pollen sterility which can be significant under certain stress conditions, like cool temperatures just prior to and during flowering and low light intensity under glasshouse conditions, appears to be due to the alien translocation. Thus, we have research in process to shorten the 7E segment, yet retain the YD resistance, and hopefully other disease resistance genes that might be on the 7E segment. The strategy we have employed uses the ph mutant on chromosome 5 to increase the potential for recombination to occur between the wheat 7D chromosome and the 7D/7E translocation. Lines were obtained which were homozygous for the Ph1b deletion and heterozygous for 7D and the wheatgrass translocation. Three molecular markers were used to identify lines which appeared to have lost one or more of these markers yet remained resistant based upon inoculation with BYDV-PAV and CYDV-RPV. Following a series of selections in successive generations several lines have been identified which appear to be resistant and have a shorter translocation. Currently progeny testing is being conducted to ensure that these lines are YD resistant.

In addition to field evaluations and identification of DNA markers for marker assisted selection, research is underway to more fully understand the mechanism of this wheatgrass-derived YD resistance. This work has centered on CYDV-RPV resistance because these lines have a very high level of resistance to CYDV and moderate to good resistance to BYDV. To study this resistance the kinetics of BYDV and CYDV accumulation was determined in a susceptible wheat line and P29 (Balaji et al. 2003). In that analysis BYDV-PAV had accumulated to significant levels by four days post inoculation compared with eight days for CYDV. The ability of BYDV to replicate earlier and at faster rates may be why BYDV-PAV is more virulent than other YDV species and consequently why it is a much more serious economic problem than CYDV. Anderson et al. (1998) postulated that resistance to BYDV and CYDV was due to a block in virus movement. Recently Wiangjun and Anderson (2004) have examined this in more detail and have shown that within the leaf area infested with CYDV-RPV viruliferous aphids CYDV replicates to equal levels in CYDV resistant wheat (P29), a susceptible wheat line (Abe) and the resistance donor *Th. intermedium*. It is clear that the resistance is not inhibiting virus replication. However, CYDV was not detected in uninfested leaves from these same resistant plants but was detected from the susceptible Abe plants demonstrating that virus movement was blocked in P29. Recently, *in situ* hybridization data has shown that within the infected leaf many cell types can be infected by CYDV viruliferous aphids. In the resistant line P29 CYDV showed limited cell to cell movement within cells in the vascular bundle and was detected in only a few sieve element cells

in these infected leaf sections. This is in contrast to Abe, the susceptible line in which all the sieve element cells in several sections contained CYDV. It appears that *Bdv*3 encoded CYDV resistance is the consequence of a very significant reduction in the ability of CYDV to move through the sieve cell plates.

REFERENCES

Anderson JM, Bucholtz DL, Greene AE, Francki MG, Gray SM, Sharma H, Ohm HW, Perry KL (1998) Characterization of wheatgrass-derived barley yellow dwarf virus resistance in a wheat alien chromosome substitution line. Phytopathology 88:851–855

Balaji B, Bucholtz DL, Anderson JM (2003) Barley yellow dwarf virus and cereal yellow dwarf virus quantification by real-time PCR in resistant and susceptible plants. Phytopathology 93:1386–1392

Bretell RIS, Banks PM, Cauderon Y, Chen X, Ceng ZM, Larkin PJ, Waterhouse PM (1988) A single wheatgrass chromosome reduces the concentration of barley yellow dwarf virus in wheat. Ann Appl Biol 113:599–603

Crasta OR, Francki MG, Bucholtz DB, Sharma HC, Zhang J, Wang RC, Ohm HW, Anderson JM (2000) Identification and characterization of wheat-wheatgrass translocation lines and localization of barley yellow dwarf virus resistance. Genome 43:698–706

Francki MG, Ohm HW, Anderson JM (2001) Novel germplasm providing resistance to barley yellow dwarf virus in wheat. Aust J Agric Res 52:1375–1382

Ohm HW, Anderson JM, Sharma HC, Ayala L, Thompson N, Uphaus JJ (2005) Registration of yellow dwarf viruses resistant wheat germplasm line P961341. Crop Sci 45:805–806

Sharma HC, Ohm H, Goulart L, Lister R, Appels R, Benlhabib O (1995) Introgression and characterization of barley yellow dwarf virus resistance from *Thinopyrum intermedium* into wheat. Genome 38:406–413

Sharma HC, Ohm HW, Perry KL (1997) Registration of barley yellow dwarf virus resistant wheat germplasm line P29. Crop Sci 37:1032–1033

Stoutjesdijk P, Kammholz SJ, Kelven S, Matsay S, Banks PM, Larkin PJ (2001) PCR-based molecular marker for the Bdv2 *Thinopyrum intermedium* source of barley yellow dwarf virus resistance in wheat. Aust J Agric Res 52:1383–88

Wiangjun H, Anderson JM (2004) The basis for *Thinopyrum*-derived resistance to Cereal yellow dwarf virus. Phytopathology 94:1102–1106

A SYSTEMIC APPROACH TO GERMPLASM DEVELOPMENT SHOWS PROMISE

A. COMEAU[1,*], V.R. CAETANO[2], F. LANGEVIN[3] AND S. HABER[4]

[1]*Agriculture and Agri-Food Canada, Sainte-Foy, Quebec, Canada G1V2J3*
[2]*Embrapa, CPACT, Est. Exp. Terras Baixas, C. P. 403, Pelotas, RS 96001-970, Brazil*
[3]*CEROM, 335 Chemin des Vingt-cinq Est, Saint-Bruno-de-Montarville, Qc, J3V4P6*
[4]*CRC, Agriculture and Agri-Food Canada, Winnipeg, Man., Canada R3T 2M9*
[*]*E-mail: comeaua@agr.gc.ca*

Abstract: Plant breeding is sometimes described as an art, but the practice has been influenced a lot by the mendelian and DNA theories. Recently, quantitative geneticists have put forward provocative conclusions about how breeding should be done, and those ideas went against quite a lot of the current practice, and against some aspects of the old dogmas. Most traits are correlated to some extent; therefore, genetic improvement based on single traits or even relatively simplified approaches is counter-productive. Meanwhile one scientist from Brazil had developed empirically, between 1969 and 2005, a multi-trait based breeding and selection system by which genetic progress could be accelerated. This complex approach was enhanced by systematic use of carefully chosen complex stresses. Some Canadian scientists recently adopted a modified version of the process. Results could be imitated from a completely different «germplasm basis». Those results suggest a need to reconsider both the theory and the practice of plant breeding. The diverse methodological components can be grouped under the name of systemic approaches. Such methods are in line with the postulates and conclusions of quantitative genetics, and also with the most recent evidence about the system of network interrelations that controls the functions of the genome and of the cellular machinery. The new approach is also allowing a more significant role of genotype x environment interaction (GE) and genotype main effect plus genotype x environment interaction (GGE) in decision-making, Moreover, the systemic approach is compatible with a less static view of heredity, which includes for example epigenesis and various effects of stress on the natural recombination and mutation rate

Keywords: biotic, abiotic, disease, tolerance, resistance, pleiotropy, biodiversity, root system, BYD, BYDV, regulatory mechanisms, recombination

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 153–160

INTRODUCTION

The constant need for plant breeding comes from increasing population and from new environmental challenges, including climate change, pollution, energy efficiency, farming systems, sustainability, and new pathogens. Impacts of foods on human health may also become a factor that needs optimization. The current breeding art is more focused and more efficient for specific goals. Molecular and high tech approaches can be used to reach specific goals more rapidly but generally lack efficiency for complex goals (Asíns 2002).

In any crop species, a successful cultivar integrates good stable yield with quality, and also with resistance to abiotic or biotic stresses. Ideally the cultivar should also facilitate sustainable and environmentally sound practices, show improvements in nutritional value, or at least, avoid loss of nutritional value. This is not an easy task. The need for better cultivars will remain important, and more efficient breeding approaches are needed in response to growing world demand (Araus et al. 2002).

The definition of scientific efficiency differs widely among scientists and granting institutions. When requesting grants, scientists are often told that their research should be more focused. However, focusing on too few traits was shown to be counter-productive (Wallace and Yan 1998). Focused approaches tend to provide good publication fodder, without practical results. Any *bona fide* plant breeder knows that cultivar creation is a totally different matter. In wheat breeding, the product must meet a large number of standards. Correlations between traits and pleiotropy may either facilitate or hinder parts of the progress towards the multi-dimensional target which represents the desired cultivar.

The process developed empirically in Brazil answered, at an acceptable cost, the challenges of multi-trait approaches targeted at variable environments. This approach and its Canadian variations will be called systemic approaches because they integrate broad biodiversity, target environments and complex stresses into the process. Systemic approaches take into account the energy costs of resistance traits and select plant with optimum energy efficiency. Understanding relationships between traits can be the fruit of experience, as was the case in Brazil. But software tools can be used in order to gather more rapidly the insight that is necessary before attempting to use a systemic approach. Software tools can help the selection of parents that escape or deviate from undesirable correlations, such as resistance associated with agronomic defects.

Losses caused by stress are very large in crops, i.e. in the range of 75% of yield potential (Browning 1998). It would be all too simple to infer that genetic progress is easy through the development of resistance and tolerance to stress. Resistances have a cost (Brown 2002). Stress responses involve reallocation of limited photosynthate towards new pathways (Geiger and Servaites 1991). If the nature of stress varies over time, some of the photosynthate allocation pathways that were previously useful may become less useful or detrimental. This brings the need for tight control and also, if possible, a degree of reversibility of photosynthate allocation, for best adaptation to fluctuating conditions. At another level, a lot of new understanding is being gathered about the functioning of the genome, the proteins, RNA and DNA.

The new vision gives a key role to interactions involved in gene regulation (Ayliffe and Lagudah 2004). The function of most genes is regulated in some way or other. It is estimated that most genes are at least in part pleiotropic in their effects, due the complex web of DNA-RNA-protein interactions. Phenology genes modify a very large number of the traits of the plant (Wallace and Yan 1998). As another example, many traits are correlated with virus tolerance (Comeau and Haber 2002). In the end, all traits of the plant could be correlated, energy requirements and final overall efficiency being the common link for breeders. The only useful genetic progress that is possible is one of better efficiency of use of photosynthate allocation within a target environment which faces somewhat predictable stresses. While previous progress was based on simpler mechanisms, future research targets are also likely to aim at the less easily tractable, durable polygenic resistances and nonhost resistance mechanisms. The main avenues of future progress lie now in those areas (Ayliffe and Lagudah 2004).

Finally, stresses modify epigenetic factors, and some of those stress effects may also alter the natural recombination and mutation rate in plants. In the past, breeding has been conducted without acknowledging the possibility and potential importance of such effects. An open mind must be kept for the possibility that frequent, unexpected and useful products could arise through mechanisms that belong outside the older mendelian dogma, and yet are fully operational in natural and domesticated plants. Methods needed to verify such possibilities are now becoming available, and research in the field of epigenesis, epimutation, paramutation, DNA methylation, histone acetylation and gene silencing may prove McClintock (1978, 1984) was at least partly correct in postulating the existence of powerful «genome reorganization» mechanisms, responsive to stress, and yet to be understood.

MATERIALS AND METHODS

The new methods are adapted for single plants, with reduced need for large plots. Single-plant methods are still lacking for some quality traits. Breeder's long term experience is complemented by the use of proper software, in order to understand the relationships between parameters and germplasm in a GxE and GGE context, before choosing germplasm x stress combinations. The broadest possible germplasm base is used, with the highest possible frequency of crossing (very often F_1/F_1 and F_1/F_2 if possible). Complex stress becomes the most important part of the germplasm development process, and it is applied to F_1, F_2 and all generations. The F_1s that do not resist complex stress are destroyed. Those that resist are either crossed or submitted to a new and somewhat different complex stress in order to keep genotypes that show plasticity of response. Artificial inoculation with barley yellow dwarf virus (BYD) has been adopted as one of the best tools that can improve overall plant efficiency and root efficiency (Comeau et al. 2005). Other diseases, waterlogging, drought, and nutrient deficiencies are also added whenever possible to the stress system. Inoculation with *Fusarium graminearum*

sometimes had to be counteracted later on by a spray of Benlate, when hot humid climate threatened total destruction of germplasm. For recessive and quality traits, a near-recurrent use is made of parents that contain the desirable background. Morphological traits that correlate with stress resistance are constantly selected for; those traits include for example flexible straw, short lower internodes, stronger root anchoring, vertical flag leaf with reduced area or pointing down (Fig. 1), and long floral peduncles (Fig. 2). The germplasm is divided into groups based on expected resistance properties; each group then moves into a specific stress selection situation. Energy efficiency and plasticity of stress response are obtained by discarding BYD sensitive plants, and also by varying the nature of the stress complex. Efficiency is later confirmed in F_4-F_6 by large plot trials under a non-stress situation. Understanding trait correlations and G x E altogether poses logistic and mathematical problems. Initially we had been using only Data Desk software (Data Description Inc, Ithaca NY) in order to grasp multidimensional views of data sets. However the GGE Biplot software offers more analytical power for complex cases (Yand and Kang 2003, Yan and Tinker 2005). Good use can be made of repeatable trends of traits x genotype correlations, relative to the plasticity of germplasm behaviour across environmental variations and biotic stresses.

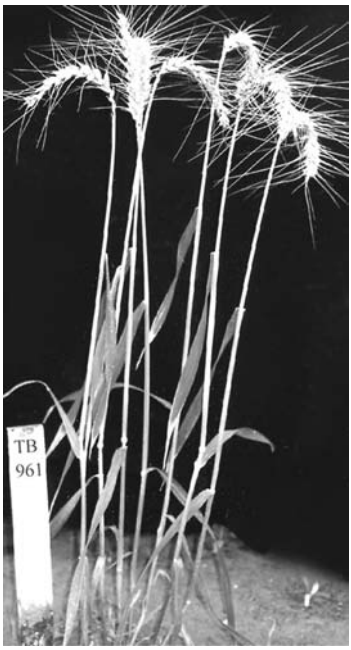


Figure 1. Erect leaves pointing downwards give better survival of the leaf tissue near the ligule, which is good for better leaf area duration, This also provides a better aeration of the canopy. Photo by Caetano

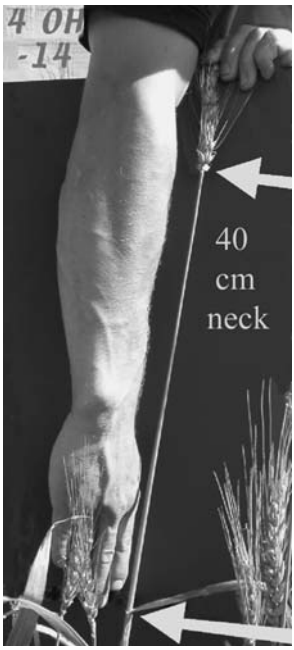


Figure 2. A wheat line with a long floral peduncle, with 40 cm between the ligule and the spike. The free peduncle offers a longer-lasting photosynthetic area that resists both drought and disease stresses. The peduncle trait is controlled by genes that are not completely tissue-specific. Photo by Comeau

RESULTS AND DISCUSSION

Correlations Between Traits, Resistance and Tolerance Across Environments

An example of decision-making basis is shown in a biplot of many traits and wheat lines (Fig. 3). The most sensitive lines for both BYD and water logging are Katepwa and HY 320; both appear as outliers. The BYD traits are correlated including BYD visual symptoms (Vsym), which is a negative trait. The most informative must be BYDtol, a weighted index of virus tolerance, calculated using all of the BYD data (Comeau and Haber, 2002). In calculating this BYDtol index, the plant height component is considered detrimental, because in humid climates, it is easy to create pseudo-tolerant wheat in which most of the virus tolerance is linked with excess plant height. In practice, the BYDtol component is used as one that can be transferred into valuable breeding germplasm. The traits from the waterlogging field trials (with presence of various root rots) are clustered (star symbols). However, traits from indoors test of hypoxia tolerance (also with presence of various root rots) are not clustered at all. Only three hypoxia-related traits are closely related to BYD and to field waterlogging reaction, namely stemg (weight of stems), Hi (harvest index) and TotBi (total plant biomass). This indoors data shows that decisions about

parameters to be observed could widely influence our understanding of complex stress response. The stem weight in indoors tests for tolerance to hypoxia goes together with BYD tolerance (BYDtol) and might help develop a predictor of other useful traits. (Fig. 3). The BYD tolerance correlates with the efficiency of mineral uptake mechanisms (Comeau et al. 2005) and also with biomass (Comeau and Haber 2002). Selection of segregating populations and pure lines assisted by a BYD stress has facilitated the identification a number of genotypes that possess remarkable tolerance to soil related problems. Some are quite tolerant to drought and/or water logging. In some cases this was associated with a better uptake of phosphorus in difficult conditions; in other cases, it was the manganese uptake that was significantly improved (Comeau et al. 2005).

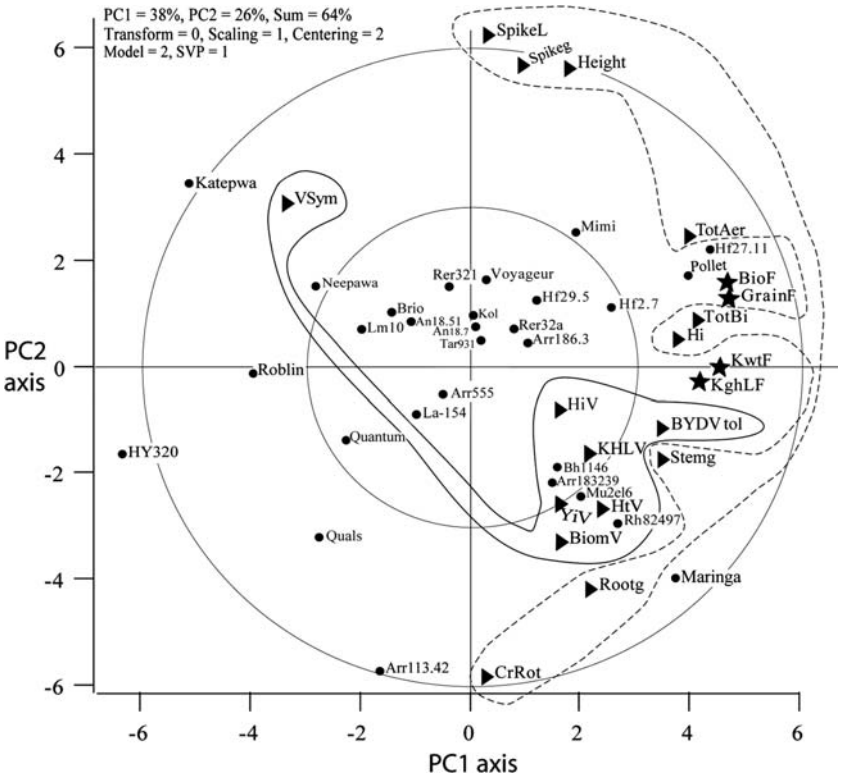


Figure 3. GGE Biplot of germplasm (29 lines, dot symbols) for 20 parameters (triangle and star symbols) including 7 under artificial BYD inoculation (Vsym, visual symptoms; HiV, harvest index; KHLV, hectoliter weight; YiV, grain yield; HtV, height; BiomV, biomass), 4 in waterlogged tests in the field (BioF, biomass; GrainF, grain yield KwtF, kernel weight KghlF, hectoliter weight), and 9 in indoors tests of tolerance to root hypoxia (SpikeL, spike length ; Spikeg, spike weight; Stemg, stem weight; Height, height of plants ; TotAer, total weight of aerial parts; TotBi, total weight of aerial and root part; Hi, harvest index; Rootg, root mass; CrRrot, crown rot score [high values undesirable])

Studying GGE plots, one can use inexpensive traits that may have indirect predictive value for other useful traits, and the software is also able to help identify lines that escape unfavorable correlations, like the one between virus tolerance and excess height or lateness (Comeau and Haber 2002). Plant morphology traits are valuable for this purpose (Cruz et al. 2001, Reynolds et al. 2000). For example, a long floral peduncle has many advantages for stress resistance (Fig. 2), but the trait correlates with other parameters, and a compromise must be found so that the final product ripens at an acceptable date and offers good yield. Peduncle length is also an indicator of mineral uptake ability in stressful conditions. The ideal length of peduncle may vary according to genetic backgrounds and environments.

Accelerated Progress in Germplasm Development

The earlier products of selection for BYD tolerance had common faults like excess height, lateness, rust sensitivity, and low protein. The systemic approach allowed, at no extra cost, the development of germplasm that avoided most of those pitfalls and resisted or tolerated all known stresses. Problems of height and lateness have largely been corrected, and rust and Fusarium head blight resistance traits have been rapidly integrated. At this point, about 3% of the produced germplasm has a satisfactory degree of resistance to all of the observed biotic and abiotic stress factors in Eastern Canada. Such plants may develop the trait known as stay-green (Silva et al. 2000). In Brazil the products of the method show an extreme degree of resistance to lodging (Cruz et al. 2001), related to better anchoring, straw flexibility, and reduction of crown disease. Crosses that offer the best resistance package may sometimes be identified by their healthy golden color. In the first year, the system operates mostly by pyramiding additive and dominant genes and/or traits. As soon as feasible, one needs to develop genotypes that pyramid essential recessive traits together with breadmaking quality, and a near-recurrent use of those parents is recommended. In some cases, progress after two crossing cycles has been exceptional. One example is improved Mn uptake, which correlates with biotic and abiotic stress resistance (Comeau et al. 2005).

One novel aspect of selection aided by stress is the possibility of increasing the rate of recombination and natural mutation through epigenetic mechanisms that respond to stress (Madlung and Comai 2004). Apparent cases have been observed in bread wheat (Haber et al. 2004). Evidence exists for tobacco (Kovalchuk et al. 2003). Stress may bring useful epigenetic changes, increasing the rate of linkage-breaking and mutation, thus widening the basis for plant breeding progress. For the sake of biodiversity, energy- and cost-efficiency considerations, the systemic approach is thus proposed as a more proper way to approach improvement for quantitative traits and plasticity of response to variations of environmental stresses. It is also proposed as the way to orient genetics and germplasm improvement towards the needs of mankind as it relates to environmental goals and human health, through improvement of use of inputs, and new ability to tackle quantitative goals that were previously beyond the reach of former breeding methods due to excessive costs and technical complexity.

REFERENCES

Araus JL, Slafer GA, Reynolds MP, Royo C (2002) Plant breeding and drought in C_3 cereals: what should we breed for? Ann Bot 89:925–940

Asíns MJ (2002) Present and future of quantitative trait locus analysis in plant breeding. Plant Breed 121:281–291

Ayliffe MA, Lagudah ES (2004). Molecular genetics of disease resistance in cereals. Ann Bot 94:765–773

Brown JKM (2002) Yield penalties of disease resistance in crops. Current Opinion in Plant Biology 5:339–344

Browning JA (1998) One phytopathologist's growth through IPM to holistic plant health. Ann. Rev. Phytopathol 36:1–24

Comeau A, Haber S (2002) Breeding for BYDV tolerance in wheat as a basis for a multiple stress tolerance strategy. In: Henry M, McNab A (eds) Barley yellow dwarf disease, recent advances and future strategies. CIMMYT, Mexico, pp 82–92

Comeau A, Langevin F, Lévesque M (2005) Root health: a world of complexity. [La santé des racines: le monde de la complexité] Phytoprotection 86: 43–52

Cruz PJ, de Carvalho FIF, Caetano VR, Silva SA, Kurek AJ, Barbieri RL (2001) Traits associated with lodging resistance in bread wheat. Ciencia Rural vol 31 N 4, ISSN 0103-8478, pp 563–568

Geiger D, Servaites J (1991) Carbon allocation and response to stress. In: Mooney H, Winner W, Pell E, Chu E (eds) Response of plant to multiple stresses, Acad Press, pp 103–127

Haber S, Gilbert J, Comeau A (2004) Stress-directed selection: inducing variation in spring wheat to enhance resistance. In: Canty SM, et al. (eds) Proc. 2nd Intl. Symp. Fusarium Head Blight, Kinko's, Okemos, MI, vol 1, p 71

Kovalchuk I, Kovalchuk O, Kalck V, Boyko V, Filkowski J, Heinlein M, Hohn B (2003) Pathogen induced systemic plant signal triggers DNA rearrangements. Nature 423:760–762

Madlung A, Comai L (2004) The Effect of Stress on Genome Regulation and Structure. Ann Bot 94:481–495

McClintock B (1978) Mechanisms that rapidly reorganize the genome. In: Reder GP (ed) Stadler Symp, Univ Missouri, Columbia, MO, vol 10, pp 25–48

McClintock B (1984) The significance of responses of the genome to challenge. Science 226:792–801

Reynolds MP, van Ginkel M, Ribaut JM (2000) Avenues for genetic modification of radiation use efficiency in wheat. J Exp Bot 51:459–473

Silva SA, Carvalho FIF, Caetano VR, Oliveira AC, Coimbra JLM, Vasconcellos NJS, Lorencetti C (2000) Genetic basis of stay-green trait in wheat. J New Seeds 2:55–68

Wallace DH, Yan W (1998) Plant breeding and whole-system crop physiology – improving crop maturity adaptation and yield. CAB International, NY, USA, 390pp

Yan W, Kang MS (2003) Cultivar evaluation based on multiple traits. In: Yan W, Kang M (eds) GGE biplot analysis: a graphical tool for breeders, geneticists and agronomists. CRC Press Boca Raton, Fla, USA, pp 121–158

Yan W, Tinker NA (2005) An integrated biplot analysis system for displaying interpreting and exploring genotype-environment interaction. Crop Sci 45:1004–1016

BREEDING HARD RED SPRING WHEAT FOR FUSARIUM HEAD BLIGHT RESISTANCE

Successes and challenges

M. MERGOUM[1,*], R.C. FROHBERG[1] AND R.W. STACK[2]

[1]*Plant Sciences Department, North Dakota State University, Loftsgard Hall, P.O. Box 5051, Fargo, ND 58105-5051, USA*

[2]*Plant Pathology Department, North Dakota State University, Fargo, ND 58105, USA*

[*]*E-mail: mohamed.mergoum@ndsu.edu*

Abstract: Fusarium head blight (FHB) or scab (caused by *Fusarium graminearum* Schwabe [telomorph *Gibberella zeae* (Schwein.) Petch]) is one of the most destructive diseases of wheat (*Triticum aestivum* L.) worldwide, causing significant reductions in grain yield and quality. In the USA, since the 1993 FHB epidemic, tremendous research efforts have been undertaken to address this major problem that causes substantial economic losses for the wheat growers, industry, and export market. The deployment of resistant varieties is the only effective, economical and environmentally safe way to control FHB in wheat. More than a decade of classical breeding efforts to develop scab resistant hard red spring wheat (HRSW) cultivars at North Dakota State University (NDSU) resulted in releasing several HRSW cultivars with varying levels of FHB resistance. Since 2000, three major HRSW cultivars with FHB resistance were released and grown on large scale in the Northern-Central plains of the USA. These are 'Alsen' (2000), 'Steele-ND' (2004), and 'Glenn' (2005). Alsen has been grown, in average, on more than one million hectares in the last 3 years. However, Alsen and most FHB resistant wheat cultivars released by other wheat programs in the USA, trace back to the Chinese line 'Sumai-3' (PI481542) or its derivatives. The HRSW breeding program at NDSU has invested substantial breeding efforts to identify, introgress novel resistance genes from other sources to enhance genetic diversity, and facilitate pyramiding these resistance genes. The ultimate objective is developing HRSW cultivars with effective and durable FHB resistance. Our efforts have yielded in releasing the HRSW cultivars "Steele-ND" in 2004 and "Glenn" in 2005. Steele-ND traces its resistance to the wheat relative species *Triticum dicoccoides* and Glenn combines both Alsen and Steele-ND resistances. This paper addresses the breeding efforts at NDSU to release FHB resistant HRSW cultivars and elite germplasm as parental material for many breeding programs worldwide; and the future challenges to keep our research efforts ahead of the FHB disease

Keywords: wheat, fusarium head blight, genetic resistance

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 161–167

INTRODUCTION

The FHB disease of cereal crops has become a very important disease in recent years. The favorable environmental conditions including wet weather during flowering and grain filling and major changes in cropping system (introduction of maize and minimum tillage) has favored disease development. Since 1993, FHB has caused serious loss of yield and quality in HRSW and durum wheat (*T. turgidum* L.) in the Northern Great Plains of the USA. Recent reports (Nganje et al. 2004) estimate combined direct and secondary economic losses caused by FHB for all crops were at $7.7 billion. Two states, ND and MN, account for about 68% ($5.2 billion) of the total dollar losses. Direct economic losses for wheat only were estimated to be $2.492 billion from 1993 trough 2001 (Nganje et al. 2004). *Fusarium graminearum* Schwabe (perfect stage: *Gibberella zeae* (Schw.) Petch) has been the principal pathogen (McMullen et al. 1997, Stack 2003). The FHB is unpredictable, and crop management generally has been ineffective for control. Chemical treatments can help reduce FHB but may not give an economic return. The only long–term and sustainable solution to FHB appears to be breeding for resistance (Miedaner 1997). Resistance to FHB in wheat is a character of highly complex inheritance (Stack 2003). Introducing complex resistance into commercial wheat and maintaining it through successive cycles of crossing to adapted but susceptible parents is a difficult task. This usually, requires continued effort using reliable and repeatable disease testing procedures. Given the time and resources for that effort, however, there is no practical or theoretical reason why such a complex character such as FHB resistance cannot be added. In fact, grain yield and quality are also complex traits and they have been part and the driving force behind every wheat breeding effort. Using classical breeding techniques and various novel technologies, the NDSU HRSW breeding project aims to (1) develop improved HRSW cultivars and germplasm which combine higher levels of resistance to FHB, superior grain yield, and bread-making quality; and (2) identify, introgress, and pyramid novel FHB resistance from diverse germplasm sources into adapted HRSW germplasm base.

MATERIALS AND METHODS

The adapted FHB resistant parents developed by the NDSU and other breeding programs and the recently developed genotypes (from the scab initiative germplasm effort) are selected for planned matings in the greenhouse crossing blocks. The segregating populations generated from crosses are screened in field nursery. Breeding cycles have now progressed such that many of the new adapted parents have FHB resistance (type II mainly) based on previous field and greenhouse results plus other agronomic and quality parameters. To introduce FHB resistance into HRSW wheat cultivars adapted to ND and neighboring regions, we have combined extensive FHB screening done in an inoculated, irrigated field nursery and intensive testing of elite materials in the greenhouse. To maintain high disease pressure, a field nursery to screen wheat lines for resistance to FHB was established. Plots

are elongated hill plots, each a single genotype, randomized within replicates. Throughout the nursery are multiple repeated check lines of known FHB reaction. Beginning at jointing stage *G. zeae*-colonized corn ("grain spawn") was distributed on the ground throughout the nursery. By the time the earliest genotypes reached anthesis, blue perithecia of *G. zeae* stage were present on the grain spawn. Light mist irrigation was applied on an intermittent cycle for a period of 3 days per week. By 3 to 3.5 weeks postanthesis FHB had developed and was scored visually on 20 individual heads per hill plot using a 0–100% scale (Stack and McMullen 1996). Grain was harvested from plots and proportion of visually scabby kernels (VSK) was determined. Deoxynivalenol (DON) levels in grain were determined by the NDSU Veterinary Sciences Laboratory using Gas Chromatographic analysis. The intensive greenhouse testing was done using the single spikelet method of FHB inoculation (Stack et al. 1997).

The advanced and elite experimental HRSW lines generated by the breeding program which have FHB resistance are tested in preliminary, advanced, and elite yield trials at 2, 4, and 6 ND locations, respectively. The agronomic data (grain yield and quality data, pests reactions including FHB due to natural infection, and shattering) and quality traits are generated from these trials. This data is also crucial to decision making on seed increase and eventual release of the new elite lines. Selected spikes/plants from the populations are sent to the winter nurseries in New Zealand to be advanced and selected for some agronomic traits such as height, maturity, shattering, and plant type.

RESULTS AND DISCUSSIONS

Using the FHB evaluation methods described above, it has been possible to produce consistently very high FHB disease pressure. This has facilitated the identification of improved lines. We have tested the FHB response in many lines representing progeny from first, second, third, and fourth breeding cycles. Some first and second cycle progeny showed good FHB resistance but none combined good FHB resistance with the agronomic traits and quality requirements that meet the commercial release. However, several advanced cycles derived lines combined those traits, and were released as germplasm (Frohberg et al. 2004, Mergoum et al. 2005b) or commercial cultivars (Mergoum et al. 2005a, 2006)

Variety Release

"Alsen" was the first NDSU spring wheat cultivar released with good FHB resistance. It was derived from the three way cross "ND674//ND2710/ND688". ND 674 and ND688 are two HRSW experimental lines developed by NDSU breeding program with good adaptation to ND wheat growing conditions and good end-use quality. Both lines are derived from 'Glupro' (PI 592759), a HRSW cultivar released in 1995 by NDSU for its very high grain protein content. ND2710 (Frohberg et al. 2004) is a HRSW experimental line developed by NDSU breeding

program from a cross involving Sumai 3. Alsen agronomic performance and disease reactions are reported in Table 1. It has a good yield potential in general, especially in eastern ND where scab disease is prevalent. Test weight and lodging resistance are excellent for Alsen and shattering resistance appears satisfactory. Alsen is moderately resistant to predominant Upper Midwest races of leaf rust (caused by *Puccinia triticina* Rob. Ex Desm), resistant to such races of stem rust (caused by *Puccinia graminis* Per.:Pers. f. sp. tritici Eriks. & E. Henn), susceptible to tan spot [caused by *Pyrenophora tritici-repentis* (Died.) Drechs], moderately susceptible to the Septoria leaf disease complex [caused mainly by *Stagonospora nodorum* (Berk.) Castellani & E.G. Germano] and to common root rot (Tables 1, 2, 3, and 4). Alsen has Fusarium head blight resistance type II expressed as reduced spreading of the disease in the spike (Table 4). Alsen has been planted on about 1 million hectares from 2002 to 2005; representing more than 30% of ND wheat acreages (N.D. Agricultural Statistics Service, USDA, 2002; 2003; 2004; 2005).

"Steele-ND" is another HRSW cultivar that was released by NDSU in 2004 with moderate FHB resistance (Mergoum et al. 2005). Steele-ND was selected from the cross 'Parshall' (PI 613587)/5/'Grandin' (PI 531005)/3/IAS20*4/H567.71//'Amidon' (PI 527682)/4/Grandin*2/'Glupro' (PI 592759). Steele-ND average FHB severity was 31.5% comparable to Alsen (28.7%) but significantly ($p < 0.01$) lower than the susceptible check 'Reeder' (58.9%) (Table 4). Visual scabby kernels of Steele -ND (26.5 %) was also very low ($p < 0.01$) compared to the susceptible check Reeder (37.2%), but slightly higher than Alsen (20.9%). Steele-ND does not include Sumai 3 in its pedigree and the source of resistance is believed to originate from the wheat relative *T. Dicoccoides*. A recombinant inbred lines (RIL) population derived from the cross of ND 735 with Steele-ND was developed for the purpose of mapping the FHB genes involved in Steele-ND. Grain yield of Steele-ND is similar to Reeder, Parshall, and Alsen (Tables 2 and 3). Steele-ND is resistant to pathotype THBL, the predominant leaf rust race in the region, and resistant to stem rust (Tables 2 and 4). Steele-ND is moderate resistant to *Septoria nodorum* and moderately susceptible to tan spot (Table 4).

Table 1. Agronomic traits and reaction to FHB and leaf diseases of Alsen and five other hard red spring wheat cultivars in North Dakota, USA, during the 1998–2000 period

Variety	Days to heading	Height	Lodging	Reaction[1]			Test weight	Protein	Grain yield
				Leaf rust	Tan Spot	FHB			
	Days	cm	1–9				Kg m^{-3}	%	Kg ha^{-1}
Butte 86	59	89	1.5	MS	MS	S	757	15.5	3507
Russ	60	89	1.8	R	R	S	743	15.1	3521
Gunner	63	89	1.3	MS	MR	MS	770	14.4	3306
2375	60	84	3.8	S	S	MS	768	14.7	3467
Grandin	62	79	1.9	MS	S	S	759	15.7	3003
Alsen	61	84	0.9	MR	MR	MR	770	15.3	3279

[1] R = resistant, MR = moderately resistant, MS = moderately susceptible, S = susceptible

Table 2. Agronomic traits and reaction to FHB and leaf diseases of Steele-ND and the most grown three major hard red spring wheat cultivars in North Dakota, USA, during the 2000–2003 period

Variety	Days to heading	Height	Lodging	Reaction[1]			Test weight	Protein	Grain yield
				Leaf rust	Tan Spot	FHB			
	Days	cm	1–9				Kg m^{-3}	%	Kg ha^{-1}
Steele-ND	61	84	2.7	R	MS/MR	MR/MS	762	15.7	3837
Alsen	59	79	1.9	MR	S	MR	762	16	3689
Parshall	60	89	1.8	MS/S	MS	MS	764	15.8	3716
Reeder	59	79	1.5	S	MR	S	770	15.5	3910

[1] R = resistant, MR = moderately resistant, MS = moderately susceptible, S = susceptible

"Glenn" is the most recent NDSU HRSW release (2005) with improved FHB resistance compared to Alsen and Steele-ND. Glenn was selected from the progeny of the ND2831//Steele-ND cross. ND2831 is a Sumai 3 derivative line that has scab resistance similar to Alsen. This cross aimed to combine sources of FHB resistance from Alsen and Steele-ND, high yield, excellent quality, and standability into one package. Data collected during the testing period indicate that Glenn provides scab resistance (Table 3) and yield potential superior to Alsen, along with improved lodging, leaf diseases resistance, and equal or slightly better milling and baking quality (Table 3). Glenn has exceptional high grain volume (Table 3), as well as excellent end-use quality for the domestic and export wheat markets. Glenn grain yield is similar to Alsen, Parshall and Reeder, but lower than Steele-ND. Grain volume of Glenn is 811 kg m-3, significantly higher than Alsen, Parshall, and Dapps. Protein content of Glenn (15.8%) is lower than Dapps (16.4%) but similar to Alsen, Parshall, and higher than Reeder (15.4%). Glenn exhibited a high

Table 3. Agronomic traits and reaction to FHB and leaf diseases of Glenn and five most grown hard red spring wheat cultivars in North Dakota, USA, during 2002–2004 period

Variety	Days to heading	Height	Lodging	Reaction[1]			Test weight	Protein	Grain yield
				Leaf rust	Tan Spot	FHB			
	Days	cm	1–9				Kg m^{-3}	%	Kg ha^{-1}
Glenn	65	87	0.7	R	MS/MR	MR/R	806	15.8	4421
Alsen	65	84	1.1	MR/MS	S	MR	770	15.6	4317
Dapps	65	91	1.2	R/MR	MR	MS	772	16.4	4209
Parshall	65	94	1.3	MS/S	MS	MS	768	15.6	4347
Reeder	66	83	0.5	S	MR	S	755	15.4	4519
Steele-ND	66	87	1.9	R/tMR	MS/MR	MR/MS	772	15.6	4552

[1] R = resistant, MR = moderately resistant, MS = moderately susceptible, S = susceptible

Table 4. Fusarium head blight (FHB) severity, Tombstone and Deoxynivalenol toxin levels; leaf rust, stem rust, tan spot, and Septoria nodorum reactions of HRSW cultivars under natural (3 locations) and artificial (7 locations) inoculation in the filed and greenhouse conditions (4 tests) in Fargo, ND from 2001 to 2004

Genotype	FHB Severity (Field test under natural infection)[1]			FHB Severity (Field test under artificial inoculation)			FHB GH	SR[2]	TS	SN
	SEV (%)	TMB (%)	DON (ppm)	SEV (%)	TMB (%)	DON (ppm)	SEV (%)		(1–5)	(1–5)
Glenn	7.6	0.7	0.4	18.9	16.0	4.0	16.3	R	3	3
Steele-ND	19.3	1.3	0.9	31.5	26.5	5.3	24.6	R	4	3
Alsen	7.0	0.9	0.8	28.7	20.9	4.8	10.8	R	5	5
Reeder	26.2	5.7	1.5	58.9	37.2	10.3	42.0	R	4	4
2398	41.8	5.4	2.0	75.2	51.7	9.9	55.5	R	-	-

[1] SEV = Severity; TMB = Tombstone; and DON = Deoxynivalenol toxin; LR = Leaf rust; SR = Stem rust; TS = Tan spot; SN = Spetoria nodorum; [2]R = esistant

level of resistance to the predominant leaf rust and stem rust races in the region. It is medium resistant to tan spot and medium susceptible to *Septoria nodorum* (Tables 3 and 4).

ACKNOWLEDGEMENTS

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0790-4-100. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative. The authors thank T. Olson (Dep. of Plant Sciences, NDSU, Fargo), for quality analysis.

REFERENCES

Frohberg RC, Stack RW, Mergoum M (2004) Registration of spring wheat germplasm ND 2710 resistant to Fusarium head blight. Crop Sci. 44:1498–1499

McMullen MP, Jones R, Gallenberg D (1997) Scab of wheat and barley: A re-emerging disease of devastating impact. Plant Dis 81:1340–1348

Miedaner T (1997) Breeding wheat and rye for resistance to Fusarium diseases. Plant Breed 116:201–220

Mergoum M, Frohberg RC, Miller JD, Rasmussen JB, Stack RW (2005a) Registration of spring wheat germplasm ND 744 resistant to Fusarium head blight leaf and stem rusts. Crop Sci. 45:430–431

Mergoum M, Frohberg RC, Miller JD, Stack RW (2005b) Registration of 'Steele-ND' wheat. Crop Sci. 45:1163–1164

Mergoum M, Frohberg RC, Olson T, Friesen TL, Rasmussen JB, Stack RW (2006) Registration of 'Glenn' wheat. Crop Sci 46: 473–474

N.D. Agricultural Statistics Service, USDA (2002) North Dakota wheat varieties

N.D. Agricultural Statistics Service, USDA (2003) North Dakota wheat varieties

N.D. Agricultural Statistics Service, USDA (2004) North Dakota wheat varieties

N.D. Agricultural Statistics Service, USDA (2005) North Dakota wheat varieties.

Nganje WE, Kaitibie S, Wilson WW, Leistritz FL, Bangsund DA (2004) Economic impacts of Fusarium Head Blight in Wheat and Barley: 1993–2001. Agribusiness and Applied Economics Report No 538

Stack RW (2003) History of Fusarium Head Blight with Emphasis on North America. In: Leonard KJ, Bushnell WR (eds) Fusarium Head Blight of Wheat and Barley. APS Press, St. Paul, MN. pp 1–34

Stack RW, McMullen MP (1996) A visual scale to estimate severity of Fusarium head blight in wheat. DSU Extension Serv. Circ. # PP–1095, 2pp

Stack RW, Frohberg RC, Casper H (1997) Reaction of spring wheats incorporating Sumai3-derived resistance to inoculation with seven Fusarium species. Cereal Res Comm 25:667–671

ADVANCES IN NITROGEN HANDLING STRATEGIES TO INCREASE THE PRODUCTIVITY OF WHEAT

K.W. FREEMAN AND W.R. RAUN*

044 N. Agricultural Hall Department of Plant and Soil Sciences, Oklahoma State University Stillwater, OK 74078

**E-mail: wrr@mail.pss.okstate.edu*

Abstract: Nitrogen Use Efficiency (NUE) currently averages 33% for wheat, and corn production around the world. Precision Sensing Technologies have the potential to increase NUE's in wheat and corn to levels exceeding 50%. This has primarily been developed via the application of two very fundamental approaches. 1. Similar to the use of yield goals, established prior to planting, predicting grain yields mid-season can be accomplished by collecting NDVI readings (V8 in corn, Feekes 5 in wheat), and then dividing by the number of days from planting to sensing. The index (INSEY or In Season Estimated Yield or YP0) essentially reflects biomass produced per day, since NDVI is an excellent indicator of plant biomass. 2. Predicting whether or not a year will be responsive to applied N is accomplished by establishing N Rich Strips in farmer fields (N applied at a rate where it will not be limiting throughout the growth cycle), but where the remaining land receives modest amounts of preplant N, but where severe N stress is not encountered. The Response Index (RI) is then computed by dividing the NDVI from the N Rich Strip by the NDVI from the farmer practice. Estimated yield is then multiplied times the RI to obtain the yield achievable with added N (YPN). The mid-season fertilizer N is determined by subtracting grain N uptake at YP0 from grain N uptake at YPN, divided by an expected efficiency factor (between 0.5 and 0.7 for topdress N). This method has proven to be very reliable in wheat and corn for obtaining maximum yields, increased NUE, and maximum farmer profit from N fertilization. Averaged over years, and locations, OSU work has shown that this methodology will on average net farmers in excess of $\$30\,ha^{-1}$. Current work is ongoing with INTA and AAPRESID (Ing. Ricardo Melchiori, and Ing. Agustin Bianchini) to further refine algorithms tailored toward Argentinean production environments (http://www.soiltesting.okstate.edu/SBNRC/SBNRC.php)

Keywords: nitrogen, yield grain

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 169–173.

INTRODUCTION

Today, strategies that increase the productivity of wheat have to be intrinsically tied to the same strategies that increase farmer profitability. With increasing fuel and natural gas prices, N fertilizer as urea will likely exceed $1.10 per kilogram within a few years. Because of this, improved N management and resultant NUE will become increasingly important all over the world in order to maintain current cereal grain production levels.

Nitrogen use efficiency can be determined using various different methods. The most common is to determine crop N uptake in the treated plots and subtract N uptake in the check plot or 0-N plot, and then divide by the rate of N applied. This method theoretically determines the percent of N in the treated plot that was derived from the fertilizer applied. Other methodologies include the use of 15N tracer techniques, whereby actual amounts taken up by the plant can be quantified. More recently, some scientists have computed N removal as a function of N applied without accounting for N taken up in the check plot. This approach is fatally flawed, and which is also an irresponsible method of accounting for fertilizer N application in cereal production. The amount of N taken up in wheat grain that comes from the soil and/or rainfall (non fertilizer N sources) can range from 40 to 60% of the total. Some authors have indicated that 50% of the N taken up in plants comes from mineralized N and atmospheric deposition. In this light, we simply cannot determine NUE without accounting for N taken up in the check, as this amount reflects how much N was delivered by the environment.

Nitrogen Recommendations using Indirect Measures

Research in Nebraska targeted the use of the SPAD 502 chlorophyll meter (Minolta Camera Col., Osaka Japan) for identifying in-season N deficiencies in corn (Blackmer and Schepers 1996). The chlorophyll meter is attached to corn leaves and measures transmittance through the leaf at 650 and 950 nm. This group was the first to calculate an N sufficiency index relative to chlorophyll meter readings from a non-N-limited area with remaining portions of the field. This work using non-N-limiting reference strips was a benchmark for all later precision agricultural methodologies that targeted improved mid-season N management in cereal production. In addition, this team in Nebraska further developed this approach, using the sufficiency index as a guide for applying mid-season N (Varvel et al. 1997). By applying their methodology, maximum yields were attained when early season N levels were adequate to maintain sufficiency indexes above 90% at the V8 growth stage. If the sufficiency index at V8 was below 90%, maximum yields could not be achieved with in-season fertilizer N applications. They further noted that early N deficiencies could be corrected using chlorophyll meters and the sufficiency index approach when deficiencies were not severe.

Indirect measurements of plants for determining variable rate N applications have been evaluated and that have been successful. Using in-season sensor (normalized

difference vegetative index or NDVI) measurements and a response index (determined by comparing a non-N limiting reference strip to the common practice), Raun et al. (2002) showed that sensing and treating each $1m^2$ in winter wheat resulted in NUE increases of 15% over that of traditional practices. The Greenseeker™ sensor has self-contained illumination in both the red (650 ± 10 nm FWHM) and NIR (770 ± 15 nm FWHM) bands. The sensor device measures the fraction of the emitted light in the sensed area that is returned to the sensor and then used to compute NDVI. This approach also resulted in the highest revenue when compared to other conventional practices using $\$0.55\,kg^{-1}$ fertilizer N pricing, roughly half of that found at this writing. Their methodology that has been developed for wheat and corn relies on the demonstrated ability to predict yield potential mid season, estimated using NDVI readings divided by the days from planting to sensing (Raun et al. 2002, www.nue.okstate.edu) and that represents growth rate, or biomass produced per day. Similar to the preplant yield goal approach, this mid-season method for determining topdress N rates is based on how much N will be removed at a given yield level. However, the difference is that they subtract the projected N uptake at the predicted yield level in the farmer practice from the projected N uptake in the non-N limiting reference strip and divide by an efficiency factor (usually between 0.6 and 0.7 for mid-season N applications) to obtain the mid-season topdress N rate. The big difference between the two methodologies is that the latter is based on specific climatic conditions encountered from planting to the time mid-season topdress N will be applied. Furthermore, environmental conditions from planting to sensing clearly change from year to year, are known to alter how much N is mineralized from soil organic matter and the amount of N in rainfall, and via the use of the sensing methodology defined, mid-season N rates can be tailored to adjust for responsive and non N responsive years. Using contributions from scientists all over the world, 14 algorithms have been developed for various regions and that include irrigated corn, dryland corn, winter wheat, spring wheat, sorghum, and bermudagrass (www.nue.okstate.edu). Each algorithm requires preplant establishment of the N rich strip or non-N-limiting reference strips developed by Blackmer and Schepers (1996), mid season NDVI sensor readings from the N rich strip and farmer practice, knowledge of planting dates, and regional yield limits (Raun et al. 2005, http://www.soiltesting.okstate.edu/SBNRC/SBNRC.php). All algorithms are free over the web, and all mathematical components of each algorithm are public property.

Applied Technologies for the Future

Modifications of the non-N limiting or N Rich strip are currently being evaluated. Engineers at Oklahoma State University have developed what is termed a Ramped N Reference Strip. This automated programmable N fertilization strip applicator can be retrofitted on most common 4-wheelers similar to the Calibration Stamp Applicator (Raun et al. 2005). Unlike the calibration stamp, this applicator has been designed to apply preplant rates ranging from 0 to 300 kg N ha^{-1}, in progressively

incremental rates of 20 kg N ha^{-1} over user defined distances (50 to 300 m). The highest rate and the rate increments can be adjusted lower depending on the crop, using different nozzles. The system that has been used in winter wheat, ramps the N rates up from 0 to 150 kg ha^{-1} in increments of 10 kg N ha^{-1} and that changes every 10 feet. Actual application rates are a function of applicator speed, sprayer pressure, and nozzle size, all of which can be adjusted according to desired rates and range. The Ramped N Reference Strip is specifically designed to assist producers in determining the mid-season fertilizer N rate by visually inspecting differences in growth mid season across the range of N rates applied. However, it should be noted that using one of various active hand-held sensors that measure NDVI is preferred, since the sensors are much more sensitive to changes than the human eye, and that can easily pinpoint the rate where mid-season growth was at a maximum. Preplant soil tests, and mid-season soil tests simply cannot provide the robust data needed to determine the appropriate mid-season fertilizer N rate, whereas the Ramped N Reference Strip is a biological N fertilization guide. Again, the visual or sensor inspection of response within the marked Ramped N Reference Strip in the middle of the season will quickly guide farmers as to whether or not added N is needed, and or how much. For example, if no visual differences in mid-season growth were observable across the Ramped N Reference Strip (0 to 150 kg N ha^{-1}), it is unlikely that there will be added response to fertilizer N. However if it were noticed that growth peaked at 100 kg N ha^{-1}, but where discernable differences were present from 0 up to 100 kg N ha^{-1} (no differences from 100 to 150 kg N ha^{-1}), the topdress rate to be applied would be around 100 kg N ha^{-1}. It is important to mention that the Ramped N Reference Strip must be applied on-top of the normal farmer practice, in order to be of use for deciphering mid-season topdress N rates.

As was discussed earlier, Raun et al. (2005) showed that mid season fertilizer N rates can be tailored to the environmental conditions encountered from planting to the time topdress N is applied. Their work employed the use of active NDVI sensors whereby readings collected from the N Rich Strip were divided by NDVI readings from the Farmer Practice to determine the response index. The response index determined soon after breaking dormancy in winter wheat using NDVI readings was highly correlated with the response index determined at harvest (grain yield in the N Rich Strip divided by grain yield in the Farmer Practice). This finding assisted them in recognizing that the response to fertilizer N changes in each field from year to year, and that could be predicted mid-season (Mullen et al. 2003). The beauty of the Ramped N Reference Strip is that it can easily be applied and marked within each field at the time of planting, and used as a visual guide for topdress N rates later in the season without the need of any NDVI sensors or chlorophyll meters. However, it should be noted that even when using the Ramped N Reference Strip, hand-held NDVI sensor readings and or chlorophyll meter readings across the range of N rates applied will be much more accurate in deciphering the exact mid-season fertilizer N rate when compared to human visual observation.

CONCLUSIONS

The use of reference strips or ramped N reference strips are expected to have a significant impact on how N is utilized for crop production throughout the world. Our failure to implement N rich or N reference strips world wide is an embarrassing, and highly irresponsible mark on our work as agronomists. For years we have understood that N in rainfall or that mineralized from soil organic matter varies drastically from year to year, yet the focus has continued to remain on preplant soil tests that did nothing to account for environmental conditions encountered during the growing season. Furthermore, we have massive amounts of data showing that yield levels vary greatly from one year to the next, even under irrigation. Mid-season evaluation of N rich strips allows farmers and agronomists to determine precisely how much N the environment delivered for free, and subsequent determination of accurate topdress N rates. Advances at Oklahoma State University have complemented this knowledge via the development of mid-season predictive models for actual grain yield that further refined the mid-season fertilizer N rates by basing N rates on the differences in projected N removal, while also accounting for how much the environment delivered for free. It should come as no surprise that this methodology is identical to that of using yield goals (preplant), but with mid-season knowledge of final yield potential. We must proceed with an aggressive extension model of this technology since it has already been proven to deliver increased nitrogen use efficiency and profitability for wheat and corn farmers in various parts of the world. Failure to extend this technology can only be blamed on academic arrogance and pride from scientists waiting to add their own scent or mark while the world starves to death. In the end, farmers could care less where these technologies were developed or who developed them, they simply want to do their very best with the limited resources they have available to them. With the advent of increasing gas prices, and ultimate fertilizer costs, farmers have to be more efficient. If they aren't, they will simply stop producing basic grains because of the lack of profitability, and in the end, the third world will ultimately pay the price due to decreased cereal production in the world. How long are we going to just sit and wait?

REFERENCES

Blackmer TM, Schepers JS (1996) Use of a chlorophyll meter to monitor nitrogen status and schedule fertigation for corn. J Prod Agric 8:56–60

Mullen RW, Freeman KW, Raun WR, Johnson GV, Stone ML, Solie JB (2003) Identifying an in-season response index and the potential to increase wheat yield with nitrogen. Agron J 95:347–351

Raun WR, Solie JB, Johnson GV, Stone ML, Mullen RW, Freeman KW, Thomason WE, Lukina EV (2002) Improving Nitrogen use efficiency in cereal grain production with optical sensing and variable rate application. Agron J 94:815–820

Raun WR, Solie JB, Stone ML, Zavodny DL, Martin KL, Freeman KW (2005) Automated calibration stamp technology for improved in-season nitrogen fertilization. Agron J 97:338–342

Raun WR, Solie JB, Stone ML, Martin KL, Freeman KW, Mullen RW, Zhang H, Schepers JS, Johnson GV (2005) Optical sensor based algorithm for crop nitrogen fertilization. Comm Soil Sci Plant Anal 36: 2783–2792

Varvel GE, Schepers JS, Francis DD (1997) Ability for in-season correction of nitrogen in corn using chlorophyll meters. Soil Sci Soc Am J 61:1233–1239

TILLAGE INTENSITY, CROP ROTATION, AND FERTILIZER TECHNOLOGY FOR SUSTAINABLE WHEAT PRODUCTION NORTH AMERICAN EXPERIENCE

T.L. ROBERTS* AND A.M. JOHNSTON

Potash & Phosphate Institute/Potash & Phosphate Institute of Canada
**E-mail: troberts@ppi-ppic.org*

Abstract: Approximately 95 million hectares (M ha) of the world's cropland is under no-till; over 40% of that area is in North America. Farmers in the Canadian prairies and the northern Great Plains pioneered wheat production in reduced tillage systems when they began experimenting with no-till in the early 1970s. Today no-till, or direct-seeding, is used on about a third of the wheat farms in the U.S. and at least half the wheat area in Canada. Most wheat growers using no-till seeding systems tend to diversify their cropping rotations to maximize production efficiency. Proper understanding of nutrient behavior in soil is necessary for appropriate fertilizer management in reduced tillage systems. Mobile nutrients like nitrogen (N) and sulfur (S) are not impacted by a lack of soil mixing to the extent that phosphorus (P) and potassium (K) are. Both P and K can become concentrated near the soil surface. This paper will examine the behavior of soil nutrients in the absence of tillage and the resulting implications for fertilizer management

Keywords: tillage intensity, crop rotation, fertilizer

INTRODUCTION

North America leads the world in no-till crop production. No-till area in the U.S. is currently about 25 M ha and Canada has 13 M ha, together accounting for 41% of the 95 M ha worldwide (Table 1). North America is also one of the world's largest producers and exporters of wheat (FAO 2005). The U.S. is the world's third largest producer and leading exporter, producing annually an average of about 60 billion metric tons (t) and exporting about 30 billion t. Canada ranks sixth in production with a yearly average of 26 billion t and third in exports at almost 18 billion t.

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 175–187.

Table 1. Extent of no-tillage adoption worldwide, 2004/05

Country	No-till area, million ha
USA	25.3
Brazil	23.6
Argentina	16.0
Canada	13.4
Australia	9.0
Paraguay	1.7
Indo-Gangetic-Plains	1.9
Bolivia	0.6
South Africa	0.3
Spain	0.3
Venezuela	0.3
Uruguay	0.3
France	0.2
Chile	0.1
Colombia	0.1
China	0.1
Others (estimate)	1.5
Total	94.6

(J. Hassell, Conservation Technology Information Center, personal communication)

The northern Great Plains has a total area of about 125 M ha, with some 52 M ha in crop production (Padbury et al. 2002). Wheat (*Triticum aestivum L.*) is the dominant crop grown in the region, followed by barley (*Hordeum vulgare L.*) and oats (*Avena sativa L.*) as cereal grains. Corn (*Zea mays L.*) is a dominant crop only in the southern regions where climatic conditions allow its production. Canola (*Brassica* species) is the foremost oilseed crop in the region, grown mainly on the Canadian prairies. Grain legumes (dry pea [*Pisum sativum L.*] and lentil [*Lens culinaris L.*]) are growing in interest as a crop diversification option, but still only represent a very small proportion of the cropping mix.

Environmental conditions on the northern Great Plains are described as severe by most, given the cold winters and hot summers. However, the greatest limiting factor to production is likely the amount and distribution of precipitation. Annual precipitation ranges mostly from 300 to 500 mm, with 165 to 300 mm falling during the April to July growing season (Padbury et al. 2002). The frost-free season ranges from 83 to 157 days, representing a major diversity in crop production potential. The soils in much of the northern Great Plains are frozen for 4 to 6 months of each year, minimizing microbial activity, nutrient release and crop residue decomposition.

Farmers in the Canadian prairies and the northern Great Plains pioneered wheat production in reduced tillage systems when they began experimenting with no-till in the early 1970s. Today no-till, or direct-seeding (i.e. no tillage prior to seeding and minimum tillage at seeding), is used on about a third of the wheat farms in the

U.S. and at least half the wheat area in Canada. Most wheat growers using no-till or direct-seeding systems tend to diversify their cropping rotations to maximize production efficiency.

Erosion control is one of the main driving forces in the adoption of no-till in much of the world. While erosion control is also important in the northern Great Plains wheat growing region, no-till adoption was also driven by the need to improve moisture use efficiency (Brandt 1992, Lafond et al. 1992; Peterson et al. 2001). The semiarid climate of the Canadian prairies is ideal for producing high protein wheat, but the region's moisture limitations have made no-till cropping systems especially attractive and economical (Zentner et al. 2002). Almost all of Canada's wheat production is in the Canadian prairies.

The Canadian prairies have about 30 M ha of cultivated land, which can be divided into five main climatic/soil zones (Fig. 1). The Brown soil zone has about 21% of the cultivated land, the Dark Brown zone 22%, and the remainder in the more humid Black and Gray soil zones. Mean annual precipitation ranges from about 300 to 400 mm in the Brown and Dark Brown soil zones to 425 to 475 mm in the Black and Gray zones.

Spring wheat is the principal crop in all soil zones. Producers have historically selected rotations that included high proportions of wheat and summerfallow, but fallow has been declining steadily since the mid 1970s (Campbell et al. 1990, 2002) while the area under no-till and reduced tillage has been increasing (Fig. 2). Fallow frequency ranged from once every two years in the Brown soil zone to one of four years in the Black soil zone, in direct relation with moisture availability. However, with the better moisture retention with a no-till cropping system, growers have been able to greatly diversify their rotations and increase cropping intensity (Table 2). Growers can now incorporate cereals (spring and winter wheat, durum wheat, barley), oilseeds (canola, flax, mustard, sunflower), pulse crops (field peas, lentils, chick peas), and forages into their rotations. Wheat still dominates in the

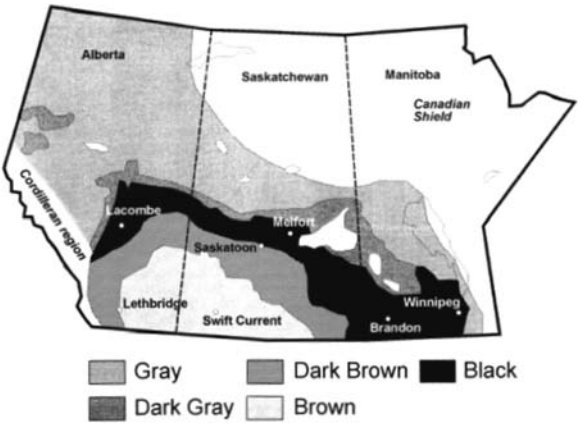


Figure 1. Soil zones of the Canadian prairies

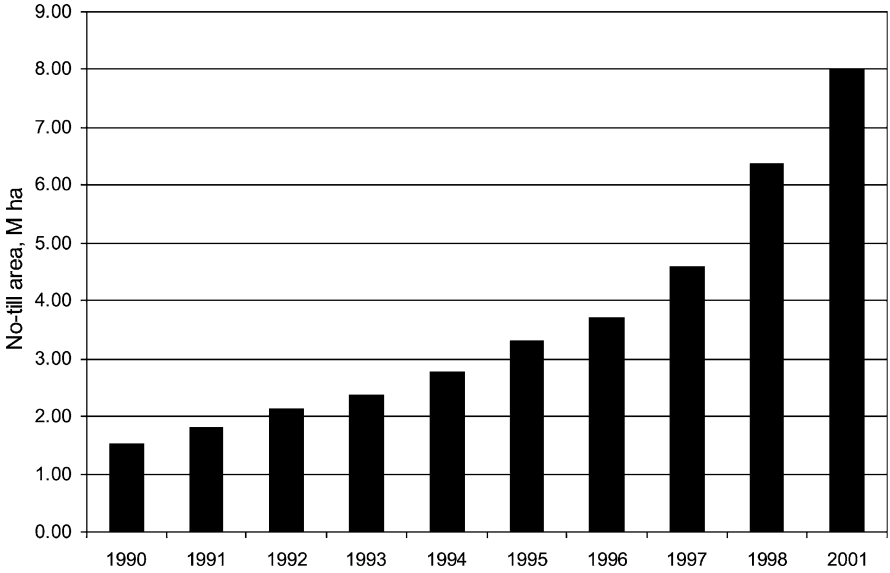


Figure 2. No-till area in the Canadian prairies (B. McClinton, Saskatchewan Soil Conservation Association personal communication)

Table 2. Trends in cropping intensity in the Canadian prairies Average rotation length*

Soil zone	1976	1980	1985	1990	1995	1998
Brown	1/1.1	1/1.1	1/1.3	1/1.3	1/1.3	1/1.6
Dark Brown	1/1.4	1/1.5	1/2.1	1/2.2	1/3	1/4
Black and Gray	1/2.2	1/2.6	1/4.9	1/4.9	1/6.7	1/10

* Interpret rotation 1/1.1 as one year fallow to 1.1 year in crop.
(Campbell et al. 2002)

rotation, but the improved water conservation from no-till gives growers greater flexibility in their cropping systems in any given year and oilseeds and pulse crops are now routinely part of a wheat based rotation (Miller et al. 2001, Campbell et al. 2002, Miller et al. 2002, Johnston et al. 2002).

SOIL CHANGES RELATED TO REDUCED TILLAGE

Tillage accelerates the natural processes of soil degradation; erosion, salinization, and acidification rates increase, while the amount and quality of organic matter decreases. Soil organic matter breaks down faster with frequent tillage, often resulting in a loss of plant nutrients. Regular tillage can also break down soil structure and tilth, which reduces moisture-holding capacity and water infiltration rates (Malhi et al. 2001).

When tillage is reduced, greater crop residues accumulate on the soil surface minimizing wind and water erosion and improving the quality of the soil. Crop residues on the soil surface increase water infiltration and reduce evaporation losses, reduce nutrient losses through erosion, and also lower the surface temperature. Cooler soil temperatures will slow nutrient release from soil organic matter, reduce diffusion of nutrients to the plant roots, and can affect root growth. In the absence of frequent tillage, mineralization is slowed and the release of plant nutrients declines, making fertilization more important in producing higher yields.

When crop residues accumulate in and on the soil surface because of less tillage, readily-decomposable plant residues and the active fraction of the soil organic matter eventually increase. Initially, when no-till is first adopted the increased carbon (C) from the crop residues causes immobilization of soil N as decomposing microorganisms use soil N to maintain their C:N ratios during the decomposition process. With time the turn-over, or breakdown, of soil organic matter reaches a new equilibrium and the pool of potentially mineralizeable N increases resulting in more plant-available nitrate (NO_3)-N and ammonium (NH_4)-N. This transition period may last several years, during which band placement of nutrients below the residue-covered surfaces becomes very important.

Most plant-available N in the soil is in the water-soluble NO_3 form, which means it can leach and moves throughout the soil profile with moisture. Sulfate (SO_4)-S is also water-soluble and can move within the soil profile, although under acidic soil conditions some SO_4 can be also adsorbed to soil colloids. Soil P and K tend to be immobile in the soil because of their reaction with calcium (Ca), magnesium (Mg) and other soil minerals, and/or soil charge (cation exchange capacity [CEC]). Without tillage and soil mixing, immobile nutrients may accumulate at the soil's surface (0–5 cm). An understanding of how nutrients move and react in the soil is necessary for proper fertilizer management in reduced tillage systems.

Soil pH may decline as soil C (i.e. organic matter) levels increase under reduced tillage systems. Changes will be proportional to the changes in organic matter and the initial pH of the soil. For example, a 26% increase in soil C content was accompanied by a decline of 0.5 pH units in a gray soil in western Canada after 10 years of no-till management (Arshad et al. 1990). Soil pH impacts nutrient availability of P and some micronutrients.

Nutrient stratification is an important concern in the management of P and K in zero-till systems. These immobile nutrients tend to accumulate in the soil surface at the depth of application. This is illustrated with the data in Fig. 3 from a Black, alkaline soil in Manitoba. Soil samples were taken at the end of a 4-year study where P was banded (58 kg P_2O_5 ha^{-1}) and K was broadcast (120 kg K_2O ha^{-1}). The lack of soil mixing during the 4 years caused P and K to accumulate where they were originally placed.

Similar observations were made in a Brown soil in Saskatchewan. Selles et al. (1999) found that after 12 years, converting from conventional till wheat-fallow to no-till continuous wheat resulted in an accumulation of plant-available P in the upper 0–1 cm of surface soil. However, this was not the case for no-till fallow wheat

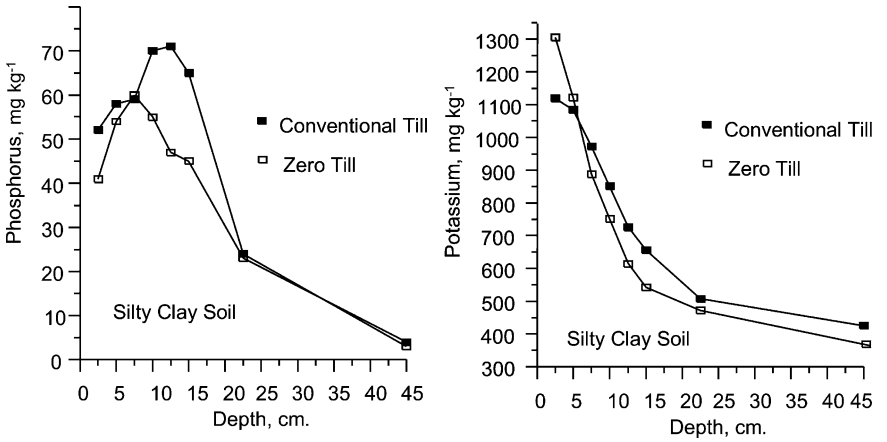


Figure 3. Effect of zero and conventional tillage on distribution of P and K in a silty clay soil in Manitoba (adapted from Grant and Bailey, 1994)

or conventional till continuous wheat. This specific treatment change was attributed to the accumulation of surface residue and lack of decomposition in no-till. The increased concentration of P at the soil surface did not result in greater P uptake by the wheat, but this was probably because starter P was used at seeding and P release from organic matter was slow due to cool soil temperatures in spring.

When soil conditions are dry, nutrients near the surface may be positionally unavailable for plant uptake. This can be a common problem in prairie soils where precipitation is limited and soils are low in P. However, it can easily be corrected by the use of starter fertilizer placed in the seed row.

Although N and S are mobile in the soil, tillage can also impact their distribution within the soil profile. A study in Manitoba on a fine sandy loam found that NO_3-N was higher in no-till than conventional till in the 0–7.5 cm depth, presumably due to release from organic residues retained near the soil surface and retention of residual N from fertilizer application under the dry conditions of the study. Similar results were found in the surface 2.5 cm in a silty clay soil. Nitrate-N also accumulated in the 60–120 cm depth in both tillage systems and soils. Other researchers in the Canadian prairies have reported no effect of tillage system on soil NO_3-N and SO_4-S to a depth of 60 cm (Malhi et al, 1992).

FERTILIZER MANAGEMENT

Fertilizer management with no-till seeding requires careful attention to fertilizer placement to optimize fertilizer use efficiency by the crop (Johnston, 2002). Soil characteristics, climate, crop type, and agronomic practices, including fertilizer application method impact the efficiency of nutrient use.

Nitrogen is the nutrient most commonly limiting crop production world wide, followed by P and K. Broadcasting N onto the residue covered surface is not the most efficient method of application because of the potential for immobilization by surface residues and volatilization losses of N (Malhi and Nyborg 1992). In-soil band placement of N is usually the most effective means of minimizing immobilization of N in no-till crops, but application of all the crop's fertilizer requirements at seeding can be challenging. Similarly, the application of P and K in bands either with, or close to the seed minimizes tie-up by the soil and increases early season uptake by the crop, especially when applied as starter fertilizer.

Fig. 4 illustrates the effectiveness of starter P over a 31-year period in southern Saskatchewan in a fallow-wheat-wheat rotation. Phosphorus application ($15\,kg\ P_2O_5\ ha^{-1}$) produced on average $342\,kg\ ha^{-1}$ more grain for wheat grown on fallow and 197 kg/ha more grain for wheat grown on stubble. The yield variability over the years was closely related to spring weather conditions and occurred although soil test P had doubled over the 31 year period (Fig. 5). Greatest P response occurred when the soils were cool and moist in the spring. Root growth and P movement in soil and uptake by plants is hindered under low soil temperatures.

Although all the P requirements for wheat can be safely applied in the seed row as a starter, that is not the case for high rates of N or K. Placement of high amounts of these nutrients directly with the seed often causes reduced germination and delayed emergence resulting in poor stands and yield loss. General recommendations used to suggest that no more than $45\,kg\ ha^{-1}$ of N as ammonium nitrate or $22–28\,kg\ ha^{-1}$ of urea N could safely be applied with the seed. These recommendations were appropriate for seeding equipment which placed the seed and fertilizer in close contact, but are not appropriate for seeding equipment which causes some scatter between seed and fertilizer (pneumatic or airseeder), or which can precisely place fertilizer away from the seed.

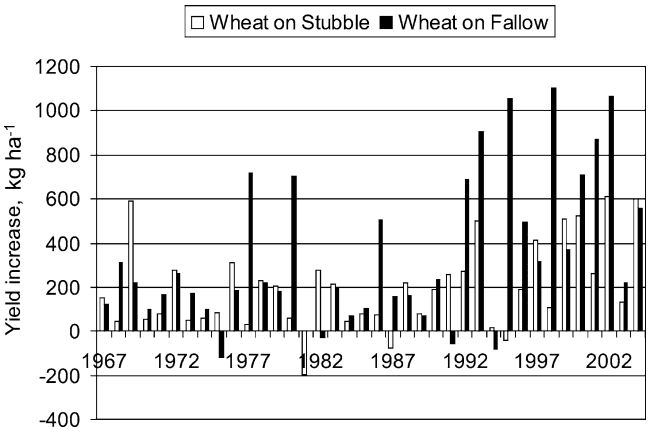


Figure 4. Yield increase in response to starter P application in a Saskatchewan fallow-wheat-wheat rotation, 1967–2004. (R.P. Zentner, Agriculture and Agri-Food Canada, personal communication)

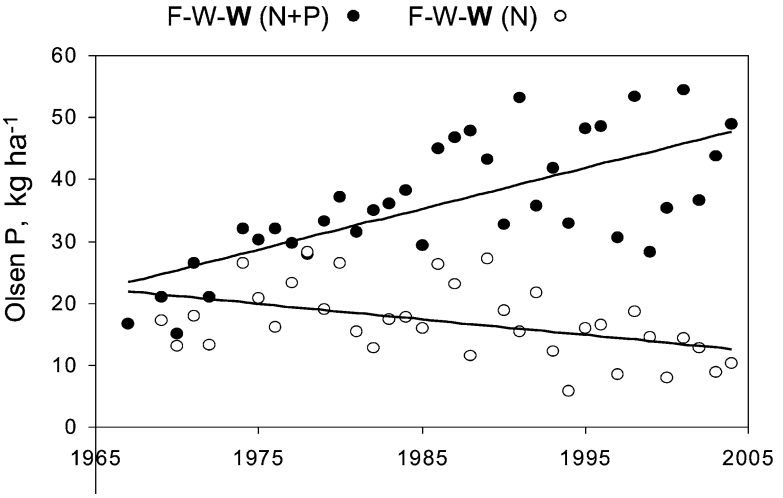


Figure 5. Influence of starter P fertilizer application on soil test P levels in the wheat phase of a fallow-wheat-wheat rotation in Saskatchewan, 1967–2004 (R.P. Zentner, Agriculture and Agri-Food Canada, personal communication)

Many factors influence how much fertilizer can be safely applied with the seed. These include: row spacing, seed bed utilization (SBU), soil texture, soil moisture, soil variability, fertilizer placement, seed furrow opener, fertilizer source, and crop. The amount of fertilizer that can safely be applied in the seed row decreases as row spacing increases. With wider rows, at a given rate per hectare the fertilizer is more concentrated and is in greater contact with the seed. This is more of a concern with N than with P. Research in Saskatchewan and Manitoba have shown that direct-seeded wheat produced similar yields for row spacing ranging from 10 to 30 cm and that higher concentrations of seed-placed P in wider rows had no effect on yield (Lafond et al. 1996).

Seed bed utilization is a measure of the amount of soil used for applying fertilizer (Roberts and Harapiak 1997). It is calculated as follows:

$$\%SBU = \frac{width\ of\ seedrow}{row\ spacing} \times 100$$

Heavier textured soils tolerate more seed row N because the increased cation exchange and water holding capacity reduce ammonia toxicity, a major cause of germination and seedling damage. Saskatchewan guidelines for the amount of urea N that can safely be applied with the seed, assuming good to excellent seedbed moisture, are shown in Table 3. Application rates for ammonium can be increased by about 25 %. Ammonium nitrate is less damaging to the seed than urea. It has a higher salt index than urea, but does not add to ammonia toxicity. Higher rates of N may be tolerated if CEC is high and seedbed moisture is excellent. Guidelines in

Table 3. Approximate safe rates of urea N (kg ha^{-1}) that can be safely applied with wheat and other cereal grains

	2.5 cm spread (Disc or knife) Row spacing, cm			5 cm spread (Spoon or hoe) Row spacing, cm			7.5 cm spread (Sweep) Row spacing, cm		
Soil Texture	15	23 SBU	30	15	23 SBU	30	15	23 SBU	30
	17%	11%	8%	33%	22%	17%	50%	33%	25%
Light (sandy loam)	22	17	17	34	28	22	45	34	28
Medium (loam to clay loam)	34	28	22	45	39	34	56	45	39
Heavy (clay to heavy clay)	39	34	34	56	45	39	67	56	45

(Henry et al. 1995)

North Dakota suggest that maximum seed row N can range from 67 to 112 kg ha^{-1} when using an air seeder (60 to 100 % SBU) in a heavy textured soil.

Many growers on the northern Great Plains have adopted the use of specialty seeding equipment that places fertilizer in a separate band from seed to avoid seed germination and emergence problems. Most commonly used are sidebanding openers mounted on pneumatic air drills, which provide a fertilizer band that is usually 3–4 cm to the side and 4–5 cm below the seed row. Results from field trial evaluation of these openers indicates that they all perform very well, as long as they are properly adjusted for the seeding implement shank angle and the soil conditions (Johnston et al. 2001). Both dry (urea) and gaseous N (anhydrous ammonia) sources, the two main N fertilizer forms used by the region's farmers, have been used when no-till seeding (Johnston et al. 1997). Using anhydrous ammonia is more common in the higher moisture regions, where N rates required to optimize wheat yields are higher.

Sulfur is the third most deficient nutrient in northern Great Plains, following N and P. It is not only important for optimizing yields, but S is also an important component of several amino acids and therefore affects the quality of bread wheat. Sulfur deficiencies are estimated to cover approximately 30% of the cultivated acreage in the Canadian Prairies and S soil testing is often unreliable in predicting S status of a field (Grant et al. 2004).

Conventional soil testing that measures soluble SO_4-S, the form of S available to plants, is problematic because of the inherent variability of SO_4 in the field and the variability of mineralization of organic S, which usually accounts for 95% of the total S in the soil. Soil testing for S is most reliable at predicting non-responsive soils that contain high amounts of S.

Sulfur is normally applied as elemental S or in the SO_4 form. Elemental S must be oxidized by microbial processes before being used by plants. The rate of conversion is dependent on characteristics that enhance microbial activity (e.g. temperature,

moisture, aeration, and pH). Oxidation rate generally increases with increasing soil temperature and decreases with very low or very high moisture. Particle size of the elemental S is also important; the smaller the particle size the faster the oxidation. Dispersion of the S particles is also an important factor in increasing the rate of oxidation.

Application of elemental S in the spring at or near planting is not recommended for annual crops, because the oxidation rate is too slow to meet the crop's S requirements. Mixtures of bentonite and elemental S are available that are intended to increase the dispersion of the S particles, thus increasing the oxidation and release of SO_4.

Compared to oilseeds, wheat is generally considered to have a low metabolic demand for S, however yield increases to applied S fertilizers can be dramatic. Doyle and Cowell (1993) reviewed studies from field experiments conducted on S-deficient soils in the more humid regions of the Canadian prairies and reported yield increases ranging from 10 to 90% on soils that had never been fertilized with S and 8 to 60% on soils that had a history of S fertilization (Table 4).

Sulfur fertilization is usually most effective when applied with adequate amounts of other nutrients. For example, the application of 22 kg S ha^{-1} in a Gray soil increased wheat yields by 10% relative to the control compared to a 30% increase when applied with N and P (Table 5). Applying N and P without S resulted in a 7% yield increase. Whether with S or other nutrients, balanced fertilization is critical for the production of wheat.

Nutrients must be applied in adequate amounts and in balanced proportions according to crop needs and soil availability. Table 6 compares yields of fallow

Table 4. Average yield response of wheat to S application in Alberta soils that have never received S fertilization and soils that have received S continuously for 20 years

Location		Control	Fertilized*	Yield increase,	No. of trials
		kg ha^{-1}		%	
No previous S application					
Gray Wooded Soils†		1422	1619	14	12
Breton‡		949	1830	93	20
U of A§		2482	2731	10	8
20-year history of S application					
Breton‡	1	774	1178	52	5
	2	2059	2225	8	5
	3	1690	2737	62	5
	4	2523	3641	44	5
U of A§	1	3379	3659	8	4
	2	1999	2023	1	4

* S applied at 15 kg ha^{-1} as Na_2SO_4.
† Average total S = 123 mg kg^{-1}.
‡ Average total S = 100 mg kg^{-1}.
§ Average total S = 670 mg kg^{-1} (Doyle and Cowell 1993).

Table 5. Effect of N, P, and S fertilization on wheat yields in Alberta

Treatment	N	P_2O_5	S	Wheat yield
			kg ha^{-1}	
1	0	0	0	2310
2	0	0	22	2550
3	18	22	0	2480
4	18	22	22	3020

(Doyle and Cowell 1993)

wheat in a fallow-wheat rotation with a 3-year fallow-wheat-wheat rotation and continuous wheat in a Dark Brown soil in southern Alberta fertilized with low rates of N and P (Campbell et al. 1990). Highest wheat yields were obtained in the wheat grown on fallow in the fallow-wheat rotation and when both N and P were applied. While yields declined in the wheat grown on stubble, in all cases highest yields occurred only when both N and P were applied together.

In the above example, K was not required to balance crop nutrition because the soils in southern Alberta are rich in plant available K. In fact, most of the wheat growing soils of the northern Great Plains are well supplied with K and normally would not be expected to respond to K fertilization. Additionally, most of the K taken up by wheat is contained in the straw and in direct seeding operations where the straw is retained in the field, little K is exported with grain, further lessening the need for supplemental K fertilization. Despite this, some high K soils in the northern Great Plains do respond to fertilization with muriate of potash (KCl).

Wheat response to KCl fertilization in high K soils may be partially attributed to restricted K diffusion to plant roots when soils are cool in the early spring, but also to the chloride (Cl) contained in the potash. Numerous studies in the northern Great Plains have demonstrated that wheat responds to Cl fertilization (e.g. Fixen 1993, Lamond et al. 1999, Grant et al. 2001); however, magnitude and frequency of the response varies with cultivar and is often related to disease pressure.

Table 6. Influence of N and P fertilization in wheat grown on fallow and stubble in a Dark Brown soil in southern Alberta

Fertilizer, kg h*a*$^{-1}$		Rotation sequence, 13-yr mean yield, kg h*a*$^{-1}$			
		Wheat grown on fallow		Wheat grown on stubble	
N	P	F-W	F-W-W	F-W-W	Continuous W
0	0	2775	2332	1203	1156
0	20	2802	2641	1176	1284
45	0	2722	2460	1519	1505
45	20	3031	2654	1908	1747

(Campbell et al. 1990)

CONCLUDING COMMENTS

Understanding soil nutrient behavior and its implications for fertility management is important for maximizing nutrient use efficiency and wheat production in no-till cropping systems. Soil testing is one of the best available tools to estimate soil nutrient levels and to make appropriate fertilizer recommendations. To be most effective, soil testing must be used appropriately and in such a way as to recognize the natural variability that exists in fields. Intensive soil sampling and/or nutrient management by soil zones utilizing GPS (global positioning systems) and GIS (geographic information systems) to map and track soil testing data are useful tools that can assist farmers in the nutrient management of their wheat.

REFERENCES

Arshad MA, Schnitzer M, Angers DA, Ripmeester JA (1990) Effects of till vs. no-till on the quality of soil organic matter. Soil Biol Biochem 22:595–599

Brandt SA (1992) Zero vs. conventional tillage and their effects on crop yield and soil moisture. Can J Plant Sci 72:679–688

Campbell CA, Janzen HH, Bowren KE (1990) Crop rotation studies on the Canadian Prairies. Research Branch, Agriculture Canada. Publication 1841/E

Campbell CA, Zentner RP, Gameda S, Blomert B, Wall DD (2002) Production of annual crops on the Canadian prairies: trends during 1976–1998. Can J Soil Sci 82:45–57

Doyle PJ, Cowell LE (1993) Sulphur. In: Rennie DA, Campbell CA, Roberts TL (eds) Impact of Macronutrients on Crop Responses and Environmental Sustainability on the Canadian Prairies. Published by the Canadian Society of Soil Science, Ottawa, Ontario. Chapter 6, pp 202–250

FAO (2005) Agricultural data FAOSTAT. Statistical database, Food and Agriculture Organization of the United Nations, http://faostat.fao.org/

Fixen PE (1993) Crop responses to chloride. Adv Agron 50:107–150

Grant CA, Bailey LD (1994) The effect of tillage and KCl addition on pH, conductance, NO_3-N, P, K and Cl distribution in the soil profile. Can J Soil Sci 74:307–314

Grant CA, McLaren DL, Johnston AM (2001) Spring wheat cultivar response to potassium chloride fertilization. Better Crops 85(4):20–23

Grant CA, Johnston AM, Clayton GW (2004) Sulphur fertilizer and tillage management of canola and wheat in western Canada. Can J Plant Sci 84:453–462

Henry L, Harapiak J, Ukrainetz H, Green B (1995) Revised guidelines for safe rates of fertilizer applied with the seed. Farm Facts. Saskatchewan Agriculture and Food. ISSN 0840–9447 SCR1194

Johnston AM (2002) Fertility issues and long-term no-till. In: Proceedings of the 14th Annual Meeting, Conference and Trade Show of the Saskatchewan Soil Conservation Association, 13–14 February 2002, Regina, SK (http:// ssca.usask.ca/conference/2002proceedings/ø2-proce.html)

Johnston AM, Lafond GP, Harapiak JT, Head WK (1997) No-till spring wheat and canola response to side banded anhydrous ammonia at seeding. J Prod Agric 10:452–458

Johnston AM, Lafond GP, Hultgreen GE, Hnatowich GL (2001) Spring wheat and canola response to nitrogen placement with no-till side band openers. Can J Plant Sci 81:179–186

Johnston AM, Tanaka DL, Miller PR, Brandt SA, Nielsen DC, Lafond GP, Riveland NR (2002) Oilseed Crops for Semiarid Cropping Systems in the Northern Great Plains. Agron J 94:231–240

Lafond GP, Domitruk D, Bailey KL, Derksen DA (1996) Effects of row spacing, seeding rate and seed-placed phosphorus on wheat and barley in the Canadian prairies. Better Crops 80(4):20–22

Lafond GP, Loeppky H, Derksen DA (1992) The effects of tillage systems and crop rotations on soil water conservation, seedling establishment and crop yield. Can J Plant Sci 72:103–116

Lamond R, Martin V, Maxwell T, Bowden R, Duncan S (1999) Chloride fertilization on winter wheat. Better Crops 83(4):21–23

Malhi SS, Nyborg M (1992) Placement of urea fertilizer under zero and conventional tillage for barley. Soil and Tillage Research 23:193–197

Malhi SS, McAndrew DW, Carter MR (1992) Effect of tillage and N fertilization of a Solonetzic soil on barley production and some soil properties. Soil and Tillage Research 22:95–107

Malhi SS, Grant CA, Johnston AM, Gill KS (2001) Nitrogen fertilization management for no-till cereal production in the Canadian Great Plains: a review. Soil and Tillage Research 60:101–122

Miller PR, McConkey BG, Clayton GW, Brandt SA, Staricka JA, Johnston AM, Lafond GP, Schatz BG, Baltensperger DD, Neill KE (2002) Pulse Crop Adaptation in the Northern Great Plains. Agron J 94:261–272

Miller PR, McDonald CL, Derksen DA, Waddington J (2001) The adaptation of seven broadleaf crops to the dry semiarid prairie. Can J Plant Sci 81:29–43

Padbury G, Waltman S, Caprio J, Coen G, McGinn S, Mortensen D, Nielsen G, Sinclair R (2002) Agroecosystems and land resources of the Northern Great Plains. Agron J 94:251–261

Peterson GA, Westfall DG, Peairs FB, Sherrod L, Poss D, Gangloff W, Larson K, Thompson DL, Ahuja LR, Koch MD, Walker CB (2001) Sustainable dryland agroecosystem management. Technical Bulletin, TB01–2. Colorado State Univ. Exp. Stn., Fort Collins

Roberts TL, Harapiak JT (1997) Fertilizer management in direct seeding systems. Better Crops 81(2): 18–20

Selles F, McConkey BG, Campbell CA (1999) Distribution and forms of P under cultivator- and zero-tillage for continuous- and fallow-wheat cropping systems in the semiarid Canadian prairies. Soil and Tillage Research 51:47–59

Zentner RP, Wall DD, Nagy CN, Smith EG, Young DL, Miller PR, Campbell CA, McConkey BG, Brandt SA, Lafond GP, Johnston AM, Derksen DA (2002) Economics of Crop Diversification and Soil Tillage Opportunities in the Canadian Prairies. Agron J 94:216–230

COMPARATIVE EFFECTIVENESS OF UREA AND CALCIUM AMMONIUM NITRATE FOR WHEAT FERTILIZATION IN SOUTH-WESTERN BUENOS AIRES (ARGENTINA)

M.M. RON[1,*] AND T. LOEWY[2]

[1] *Departamento de Agronomía, Universidad Nacional del Sur (UNS). San Andrés 800. (8000) Bahía Blanca. ARGENTINA Tel.: 0291-4595126*

[2] *INTA EEA Bordenave. Ruta Prov. 76. Km 36,5. (8187) Bordenave. ARGENTINA*

[*] *E-mail: mmron@criba.edu.ar*

Abstract: The objective of this paper was to report on the relative effectiveness of urea (U – 46% N) and calcium ammonium nitrate (CAN – 27% N) for wheat fertilization in southwestern Buenos Aires Province (Argentina). Five experiments were carried out between 1996 and 1998. Treatments were N rates (0 to 45/60 kg ha^{-1}) and sources (U and CAN), applied broadcast at tillering. Crop variables studied were: crop yield (CY), protein grain concentration (CPG) and N yield (NY). Effect of sources was analyzed by comparison of crop variables for individual treatments and by the calculation of two parameters i) efficiency ratio (ER) and ii) substitution value (SV). CAN treatments gave higher values of CY and NY in 4 of the 5 experiments and of CPG in 50% of the cases. However, none of these were significant. Both ER and SV varied with experiments. ER was around 1.35, 1.25 and 0.98 for CY, NY and CPG. This means on average CY response was 35% higher for plots fertilized with CAN, with very little reduction of CPG. SV was around 1.5 for CY, i.e. U attained the same CY as CAN if U rates were increased by 50%. An advantage of using SV for CY is that direct comparisons with price ratios can be made for a simple economic analysis. CAN superiority was related not only to a greater N apparent recovery but also to increased physiological efficiency. This could be explained through the different availability in time of the two sources

Keywords: nitrate, fertilization

INTRODUCTION

Fertilization technology is aimed at increasing fertilizer efficiency and crop profitability, while reducing the impact on the environment. The choice of source is an aspect to be considered. Comparison of nitrogen sources is frequently included in

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 189–195.

crop fertilization research (Howard et al. 2002). Field experiments are the essential tool to provide a realistic evaluation of the performance of different sources. Ideally, the evaluation should be carried out on a range of climate, crop and soil characteristics. The cost of obtaining this detailed information would be prohibitive if it had to be repeated every time a new fertilizer product is put on the market. Therefore, bridging experiments are carried out in which agronomic efficiency of various sources is tested with relationship to a standard product.

Different parameters can be used for the comparisons, as explained by Rajan et al. (1996), when contrasting effectiveness of phosphate rock with hidrosoluble fertilizers. The methods have been applied to alternative techniques for wheat fertilization in the area, such as forms of phosphorus application (Ron and Loewy 2000a) and time of nitrogen application (Ron and Loewy 2000b). Nitrogen sources could be compared in the same way using urea as the standard fertilizer.

The objective of this paper was to report on the relative effectiveness of urea (U – 46% N) and calcium ammonium nitrate (CAN – 27% N), as N sources for wheat in southwestern Buenos Aires.

MATERIALS AND METHODS

The study area is situated in southwestern Buenos Aires Province (Argentina), between 500 mm and 700 mm isohyets. It has an area of about 4.5 million hectares. Wheat is the main crop grown under rainfed conditions in rotation with pastures and summer crops. Every year about 1 million hectares are sown with this cereal, rendering 20% of the total production in the country. The climate is temperate, the average yearly temperature being 15 °C. Spring and autumn are the typical rainy seasons. Soils are mostly Mollisols, main great groups being Haplustols and Argiustols in the west and Hapludols and Argiudols in the east (Zalazar Lea Plaza and Moscatelli 1989).

Five wheat experiments were carried out between 1996 and 1998. The design was of 3 complete randomized blocks. Treatments were N rates (ranging from 0 to 46 kg ha^{-1}) and sources (U and CAN), applied broadcast at tillering. In 1998 the experiment included a higher N rate (60 kg ha^{-1}) and the treatments were also applied at sowing (Table 1). The following crop variables were evaluated: crop yield (CY), protein grain concentration (PGC) and N yield (NY).

Analyses of variance were performed to study the effects of treatments. When the F-test from the ANOVA was significant for treatment effects a critical least significant difference (LSD) value was calculated for planned mean comparisons: check vs. fertilised plots and U vs. CAN, for the same rate.

Effect of sources was analyzed by comparison of crop variables for individual treatments and by the calculation of two parameters i) efficiency ratio (ER), i.e. the quotient between CAN and U agronomic efficiencies and ii) substitution value (SV), which is defined as the ratio of U and CAN rates, rendering the same response. For a continuous analysis, a linear relationship between crop variables and N rates and a constant effect of U relative to that of CAN – within the N range covered

Table 1. Characteristics of experiments

Experiment	Year	Location	Crop	Cultivar	N rates (kg ha^{-1})	Time of application
1	1996	Bordenave	Durum wheat	Buck Cristal	0-30-45	Tillering
2	1997	Bordenave	Durum wheat	Buck Cristal	0-27-46	Tillering
3	1997	17 de agosto	Durum wheat	Buck Cristal	0-27-46	Tillering
4	1997	Gascón	Bread wheat	ProINTA Federal	0-27-46	Tillering
5	1998	Bordenave	Durum wheat	Buck Topacio	0-30-60	Sowing-tillering

by the experiments - was assumed. For each experiment, ER was calculated for individual N rates and as the quotient between the slopes of linear fits for response to CAN-N and U-N. SV was estimated by combining the data for the two sources in a function of the form:

$$(1) \qquad y = b_0 + b_1(SV \times N),$$

where y: crop variable, N: N rate kg ha^{-1}, b_0 and b_1 coefficients, and SV = 1 for U. SV for CAN was estimated from the data by successive approximation as described by Colwell and Goedert (1988). Statistical significance of SV was tested as explained in a previous paper (Ron and Loewy 2000a).

RESULTS AND DISCUSSION

Crop yields reflected weather conditions in the three years: in 1996 spring rains were delayed while in 1997 precipitations were above average creating good soil moisture conditions during the growing season. Effect of treatments was significant in 4 of the 5 experiments for CY and NY, while PGC responded significantly in only 2. CAN treatments gave higher values of CY and NY in 4 of the 5 experiments and of PGC in 50% of the cases. In 1997, U was more efficient in one case, in which fertilizer was applied ten days before the other two experiments. However, none of these were significant (Table 2). Mean CY without N was 2771 kg ha^{-1} while mean yields for the CAN treatments were 3523 and 3675 kg ha^{-1}, for increasing N levels. The corresponding CY with U were 3361 and 3469 kg ha^{-1}.

Both ER and SV varied with experiments. ER was around 1.35, 1.25 and 0.98 for CY, NY and PGC. This means on average CY response was 35% higher for plots fertilized with CAN, with very little reduction of PGC. SV was around 1.5, 1.3 and 0.95 for CY, NY and PGC. Fig. 1 shows the only site and variable in which addition of SV to the linear equation was significant. In the figure U attained the same CY as CAN if U rates were increased by 50%.

Table 2. Effect of N source and rate on crop yield (kg ha^{-1}) and protein grain concentration (%) in the five experiments

N treatment	Crop yield						Protein grain concentration					
	1	2	3	4	5S	5T	1	2	3	4	5S	5T
Check	1800	2742	3014	3744	2663		12.1	9.0	8.2	12.0	9.6	
U-1	2252	3489	4091	3747	3212	3375	13.2	9.1	9.4	13.5	11.8	11.0
U-2	2271	3788	4127	4216	3268	3142	13.5	9.6	9.6	13.8	11.1	11.2
CAN-1	2336	3683	3813	4047	3674	3587	13.1	9.1	8.8	13.7	11.0	11.1
CAN-2	2575	3983	3942	4375	3511	3666	13.2	9.2	9.6	13.9	12.5	11.4
P<F	*	**	***	*	ns		ns	ns	***	*	ns	
CV (%)	13	8	9	8	21		7	5	7	5	9	
LSD (0.05)	514	481	547	575					1.1	1.2		

U: urea, CAN: calcium ammonium nitrate; 1 and 2 N levels

*, **, *** Treatments significant at the 0.05, 0.01 and 0.001 levels, respectively 5S – 5T: fertilizers applied at sowing or tillering

Constant values of ER derived from linear fits were similar to SV (Table 3). This is usually the case when application rates are comparatively low. However, ER, a vertical comparison, has a different meaning from SV, a horizontal comparison (Chien et al. 1990).

An advantage of using SV for CY is that direct comparisons with price ratios can be made for a simple economic analysis, e.g. when it equals 1.5, U is the cheaper source if CAN-N: U-N price ratio is greater than 1.5 (Fig. 2). Expressed as the commercial fertilizer this means a CAN:U price ratio of 0.87. SV values greater than 1 for NY suggest CAN was more environmentally friendly in most experiments.

The better performance of CAN relative to U has been accounted for by differences in ammonia volatilization from the two sources (Sommer and Jensen 1993) and weather and soil conditions around the time of N application (Gately 1994). The

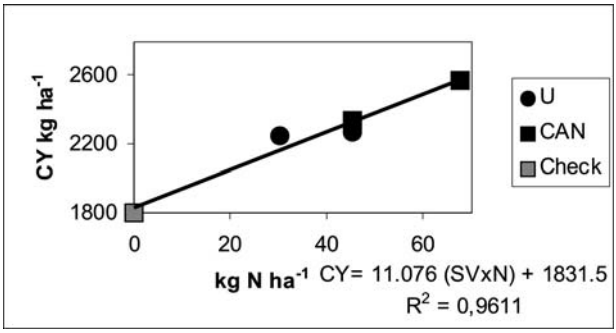


Figure 1. Mean values of crop yield for 5 treatments and linear fit (Eq. 1), Bordenave 1996. Substitution value (SV) U-N:CAN-N = 1.5

Table 3. Efficiency ratio (ER), and substitution values (SV) of CAN relative to U, calculated from linear fits for 3 crop variables in the five experiments

Expt.	Crop Yield						Protein Grain Concentration						Nitrogen Yield					
	1	2	3	4	5S	5T	1	2	3	4	5S	5T	1	2	3	4	5S	5T
ER	1.56	1.19	0.82	1.41	1.40	2.10	0.75	0.31	0.94	1.07	1.93	1.12	1.28	1.04	0.85	1.23	1.67	1.63
SV	1.50	1.26	0.76	1.50	1.90	2.40	0.75	0.44	0.82	1.10	1.50	1.10	1.25	1.09	0.77	1.30	1.80	1.80

1 and 2 N levels 5 S – 5Tfertilizers applied at sowing or tillering

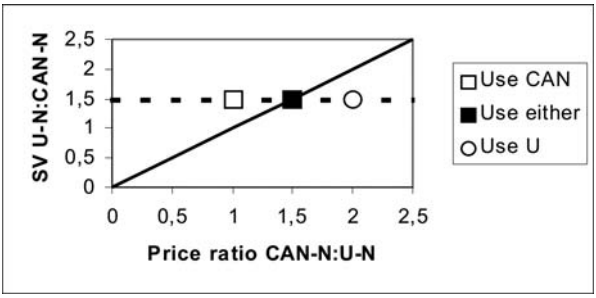


Figure 2. Decision model for choosing the more profitable N-source, SV as in Figure 1. (Adapted from Chien et al. 1990)

former cause does not seem the more relevant for winter crops under conventional tillage in the area.

In this paper SV was higher for CY than for NY in 4 experiments with fertilizers applied at tillering. This indicates CAN slight superiority was related not only to a greater N apparent recovery but also to increased physiological efficiency. This is consistent with nitrogen being more readily available from CAN immediately after application. Effect of different availability in time of the two sources may be reduced by timely rainfall (Howard et al. 2002). Differences between sources tended to be smaller when weather conditions were more favourable (1997). Moreover, U was more efficient than CAN in the site which was fertilized earlier that year. On the other hand, the only significant SV value was found for the drier year (1996).

In conclusion, N sources may determine different agronomic and physiological efficiencies for wheat in south-western Buenos Aires. Less efficient sources may be more economical if nitrogen cost is lower but this implies a different impact on the environment. An earlier application of U at tillering seems an option in order to counteract reductions of effectiveness due to a lag in nitrogen availability. This should be considered when designing comparative experiments between the sources studied in this paper.

ACKNOWLEDGEMENTS

Funds for conference registration of the first author were provided by CIMMYT and ICARDA. This work was partially supported by PROFERTIL S.A. through a Co-operation Contract for Research and Development with FUNS (FUNDACIÓN DE LA UNIVERSIDAD NACIONAL DEL SUR).

REFERENCES

Chien SH, Sale PWE, Friesen DK (1990) A discussion of the methods for comparing the relative effectiveness of phosphate fertilizers varying in solubility. Fert Res 24:149–157

Colwell JD, Goedert WJ (1988) Substitution rates as measures of the relative effectiveness of alternative phosphorus fertilizers. Nutrient Cycling in Agroecosystems 15:163–172

Gately TF (1994) A note on urea versus calcium ammonium nitrate for winter wheat. Ir J Agr Food Res 33:193–196

Howard DD, Newman MA, Essington ME, Percell WM (2002) Nitrogen fertilization of conservation-tilled wheat. I. Sources and application rates. J Plant Nutr 25:1315–1328

Rajan SSS, Watkinson JH, Sinclair AG (1996) Phosphate rocks for direct application to soils. Adv Agr 17:77–159

Ron MM, Loewy T (2000a) Effect of phosphorus placement on wheat yield and quality in south-western Buenos Aires (Argentina). Comm Soil Sci Plant Anal 31:2891–2900

Ron MM, Loewy T (2000b) Factores de eficiencia del N aplicado en trigo, a la siembra o macollaje. 17mo Congreso Argentino de la Ciencia del Suelo, Mar del Plata, Abril de 2000

Sommer SG, Jensen C (1994) Ammonia volatilization from urea and ammoniacal fertilizers surface applied to winter wheat and grassland. Nutrient Cycling in Agroecosystems 37:85–92

Zalazar Lea Plaza J, Moscatelli G (1989) Mapa de suelos de la Provincia de Buenos Aires. S.A.G. y P. - PNUD Arg. 85/019. INTA-CIRN. p 525

DYNAMICS OF ROOT DEVELOPMENT OF SPRING WHEAT GENOTYPES VARYING IN NITROGEN USE EFFICIENCY

J.M. HERRERA*, P. STAMP AND M. LIEDGENS

Institute of Plant Sciences – Swiss Federal Institute of Technology Universitatstrasse 2 – Zurich – Switzerland

**E-mail: juan.herrera@ipw.agrl.ethz.ch*

Abstract: Three spring wheat genotypes (cv. Albis and Toronit and the experimental line L94491) identified to vary in nitrogen use efficiency characteristics, were studied in lysimeters under two levels of N supply (0 and 250 Kg N ha^{-1}) in 1999 and 2000. No. of roots cm^{-2} were obtained from regular minirhizotron observations at soil depths of 0.10, 0.25, 0.45, 0.80 and 1.00 m and fitted to a logistic equation. The parameters of the logistic model were influenced by all study factors, indicating a high plasticity of the root system of spring wheat to respond to different soil conditions. A single main genotype effect was observed among all tested factors: the asymptotic no. of roots cm^{-2} was significantly higher for Toronit than Albis and especially L94491 in the topsoil (0.10 and 0.30 m). Contrastingly, the N supply modified the asymptotic growth in 1999 at 0.10 m and in both years at 0.25 m as well as the root growth pattern at 0.80 in 1999 and at 0.10 m and 0.25 m soil depth in both years

Keywords: root development, nitrogen

INTRODUCTION

Although the roots are the first and main plant organs to respond to changes in soil properties, little is known about the relevance of rooting characteristics for N uptake. Genotypic differences in N uptake were often found in wheat (e.g. Le Gouis et al. 2000). Although the roots were seldom studied in those experiments, the differences were often attributed to the root system. The time course of the root development of spring wheat genotypes, differing in N uptake or use, may be important for identifying the physiological base of these differences. Early root development has been identified to enhance N uptake in sandy soils in Mediterranean environments

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 197–201

(Liao et al. 2004). In the present study, the course of root development at low and high N supply was analyzed for spring wheat genotypes, known to vary in nitrogen use efficiency (Noulas et al. 2004).

MATERIALS AND METHODS

A lysimeter facility in the Swiss midlands (47° 26′ N, 8° 40′E) was used in 1999 and 2000. The basic lysimeter unit was a watertight, double-walled Fiberglass container. The inner surface area of the container was 1.00 m^2 and the length of the soil column 1.10 m. Each lysimeter contained minirhizotrons for the observation of roots. The minirhizotrons were 1.20 m long with an external diameter of 60 mm and were placed horizontally in the lysimeters. The Swiss spring wheat (*Triticum aestivum* L.) cultivars Toronit and Albis and the experimental line L94491 were used in the present study. Sowing dates were 15 March 1999 and 23 March 2000. The seeding rate was 420 seeds m^{-2} and sowing depth was 20 to 30 mm. Before sowing in each year, fertilizer was applied at rates of 60 kg ha^{-1} of Foskal® (7, 20, 1, 4 and 2 kg ha^{-1} P, K, Mg, Ca and S, respectively) and 20 kg N ha^{-1}, supplied as ammonium nitrate (NH_4NO_3). Half the lysimeters received no additional N (low N supply, LN), while the rest, between the beginning of tillering (BBCH stage 20–21) and flowering (BBCH stage 60) received 250 kg N ha^{-1} (high N supply, HN), split into four applications of NH_4NO_3 (90, 40, 60 and 60 kg N ha^{-1}). The experimental layout in each year was a randomized complete block design with four replications.

Shoots in the whole lysimeter were cut at ground level at physiological maturity (BBCH stage 92 or later), dried at 65 °C for 48 h and separated into grains and straw. The N concentration of the grains and the straw of the subsamples was analyzed with a LECO CHN-1000 auto analyzer (LECO Corporation, St Joseph, MI, USA) and used to estimate shoot N off take. Root images were recorded at the minirhizotron – soil interface using a special camera system (Bartz Technology Co., Santa Barbara, CA, USA). The response variable was the cumulative number of roots cm^2 (CNR), since root decay was not considered. The raw data of CNR suggested that a logistic equation (Hunt 1982) is a good approximation of the pattern observed for CNR over time. The logistic model is characterized by three parameters: MNR, the asymptotic limit of CNR, 50MNR is the time, at which 0.5 of the asymptotic value is reached, and 50/75MNR is the time lag between 50MNR and the time at which CNR reaches 0.75 of the asymptotic value. A particular advantage of this modeling approach is that the parameters can be interpreted biologically: MNR indicates the approximate magnitude of root development, while 50MNR and 50/75MNR indicate the approximate time course of root development. The CNR values available for each plot x depth combination are repeated measures over time. One approach to analyzing such data is to extract the parameters of a suitable parametric model and analyze their variance (Meredith and Stehman 1991).

RESULTS

At LN, the three genotypes had a similar shoot N off take, while at HN the N off take by Albis was the lowest (Table 1). Toronit had the highest grain yields with the lowest N concentrations, whereas L94491 had the highest grain N concentration, irrespective of the year and the level of NS (data not shown).

Table 1. Means of N uptake as influenced by genotype, year and N supply

Genotype	1999		2000	
	N supply (kg N ha^{-1})			
	20	270	20	270
	Shoot N uptake (g m^{-2})			
Albis	9.28	24.57b†	19.53	26.71b
L94491	9.44	27.83a	19.74	33.07a
Toronit	9.74	29.75a	20.16	32.03a

† Means followed by different letters are significantly different according to a pair-wise t-test ($p < 0.05$).

Table 2. Means of the parameters of the logistic equation of root development of spring wheat genotypes at different soil depths in 1999 and 2000

Soil depth (m)	Genotype	1999	2000	1999	2000
		MNR† (roots cm^{-2})		50MNR (days)	
0.10	Albis	3.41	3.78b‡	58	53
	L94491	2.57	3.25b	49	53
	Toronit	3.38	5.61a	52	54
0.25	Albis	1.97b	3.29ab	58	50
	L94491	2.14b	2.52b	54	45
	Toronit	3.47a	3.97a	58	44
0.45	Albis	1.67	3.23	71a	44
	L94491	1.26	3.35	61b	43
	Toronit	1.30	3.07	64a	43
0.80	Albis	1.49	2.90	70	50
	L94491	1.65	2.92	67	50
	Toronit	1.84	2.95	68	49
1.00	Albis	1.94	4.38	73	97
	L94491	4.41	3.69	90	110
	Toronit	2.40	3.99	81	86

† Parameters of the logistic equation: MNR, maximum CNR; 50MNR, time at which the cumulative CNR is 0.5 of the maximum. Data for 50/75MNR not shown due to lack of significant differences.

‡ Means followed by different letters are significantly different according to a pair-wise t-test ($p < 0.05$).

There was no significant interaction between genotype and NS for any of the parameters of the logistic equation used to fit CNR to days after sowing. The effect of genotype on the maximum number of roots cm^{-2} (MNR) was observed only in the top soil (0.10 and 0.25 m). Toronit produced more roots (higher MNR) than Albis and L94491 at 0.25 m in 1999 and than Albis at 0.10 m and than L94491 at 0.10 m and 0.25 m in 2000 (Table 2). However, such differences diminished in deeper soil layers. L94491 had a lower 50MNR from 0.10 to 0.80 m in 1999. Albis, in contrast, tended to have the highest values of 50MNR and 50/75MNR (data not shown) at most of the soil depths. However, a significant effect of genotype on the time course of root development was observed only in 1999 at 0.45 m (Table 2).

DISCUSSION

Albis required less N than the other two genotypes due to its lower grain yield. The lower grain yield of Albis was not associated with lower straw yields, suggesting a potential post-anthesis limitation of growth. Although, the roots of Albis tended to develop relatively late in the growing season than L94491 and Toronit, these differences were seldom significant (Table 2). The greater amount of roots of Toronit in the top soil was of no advantage for shoot N uptake compared to L94491. However, Toronit had systematically higher grain and straw yields compared to Albis and L94491. This is in line with the negative correlation between grain yield and grain N concentration often reported for wheat (Slafer et al. 1994). The root development of Toronit did not decrease at any of the studied depths (Table 2). Therefore, the lower N concentration in the grains of Toronit is probably a consequence of dilution (Slafer et al. 1994) rather than a lower ability to forage for N resources associated with weaker root development (Table 2). In conclusion, the genotypes differed in the number of roots developed at the topsoil and to a minor extent on the time course of root development but this was little related to their known N efficiency traits at the shoot level.

ABBREVIATIONS

CNR cumulative number of roots cm^{-2}
HN high N supply (270 kgN ha^{-1})
LN low N supply (20 kgN ha^{-1})
MNR, 50MNR, 50/75MNR parameters of the logistic equation
NS nitrogen supply. BBCH: Universal growth stage scale (Lancashire et al. 1991).

REFERENCES

Hunt R (1982) Plant growth curves. Edward Arnold, London, UK

Lancashire PD, Bleiholder H, Langelüddecke P, Stauss R, Van den Boom T, Weber E, Witzenberger A (1991) A uniform decimal code for growth stages of crops and weeds. Ann Appl Biol 119:561–601

Le Gouis J, Béghin D, Heumez E, Pluchard P (2000) Genetic differences for nitrogen uptake and nitrogen utilization efficiencies in winter wheat. Eur J Agron 12:163–173

Liao M, Fillery IRP, Palta JA (2004) Early vigorous growth is a major factor influencing nitrogen uptake in wheat. Funct Plant Biol 31:121–129

Meredith MP, Stehman SV (1991) Repeated measures experiments in forestry: focus on analysis of response curves. Can J Forest Res 21:957–965

Noulas Ch, Stamp P, Soldati A, Liedgens M (2004) Nitrogen Use Efficiency of Spring Wheat Genotypes under Field and Lysimeter Conditions. J Agron Crop Sci 190:111–118

Slafer GA, Satorre EH, Andrade FH (1994) Increases in grain yield in bread wheat and associated physiological changes. In: Slafer GA (ed) Genetic improvement of field crops. Marcel Dekker NY, USA. pp 1–68

SITE-SPECIFIC QUALITY MANAGEMENT IN WHEAT RESULTS FROM THE 2003 FIELD TRIALS IN ARGENTINA

R. BONGIOVANNI*, A. MÉNDEZ, W. ROBLEDO, M. BRAGACHINI, F. PROIETTI, F. SCARAMUZZA AND F. SPERANZA

National Institute of Agricultural Technology, Manfredi Experiment StationRuta 9 km 636 (5988) Manfredi, Argentina

**E-mail: rbongiovanni@correo.inta.gov.ar*

Abstract: Wheat fields in the semiarid region of Argentina are spatially variable in soil nitrogen (N) fertility and crop productivity. By accounting for spatial variation in soil N levels, variable-rate fertilizer application may improve crop yield and quality, and N use efficiency within fields. Therefore, there is interest in applying variable rates of fertilizer N across the landscape. The general objective of this research is to determine relationships among yield, protein and N rates using spatial regression analysis to yield monitor data as a means to optimize variable rate nitrogen fertilizer strategies in wheat for high protein levels in grain. The data were drawn from an on-farm N trial of 10.2 ha within a 44 ha field conducted in Manfredi, Córdoba, Argentina in 2003. The experimental design was a complete block strip trial that included two different types of soils in terms of landscape (hilltop and lowland) and two different antecessor crops (corn and soybeans). The fertilized strips were wider than the combine platform width, with zero N application as the control, and five other rates of N (12, 37, 62, 88 and 112 kg ha^{-1}). Grain samples were collected directly from the grain flow of a combine harvester, analyzed for quality in the laboratory and converted into a GIS layer, together with the yield monitor data. Yield and grain quality variability was observed across the field, and among treatments. The combination of yield maps, soil moisture and protein content can help to determine management zones in order to maximize economic benefit. This approach offers opportunities to optimize grain protein on a site-specific basis by accounting for spatial variability of N fertility within individual fields

Keywords: GIS, yield grain, yield quality

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 203–208.

INTRODUCTION

Within-field variation in grain protein of cereal crops has been observed in North America (Long et al. 2000, Engel et al. 1999), Europe (Reyns et al. 2000, Stafford 1999, Delin 2004) and Australia (Low et al. 1997, Kelly et al. 2001, Stewart et al. 2002, Norng et al. 2005).

These papers suggest that grain protein content at harvest is a useful indicator of N nutrition adequacy for wheat, once a critical level has been established. Producers in Argentina are encouraged to produce high protein wheat by an economic incentive based on protein concentration, i.e., there is a 2% price bonus for each percentage of protein above 11% (Cuniberti 2004). Protein concentration of grain is greatly influenced by the level of N fertility, which varies spatially within fields (Engel et al. 1999, Long et al. 2000). Therefore, a challenge facing growers is optimizing protein content while taking into account that variability.

Even though researchers acknowledge the presence of spatial dependence in these studies, there is no adequate spatial treatment of the problem. The approach taken in this paper is based on a spatial econometric methodology (Anselin 1988) to carry out statistical inference for the response function in the yield and protein models, in order to account for the effects of spatial autocorrelation and spatial heterogeneity in the field, with the purpose of obtaining more efficient parameter estimates.

The specific objectives of this research are: 1) to map wheat protein content in order to determine the amount of variation within the field, 2) to find the relationships between yield and quality for different growing conditions of the field, 3) to estimate the amount of N required to optimize wheat yield and protein levels, based on N rates, landscape positions, and previous crops, 4) to divide the landscape into management zones in which fertilizer is applied according to these N factors, and 5) to demonstrate the potential usefulness of the proposed strategy by comparing the wheat yield and protein levels obtained from spatially variable vs. uniform N application.

Our approach was to use grain protein concentrations as an indicator of N sufficiency vs. deficiency in wheat, and then examine whether grain protein mapping could be used as a tool to aid in making N fertilizer recommendations. The appeal of this concept is that a new protein sensor has recently become available for the Argentine market in 2006 for mapping wheat grain protein.

MATERIALS AND METHODS

N response data was collected from strip trials at a farm, located at 63° 44′ 51″ of longitude W and 31° 52′ 47″ of latitude S in Manfredi, Córdoba, Argentina, for the 2003 crop season. The experimental design for the trials was a complete block strip trial with paired control that included four management zones: two different types of soils in terms of landscape and two previous crops (CH=Corn, Hilltop, SH=Soybean, Hilltop, CL=Corn, Lowland, and SL=Soybean, Lowland). The strips were wider than the platform width, with zero N application used as the control,

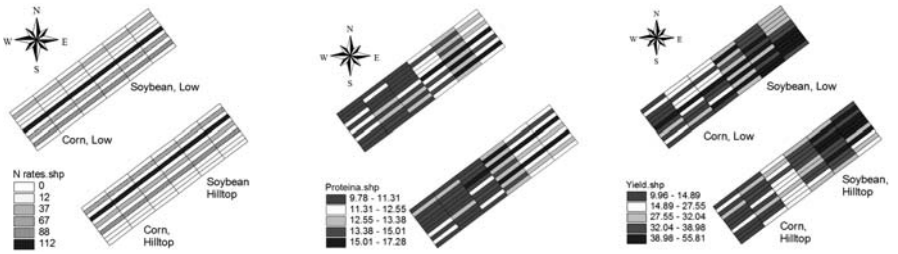


Figure 1. Experimental design. N rates (left), protein content (center) and yields (right)

and five other rates of N: 12, 37, 67, 88 and 112 kg ha^{-1}. The N rates were constant in each strip (Fig. 1). The source for N was Urea Ammonium Nitrate (UAN). All treatments included a previous uniform fertilizer rate of Monoammonium phosphate (MAP) and of Ammonium Thiosulphate (Sol Plus) in order to avoid other yield limiting factors.

Wheat yield was measured with a combine equipped with a yield monitor with GPS. Grain yield was measured in one-second intervals of time, and at the same time, grain was manually sampled in 90-m intervals of distance, from the outlet of the auger that delivers grain into the combine's bin. The combine's GPS receiver was used to georeference the location of each sample. Grain samples were analyzed in a laboratory for protein concentration (Kjehldahl-method).

In order to obtain a balanced design, the original data yield points (2654 observations) were spatially averaged. This was executed in the software SSToolbox™, creating 14 by 90 meter grids over the observations to match the protein observations. Finally, and after averaging the data within each grid, a layout of 132 regular polygons was obtained. A Spatial Error model (Anselin 1988) by management zones was used to estimate the relationships among yield, protein and N rates.

The objective of the optimization problem was to find the economically optimal N rates (EONR's) that maximize profit by management zone, as a function of yield and protein, considering a 2% premium or penalty if protein level is above or below 11%:

$$\underset{N}{Max}\ \text{Net Returns (N)} = \underset{N}{Max}\begin{bmatrix}(\text{Price}\times 0.98)\times \text{E[Yield(N)]if E[Yield(N)]} < 11\% \\ (\text{Price}\times 1.02)\times \text{E[Yield(N)]if E[Yield(N)]} > 11\%\end{bmatrix}$$

RESULTS AND DISCUSSION

Visual symptoms of N deficiency were clearly apparent between the control and treatments receiving N fertilizer. Absolute grain yield vs. protein response curves illustrate the diversity of yield and protein levels achieved in this study (Fig. 2).

There was a quadratic correlation ($R^2 = 0.81$) between grain yield (Y) and protein concentration (P) only for high yields in the zones where soybean was the previous crop; whereas there was a direct, linear relationship in the zones with corn

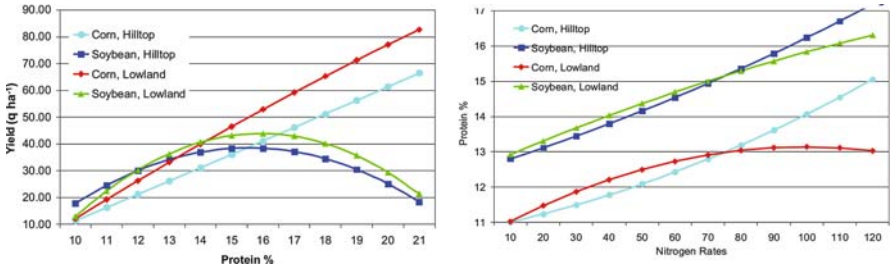


Figure 2. Absolute grain yield vs. protein (left) and grain protein content vs. nitrogen rates (right) relationships for wheat by management zones

as the antecessor. The different shape of the curves by previous crops shows that when soybean was the antecessor, there was more available nitrogen to the plant (between 50 and 60 kg ha^{-1} more) than with corn. The estimated functions were: $Y_{CH} = -36.83057 + 4.71819P + 0.00957P^2$; $Y_{SH} = -124.97525 + 21.04068P - 0.67681P^2$; $Y_{CL} = -68.52581 + 8.83020\ P - 0.07755P^2$; $Y_{SL} = -179.692370 + 28.05901P - 0.88027P^2$. In this model the intercept, the linear and the quadratic terms were significant at the 1% significance level, although the management zones and their interaction terms proved to be not significant at standard significance levels.

The relationship between protein and nitrogen rates was also established (Fig. 2, right). The quadratic polynomial type-function provided a good fit of grain protein vs. N rates relationships. The estimated functions were: $P_{CH} = 10.80967 + 0.01867N + 0.00014N^2$; $P_{SH} = 12.50519 + 0.02886N + 0.00008N^2$; $P_{CL} = 10.52919 + 0.05264N - 0.00026N^2$; $P_{SL} = 12.52247 + 0.04083N - 0.00008N^2$.

The intercept, the linear and quadratic terms, and the management zones where significant at the 1% significance level, although the interactions of N and N^2 with management zones where not significant. The highest protein levels were associated with the highest N rates and vice versa ($R^2 = 0.94$). The protein content was highest for the management zones that had soybean as the previous crop, which provided 75 kg ha^{-1} more N to the soil than corn in the Hilltop and 9 kg ha^{-1} more N in the Lowland (at a 3-meter depth). This further indicated that the concentration of protein in grain is related to the content of N in the soil.

In general, the quadratic model appears to better describe protein vs. N relationships where water was available (Lowland). In the Hilltop, where growth was most affected by the lack of water during the grain-fill period, the signs of the N^2 coefficient estimates are positive, leading to a corner solution. The response curve is not well-behaved in the Hilltop, due to low soil water availability.

Within-field variations in protein are due to both differences in yield and plant-available soil nitrogen. When nitrogen is limiting for both yield and protein, protein and yield are likely to be positively correlated. When nitrogen only limits protein, protein and yield are instead negatively correlated. In either case fertilization demand is higher in areas with low protein. A positive correlation, however,

indicates that fertilization was below optimum at most sites, whereas a negative correlation indicates that fertilization was near or above optimum at most sites.

The estimated coefficients of grain yield (Y) response to N ($R^2 = 0.85$) were: $Y_{CH} = 15.310074 + 0.124517N + 0.000421N^2$; $Y_{SH} = 32.573887 + 0.131355N - 0.000595N^2$; $Y_{CL} = 15.673268 + 0.233808N - 0.000229N^2$; $Y_{SL} = 33.912811 + 0.163505N - 0.000515N^2$. In this model the intercept, the linear term and the management zones were significant at the 1% significance level, although the quadratic and their interaction terms proved to be not significant at standard significance levels. This is probably due to the fact that the maximum physical response was not achieved at the used rates in the lowland areas.

The optimization problem described above achieved corner solutions when the EONR's where estimated as a function of yield and protein. Therefore, the choice was for the EONR's that optimized wheat yields: 0, 61, 112 and 112 kg ha^{-1} for Hilltop Corn; Hilltop Soybean; Low Corn; and Low Soybean, respectively. Net price of wheat used was 36.08 \$ q^{-1} (including a 3% trade cost) and the net price of N from urea was 2.10 \$ kg^{-1} (with a 15% interest rate for six months).

CONCLUSIONS

The spatial auto-regression results show that the relationship between protein and yield was statistically significant, which implies that protein sampling is economically feasible and that management decisions can be made using these relationships.

One potential use of grain quality information in Argentina would be to identify protein, oil content and other characteristics in the field, and use that information to harvest selectively and route loads to different markets. Producers under contracts could harvest strips through several fields to find wheat that would satisfy the protein requirements of a contract and thereby avoid the substantial penalties for delivering low protein product (Cuniberti 2004). In the longer run, it is possible to identify landscape areas that consistently produce grain with specific characteristics (e.g., higher protein on hilltops) and harvesting those separately. Another use of the grain quality information would be to identify nitrogen deficient and excess areas. Grain protein is often highly correlated with the N status of the plant. Low grain protein is often a good indicator of inadequate nitrogen. Grain protein could be one layer in the crop GIS used to generate N management strategies.

The Zeltex AccuHarvest On-Combine Grain Analyzer was recently introduced to Argentina and is installed on a combine used by INTA. This technology opens new opportunities for farmers in Argentina, because the protein map obtained in this study will be done with a semi-automated device. Nevertheless, protein collection will still be costly in the near future. Market price in Argentina is USD 17,000. Despite this high price, results from this study suggest that in the longer term, gains can be made from the collection of protein data which would lead to the improvement of management decisions.

The wheat data showed that the protein/yield relationship exists and is consistent to within 90 m, which would allow farm managers to make variable-rate

N management decisions. However, before investing in on-the-go grain quality monitors, the potential benefits will have to be weighed against the input costs. This study has shown that the protein-yield relationship was significant given that there is enough variability in the original yield and protein data.

REFERENCES

Anselin L (1988) Spatial Econometrics: Methods and Models. Kluwer Academic Publishers, Dordrecht, Netherlands

Cuniberti M (2004) Nuevo estándar de comercialización de trigo. INTA Marcos Juárez, Feb/2004

Delin S (2004) Within-field variations in grain protein content – relationships to yield and soil nitrogen and consistency in maps between years. Precis Agr 5:565–577

Engel RE, Long DS, Carlson GR, Meirer C (1999) Method for precision N management in spring wheat: I. Fundamental relationships. Precis Agr 1(3):327–338

Kelly RM, Strong WM, Jensen T, Butler D (2001) Strategies to interpret the yield map: defining yield-limiting factors. In: Proceedings of the 10th Australian Agronomy Conference, Hobart. Australian Society of Agronomy. http://www.regional.org.au/au/asa/2001

Long DS, Engel RE, Carlson GR (2000) Method for precision nitrogen management in spring wheat: II. Implementation. Precis Agr 2(1):25–38

Low R, Moore G, Nicolas M, Williams B (1997) An Investigation of Barley Grain Protein Variation. In: McBratney AB, Whelan BM (eds) Proceedings of the 2nd Australian Symposium on Precision Agriculture. Australian Centre for Precision Agriculture, Sydney

Norng S, Pettitt AN, Butler D, Strong WM (2005) Investigating the relationship between site-specific yield and protein of cereal crops. Precis Agr 6:41–51

Reyns P, Spaepen P, De Baerdemaeker J (2000) Site-specific relationship between grain quality and yield. Precis Agr 2(3):231–246

Stafford JV (1999) An investigation into the within-field spatial variability of grain quality. In: Stafford JV (ed) Precision Agriculture '99: Proceedings of the 2nd European Conference on Precision Agriculture. Sheffeld Academic Press, Sheffeld, UK, pp 353–361

Stewart CM, McBratney AB, Skerrit JH (2002) Site-specific durum wheat quality and its relationship to soil properties in a single field in northern New South Wales. Precis Agr 3:155–168

CORN AND SOYBEAN RESIDUE COVERS EFFECTS ON WHEAT PRODUCTIVITY UNDER NO-TILLAGE PRACTICES

M. BARRACO[1,*], M. DÍAZ-ZORITA[2] AND G. DUARTE[3]

[1]*General Villegas Experimental Station, INTA, CC 153, (6230) General Villegas, Buenos Aires, Argentina*
[2]*CONICET and University of Buenos Aires, Cátedra de Cereales, Av. San Martín 4453, (1417) Capital Federal, Argentina*
[3]*DZD & Asoc., M. Cachau s/n, (6237) América, Buenos Aires, Argentina*
[*]*E-mail: mbarraco@correo.inta.gov.ar*

Abstract: Wheat (*Triticum aestivum* L.) grain yields under no-till production systems have been shown to be reduced in the presence of maize (*Zea mays* L.) residues. It has been suggested that sowing a greater density of wheat seeds or removing maize residues from the planting rows contributes to avoid this problem. However, the causal factors and the mechanism that produce reductions in wheat yields are no clearly defined. Our objective was to determine the effects of different volumes of maize or soybean [*Glycine max* (L.) Merrill] residues on no-tillage wheat establishment and production under field conditions on a Typic Hapludoll from the Pampas region of Argentina. The study was performed during the 2002, the 2003 and the 2004 growing seasons. Two treatments [residue volume (0, 4, 8 and 16 Mg ha^{-1}) and crop residue (maize and soybean)] were imposed after sowing wheat at low and high plant densities, 301 and 396 seed ha^{-1}, respectively). The previous crop was sunflower (*Helianthus annus* L.) and the residues were applied on the soil surface immediately after planting and fertilizing with 125 kg ha^{-1} of Nitrogen. Independently of the quality of the residues and the sowing density, wheat plants m^{-2}, spikes m^{-2} and grain yields ha^{-1} decreased when residue volume increased. In general, lower soil temperatures values and variability were observed when increasing the volume of residues. The presence of large amounts of maize or soybean residues causes the reduction in no-tillage wheat productivity (plant stand and numbers of spikes). However, only maize residues causes significant reductions in grain yields, independently of the seeding rate. The absence of significant differences in soil temperature measurements between residues allows us to think that the effects on surface soil temperature are not the main factor explaining the reduction in wheat grain yields in the presence of maize residues. Increasing the seeding rate can contribute to ameliorate the grain yield reduction in the presence of maize residues but further research is required for explaining the reasons for the behavior of the crop

Keywords: no-tillage practice, grain yields, corn and soybean residue

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 209–216.

INTRODUCTION

Wheat grain yield reductions are often described in crops when planted after maize crops under no-till production practices. For example in the 2001 growing season, and under extensive production systems in the north west region of the Buenos Aires province (Argentina), Satorre (pers. comm.) described that mean wheat yields after soybean crops was 2952 kg ha^{-1} while it was 2357 kg ha^{-1} when the crop followed maize. Similar results were obtained in the sandy pampas region (Argentina) with mean yields of 5301 and 4750 kg ha^{-1} for wheat crops following soybean and maize crops, respectively (Díaz-Zorita, unpublished). The negative effects of the presence of maize residues on wheat yields are generally justified because of its volume and spatial distribution (Staricka et al. 1991), and also in response of the color of the residues and their effects on soil temperatures. In the presence of residue cover, the amplitude in soil temperature is lesser than in a soil without them (Kiger and Grove 1999). Because of a high C:N ratio, maize residues have a slow decomposition rate compared with other crops such as soybean. This effect can immobilize a relevant amount of N reducing its availability for wheat crops seeded after maize crops.

The origin of the wheat yield reduction could be due to interferences during seeding, changes in soil temperature or in other properties during the decomposition of maize roots and surface residues. However, the available information is not enough to discriminate between the physical interference of maize residues during the seeding operation and other effects related with its presence during the crop growth and yield.

Our hypothesis is that high amounts of crop residues, independently of its composition (i.e. maize or soybeans) result in combined factors (i.e. soil temperature and other physical restrictions) during seeding and growing wheat stages that interfere with the normal crop growth.

The objective of this study was to determine the effects of similar levels of maize or soybean residues on wheat establishment and grain yields when applied after seeding the crops in the absence of residues under no-till practices.

MATERIALS AND METHODS

The study was performed at the INTA General Villegas Experimental Station (Drabble, Buenos Aires, Argentina) during the 2002 to 2004 growing seasons on a Typic Hapludoll [Soil organic matter: 28.8 ± 1.1 g kg^{-1}, soil extractable P (Bray Kurtz 1): 18.3 ± 2.0 mg kg^{-1} and water pH: $6.0 \pm 0, 1$] under continuos no-till practices since 1997. In each season, and with the purpose of avoiding soil effects related with the decomposition of the roots of the previous crop, wheat crops (var. Klein Escorpion in 2002 and 2003, var. Buck Guapo in 2004) were sowed following sunflower (*Helianthus annus* L.) and in the absence of aboveground residues capable of interfiring with the seeding operation. Two wheat seeding rates were used (low rate: 301 seeds m^{-2} and high rate: 396 seeds m^{-2}). Fungicide treated seeds was always utilized. After seeding, 2 main treatments were applied on the surface of the

soils: (a) residue type (maize or soybean) and (b) amount of residue (0, 4, 8 and 16 Mg ha^{-1}). In each season, the residues were collected after the harvest of the crops (fall) and cut in small uniform pieces of approximately 20 mm long (MTD Yard Machines® model 135212).

At seeding (last week of June), and before the surface application of the residues, 125 kg ha^{-1} of N (urea) were broadcasted. At the beginning of tillering (August), all the treatments were fertilized with 250 kg ha^{-1} of ammoniun sulphate.

Crop plant stands counts were performed 15 days after crop emergence based on a 2 m^2 sampling area within each plot. At physiological maturity, grain production and yield components (single grain weight, number of grains and number of spikes) were determined by manual harvesting of in 4 m^2 in each plot.

During the 2002 and 2003 growing seasons soil temperature (0 to 5 cm depth) at 9:00 AM and 4:00 PM was registered during the first week after seeding (July), during tillering (September) and at flowering (October). In each treatment, the daily mean soil temperature was calculated from the average value between the 9:00 AM and the 4:00 PM measurements. The relative mean soil temperature (RMST) was calculated from the difference between the mean soil temperature of each treatment (ST_i) and the untreated control (ST_0) relative to ST_0,

$$\text{RMST } (\%) = (ST_i - ST_0)\ {ST_0}^{-1} \times 100$$

The thermal amplitude was calculated based on the absolute soil temperature difference between the 4:00 PM and the 9:00 AM measurements. The relative thermal amplitude in soil temperature (RSTA) was calculated from the difference between the daily soil thermal amplitude of each treatment (STA_i) and the untreated control (STA_0) relative to STA_0,

$$\text{RSTA } (\%) = (STA_i - STA_0)\ {STA_0}^{-1} \times 100$$

In each seeding rate, the treatments were located based on a randomized complete block design with 10 m^2 plots. The results were analyzed using ANOVA, LSD means differences test ($p < 0.05$) and regression analysis procedures (Analytical Software 2000).

RESULTS AND DISCUSSION

Plant Stand

Independently of the wheat seeding rates, both the amount and the type of residue covers modified the established plant stands (Table 1). In the 2002 growing season, the number of plants was strongly and negatively related with the amount of surface residues ($r^2 = 0.98$) for both types (maize or soybean). However, maize residues reduced more the crop establishment than soybean residues did, 15.7 to 10.5 and 10.7 to 8.4 plants m^{-2} (Mg $ha^{-1})^{-1}$, for low and high seeding rates, respectively. Increasing the seeding rates allowed to obtain a similar quantity of established

Table 1. Wheat plant stand per square meter 15 days after emergence depending of seeding rates, residue cover type and amount. In each season, seeding rate and residue type (maize or soybean), different letters show significant differences between residue cover amounts ($p < 0.05$)

Growing season	Residues (Mg ha^{-1})	Wheat seeding rate (plants m^{-2})			
		301		396	
		Maize	Soybean	Maize	Soybean
2002	0	264 a		386 a	
	4	206 ab	217 ab	295 b	320 b
	8	177 bc	184 ab	277 b	283 b
	16	91 d	126 cd	123 d	208 c
2003	0	194 a		228 ab	
	4	157 ab	192 a	216 ab	238 a
	8	142 b	166 ab	180 bc	200 ab
	16	147 b	154 ab	145 c	182 bc
2004	0	211 ab		232 a	
	4	205 ab	205 ab	222 a	245 a
	8	199 ab	184 ab	198 a	249 a
	16	142 b	177 ab	184 a	251 a

plants in the presence of 4 and 8 Mg ha^{-1} compared with the control without surface residues for the low seeding rate treatments. In the 2003 growing season, although the establishment of the crop was reduced in the presence of surface residues, the relationship was significant for the high seeding rate ($r^2 = 0.97$ and $r^2 = 0.80$ in the presence of maize and soybean surface residues, respectively). Maize surface residues reduced more the wheat establishment than soybean residues at a rate of 2.6 to 0.2 and 5.4 to 3.3 plants m^{-2} (Mg ha^{-1})$^{-1}$, for the low and the high seeding rates, respectively. Increasing the seeding rate allowed to obtain a similar crop establishment than the one observed in the control without residues for all the treatments with the exception of the 16 Mg ha^{-1} of surface maize residues. In the 2004 growing season, it was observed a significant plant stand reduction when increasing the maize and soybean residue cover in the low seeding rates treatments, 4.4 and 2.5 plants m^{-2} (Mg ha^{-1})$^{-1}$, respectively. In the high seeding rates, only the presence of maize residues reduced the establishment of the wheat crops with 3.1 plants m^{-2} (Mg ha^{-1})$^{-1}$.

Grain Production and Yield Components

The number of spikes per square meter varied between 316 to 495 and 351 to 565 for the low and the high seeding rates, respectively (data not shown). In the 2002 growing season, only in the treatments with low seeding rates and in the presence of 16 Mg ha^{-1} of maize residues, the number of spikes was lower than in the control treatment without residue cover. In the following season, the same effect

was observed also in the presence of 8 Mg ha^{-1} of residues. However, in the 2004 growing season, there were no differences in the number of spikes between treatments and furthermore, in the presence of the higher residue cover with soybeans this yield component was greater than the control.

In the 3 growing seasons, there were not significant differences in single grain weights among treatments (data not shown). Only in the 2003 growing season less number of grains per square meter was observed in the presence of 8 and 16 Mg ha^{-1} of residues, independently of their type (maize or soybean) and seeding rate.

Wheat yields varied between 2437 to 4885 and 2813 to 4985 kg ha^{-1} for the low and the high seeding rates, respectively (Table 2). In the 2002 growing season, lower yields were observed in the presence of 16 Mg ha^{-1} of maize residue cover in the low seeding treatments. A similar trend was described for the high seeding treatments but the differences were not significant ($p < 0.05$). Increasing the amount of maize residue cover reduced grain yields at rates of 41.0 and 30.6 kg (Mg residues)$^{-1}$ for the low and the high seeding rates, respectively. The available information was not enough for describing differences in yields due to the presence of soybean residues. In the 2003 growing season, and in the treatments with low seeding rate, the presence of 8 and 16 Mg ha^{-1} of maize residues reduced the grain yields. A similar trend was observed for the high seeding rate treatments, but with no significant differences ($p < 0.05$). Increasing the maize residue cover reduced wheat yields at rates of 61.0 and 22.0 kg (Mg residues)$^{-1}$ for the low and the high seeding rates, respectively. In the case of soybean residues, the mean yield reduction rate was of 39.0 kg (Mg residue)$^{-1}$ for the low seeding rate. No significant effects of the presence of

Table 2. Wheat grain yield (kg ha^{-1}) depending of seeding rates, residue cover type and amount. In each season, seeding rate and residue type (maize or soybean), different letters show significant differences between residue cover amounts ($p < 0.05$)

Growing season	Residues (Mg ha^{-1})	Wheat seeding rate (plants m^{-2})			
		396		396	
		Maize	Soybean	Maize	Soybean
2002	0	3243 a		3329 a	
	4	2703 ab	2883 ab	3417 a	3040 a
	8	3020 ab	2983 ab	2813 a	3213 a
	16	2437 b	3087 ab	2940 a	3405 a
2003	0	4558 a		4017 a	
	4	4317 ab	4885 a	3905 a	3245 a
	8	3655 b	4022 ab	3329 a	4065 a
	16	3624 b	4120 ab	3700 a	3672 a
2004	0	4084 ab		4469 ab	
	4	3967 ab	3955 ab	4459 ab	4412 ab
	8	4296 ab	4471 ab	4018 bc	4354 ab
	16	3801 b	4751 a	3503 c	4985 a

soybean residues on wheat yields for the high seeding rates was observed. In the 2004 growing season, only the presence of 16 Mg ha^{-1} of maize residues reduced the grain yields for both seeding rates. When increasing the maize residue cover, the yields decreased at rates of 14.0 and 65.0 kg (Mg residue)$^{-1}$ for the low and the high seeding rates, respectively. Although the number of spikes per plant was greater when increasing the amount of residue cover (data not shown), it was not enough for compensating the plant stand reduction described in the presence of high amounts of residues.

Taking into account that all the residues were broadcasted after seeding the crops, we can conclude that the described wheat yield reduction in presence of maize residues is due to its presence during the growing season and because of the characteristics of the residue and its effect on the crop and soil environment. In this study, we applied high N fertilization levels above the requirements of the crops and nutrient deficiency symptoms were not detected in the crops. Thus, we assume that possible effects of residue decomposition on soil mineralization process could be descharged. Increasing the seeding rate could be a crop management practice to reduce the negative effect of high amount of residues on wheat yields. However, the available information is still inconclusive and further studies are required.

Soil Temperature

The large amount of residues cover reduced the mean soil temperatures during tillering (only 2002 growing season), and also at flowering (Figs. 1 and 2). However, there were not significant differences in soil temperature value during the early

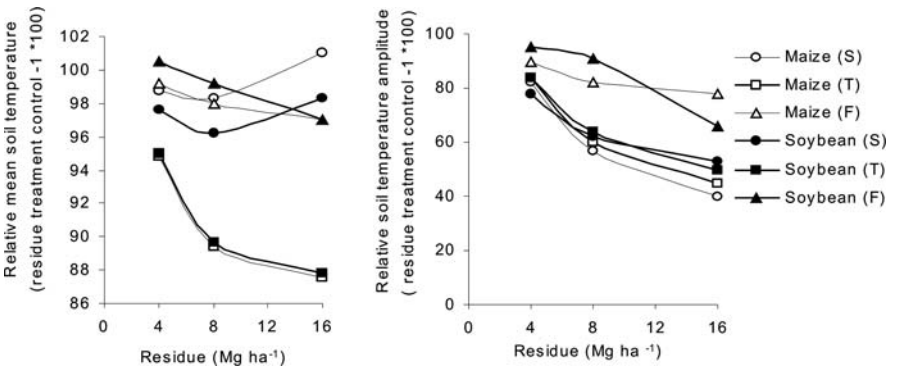


Figure 1. Effects of three amounts of maize and soybean residues on relative mean soil temperature (0 to 5 cm depth) and temperature amplitude during the first week after seeding (S), during tillering (T) and at flowering (F) of no-till wheat crops. The mean soil temperature in the treatments without residue cover was of 8, 0°C, 12, 9°C and 18, 8°C for S, T and F, respectively. Mean of 2 seeding rates in the 2002 growing season

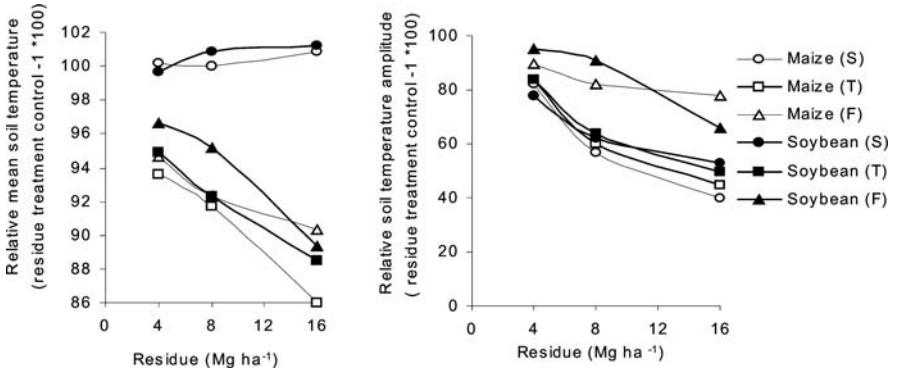


Figure 2. Effects of three amounts of maize and soybean residues on relative mean soil temperature (0 to 5 cm depth) and temperature amplitude during the first week after seeding (S), during tillering (T) and at flowering (F) of no-till wheat crops. The mean soil temperature in the treatments without residue cover was of 6, 0°C, 15, 7°C and 20, 7°C for S, T and F, respectively. Mean of 2 seeding rates in the 2003 growing season

vegetative growth of the crops. The presence of residues reduced the soil temperature amplitude when compared with the control without residues, mainly during the establishment and tillering growing stages of the crops. The greater effects on soil temperature due to increasing the residue cover were more relevant in the presence of maize residues than when soybean residues were applied (Figs. 1 and 2). These observations suggest that changes in soil surface temperature in the presence of different levels of residue cover could not be the main environmental factor explaining the described changes in plant stand and grain yields. However, further research is required to understand the potential effects of the residues on the duration of below freezing temperatures during winter and their effect on plant survival and growth.

CONCLUSIONS

We conclude that high amounts of maize or soybean residue cover reduces plant establishment and the number of spikes produced for no-till wheat crops. However, only the presence of maize residues significantly reduces the grain yields. The practice of increasing the seeding rate can contribute to ameliorate this reduction.

The major effects on the crops were described during its establishment stages reducing the plant stand and tillering. However, we can not explain the differences due to soil temperature changes during the growth of the crop. Further research is required for adjusting the contribution of crop seeding rates and also for describing potential changes in temperature, mainly freezing duration, within the residue layer.

REFERENCES

Analytical Software (2000) Statistix 7. User's manual. Analytical Software, Tallahassee, FL. USA. p 359

Kiger CE, Grove JH (1999) Corn residue placement influence on no-tillage winter wheat development. Agron Abstr 91:290

Starikca JA, Allmaras RR, Nelson NW (1991) Spatial variation of crop residue incorporated by tillage. Soil Sci Soc Am J 55:1668–1674

GENETIC ASSESSMENT OF THE ROLE OF BREEDING WHEAT FOR ORGANIC SYSTEMS

K. MURPHY AND S.S. JONES*
Washington State University
**E-mail: joness@wsu.edu*

Abstract: Organic farming as an agricultural system includes substantially different growing methods and guidelines than conventional farming systems. Plant breeding programs typically breed and select varieties under a farming system that includes the use of agronomic practices not permitted under organic farming standards. One objective of our research is to quantify the overall differences in yield of winter wheat when grown under two contrasting systems, organic and conventional. Thirty-five advanced breeding lines were evaluated in five locations in Washington State in both organic and conventional plant breeding nurseries. These breeding lines showed a statistically significant difference in change of rank for yield and genotype by system interactions between the two systems at four of five locations. Another objective of our research is to evaluate historical and modern wheat varieties under organic conditions to identify traits that confer adaptation and optimal yields in organic conditions. Analysis of variance shows no significant difference in the top yielding historical varieties and the modern varieties when grown in organic conditions, indicating that traits important to yield in organic systems can be found in historical wheat varieties that were selected under low input conditions. These results support the argument for the need to select varieties for organic agriculture in an organic production system in order to optimize yield potential

Keywords: organic systems, breeding program, grain yield

INTRODUCTION

Organic farming requires a cropping system approach that differs significantly from conventional farming. Differences in soil biology, weed management, nutrient management and disease control all combine to alter the growing environment of wheat. Plant breeders selecting varieties for organic farmers must determine if the best varieties in a conventional system are also the best varieties in an organic

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 217–222.

system. If the best varieties are similar in both farming systems, then selection of varieties for organic agriculture can occur reliably within a conventional system. If the best varieties are significantly different, then selection of varieties for organic agriculture should occur within organic management systems.

Although there are no direct comparisons of organic and conventional plant breeding systems, evidence from other cropping systems points to a need for a separate breeding program to develop varieties that do particularly well under organic management. Several studies have shown that breeding for low input agriculture would be most efficient if selection is conducted in low input environments (Atlin and Frey 1990, Smith et al. 1990, Simmonds 1991, Ceccarelli 1994). These studies suggest that the best varieties for low input agriculture are not the same varieties that are the best in high input conditions.

O'Leary and Smith (1999) suggest that a monoculture cropping system is not an optimal environment in which to select corn types for clover/bean intercropping systems. O'Leary and Smith (1999) found a statistically significant interaction of varieties in different systems, suggesting both a genotype X cropping system (G X S) interaction and a change in the ranking of the yields. This indicates that selection in monoculture will not necessarily identify cultivars or breeding lines best suited to an intercropped system. Other studies however, have concluded that the evaluation of genotypes under mono-cropping provides sufficient information to select varieties efficiently for intercropping systems and therefore there is no need to maintain separate breeding programs for intercropping or double cropping (Panter and Allen 1989, Santalla et al. 2001).

Several studies have shown that no significant difference exist among cultivar performance grown in different systems, including tillage systems (Rao and Dao 1994, Weisz and Bowman 1999, Carr et al. 2003) and nitrogen input level systems (Hasegawa 2003). These studies indicate that different farming systems do not require separate breeding programs with different objectives and selection methods. To optimize breeding for salinity-stressed environments, however, Kelman and Qualset (1991) suggest that selection in low salinity environments would produce cultivars with high yield potential for environments with moderate salinity stress. The conclusions reached by the authors of these studies demonstrate the complexity of genotype X system interactions and indicate that individual systems should be evaluated to determine the selection benefits of maintaining separate breeding programs.

The objective of our current research is to determine 1) the need for an organic breeding program for wheat and; 2) the traits responsible for increasing yield of wheat varieties in organic systems.

MATERIALS AND METHODS

Experiment 1 – System by Genotype Interaction

The experiment was a split-block design, with organic and conventional systems as the whole plot factor and 35 wheat genotypes as the subplot factor. Thirty

advanced (F6–F8) soft white winter wheat breeding lines and five commonly grown control varieties were planted in five locations (site/years) in Washington State. Each location consisted of two nurseries, one conventional and one organic, and each nursery contained 35 genotypes replicated four times in a randomized complete block design. The organic nursery was fertilized with PerfectBlend 4-4-4 organic fertilizer at the rate of 7.3 kg ha^{-1}, drilled with the seed at planting. The conventional nursery was fertilized with 16.3 kg ha^{-1} of N, 3.6 kg ha^{-1} of Phosphate and 2.7 kg ha^{-1} of Sulfur and incorporated into the soil within a week of planting. Plots consisted of seven rows, 2.5 m long and 1.25 m wide.

Data were analyzed using analysis of variance software PROC GLM (SAS Institute, Cary, NC). Levene's test was used to test for homogeneity of variance for both environment and system and normality was checked using PROC Univariate (SAS Institute, Cary NC).

Experiment 2 – Yield Evaluation of Historical Varieties

Fifty-six historical and seven modern spring wheat varieties were grown under low-input conditions in two locations in Pullman, WA in a randomized complete block design with three replications in one nursery and four replications in the second nursery. The nursery was fertilized with Perfect Blend 4-4-4 organic fertilizer at the rate of 7.3 kg ha^{-1}, drilled with the seed at planting. Weed control was conducted using a rotary harrow. No herbicides or hand weeding were used for weed control.

RESULTS AND DISCUSSION

Analysis of variance shows highly significant ($p < 0.01$) genotype X system interactions for winter wheat yield in four of the five locations, suggesting that the highest yielding varieties in a conventional farming system are not the highest yielding varieties in an organic farming system. Yield is an excellent general indicator of the important interactions of many different genetic and environmental factors and can be used as an appropriate measurement of genotypic response to system-specific factors. Fig. 1 shows the rankings for yield in organic vs. conventional systems in Douglas County, WA and Fig. 2 shows the rankings for yield in organic vs. conventional systems in Whitman County, WA.

Fig. 3 shows the yield (g $plot^{-1}$) of spring wheat varieties in low-input conditions that were widely grown in the Pacific Northwest region of the US from the 1860's to present. The results show no significant difference between the highest yielding several historical varieties and the seven modern varieties. This indicates that some historical varieties contain traits that confer adaptedness and potential for improved yield to organic conditions. These traits include nitrogen-use efficiency, disease resistance, weed competitiveness and weed tolerance. Evaluation of these traits in organic conditions and incorporating these traits into modern varieties through selection in organic farming systems will be a critical step in developing cultivars suited to organic agriculture.

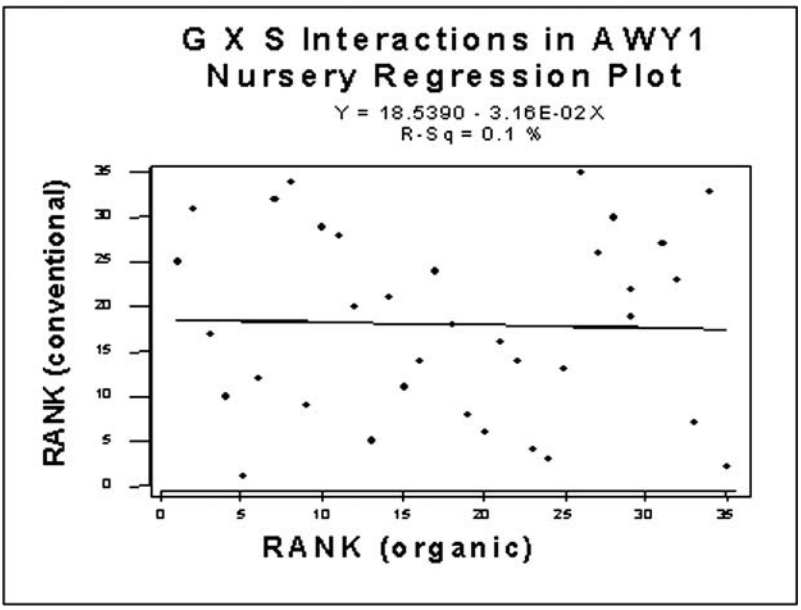


Figure 1. Regression plot of wheat genotype ranking between two systems, organic and conventional, for 35 genotypes in Douglas County

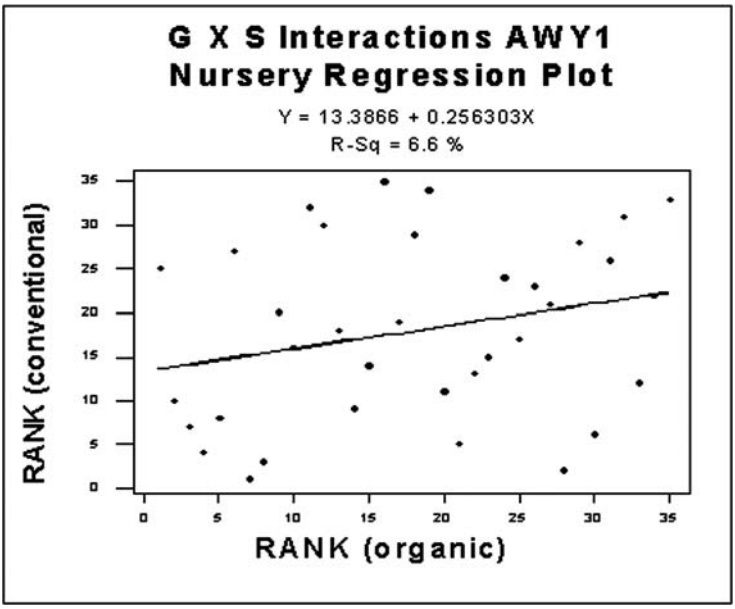


Figure 2. Regression plot of wheat genotype ranking between two systems, organic and conventional, for 35 genotypes in Pullman

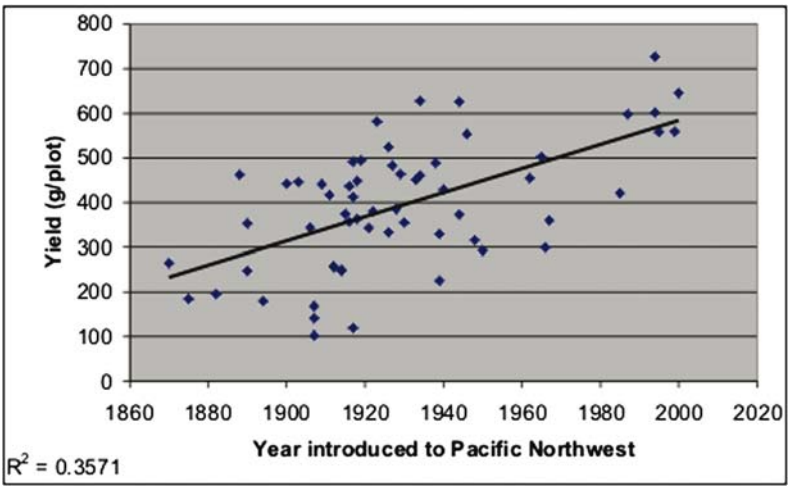


Figure 3. Yield of historical and modern spring wheat varieties under low-input conditions with organic fertilizer

The chemical intensive agricultural system of the green revolution was not successful in large creating large increases in grain yield until new varieties suited to the different production system became available. A similar situation is presenting itself with the emergence and rapid growth of organic agriculture. Without crop varieties suited to the unique conditions present in organic systems, organic agriculture may not realize its full potential as a viable alternative to conventional agriculture.

ACKNOWLEDGEMENTS

This research is funded by the Organic Farming Research Foundation and Washington State University Center for Sustaining Agriculture and Natural Resources.

REFERENCES

Atlin GN, Frey KJ (1990) Selecting oat lines for yield in low-productivity environments. Crop Sci 30:556–561

Carr PM, Horsley RD, Poland WW (2003) Tillage and seeding rate effects on wheat cultivars: I. Grain production. Crop Sci 43:202–209

Ceccarelli S (1994) Specific adaptation and breeding for marginal conditions. Euphytica 77:205–219

Hasegawa H (2003) Crop ecology, management & quality: High-yielding rice cultivars perform best even at reduced nitrogen fertilizer rate. Crop Sci 43:921–926

Kelman WM, Qualset CO (1991) Breeding for salinity-stressed environments: recombinant inbred wheat lines under saline irrigation. Crop Sci 31:1436–1442

O'Leary N, Smith ME (1999) Breeding corn for adaptation to two diverse intercropping companions. American Journal of Alternative Agriculture 14:158–164

Panter DM, Allen FL (1989) Simulated selection for superior yielding soybean lines in conventional vs. double-crop nursery environments. Crop Sci 29:1341–1346

Rao SC, Dao TH (1994) Straw quality of 10 wheat cultivars under conventional and no-till systems. Agron J 86:833–837

Santalla M, Rodino AP, Casquero PA, Ron AMd (2001) Interactions of bush bean intercropped with field and sweet maize. Eur J Agron 15:185–196

Simmonds NW (1991) Selection for local adaptation in a plant breeding programme. Theor Appl Genet 82:363–367

Smith ME, Coffman WR, Barker TC (1990) Environmental effects on selection under high and low input conditions. In: Kang MS (ed) Genotype-by-Environment interaction and plant breeding. Dept of Agron., Louisiana Agric. Expt. Stn., Baton Rouge, USA, pp 261–272

Weisz R, Bowman DT (1999) Influence of tillage system on soft red winter wheat cultivar selection. J Prod Agr 12:415–418

A NOVEL VARIETY MANAGEMENT STRATEGY FOR PRECISION FARMING

A.A. ROMANENKO*, L.A. BESPALOVA, I.N. KUDRYASHOV AND I.B. ABLOVA

Krasnodar Lukyanenko Research Institute of Agriculture,
**E-mail: kniish@kniish.ru*

Abstract: In the Northern Caucasian region the development and deployment of improved varieties and farming systems has lead to increased winter wheat yield and yield-stability. A key element of this success has been an integration of strategies aimed at increasing economic returns to wheat growers through improved crop management and more optimal matching of varieties and management. Advice is provided to farmers based on their planned level of expenditure on inputs such as fertilizer and pesticides, projected income, the scale of their enterprise, and the production system they will use which is greatly influenced by their level of mechanization. Integration of this information is used to assist them in selecting the best mix of varieties for their farm. To manage risk and account for year-to-year variation caused by genotype by environment interaction, between 3 and 6 winter wheat varieties best suited to their enterprise will be chosen based on long term data

Development of an Agrotechnological Varietal Passport for each variety is an important tool used in the matching of varieties to farm enterprises. The Varietal Passports outline data on the relative performance of varieties following different preceding crops, adaptation to different environment types, optimal sowing date, growing period, pest and disease responses, resistance to abiotic stresses, inherent grain quality characters, suitability for machine harvesting (determined by lodging and shedding resistance), optimal sowing rate, mineral fertilizer requirement, genotype-pesticide response and typical gross margins

Our novel variety management policy is based on crop pre-adaptation, considering poor long-term climate predictability, and our target that a variety should cover not more than 15% of the region's sowing area. This variety management policy has allowed the benefits of ongoing scientific advances in both improved varieties and management practices to be captured by the farmers of the Krasnodar region. Over the last two decades a consistent rise of winter wheat yield over the more than one million hectares in the Krasnodar region has increased mean yields from 3.3 t ha^{-1} to 4.6 t ha^{-1}

Keywords: maximize yields, genotype × environment responses

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 223–231

INTRODUCTION

The fundamental role of breeding science in the release of improved varieties and their contribution to increasing total grain production is widely acknowledged (Lukyanenko 1973, Borojevich 1984, Vasilchuk 2001, Balla et al. 1987, Bespalova 1998, Valkoun et al. 1987, Nettevich 2002). According to these authors' estimates, annual yield increases over the last three decades of the 20th century varied from between 0.5 to 1.8%. This increase was largely due to parallel development of more intensive crop management systems and release of improved varieties better suited to these intensive management systems (Romanenko and Lenkova 2004). Continual intensification of agricultural production over more than 50 years has led to environmental degradation and an almost exponential growth in inputs of non-renewable resources per additional unit of agricultural produce (Zhuchenko, 1994, 2001, 2004, 2004a).

The Southern Federal District of Russia including the Krasnodar region is the most favorable area in Russia for achieving high wheat yields. At the same time it is the zone that has suffered the most acute environmental degradation from intensive land use, reduced fertility and moisture levels following high input break crops such as sunflower and maize and macro- and micronutrient imbalances. The high proportion of the cropping area planted to grain crops, increasing application of pesticides and their mutagenic and selective effect on the complex of wheat pathogens hinder further high quality yield growth (Molchan 2002).

Economic changes in Russia have seen a huge range in the uptake of improved management practices and investment in mechanization among grain producers. These differences translate to a large range in wheat yields, even within production regions. For example, at Novokubanskij rayon (farmland area with near 30000 ha of wheat, in Krasnodar region) average winter wheat yields are 6.0 t ha^{-1}. However within this area some farms regularly achieve 7.0–8.0 t ha^{-1}, while other farms only achieve 30 to 40% of a varieties potential yield (around 2.5–3.0 t ha^{-1}). These differences arise because varieties with the highest yield potentials can only achieve this potential with high levels of input, where they show the greatest advantage compared to less productive varieties. At the same time, intensive cropping practices are justified only when the level of inputs is consistent with the environment and potential of the grown variety (Vasilchuk 2001). If levels of input are in excess of what can be used by a variety in a particular environment, wheat yields may actually fall in spite of increased inputs. According to Zhuchenko (1994), effective implementation of intensive precision farming must be based on an integrated understanding of the key factors driving crop yield, and how they interact and continued adoption of new technology and beneficial interactions. Hence, in addition to the breeding of improved wheat varieties and better crop management practices, further growth of wheat yields will depend on optimizing combinations of the genetic peculiarities of varieties and the conditions in which they grow. That is why our aim to understand and maximize average yields in presence of genotype × environment interactions is an important aspect for increasing winter wheat yields (Shevelukha and Morozova 1986).

Our novel variety management policy, having as its core the principle of mosaic placement of varieties (i.e. the distribution of genetically and biologically distinct varieties inside of a region), aims to maximize yields across farms and regions through utilization our understanding of the genotype × environment responses of varieties within farming systems.

MATERIALS AND METHODS

Development of variety passports at the Krasnodar Lukyanenko Agricultural Research Institute (lat. 45 °N, long. 38 °E, altitude 24 m) was initiated in 1995. Experiments were carried out on a 36 hectare trial area divided between eight-field crop rotation treatments. More than 50 varieties have been studied over the following ten years. Each year, 20–26 varieties are studied in a six-factor field experiment, including most of the important agronomic factors effecting grain yield and quality. These include: (1) previous crop (perennial grasses, sunflower and winter wheat), (2) sowing date (early optimal, late optimal, late), (3) fertilizer application (nothing, 10 kg N + 25 kg P + 25 kg K ha^{-1} to the seedbed, 40 kg N + 75 kg P + 75 kg K ha^{-1} as basic fertilizer), (4) early spring nitrogen dressing (0, 35 and 70 kg N ha^{-1}), (5) late spring nitrogen dressing (0, 35 and 70 kg N ha^{-1}), (6) fungicide protection (zero, one and two treatments).

Inclusion of the most important agronomic factors in the field experiment each year produced a wide range of levels of the studied traits (e.g. yields ranging from a minimum of 0.8 t ha^{-1} to a maximum of 11.2 t ha^{-1}). Experimental analyses were based on Statgraphics Plus 4.0 Draper-Lin small composite design (Manugistics, Inc. 1997). Designs allowed accurate estimation of linear and nonlinear effects of factors on the studied trait as well as all first-order interactions between factors. Determination coefficients of derived regression models generally exceeded 95%. Data were also processed by means of discriminant, cluster, correlation, variance and regression analyses.

RESULTS AND DISCUSSION

The principal prerequisite for introduction into commercial production of our Mosaic placement of varieties has been the steady stream of high-performance varieties developed by the Krasnodar wheat breeding program, characterized by broad genetic diversity and varying responses to environmental factors. The second prerequisite is considered the broad scale of farming systems research providing an understanding of the contribution of a wide spectrum of, often interacting, factors to grain yield and quality in commercial production. Our multi-environment variety testing network provides additional valuable data to further refine our recommendations for areas with stresses not present in trials on our research station.

In a region like Krasnodar, characterized by a large range in levels of mechanization, crop rotations, inputs of fertilizers and pesticides, achieving yield growth

across a whole farm or region depends on a diverse set of varieties adapted to the range of production systems in which they will be grown. Matching of varieties to production systems requires detailed understanding of the response of individual varieties to each of these systems. One of the most important characteristics of a variety determining its place in the crop production system is its response to the intensity of management due to the resulting differences in soil fertility, and pest and disease intensity, achieved by these different levels of input (Kudryashov and Bespalova 2000). Low input production systems generally produce lower winter wheat yields, while higher yields are usually achieved with higher input levels. This is true for most varieties, although they differ in their level of response to higher inputs. Some varieties clearly show a greater than average response to increasing input levels while others show better maintain yield levels at lower input levels. Of the varieties presented in Fig. 1, the semi-dwarf variety Kroshka shows the greatest yield response to higher input levels. The variety Pobeda 50 also shows a positive response to higher inputs and, although it is lower yielding at all input levels than Kroshka, its superior grain quality offers some compensation in levels of return to growers. Conversely, yields of varieties Zimorodok, Knyazhna and Ekho actually decrease at higher input levels. Due to this negative response, these varieties are higher yielding than Pobeda 50 and Kroshka in low input systems but lower yielding at average yield levels below 4.0–5.0 t ha^{-1}.

Application of appropriate levels of input to optimize the grain yield of a variety and maximize economic returns to farmers will be, in some cases, mean restricting input levels and also will have the benefit of reduced environmental pollution. An

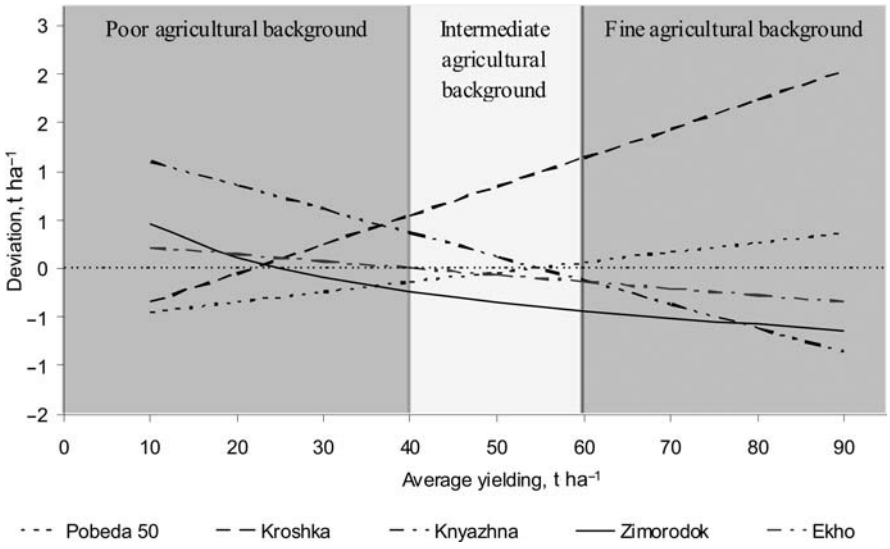


Figure 1. Grain yield of a set of wheat varieties (expressed as deviation from mean yield of all varieties) across a range of average yield levels related to levels of input. Krasnodar, 1996–1998

understanding of this principle is demonstrated in the Krasnodar mantra relating to fertilizer application that it should do no harm. Generally, early spring nitrogen dressing results in good yield responses. However, varieties Doka, Lastochka and Veda show a yield response with early spring dressing only following a holy clover forecrop. Subject to application rate, early spring dressing caused a 0.5-1.3 t ha^{-1} yield decrease in our experiments (Fig. 2). Nitrogen application at the booting stage resulted in yield increase of 0.23 t ha^{-1} with 35 kg N ha^{-1} and a 0.46 t ha^{-1} increase with 70 kg N ha^{-1}. Thus, Doka and similar varieties do not require nitrogen dressing at the resumption of spring growth following annual and perennial grasses, rape and pea forecrops.

Year to year variation and unpredictability in the stresses experienced by wheat crops poses a threat to yield stability, since they can negatively affect the whole winter wheat production area. The relative yield performance of the diverse range of varieties grown in Krasnodar changes every year (Table 1), but provides greater yield stability in region wide and farm scale. Growing a set of varieties differing in growing period, grain filling rate, etc. means that not all crops will be affected equally by any climatic or other stress situations, and yield losses will be restricted to the area with the variety or varieties most affected by the given stress. Use of varieties with different growing periods also spreads the harvest period over a longer time making it easier to harvest each variety closer to its optimum harvest time.

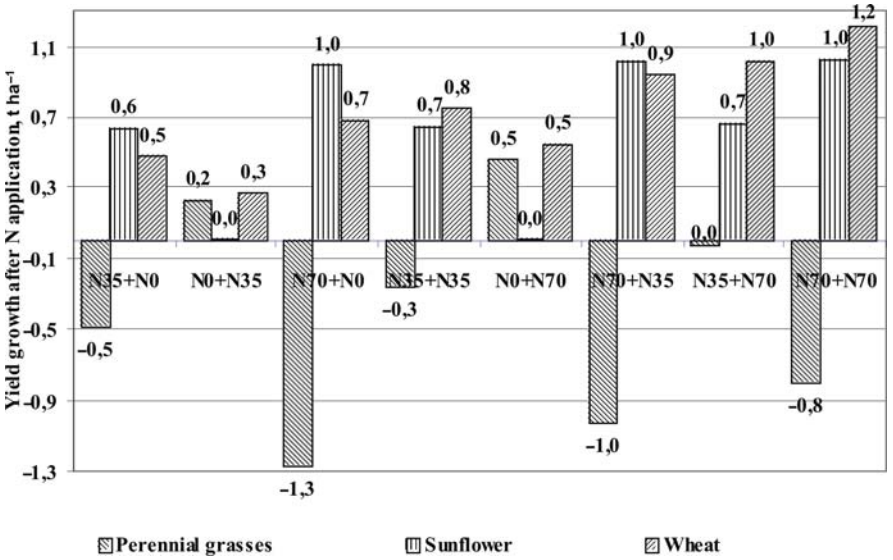


Figure 2. Grain yield response of variety Doka to N dressing following different rotation crops. Krasnodar, 2003–2005

Table 1. Yield of wheat varieties of different maturity groups, t ha^{-1} at Krasnodar from 2001–2004

Maturity group	Variety	2001	2002	2003	2004
Ultra-early	Yugtina, Russa, Yubilejnaya 100, etc.	7,18	6,94	4,55	5,89
Early	Pobeda 50, Batko, Starshina, PalPich, Kroshka	7,80	6,92	6,00	6,22
Mid-season	Lira, Krasnodarskaya 99, Zimorodok, Ekho, Doka	6,75	7,62	5,97	6,35
Mid-late	Knyazhna, Polovchanka, Krasota	6,61	7,03	6,68	5,89

Diversity in genes for disease resistance and tolerance among the varieties helps not only to decrease levels of yield loss due to disease pressure but also to minimize the risk of harmful new mutations to virulence that overcome a single resistance gene deployed over a large proportion of the cropping area. Maintenance of high levels of effective disease resistance through deployment of such a diversity of resistance genes is a major contribution to protecting the yield potential of the variety itself as well as indirectly protecting the yield of all varieties across the entire cropping area.

In the case of the variety PalPich with good grain quality, yellow rust and *Septoria tritici* resistance, moderate susceptibility to leaf rust and powdery mildew, the effect of fungicide application on grain yield was insignificant in all years in which it was studied (Fig. 3). Due to its earliness and high rate of grain filling this variety escapes leaf diseases and does not require fungicide protection. This demonstrates the value of quicker maturing varieties in avoiding disease pressure and improving yield stability. The need to protect crops from disease through fungicide application can also be greatly influenced by previous crops (sunflowers, sugar beet) which lower soil moisture levels. This variety is recommended following such forecrops.

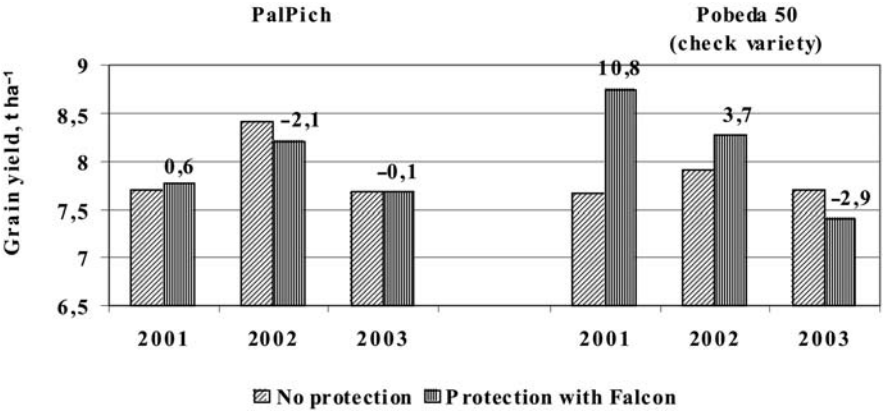


Figure 3. Fungicide (Falcon, Bayer Crop Science) protection effect on PalPich's grain yield. Krasnodar, 2001–2003 (holy clover as a forecrop)

The variety Tanya with the highest yield potential of 12 t ha^{-1} shows its greatest productivity at optimal sowing dates. Delay of sowing causes decrease of the variety's advantage over other varieties from 11–13 percent down to 5 percent at late sowing (Fig. 4).

The variety Vostorg, typical of the majority of varieties in Krasnodar, shows a negative yield response to delayed sowing (Fig. 5). However, when sown on November 4 (one month later than optimum) although showing a yield decrease, maintains higher yield levels relative to the average yield of 22 varieties included in the experiment, with a maximum advantage of 111 percent.

Based on these data and considering farmer practices and preferences, we designed strategies to help them achieve optimal grain quality, yield and yield stability in their farming system. A multilevel system of winter wheat adaptation starts with macro-level zoning, i.e. offering to each zone and sub-zone a set of

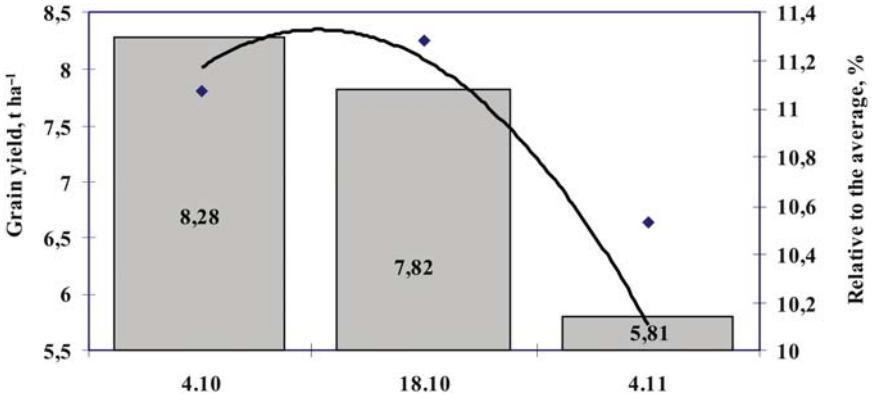


Figure 4. Sowing date response of variety Tanya. Krasnodar, 2002–2004

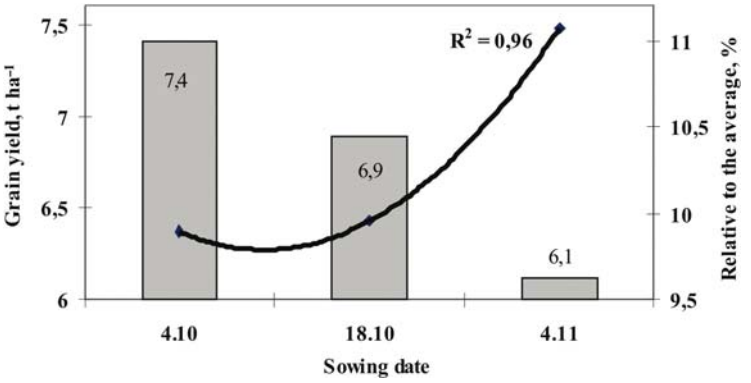


Figure 5. Sowing date response of variety Vostorg, Krasnodar, 2002–2004

biologically and economically different varieties representing a polymorphic series. The varieties offered to each zone show a high level of reliability due to the particular combination of traits underpinning their high adaptability. At the same time, breeding targets are not set so high that all varieties possess high levels of resistance or tolerance to stresses that are not limiting to yield in a given zone. The set of varieties recommended for a certain area is determined by yields over the preceding 3–5 years, proportion of the area sown to each variety in the previous season and growing period (i.e. probable harvest timing) and incidences of pests and diseases, etc. A good example of the success of our variety management policy can be seen in the Novokubanskij farmland subzone. This winter wheat growing area of 33000 ha, averages the highest yields of any subzone in the Krasnodar region. The set of recommended varieties for this subzone is made up of those most responsive to high input levels, covering a range of different maturity groups, responsiveness to high input levels and ability to perform at average yield levels of 7–10 t ha^{-1}. On about 55% of the area (after grain and silage maize, peas, perennial grasses) the varieties sown (Delta, Deya, Zimorodok, Batko, Selyanka, Lira) were resistant or moderately resistant to *Fusarium* head blight (FHB). Use of FHB resistant varieties in this subzone is an important aspect of the variety management policy, which helps to produce mycotoxin-free grain. In addition, some of the FHB resistant varieties are adapted to later sowing dates. In 2005, about 4% of the area was planted to the new variety Fisht suitable for sunny southern slopes near the forestland, where leafhopper damage may occur and lead to the spread of virus diseases (currently a serious issue in this subzone). Due to this set of precisely placed varieties, in 2005 Novokubanskij farmland produced average wheat yields of 5.9 t ha^{-1}, exceeding the average yield across Krasnodar by 1 t ha^{-1}.

CONCLUSION

This approach of targeted placement of varieties on macro, meso and micro-levels has enabled the Krasnodar wheat industry to:

1) avoid one variety monopolizing wheat plantings and the inherent risks this poses, even if it is an outstanding variety;
2) gain additional produce due to genotype × environment interaction;
3) use in each farmland subzone and each farm an optimized variety placement structure and a set of interchangeable varieties to maximize yields and minimize production risks;
4) diversify commercial variety assortment;
5) adapt crop management practices to the biological peculiarities of the varieties grown;
6) conduct on-the-fly systematic multilevel variety change;
7) reduce the risk of damaging disease epiphytotics;
8) incorporate a pre-adaptive policy in variety release decisions;
9) respond dynamically to changing market signals, varieties and uptake of technology by farmers.

REFERENCES

Balla L, Szunics L, Siladi D (1987) Geneticheskij progress v selektsii pshenitsy. Voprosy selektsii i genetiki zernovykh kultur: 289–297

Bedö Z, Lang L, Veisz O, Vida G (2003) Breeding of winter wheat (*Triticum aestivum* L.) for different adaptation types in multifunctional agricultural production. Vestnik regionalnoj seti po vnedreniu sortov pshenitsy i semenovodstvu: 99–105

Bespalova LA (1998) Selectsia polukarlikovykh sortov ozimoj myagkoj pshenitsy. Nauchnyj doklad: 1–50

Kudryashov IN, Bespalova LA, Konopkin SO (2000) Otsenka reaktsii sortov ozimoj pshenitsy na uroven agrofona. Sbornik nauchnykh trudov: 130–135

Lukyanenko PP (1973) Izbrannye trudy: 111–230

Molchan IM (2002) Poslovitsa ne naprasno molvitsa. Kulturnye rasteniya dlya ustojchivogo selskogo khozyajstva v XXI veke (immunitet, selektsia, introduktsia): 233–245

Molchan IM (2004) Lisitsin i sovremennoe semenovodstvo. Sbornik trudov: 147–165

Nettevich ED (2002) Itogi selektsii osnovnykh zernovykh kultur k nachalu 3-go tysyacheletiya (analitich-eskij obzor Gosreestra selektsionnykh dostizhenij za 2000-j god): 1–45

Romanenko AA, Lenkova MI (2004) Ecologo-economicheskaya effectivnost ispolzovaniya zemli: p 269

Shevelukha VS, Morozova AV (1986) Zakonomernosti I puti upravleniya formirovaniem zerna zlakov: 19–86

Tyuterev SL (2000) Sovershenstvovanie khimicheskogo metoda zashity selskokhozyajstvennykh kultur ot semennoj i pochvennoj infektsii: p 251

Valkoun J, et al. (1987) Povyshenie geneticheskogo potentsiala ozimoj pshenitsy v CSSR. Voprosy selektsii i genetiki zernovykh kultur: 307–316

Vasilchuk NS (2001) Selektsia yarovoj tverdoj pshenitsy: 1–119

Zhuchenko AA (1994) Strategiya adaptivnoj intensificatsii selskogo khozyajstva: 148

Zhuchenko AA (2001) Adaptivnaya sistema selektsii rastenij (ecologo-geneticheskie osnovy). 1:780

Zhuchenko AA (2004) Rol selektsii i semenovodstva rastenij v adaptivnoj sisteme selskokhozya-jstvennogo prirodopolzovaniya. Sbornik trudov: 88–121

Zhuchenko AA (2004a) Vozmozhnosti sozdaniya sortov i gibridov rastenij s uchetom izmeneniya klimata. Materialy nauchno-prakticheskoj konferentsii, Saratov: 10–16

YIELD PERFORMANCES OF CEREAL VARIETIES IN VARIOUS CROP ROTATIONS UNDER MEDITERRANEAN DRYLAND AREAS

M. AVCI*, T. AKAR, K. MEYVECI, M. KARACAM AND D. SUREK

Central Research Institute for Field Crops. P.O.Box: 226 Ulus-Ankara Turkey,
Fax: (+90) *312 2878958*
**E-mail: muzafavci@yahoo.com*

Abstract: Because most of the dryland cereal varieties were improved under fallow/cereal rotation system, their performances in other cropping systems were questionable and reported unsatisfactory by some farmers. Therefore, the objective of this study was to investigate the performances and adaptation of newly registered varieties in different two course rotations for targeted recommendations. 12 cereal varieties were tried on 9 different 23-year-crop rotations plots for three consecutive years, 1999/2000, 2000/2001, 2001/2002. The varieties were 4 barley (malting two rowed Aydanhanım, and feeding Tarm and six rowed Çetin and Avci), 4 durum wheat (Altın, Ankara, Altıntaş, and Yılmaz), and 4 bread (Dağdaş, Gün, Kırgız and Mızrak) wheat. The cereal varieties were rotated with fallow, wheat, winter vetch, winter lentil, sunflower, safflower, lentil, chickpea and barley/vetch mixture. Grain and biomass yields, plant height, harvest index, kernel per spike, kernel weight and spike number per square meter were traits determined. Biplot analysis showed that responses of cereal varieties varied in dry (2001), wet and cold (2000) and normal (2003) seasons. Overall evaluations of responses indicated that some varieties adapted more to certain rotations than other varieties such as Aydanhanım for Safflower/cereal rotation. There was a general tendency that Tarm and Gün varieties performed well in winter cold and dry seasons in all rotations. Dağdaş yielded pretty well following winter legumes and winter legume/cereal mixture except dry season. Six rowed barleys, Altıntaş and Yılmaz, were better in mild winter season in all rotations. Traits correlations indicated that spike number per square meter was always positively correlated with grain yields for all experimental seasons. While all yield components had positive contribution to the grain yields in wet season, kernel weight and kernel per spike had negative contribution to grain yields in the dry season. General evaluation showed that cereals succeeding chickpea and spring lentil crops were leading in terms of height, biomass, harvest index and grain yields, except cereals following fallow which were exceptionally superior in dry season. They also had higher kernel weight in dry and normal seasons

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 233–241

Keywords: Dryland crop rotations, lentil, chickpea, sunflower, safflower, fallow, wheat, cereal, traits, biplot

INTRODUCTION

Results of crop rotation research during 1982 to 1996 indicate that fallow-wheat is outstanding and this is closely followed by winter vetch-wheat rotation. Continuous wheat and safflower-wheat have always lowest yields. The other rotations (sunflower, spring and winter lentils) are similar as regard to wheat yields. All rotation systems deteriorate wheat yields in response to production years. The least loss in the yield is recognized with the continuous wheat and spring lentil-wheat. The other rotations are similar in terms of yield decline. The deterioration rates for the rotations are negatively correlated with soil organic matter (Avci et al. 1998). This trend shows that some of the crop rotation systems particularly spring lentil-wheat tend to outperform wheat-fallow and other rotations in terms of grain yields.

Large genotype x year and genotype x location interactions arising from unpredictable rainfall are features of dry environments. However, the former is more important and dominant in majority of cases. Besides these interactions, the crop rotation, which includes different agronomic managements and crop effects, has profound influence on crop yields perhaps more than those interactions.

In variety improvement studies in Central Anatolia, advanced lines have been selected in fallow-cereal rotation system. Therefore, their performances in fallow-cereal system are superior or expected to be superior as regard to other cropping systems. In annual cropping systems, the previous crops leave completely different agronomic conditions to the succeeding cereal crops as compare to fallow (Meyveci and Munsuz 1987). As a result, the cereal varieties show differential yield responses depending on the types of previous crops and management they receive. The improved barley and wheat varieties, although they improved in fallow system, considerable yield depressions of some varieties in farmer fields are reported. Therefore, information on the performances of different wheat and barley varieties is needed for the right recommendation of the varieties to particular crop sequences. The objective of this study was to investigate wheat and barley varieties in terms of adaptation to various crop rotation systems.

MATERIALS AND METHODS

Climate and Weather Conditions

The Central Anatolian plain is a dryland area. The average seasonal rainfall is 370 mm ranges roughly from 250 to 450 mm, depending on the locality. Scarcity of the rainfall and its uneven distribution over seasons are the most important properties of the region.

The fall of the first growing season (1999–2000) was very dry, January, February and March were 2–3 °C colder than average without any crop damage. The emergence of crops was slow and completed in winter months. The annual rainfall was 424 mm higher than average.

The weather in 2000/2001was very dry and hot. The annual rainfall was 230 mm. Only May rainfall (75 mm) was effective for the crop growth. The emergence of crops was completed before winter.

The 2001/2002 growing season was very rainy in November, December and April. Seasonal rainfall was 446 mm. The winter months were very cold causing crop damage. It dropped to −15 °C several times.

Experimental Rotations and Cereal Varieties

The rotation experiment was located at Turkey with lat. 39° 40′ N, long. 39° 39′ E, and altitude 1050 m. In the experiment, nine-two year rotations of three main types were tested. i) M*onocrop wheat*: fallow-wheat (FAL) which is the traditional cropping system in the region and continuous wheat (WH). ii) *Winter legumes-wheat* rotations: winter lentil (WL) and winter vetch (WV)-wheat rotations. iii) *Spring crops-wheat rotations*: They involved sunflower (SUN), safflower (SAF), spring lentil (SL), chickpea (CH), and mixture of vetch and barley (MIX)-wheat/barley. Two field courses of rotations were employed in order to get data each year.

The experiment was established in 1982 and still continuing. The design of experiment was strip blocks with three replications. Vertical plots were rotations and horizontal plots were cereal varieties. The history of experimental site was fallow-wheat before to start of rotation experiment.

The varieties used were four barley varieties which were two rows malting: Aydanhanım (AHAN) and feeding Tarm-92 (TAR); two 6 rows of feeding: Avci (AVC) and Cetin (CET); four durum: Altın (ALT), Ankara-98, (ANK), Altıntaş (ATAS); and four bread wheat: Yılmaz (YIL), Dağdaş (DAG), Gun-91 (GUN), Kırgız (KIR), and Mızrak (MIZ). They were investigated in terms of grain and biomass yields, plant height, harvest index, grains per spike, kernel weight and spike number per square meter.

The variety grown in each rotation were shown in the text and figures as combination of rotation and then variety abbreviations such as FALAHAN express the performance of Aydanhanım in fallow-cereal rotation.

Agronomic Practices

Fallow covers 14 months with several tillage practices to conserve moisture. Wheat was planted in the period from 15 September to 15 October and harvested in the late July or in the early August. Winter legumes (vetch and lentil) were planted in October and harvested in May. Spring crops were planted with cereal drill in April

or early in May, spring lentil and cumin were harvested in June, chickpea in late July and sunflower and safflower harvests were in August or in early September.

All crops were fertilized with commercial doses of N and P. Weeds were controlled by hand and suitable herbicides.

A free add-in for Excel by Lipkovich and Smith (2002), of Virginia Tech. was used to draw biplot (Gabriel 1971) graphics.

RESULTS

Traits versus Rotation-Genotype Combinations

1999/2000 season

In this season, the grain yield was averagely 3.3 t ha^{-1} having 4.1 t ha^{-1} maximum and 2.2 t ha^{-1} minimum values. The average values of plant height, biomass, harvest index, spike per square meter, grains per spike and kernel weight were 85 cm, 835 g m^{-2}, 36%, 266 grains m^{-2}, 31 grains $spike^{-1}$, 41 g respectively. Their respective ranges (difference between maximum and minimum) were 94, 1292, 61, 754, 42, 39.

The results of biplot analysis, accounted for 71% of variation, indicated that SLAVC, CHAVC, CHCET were the leading rotation-genotype combinations in terms of plant height, biomass, grain yield and harvest index. CHANK, FALCET and SLGUN were the succeeding combinations. CHCET and SUNATAS were superior in kernel weight and grains per spike. Highest spike per square meter was obtained with FALAVC, WVGUN and MIXTAR (Fig. 1). Here it is interesting to note that although Cetin is 6 rowed barley which is normally lower kernel weight than two row-barleys, it turned out to be highest kernel weight in CH rotation.

2000/2001 season

The average grain yield of this season was 0.7 t ha^{-1} ranging from 0.05 to 2.2 t ha^{-1}. The averages for the traits were 58, 1190 g, 15%, 102 spike per square meter, 28 grains per spike and 28 g. Their respective ranges were 64, 1190, 33, 361, 59, 36.

The season was extremely dry and the respective yields were very low below 1 t ha^{-1} except for fallow-cereal rotation which yielded approximately 2 times more than annual cropping syatems. Explaining 71% of variation due to all measured traits, biplot analysis showed that except for Ank-98, all the genotypes, Tarm and Cetin being the first ranked, grown after fallow reached highest height, biomass and grain yields. Second highest combinations were WLATAS and WLAHAN in this respect.

The high values of spike per square meter were obtained with durum varieties after fallow such as FAL altın, FALMIZ, FALATAS and FALYIL Kernel weight and grains per spike showed different pattern. Highest values were found with FALANK, SUNYIL, MIXALT and FALAHAN, respectively (Fig. 2).

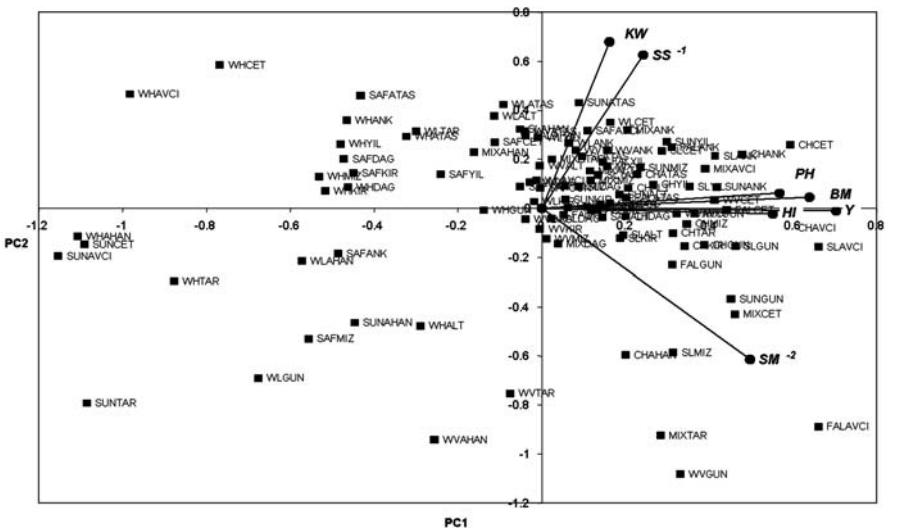


Figure 1. Biplot display based on the 1999–2000 rotation trial. PC1 and PC2 are first and second principal components. FAL, WL, SUN, SL, WV, CH, SAF, WH and MIX stands for abbreviations of rotating phases (crops) with wheat: fallow, winter lentil, sunflower, spring lentil, winter vetch, chickpea, safflower, wheat and cereal-vetch mixture. AHAN, TAR, AVC, CET, ALT, ANK, ATAS, YIL, DAG, GUN, KIR, MIZ are the abbreviations of 4 barley, 4 durum and 4 bread wheat varieties, respectively. Performances of a variety in any crop rotation are shown by combinations of rotation and variety abbreviations e.g. FALGUN is the performance of Gun variety in fallow/wheat rotation. KW: kernel weight, SS^{-1}: grain number per spike, PH: plant height, BM: biomass, Y: grain yield, HI: harvest index, SM^{-2}: spike per square meter

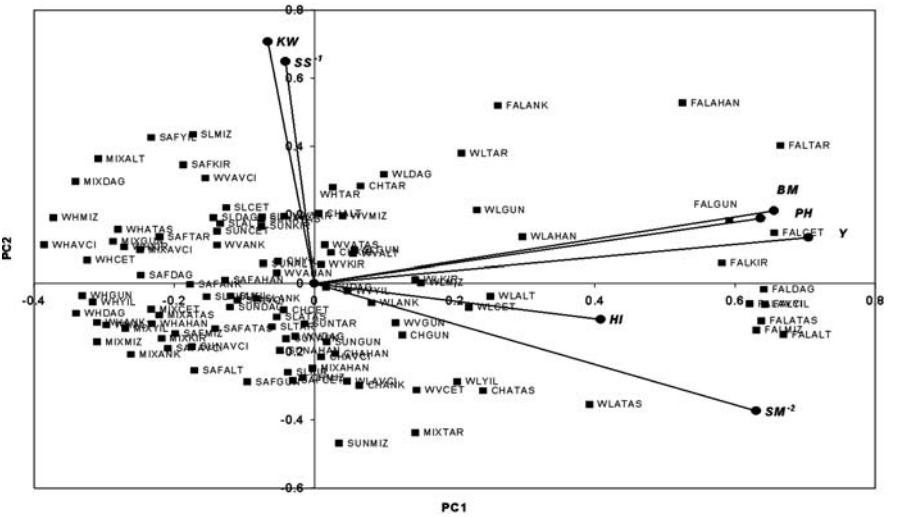


Figure 2. Biplot display based on the 2000/2001 rotation trial. See Fig. 1 for labels details

2001/2002 season

The average of grain yield was 3.1 t ha^{-1} ranging from 5.2 to 1.2 t ha^{-1}. The average values of the traits were 81cm, 839 g, 33%, 294 spikes per square meter, 36 grains per spike and 40.7 g, respectively. Their respective ranges were 39, 993, 32.7, 294, 36, and 27.5.

SUNgun and SLgun were reached to highest grain yields, Biomass, height, spike per square meter and grains per spike. These combinations were followed by SUNtar, SLtar, SLmız and SUNmız.

High harvest index was obtained with FALgun and FALyıl. Highest kernel weight was with FALalt, FALank, WLank, WHank and SLavcıand FALgUn (Fig. 3).

Traits Correlations

The vectors of the plant height, biomass and grain yields in Fig. 1, 2 and 3 were very close and projected to the same direction. This showed that all these traits were closely and positively correlated in all three seasons. Likewise, kernel weight and grains per spike were correlated highly and positively for the first two seasons but because the vectors of these traits had narrow angle and projected to reverse direction for 2001/2002 they had highly and negatively correlated. For the first two seasons kernel weight and grains per spike, and for the third season only, kernel weight was correlated highly and positively with spike per square meter. Third year's grains per spike had positive and medium high correlation with spike per square meter.

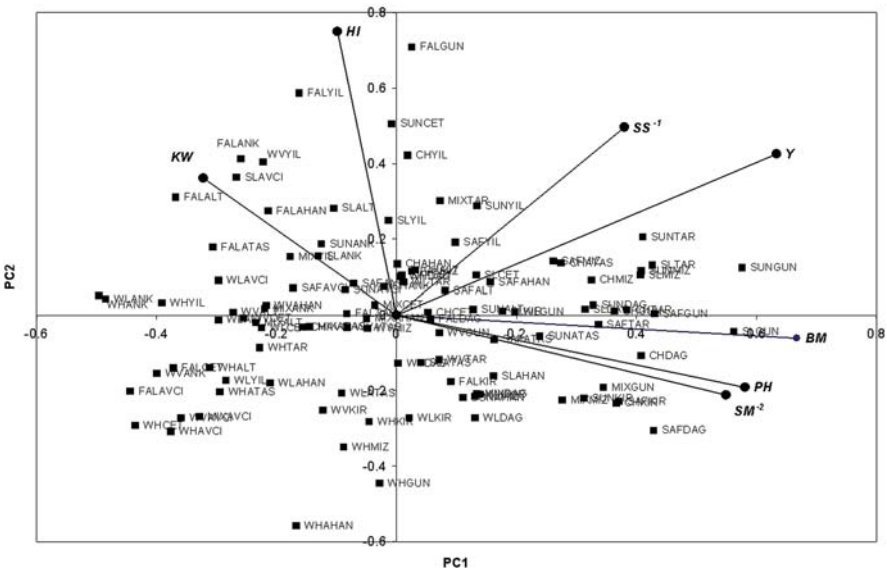


Figure 3. Biplot display based on the 2001/2002 rotation trial. See Fig. 1 for labels details

The group of plant height, biomass and grain yield had medium to high positive correlations with spike per square meter for all season. While all components contributed positively to grain yield formation in 1999/00, only kernel weight and grains per spike did not have any contribution in 2000/01 and only kernel weight had negative impact in 2001/02 (Fig. 1, 2 and 3).

Kernel weight and grains per spike had very weak correlation with grain yield in dry season (Fig. 2), however, one or both of those components had moderate to high positive (grains per spike) or negative (kernel weight) correlations with grain yields in other wet seasons (Fig. 1 and 2). This may imply that spike per square meter contributed much to grain yield in dry season. The yield increase in wet seasons originated from contributions of one or both of grains per spike and kernel weight increases in addition to spike per square meter.

Traits versus Rotations

CH, SL, FAL and MIX rotations were leading in terms of height, biomass, harvest index and grain yields as indicated by biplot analysis (Fig. 4). WL, SL and WH had higher kernel weight and grains per spike in 1999/00 season, mostly due to lowest spike per square meter in those rotations excluding SL (Fig. 4A).

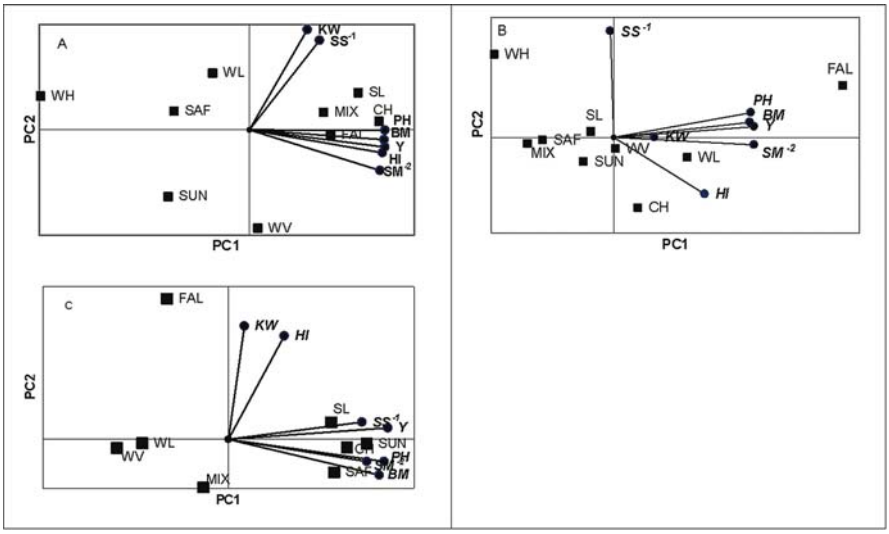


Figure 4. Biplot figures displaying traits vs. rotations for 1999–2000 (A), 2000/2001 (E) and 2001/2002 (C) rotation trials. PCi and PC2 are first and second principal components. FAL, WL, SUN, SL, WV, CH, SAF, WH and MIX stands for abbreviations of rotating phases(crops) with wheat: fallow, winter lentil, sunflower, Spring lentil, winter Vetch, chickpea, safflower, wheat and cereal-Vetch mixture. KW: kernel weight, SS^{-1}: seed number per spike, PH: plant height, BM: biomass, Y: grain yield, UI: harvest index, SM^{-2}:spike per square meter

The FAL and WL rotations were dominating in terms of all traits except for harvest index and grains per spike. These traits were highest with CH and WH in 2000/01 (Fig. 4B).

In 2001/02 season, SUN, CH, SL and SAF were superior in all traits with the exception of kernel weight and harvest index. For the latter traits, FAL came the first having overwhelmingly higher kernel weight and harvest index and lower spike per square meter than other rotations (Fig. 4C).

The overall evaluation indicated that CH and SL (spring pulses-cereals) in wet seasons, FAL in dry season were superior and high in all traits.

DISCUSSION

High summer rainfall and more than average seasonal rainfall in 1999/2000 diminished the fallow advantage in soil moisture accumulation. All these facts reflected in yield, making SL and CH rotations over yield FAL and other rotations. In case of high seasonal rainfall in crop cycle, fallow did also not showed its advantage in terms of yield. Long-term rotation effects also played major role in this result. Previous 16 year-analysis of these rotation systems showed that all rotation systems caused yield decrease in wheat in response to number of application years of rotations. The SL rotation was the one of the least deleterious systems on wheat yields (Avci et al. 1998).

The 2000/01 season was the reverse of 1999/2000 due to high winter temperatures and low and ineffective rainfall. Cereal yields in plots of FAL rotation were 2 to 3 times more than cereal yields in annual crop rotations, which were between 0.2 to 1 t ha^{-1}. This was mainly for high fallow efficiency in water accumulation and utilization of this accumulated moisture during 1999/2000 dry spring months in which rapid crop growth and peak moisture demand took place.

In 2001/02, winter dead (number of plant in spring x 100/number of seed planted) in barley and wheat crops resulted in lower yields particularly in barley. The kill rates were 50–55% for bread wheat, 55% for TARM barley, 75–84% for malting and two row barleys, 55–60% for durum wheats. Lower fallow efficiency due to previous year's drought and high crop phase rainfall during spring eliminated the fallow advantage in soil moisture accumulation. At planting time (October, 2001) of wheat, there were not much difference between FAL and other rotations in terms of soil moisture. Consequently, SUN, SL and CH rotations with Gun, Tarm and Dagdas varieties turned out to have high yields.

The WH rotation was the worst rotation regarding yield. The emergence in this rotation became a problem after 10 years of rotation period. Weak, sparse emergence and discoloration, and drying of tips of seedlings were observed every year for each variety. This may result from toxic matters to seedling and germinating seeds, produced during decomposition of straw incorporated into seedbed during tillage. This hypothesis was corroborated by obtaining normal emergence when direct stubble-in planting causing no stubble-seedbed incorporation. The lower emergence

and lower spike per square meter with WH rotation mostly resulted in higher kernel weight and grains per spike in some varieties which were generally durum wheat and two rows barley.

REFERENCES

Avci M, Eyuboglu H, Avcin A, Meyveci K, Karaca M (1998) Crop yields and soil properties as influenced by long term dryland crop rotations in Central Anatolia. In: Jones MJ (ed) The challenge of production system sustainability. Long term studies in agronomic research in dry areas. Abstracts of Presentations and Workshop Conclusions. ICARDA, Aleppo, Syria. ISBN: 92-9127-088-1, p 55

Gabriel KR (1971) The biplot-graphic display of matrices with application to principal component analysis. Biometrika 58:453–467

Lipkovich I, Smith EP (2002) http://www.stat.vt.edu/facstaff/epsmith.html

Meyveci K, Munsuz N (1987) Soil moisture and inorganic nitrogen forms in two year rotations in Central Anatolia In: Proceeding of Turkish Cereal Symposium, TÜBITAK-Uludag University, Oct. 6–9, 1987, Bursa, Turkey. pp 135–147

HERBICIDE TOLERANCE IN IMIDAZOLINONE-RESISTANT WHEAT FOR WEED MANAGEMENT IN THE PACIFIC NORTHWEST U.S.A.

D.A. BALL* AND C.J. PETERSON

Oregon State University, Columbia Basin Agricultural Research Center, Pendleton, Oregon, and Department of Crop and Soil Sciences, Corvallis, Oregon, U.S.A.
**E-mail: daniel.ball@oregonstate.edu*

Abstract: Winter wheat ranks high in importance as an agricultural crop in the Pacific Northwest states of Washington, Idaho, and Oregon. Winter annual grass weeds such as jointed goatgrass (*Aegilops cylindrica*), downy brome (*Bromus tectorum*), feral rye (*Secale cereale*), wild oat (*Avena fatua*) and Italian ryegrass (*Lolium multiflorum*) have the same life-cycle as winter wheat and are difficult to control in conventional wheat production systems. These weeds annually account for millions of dollars of lost wheat production and reduced quality (i.e. discount by impurities). There has been only moderate success in controlling winter annual grasses in wheat by utilizing multiple-year crop rotations with spring crops and fallow periods, and with chemical control. Selective herbicides have been available for chemical control of downy brome, Italian ryegrass, and wild oat. However, before use of imazamox herbicide with imidazolinone-resistant (CLEARFIELD*) wheat, there was no herbicide that could selectively control jointed goatgrass, feral rye, or volunteer cereals in winter wheat. The first commercial release of an imidazolinone-resistant winter wheat variety in the Pacific Northwest was made during the 2003 growing season. Plant breeders in the Pacific Northwest are continuing to develop imidazolinone-resistant winter wheat varieties adapted to a range of Pacific Northwest production regions

One issue of concern for wheat varietal development is that single-gene tolerance to imazamox in CLEARFIELD* varieties can sometimes show visible crop injury, and possibly, yield reductions in response to herbicide applications. Crop tolerance can vary with time of herbicide application relative to wheat stage of growth, environmental conditions that reduce the wheat plant's ability to metabolize imazamox and, possibly, with specific wheat varieties. A multiple year and location study was conducted to evaluate imazamox tolerance in CLEARFIELD* wheat lines being developed for Pacific Northwest production regions. Results indicate that during specific years and locations, single-gene CLEARFIELD* varieties differed slightly in their relative tolerance to imazamox. The most important determinant of crop tolerance was related to herbicide application rate and timing

Keywords: clearfield, herbicide tolerance

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 243–250

INTRODUCTION

In the Pacific Northwest (PNW), winter annual grass weeds such as jointed goatgrass (*Aegilops cylindrica*), downy brome (*Bromus tectorum*), feral rye (*Secale cereale*), Italian ryegrass (*Lolium multiflorum*), and wild oat (*Avena fatua*) account for millions of dollars of lost wheat production and reduced quality through higher impurities. These weeds have the same, or very similar, life-cycles as winter wheat and are difficult to control in conventional wheat-fallow rotations. The development of winter wheat, marketed under the trade name CLEARFIELD*, provides growers with a new herbicide system for selective, in-crop control of previously uncontrolled jointed goatgrass (Ball et al. 1999), feral rye, volunteer cereals, and other winter annual grass weeds in winter wheat (Clemmer et al. 2004, Geier et al. 2004).

Imazamox (trade name Beyond® in the USA) is the herbicide used for weed control in the CLEARFIELD* wheat system. Imazamox is a broad-spectrum herbicide (grass and broadleaf weeds) that provides post-emergence and some in-season residual weed control. Imazamox, and similar, imidazolinone herbicides, inhibit the activity of the enzyme, acetolactate synthase (ALS), also known as acetohydroxyacid synthase (AHAS), which is necessary for the biosynthesis of the branched chain amino acids valine, leucine, and isoleucine (Anderson 1996). In response to an imazamox herbicide application, ALS is inhibited in susceptible plants depriving them of the previously mentioned, essential amino acids (Frihauf et al. 2005). This causes the eventual death of treated plants. The ALS enzyme is unique to bacterial and plant species and is not found in the animal kingdom. The gene providing herbicide resistance was derived through a sodium azide (pH 3) induced mutation of the French cultivar 'Fidel' (Newhouse et al. 1992). The mutation event resulted in an altered form of the ALS enzyme that is not inhibited by the herbicide at normal herbicide application rates. CLEARFIELD* varieties are non-genetically modified organisms (GMO), as no foreign DNA was introduced or inserted during the development process.

The first publicly-developed CLEARFIELD* winter wheat varieties released in the United States, were 'Above' (from Colorado State University) and 'AP502 CL' (marketed by AgriPro Wheat) in 2001. These two hard red varieties are best adapted to areas of the central Great Plains. In 2003, seed of the first CLEARFIELD* variety developed for the PNW was marketed to growers by General Mills. Since that time, two CLEARFIELD* varieties were developed and released by Oregon State University, and one by the University of Idaho. These have been rapidly adopted by growers in the PNW and acreage of publicly-developed CLEARFIELD* varieties are estimated to be approximately 400,000 acres for 2006. All winter wheat CLEARFIELD* varieties released in the U.S. to date are based on single-gene resistance to the herbicide.

An unknown in the development of CLEARFIELD* varieties was whether the altered ALS gene would respond similarly to herbicide applications in different genetic backgrounds. A further concern was possible interactions with environmental conditions, such as factors that impact herbicide applications, plant

metabolism, and post-application plant stress. A three-year study was conducted to evaluate performance of CLEARFIELD* varieties under varying herbicide rates and application dates at two locations in Oregon.

MATERIALS AND METHODS

Field study sites were established near the Columbia Basin Agricultural Research Center in Pendleton, Oregon U.S.A (lat. 45° 43′ N, long. 118° 33′ W) and at a Research Center site near Moro, Oregon (lat. 45° 29′ N, long. 120° 43′W). Plots were established on land that had been fallowed the previous year. The 20-year average annual precipitation at the sites is 442 mm and 271 mm for Pendleton and Moro, respectively. Plots were established at typical commercial seeding rates and planting dates in autumn of 2002, 2003, and 2004. At both sites, evaluations of imazamox tolerance were made on soft white winter CLEARFIELD* varieties ORCF-101 and ORCF-102 from Oregon State University; Idaho 587 from the University of Idaho; ClearFirst™ from General Mills; and the soft red winter wheat line Cv. 9804 (aka FS-4), a derivative of the French variety Fidel. The line Cv. 9804 was the tolerance trait donor line for ORCF-101, ORCF-102, and Idaho 587 lines. The tolerance trait in the line ClearFirst was an independent mutation event. The tolerance trait resides on the *als1* locus (D-genome homeologue) in ORCF-101, ORCF-102, Idaho 587, and Cv. 9804. In the line ClearFirst, the trait resides on the *als2* locus (B-genome homeologue). Both traits have demonstrated comparable tolerance in field trials in the PNW (Dahmer, pers. comm.)

The herbicide treatment plan included an early treatment timing when wheat was in the four-leaf stage, and a second treatment timing when the wheat was in the 5–7 leaf stage with 1–2 tillers. Treatment dates varied with year and site depending on stage of wheat growth. The recommended commercial use rate of imazamox for CLEARFIELD* wheat ranges from 35 to 52.5 g active ingredient per ha (g a.i. ha^{-1}). The rates applied in these trials ranged from 35 to 105 g a.i. ha^{-1}, which included the recommended use rates and twice the recommended use rates. Treatments included a non-ionic surfactant at 0.25% v/v and a 32% UAN nitrogen solution at 2.5% v/v, applied either early (3–4 leaf wheat) or late (5–7 leaf wheat) post-emergence. Treatments at both sites were applied with a hand-held CO_2 pressured sprayer delivering 180 l ha^{-1} at 207 kPa. Individual plots were 1.5 by 4.5 m in a factorial design in 2003, and a split-plot design in 2004 and 2005 in which herbicide was the main factor and cultivar was the sub-factor, with 4 replications in blocks, each year. Visible crop injury (0 to 100%) was evaluated twice after the late herbicide application treatment. Evaluation times are denoted as being 30 and 60 days after late application treatment (DAT), but are approximate evaluation times after treatment, only. Wheat grain yield was taken each year by harvesting the entire 1.5 m by 4.5 m plot area with a plot combine. Yields were only obtained at Moro in 2005 due to production problems unrelated to the experiments. Kernel weights were measures as an indicator of grain quality in the 2003 through 2005 Pendleton trials.

Data from the two locations were analyzed separately over years using SAS GLM (SAS Institute, Inc. 2004). All analyses used the $p < 0.05$ level for tests of significance. The 2003 trial at Moro was abandoned due to poor stands and yield data was not collected at Moro in 2004. Kernel weight data was collected for Pendleton trials in 2004 and 2005. Mean squares from analysis of variance for crop injury, grain yield, and kernel weight are presented in Table 1.

RESULTS AND DISCUSSION

Significant herbicide injury was observed in the CLEARFIELD* varieties at the 105 g a.i. ha^{-1} rate at both Pendleton and Moro (Tables 2 and 3). This rate represents approximately 2 to 3 times the recommended label rate. Visual injury was minimal at the other herbicide rates, regardless of time of application. The crop injury that occurred due to herbicide application produced a transient stunting, apparent delay in crop growth, and slight chlorosis. These symptoms mostly disappeared over time. Evidence of this diminishing injury is apparent upon comparison of the 30 and 60 DAT injury evaluations (Tables 2 and 3). This crop injury did have some significantly effect on grain yield, only at the highest herbicide application rate, particularly at Pendleton (Table 3). However, crop injury did not appear to affect kernel weights averaged over a 2-year period at the Pendleton site (Table 3). Significant differences due to herbicide treatment could not be related to weed control differences since weed populations were relatively negligible in all years.

Significant interactions between variety and treatment existed for visual injury ratings at 30 DAT. Herbicide injury was most evident on the ClearFirst variety at Moro, and injury ratings increased as the application rate increased from 52.5 to 105 g a.i. ha^{-1}, particularly for the earlier application dates. Injury also was evident on the other varieties, but to a lesser degree than for ClearFirst (Tables 2 and 3). Except for the ClearFirst variety, the remaining CLEARFIELD* varieties, including the parent Cv. 9804 line, exhibited a similar response to increasing herbicide application rates each year. There was some evidence of variation in crop injury among varieties in individual years and location, but, with the exception of ClearFirst, there was no consistent pattern of response.

Results of analysis of grain yield indicated that, over test years and 2 locations, all varieties responded similarly to herbicide applications. There was some reduction in grain yield at the 105 g herbicide application rate at the Pendleton site (Table 3). Analysis of grain yield indicated a significant year by herbicide treatment interaction at Pendleton. However, no variety x herbicide treatment interaction occurred with respect to yield. In general, all varieties yielded higher at Pendleton than at Moro, due to greater precipitation at Pendleton. At Moro, ORCF-101 was the highest yielding cultivar over all years. At Pendleton ORCF-102 was the highest yielding cultivar. At both locations, Idaho 587 was the second highest yielding cultivar and had the greatest average yield across locations. In the untreated plots, grain yields of ORCF-101, ORCF-102, and Idaho 587 were comparable to that of the

Table 1. Mean squares from analysis of variance for crop injury at 30 and 60 days after application of treatments (DAT), grain yield, and kernel weight after imazamox treatments at Moro and Pendleton, Oregon, 2003–2005

	Moro				Pendleton[1]				
	Df	30 DAT	60 DAT	Yield[2]	Df	30 DAT	60 DAT	Yield	Kernel Wt
Year	1	139.4**	6.7		2	8148**	1779.7**	27521616**	514.9**
Rep	3	98.0**	22.0*	5775842**	3	110**	8.7	1625167*	1.3
Year x Rep	3	12.9	5.6		6	51	10.7	2537881	0.7
Cultivar	4	307.3**	9.6	42350448**	4	60**	7.8	15505990**	421.0*
Treatment	8	314.7**	145.3**	500685**	6	4921**	619.4**	4152744**	4.2
Cultivar x Treatment	32	38.9**	5.8	103857	24	34**	2.7	452510	1.2
Cultivar x Year	4	4.1	1.5		8	53**	3.6	2688959**	25.8**
Treatment x Year	8	32.1	9.1		12	2403**	447.2**	1730976**	19.9**
Cultivar x Treatment x Year	32	36.6**	2.6		48	29**	3.6	412877	2.4
Pooled error	264	19.8		185359	293	17	4	561390	3.9

*,** Significant at $P < 0.05$ and $P < 0.01$, respectively. Note: df for pooled error is 132 for grain yield at Moro.

[1] Pendleton seed yields are average of 3 years, and kernel weights are the average for 2 crop years.

[2] Moro yield is 2005 only.

Table 2. Mean crop injury and grain yield of imidazolinone resistant (IMI)-wheat varieties after imazamox application rates and timings. Moro, Oregon, 2004–2005

Treatment[1]	Cv. 9804			Idaho 587			ORCF-101			ORCF-102			ClearFirst			Treatment means		
	Injury		Yield[2]	Injury		Yield	Injury		Yield	Injury		Yield	Injury		Yield	Injury		Yield
	30 DAT	60 DAT		30 DAT	60 DAT		30 DAT	60 DAT		30 DAT	60 DAT		30 DAT	60 DAT		30 DAT	60 DAT	
	---%---		Kg ha^{-1}	---%---		Kg ha^{-1}	---%---		Kg ha^{-1}	---%---		Kg ha^{-1}	---%---		Kg ha^{-1}	---%---		Kg ha^{-1}
Untreated	0	0	2942	0	0	5464	0	0	5481	0	0	5027	0	0	4271	0	0	4637
35 Early	0	0	3009	0	1	5565	0	1	5599	0	0	5229	1	1	4304	0	0	4741
52.5 Early	0	0	2572	1	1	5296	1	1	5413	1	0	4876	8	1	4035	2	0	4439
105 Early	4	4	3127	8	6	5296	5	5	5649	6	6	4842	21	10	3665	9	6	4516
35 Late	0	0	2943	0	0	5716	0	0	5750	0	1	5094	1	0	4607	0	0	4822
52.5 Late	0	0	3111	3	0	5498	1	0	5918	0	0	5414	3	0	4287	1	0	4845
105 Late	2	2	3262	2	1	5229	2	1	5548	1	1	4943	6	1	4103	3	1	4617

[1] Yield is 2005 only.

Table 3. Mean crop injury, grain yield, and kernel weight of imidazolinone resistant (IMI)-wheat varieties after imazamox application rates and timings. Pendleton, Oregon, 2003–2005

Treatment[1]	Cv. 9804				Idaho 587				ORCF-101				ORCF-102				ClearFirst				Treatment Means			
	Injury		Kernel Wt.	Yield	Injury		Kernel Wt.	Yield	Injury		Kernel Wt.	Yield	Injury		Kernel Wt.	Yield	Injury		Kernel Wt.	Yield	Injury		Kernel Wt.	Yield
	30 DAT	60 DAT			30 DAT	60 DAT			30 DAT	60 DAT			30 DAT	60 DAT			30 DAT	60 DAT			30 DAT	60 DAT		
	---%---		-mg-	Kg ha^{-1}	---%---		-mg-	Kg ha^{-1}	---%---		-mg-	Kg ha^{-1}	---%---		-mg-	Kg ha^{-1}	---%---		-mg-	Kg ha^{-1}	---%---		-mg-	Kg ha^{-1}
Untreated	0	0	43	7583	0	0	44	7593	0	0	40	7157	0	0	42	8165	0	0	35	6691	0	0	41	7432
35 Early	1	1	44	7454	1	0	44	7398	1	0	41	7559	2	0	40	8088	2	1	33	6574	2	0	41	7398
52.5 Early	2	0	45	7685	3	2	45	7487	3	2	40	7414	5	1	42	7577	5	1	35	6823	4	1	41	7402
105 Early	21	7	45	7269	18	7	45	6839	23	8	40	6611	25	9	42	7392	27	7	36	6209	23	7	41	6874
35 Late	2	0	45	8048	1	0	45	7655	1	1	41	7515	1	0	41	8052	1	0	36	7112	1	0	41	7670
52.5 Late	4	1	44	7487	6	1	45	7672	3	1	40	7717	6	1	42	8089	5	1	36	6966	5	1	41	7578
105 Late	20	8	45	7241	19	7	45	7201	17	6	41	6670	21	8	42	8031	15	6	35	6627	19	7	42	7171

[1] Seed yields are average of 3 years, and kernel weights are the average for 2 crop years.

non-CLEARFIELD* check variety Stephens, which suggests that the *als1* gene does not have any negative effect on yield potential (data not shown).

Observations on crop injury, grain yield, and grain quality support a conclusion that herbicide tolerance from the *als1* locus tolerance trait (D-genome homeologue) among PNW breeding lines will likely be similar, so extensive, multi-year screening for herbicide tolerance during the varietal development process may not be necessary. The ClearFirst variety, with the herbicide tolerance trait residing on the *als2* locus (B-genome homeologue), is an exception. For this line, there was more herbicide injury at the high herbicide application rate, and seed yield and kernel weights were lower than with other, presumably better adapted lines, even when no herbicide was applied.

ACKNOWLEDGEMENTS

Portions of this research were funded by the Oregon Agricultural Experiment Station, the Oregon Wheat Commission, and BASF Corporation. The authors wish to acknowledge the support of these organizations. Special thanks, also to Sandra Frost, Oregon State University, for providing data analyses and statistical support for this study.

REFERENCES

Anderson WP (1996) Weed science: Principles and applications, 3rd edn. West Pub. Co., New York, p 200

Ball DA, Young FL, Ogg AG Jr (1999) Selective control of jointed goatgrass with imazamox in herbicide-resistant wheat. Weed Tech 13(1):77–82

Clemmer KC, York AC, Brownie C (2004) Italian ryegrass (*Lolium multiflorum*) control in imidazolinone-resistant wheat. Weed Tech 18(3):481–489

Frihauf JC, Miller SD, Alford CM (2005) Imazamox rates, timings, and adjuvants affect imidazolinone-tolerant winter wheat cultivars. Weed Tech 19:599–607

Geier PW, Stahlman PW, White AD, Miller SD, Alford CM, Lyon DJ (2004) Imazamox for winter annual grass control in imidazolinone-tolerant winter wheat. Weed Tech 18(4):924–930

Newhouse KE, Smith WA, Starrett MA, Schaefer TJ, Singh BK (1992) Tolerance to imidazolinone herbicides in wheat. Plant Physiol 100:882–886

SAS User Support Services (2004) SAS Institute Inc. Cary, NC, USA

INVESTMENT RATE OF RETURN IN WHEAT RESEARCH IN IRAN

H. ASADI

Agricultural Economist, Economical Studies Unit, Cereal Research Department, Seed and Plant Improvement Institute (SPII), Karaj, Iran, Tel: +98261(2782981), Fax: +98261(2782698)
E-mail: hormoz_asadi2004@yahoo.com

Abstract: The main objectives of this study were to:investigate agricultural budget as a portion of Gross National Production(GNP),determine the substitution value of gross production for irrigated bread wheat varieties in different regions, determine the Benefit-Cost Ratio and Investment Rate of Return for released irrigated wheat (Mahdavi, Niknejad, Atrak, Tajan, Alamoot, Zarrin, Alvand, Darab2, Marvdasht, Kavir, Chamran and shiroodi varieties), that, carried out at cereal research department in 2000.In this study for assessment of investment economic efficiency in varieties production used Benefit–Cost Ratio and Internal Rate of Return. Of course, the total cost and benefit change to present value and economic rate of return with discount rate %18.5 are estimated. Results showed that:

Benefit cost ratio of released bread wheat varieties under research condition was 25.8. Investment rate of return in newly released irrigated wheat varieties under research condition was estimated %77.8 In addition; investment on wheat research program was economical, because, rate of return was estimated more than the discount rate (%18.5). Further, benefit-cost ratio for Mahdavi, Niknejad, Atrak, Tajan, Alamoot, Zarrin, Alvand, Darab2, Marvdasht, Kavir, Chamran and shiroodi varieties under research condition were estimated 5.3, 2.5, 10.1, 45.8, 72.2, 2.8, 9.1, 1.5, 13, 4.4, 18.1 and 9.1, respectively, and the investment rate of return for the above varieties were estimated 61.7, 55.3, 91.5, 137.3, 68.4, 53.1, 70.2,35.6, 62.2,55.5,192.3 and 150.5 percent, respectively

Keywords: Wheat, Variety, Research, Economic return, Iran

INTRODUCTION

Wheat research in Iran has a history of about 70 years. In this period, 70 Wheat varieties have been released. The seed and plant improvement institute (SPII) was established in 1946, Karaj, and the Department of Cereal Research was the first

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 251–257.

department to be formed and became actives at SPII. In Iran, breeding started in 1942. Currently, irrigated wheat research activities are conducted through a team wok in 21 research center and 33 stations around the country. The main objectives of the wheat breeding program are to increase yield potential, stability and adaptation, to increase resistance/tolerance to abiotic stresses such as drought, heat, cold, salinity and pre-harvest sprouting and durable resistance to diseases and pests, with medium to high flour quality for traditional flat bread baking (saidi and et al., 2001). Investment rate of return in Ghods wheat research in Iran by using benefit-cost ratio and internal rate of return was estimated 2.95–23.5 unit and 59.03–66.6 percent, respectively. (Hagiri and Rafati, 1996). Rate of return from wheat research in Fars province in Iran with selected 203 farmers by sampling method and production function approach is estimated 43.6% (Rafati and Najafi, 1996).

The main objectives of this study were to investigate agricultural budget as a portion of Gross National Production (GNP), determine the substitution value of gross production for irrigated bread wheat varieties in different regions, determine the Benefit-Cost Ratio and Investment Rate of Return for released irrigated wheat (Mahdavi, Niknejad, Atrak, Tajan, Alamoot, Zarrin, Alvand, Darab2, Marvdasht, Kavir, Chamran and Shiroodi varieties).

MATERIALS AND METHODS

In this study the internal rate of return and benefit-cost ratio was used for estimating investment rate of return. The model for estimating is described blow:

$$Benefit-\cos t\ ratio = \left[\sum_{t=1}^{n} B_t/(1+i)^t\right] \Big/ \left[\sum_{t=o}^{n} C_t/(1+i)^t\right]$$

$$F = P(1-i)^t$$

While, Bt annual gross benefits from wheat research in year t, Ct annual costs from wheat research in year t, F investment value in year t, P Peremerly investment, T year and i was discount rate.The internal rat of return (IRR), which is the discount rate that makes the net present value (NPV) of a stream of net social benefits equal Zero. A general formulation for the estimation of the gross benefits from a new variety (B_t) can be derived as follows (Heisy and Brennan, 1991).

$$Bt = G_t P_t A_t Y_t$$

Where P_t is the price for the wheat in year t, g_t is the percentage gain in yield from the breeding program in year t, A_t is the area sown in year t and Y_t is the mean yield of new variety in year t.

In this study, data required included price of wheat in different year, agricultural research budgets, cereal research budgets, distributed seeds of different varieties in irrigated bread wheat, Average yield of new and old varieties under research

condition, recommended seed (ha). Data were obtained from the Ministry of Agriculture, Cereal Research Department in SPII, and Agricultural Support Services Company. In this study, a stream of research cost for (1982–2000) periods is estimated.

RESULTS AND DISCUSSION

The total of distributed seeds from irrigated bread wheat in different area in Iran was 123881 (ton) in 1996. The share of irrigated wheat seeds including Mahdavi, Niknejad, Atrak, Tajan, Alamoot, Zarrin, Alvand, Darab2, Chamran from the total of distributed seeds of wheat in (1997–2000) was estimated 41.4, 59.6, 67.5 and 73.4 percent. Increasing yield of new varieties (Mahdavi, Niknejad, Atrak, Tajan, Alamoot, Zarrin, Alvand, Darab2 and Chamran to witness Varieties under research condition was 5, 5.5, 7, 18, 9, 10, 9, 2 and 3 percent. Thus, research achievements the released of bread wheat varieties including, increasing yield, salinity and diseases tolerance, Limited irrigation tolerance, resistance to logging and shattering and good bread making quality.

In 1999, Gross National production(GNP), research budget and Agricultural research budget were 396040000, 1535032 and 406322 million rials in Iran, respectively. Proportion of research budget and Agricultural research budget to GNP were 0.39 and 0.1 percent, respectively (Table 1).In this study, cultivated area of irrigated wheat varieties were 1.5 and 1.73 million (ha) in (1999–2000) respectively. In 2000, total cultivated area from Tajan and Chamran varieties were 17.5 and 35.4 percent, respectively. In (1995–1999), the percentage of new wheat varieties total cultivated area was 0.4, 6.2, 21.1, 44.5 and 65.8 percent, respectively (Table 2). Substitution value of gross production for released of irrigated wheat varieties in Iran in (1995–2000) were 1438, 36256.7, 136115.8, 333144.2, 531713 and 709212 million rials, respectively.(Table 3).

Benefit–cost ratio for wheat research was estimated more than unit, in the other hand, economic benefit of released varieties were more than research costs. Benefit

Table 1. Agricultural budget as portion of GNP during (1995–2000) in Iran

Explain	GNP to market price(million rials)	Research budget (million rials)	Agricultural research budget (million rials)	Proportion of research budget to GNP(%)	Proportion of agricultural research budget to GNP(%)
1995	177065600	613218	215143	0/35	0/12
1996	233132500	691940	234602	0/3	0/1
1997	276068900	814225	344966	0/29	0/13
1998	327217000	1228507	398574	0/37	0/12
1999	396040000	1535032	406322	0/39	0/1

Source: 1. Economic report, management & programming organization.
2. Program & budget, SPII, 2000.

Table 2. Cultivated area of released irrigated wheat varieties on the basis on distributed seed (ha)

Explain	1995	1996	1997	1998	1999	2000
Total area* Cultivated	2291489	2264669	2269899	2229078	2253827	2177901
Mahdavi	3047.4	27000	52000	98800	113300	135400
Niknejad	106.2	7000	35200	47600	63600	35400
Atrak	533.2	13800	64200	112800	129200	126200
Tajan	475	28700	104500	184700	248900	302800
Alamoot	2152.9	17500	56100	100300	129400	115600
Zarrin	–	558.8	4200	18200	30600	46200
Alvand	1514.7	23600	48600	84200	105300	116700
Darab 2	–	7496.3	38400	65700	71400	56200
Marvdasht	–	956.25	24200	69600	129900	126200
Kavir	393.75	1500	4800	8000	9950	18500
Chamran	–	8631.3	40300	181700	421100	612200
Shiroodi	–	2268.8	7300	19900	31450	37000
Total varieties	8223.25	139011.5	479800	991500	1484100	1728400

Source: Research data *Agricultural Ministery

cost ratio for Mahdavi. Niknejad, Atrak, Tajan, Alamoot, Zarrin, Alvand, Darab2, Marvdasht, Kavir, Chamran and Shiroodi varieties were estimated 5.3, 2.5, 10.1, 45.8, 7.2, 2.8, 9.1, 1.5, 13, 4.4, 18.1 and 9.1, respectively. Benefit cost ratio for total wheat varieties were estimated 25.8. Investment rate of return (IRR) for total bread wheat varieties was estimated 77.8%. Investment rate of return for Mahdavi. Niknejad, Atrak, Tajan, Alamoot, Zarrin, Alvand, Darab2, Marvdasht, Kavir, Chamran and Shiroodi varieties was estimated 61.7, 55.2, 91.5, 137.3, 68.4, 53.1, 70.2, 35.6, 69.2, 55.5, 192.3 and 159 percent, respectively (Table 4).

Table 3. Substitution value of gross production for irrigated wheat varieties (Million rials)

Explain	1995	1996	1997	1998	1999	2000
Mahdavi	352	3874.5	8736	20748	26648.2	37059
Niknejad	12.3	1010.2	5947.4	10053	15044.2	9744.3
Atrak	70.3	2257.5	12295.6	27004.3	34642	39376.7
Tajan	177.7	13343.5	56881.4	125670	189673.7	268519.4
Alamoot	409.2	4132.8	15510.5	34663.7	50087	52070
Zarrin	–	146.6	1290.2	6988.8	13160.4	23122.2
Alvand	287.9	5573.4	13436.9	29099	40758.7	52565.4
Darab 2	–	362.7	2175	4651.6	5661.7	5186
Marvdasht	–	2744.4	8131.2	29232	61105	69082
Kavir	128.6	608.8	2281	4752	6619.5	14322
Chamran	–	668.8	3656	20604.8	53483.1	90482
Shiroodi	–	1532.9	5774.6	196677	34829.5	47683
Total varieties	1438	36256.4	136115.8	333144.2	531713	709212

Table 4. Benefit-cost ratio and internal rate of return for research wheat during 1995–2000

Varieties	Distributed seed(ton)	Cultivated area (000ha)	Substitution value of gross production (million rials)	Present value of cost (million rials)	Net present value of activity (million rials)	Benefit-cost ratio	Rate of return (%)
Mahdavi	35607	429.5	97418	27204	115910	5.3	61.7
Niknejad	11297	188.91	41810	25158	38366	2.5	55.2
Atrak	25696	446.73	115647	16912	153045	10.1	91.5
Tajan	65172	870.1	654265	20462	917027	45.8	137.3
Alamoot	29196	421.1	156874	39251	199243	7.2	68.4
Zarrin	8565	99.76	44708	21908	38489	2.8	53.1
Alvand	28099	379.9	141721	22998	185273	9.1	70.2
Darab2	12771	239.2	18038	66014	8767	1.5	35.6
Marvdasht	24061	43.15	170294	18366	220390	13	69.2
Kavir	3725	350.9	28713	8240	31221	4.4	55.5
Chamran	104405	1263.9	168895	12152	212933	18.1	192.3
Shiroodi	7085	97.9	109498	10616	142508	14.4	159
Total	355513	4830	1747879	152252	2401718	25.8	77.8

To conclude, investment on bread wheat research program was economical. In this study, Substitution value of irrigated Wheat varieties (Mahdavi, Niknejad, Atrak, Tajan, Alamoot, Zarrin, Alvand, Darab2, Marvdasht, Kavir,Chamran and Shiroodi) were estimated 97418, 41810, 115647, 654265, 156874, 44708, 141721, 170294, 170294, 28713, 168895, 109498 million rials.So, substitution value of irrigated wheat varieties were estimated 1747879 million rials.

In during 1995–2000, the total of distributed seeds from new irrigated bread wheat in different area in Iran for 12 varieties was estimated 355513 ton. Cultivated area of irrigated wheat varieties were 4.83 million(ha).Substitution value of gross production for released of irrigated wheat varieties were estimated 1747879 million rials. The present value of cost for irrigated wheat varieties were estimated 152252 million rials.Net present value of irrigated wheat varieties were estimated 2401718 million rials(Table 4).

CONCLUSION

According to research, not only, the use of new varieties avoid from reduce crop yield, but also, to minimize the use of production inputs (poisons), to reduce production costs, to avoid pollution of surface and ground water of environmental resources, to increase both short and long-term farm profitability. By the way, good quality varieties reduce crop wastes, and generally, benefits reach to consumers and society. Benefit cost ratio of released bread wheat varieties under research condition was 25.8. Investment rate of return in newly released irrigated wheat varieties under research condition was estimated %77.8 In addition, investment on wheat research program was economical, because, rate of return was estimated more than the discount rate(%18.5).Further, benefit-cost ratio for Mahdavi, Niknejad, Atrak, Tajan, Alamoot, Zarrin, Alvand, Darab2, Marvdasht, Kavir, Chamran and Shiroodi varieties under research condition were estimated 5.3, 2.5, 10.1, 45.8, 72.2, 2.8, 9.1, 1.5, 13, 4.4, 18.1 and 9.1, respectively, and the investment rate of return for the above varieties were estimated 61.7, 55.3, 91.5, 137.3, 68.4, 53.1, 70.2,35.6, 62.2,55.5,192.3 and 150.5 percent, respectively.

ACKNOWLEDGEMENTS

The authors would like to thank from Dr. Saidi and Eng. Akbari, the Head and deputy of cereal research department, Eng. Niamanesh, Eng. Vahabzadeh and Eng. Smailzadeh helpful comments.

REFERENCES

Heisy PW, Brennan J (1991) An analysis model of farmers demand for replacement seed. Am J Agric Econ No 73(4):1044–52

Hagiri, Rafati M (1996) Estimation of investment return in Ghods wheat research in Iran. Economic research center. Agricultural Research, Education and Extension organization

Rafati M, Najafi B (1996) Determination rate of return for wheat breeding research in Farsi province of Iran. Quarterly. J Agric Econ Studies 15:9–33

Soltani GR (1981) Engineering economic. Publications of Shiraz University

Saidi A, Akbari A, Mozzaffari J, Heidari A, Serja Azari M, Pirayeshfar B, Yazdansepas A, Torabi M, Alizadeh A, Vahabzadeh M, Asadi H, Jalal Kamali MR (2000) Iranian wheat pool. A history of wheat breeding. The world wheat book

WEB-BASED SYSTEM TO TRUE-FORECAST DISEASE EPIDEMICS

I. Fusarium Head Blight of wheat

J.M.C. FERNANDES[1,*], E.M. DEL PONTE[2], W. PAVAN[3] AND G.R. CUNHA[1]

[1]*Embrapa Trigo, Passo Fundo, RS, 99001-970, Brazil*
[2]*Dept of Plant Patholoy, Iowa State Univ. 50011, Ames, IA, USA*
[3]*ICEG, Univ. of Passo Fundo, Passo Fundo, RS, 99001-970, Brazil*
[*]*E-mail: mauricio@cnpt.embrapa.br*

Abstract: One of the challenges for modelers, besides the development of mathematical equations integrating biology and climatology, is to provide a comprehensive model delivery system through the Internet. The use of near real-time and forecast weather data is key to true-forecast disease outbreaks at local and regional basis. A prototype of such a system is proposed here to predict and forecast infection risks for Fusarium head blight (FHB) using a model previously developed. The system was designed to access and retrieve weather data from an automatic weather station and from a remote database with 7-days weather forecast for the same local. The model is initiated through a web interface and the simulation starts by selecting heading date. Once the current day is entered, the model uses forecast information to warn disease outbreaks, by combining forecast and historical weather data. Model results are presented to the user in tabular, graphical and report, which shows interpretation for the results based on an expert assessment. Once a registered user set a heading date, daily simulation updates are sent daily to email and cell phones. The prototype proved functional, is flexible and has the potential to integrate a decision support system for disease management. A farmer cooperative is extensively testing the system during the current wheat season in the state of Rio Grande do Sul

Keywords: Fusarium Head Blight, model

INTRODUCTION

Disease forecasting has become an established component of quantitative epidemiology. The mathematic description of disease dynamics is the core of several disease forecast models that have been developed in the last four decades. However,

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 259–264.

many models have not lived up to the expectations that they would play a major role and lead to a better disease management. Amongst the reasons, the presumption of a disease forecast model is that it makes projections of major events in disease development and most present forecast models do not (Seem 2001). An exciting development in this area is the possibility to use weather forecasts as input into disease models and consequently output true disease forecasts. As weather forecasts improve together with more accurate estimations of micro environmental variables useful for plant disease models, as such precipitation and leaf wetness duration, it will be possible to provide seasonal estimates of disease likelihood and forecast outbreaks. This is especially interesting for field crops for the reason that unnecessary sprays has a significant impact on production costs, and no timely applications may result in inadequate control.

The present work illustrates an approach towards that direction by the use of novel programming languages and technology for the development of a web-based system for model implementation and delivery. The case study is FHB, a disease of great concern for wheat production worldwide as well as for southern Brazilian wheat areas. Despite of all research done along many years, the control of this disease is still challenging given its complex nature (McMullen et al. 1997) and some factors as dose rate, application timing and spray quality for adequate coverage of the spike tissues are key in fungicide efficacy for a good control (Reis 1986, Picinini and Fernandes 2001). FHB forecast models are considered an important tool for the decision-making, allowing producers to timely and effectively apply fungicides in conjunction with other control strategies (McMullen et al. 1997, Xu 2003). Different approaches for modeling this disease is found in the literature and comprehensive information on several FHB models have been reviewed (Del Ponte et al. 2004).

Critical knowledge on the epidemiology of a disease needs to be available in developing a decision support system. The Epidemiology of FHB has been studied in southern Brazil since late 1980's. Climatic conditions are most suitable in that region, and disease has a periodical occurrence. The distinct climate conditions observed along the years have helped in identifying the main factors affecting regional epidemics. A mechanistic process-based simulation model, named GIBSIM, has been developed and improved along the years with previous knowledge and a series of local studies on the interaction of pathogen, host dynamics, and the environment. The model has been validated with epidemic cases observed in Passo Fundo location, Brazil. The data has been collected on experimental plots in 5-years and distinct planting dates each year. The accumulated risk infection index simulated by the model explained 93% of variation in disease severity (Del Ponte et al. 2005).In this work, GIBSIM model is the core of a web-based system designed to gather site-specific and forecast weather data and deliver true-forecasts for FHB for consultants in the Cotrijal cooperative located in Não Me Toque in the state of Rio Grande do Sul, Brazil.

MATERIAL AND METHODS

The web application, called GIBSIMWeb was developed based on the Model-View-Controller (MVC) design pattern. The model part is the business logic; the view presents images and data on WebPages; and the controller determines the overall flow of the application (Fig. 1). The server programs are: weather data management server (WDMS), database server (DBS), disease forecasting model server (DFMS), and web server (WS). WDMS consists of a module for weather data retrieval from automated weather stations located at remote sites. Data is updated at 10 minutes interval. In addition, forecast data, provided by the INPE (National Institute for Space Research) is retrieved by FTP protocol. PostgreSQL is the core of DBS and stores weather data, as well the identifiers for weather station and run-time parameters as cultivar, planting date, previous crop, etc. DBS is interfaced with WDMS and DFMS using a Java API, and with WS using an SQL module in a JSP script engine. WS retrieves information from DBS upon request by users through a client-side (web-browser) interface. In addition, it provides a simple request form

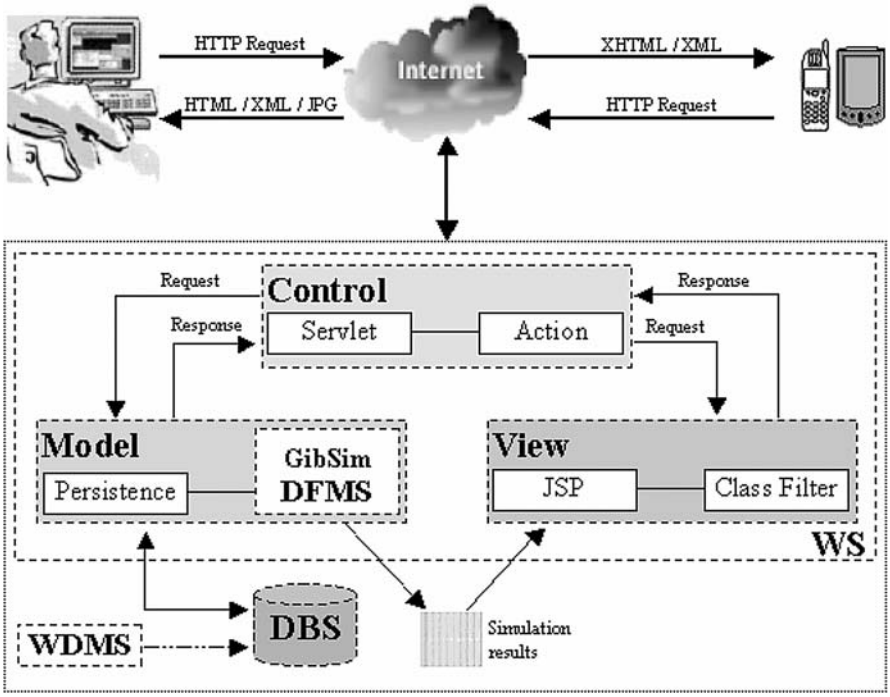


Figure 1. Architecture of the Web application designed for gathering and storing actual and forecast weather data to run a simulation model to forecast risk of Fusarium Head Blight of wheat. The server programs are: weather data management server (WDMS), database server (DBS), disease forecasting model server (DFMS), and web server (WS)

for defining the run-time parameters. The output is displayed either in textual or graphical format by using a server-side plotting script. The system is also set to deliver simulation output to cell phones and PDA. Besides the option of defining a weather station in the database, the system allows users to input their own weather data, such as precipitation, temperature, relative humidity, etc., customizing the results for site-specific conditions.

The system uses either hourly or daily weather data from DBS, and DFMS produces daily risk infection index by using near real-time and anticipated risks by combining historical with 7-day forecast weather. During the simulation, each sub-model uses data from WDMS. The daily output is a risk infection index calculated based on daily outputs from each sub-model. The forecast risk combines both historical and 7-days forecast of hourly weather data, generated by the ETA model using a grid of 40 km × 40 km. Since the model accounts for the effect of wheat development to estimate disease severity, the simulation starts at the day the first heads emerge in the field. At any time since then, actual and week ahead accumulated risk index is estimated. Once an accumulated risk level of concern is projected and the simulation is at the critical time for control, the model warns that fungicides may be needed.

RESULTS AND DISCUSSION

The GIBSIMWeb system successfully collected hourly weather data as solar radiation, temperature, precipitation and relative humidity from three automatic weather stations located within Cotrijal area and forecast data from INPE servers, and stored them in the DBS. After defining location, heading date, and cultivar, the system was set to present the results in the WebPage in a tabular, graphical and report format (Fig. 2). The table shows model output and weather variables. The graph shows the daily increase of infection index, and some environmental

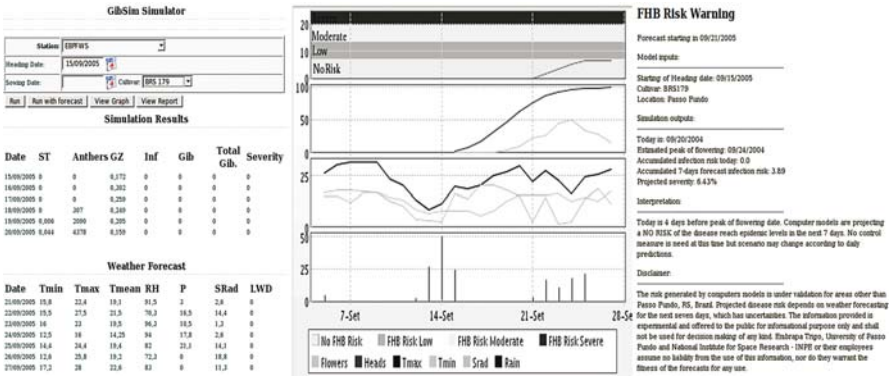


Figure 2. Computer screen showing model inputs and simulation results

variables. Infection indices and related risk were computed in a daily basis since first day of the simulation and the anticipated risk took into account the actual and forecast data. The report is a summary and interpretation of the risk of outbreaks, which may be used to base decision-making. The reports were sent to emails and cell phone provided by registered users. For those setting a specific date for heading the system run automatically in a daily basis using pre-set parameters. Numerical infection index was converted to 4 categorical levels (No, low, moderate and high epidemic risk) that based decision-making on fungicide application, along with other factors. During the wheat season of 2005 the system was tested by 32 consultants from the farm cooperative Cotrijal. The heading date in the area for the wheat varied from September 5th to 23th. The system predicted low to moderate risk for the wheat fields with the heading stage starting at the beginning of September. In contrast a moderate to high risk was predicted to wheat fields with the heading stage after September 20th. In general, the predictions obtained from the system coincided with field observations. The GibSimWeb URL is: http://inf.upf.br:8080/gib/

The system proved functional and can be easily extended to other locations where automatic stations are available with the capability to send data to DBS using the same protocol. Further on, the system may contain modules to allow a user to set weather retrieval from his own on-site automatic station directly to the DBS or from there to his computer and access a local database, besides retrieve forecast data from INPE. Therefore, the user may run the model for his location from any computer or mobile device accessing the web. The user will have the option to either make his data public or private. This would be an alternative to computerized weather stations that are more costly.

A tactical utility of the Web application for the management of FHB is the potential to improve disease control by allowing timely fungicide applications. Weather a high risk of outbreaks is anticipated, application of fungicides soon after infections, if weather permits, would help improving fungicide efficacy with a curative effect. Besides that, once weather data is available for several locations in a region, the model can be used to assess spatial variability of regional epidemic. Once long-term historical weather dataset is available for several locations in a production region, the model can be used to map climatic suitability for the epidemics. Effects of planting dates and crop rotations could be evaluated without the need of local experimentation. This system may also be used to hindcast past scenarios to test the accuracy of the system.

The modularity of the system allows the implementation of other disease models especially those requiring more complex data as hourly weather information and leaf wetness duration. The disease simulator may be easily layered with crop models such as the CERES-WHEAT from The Decision Support System for Agrotechnology Transfer (DSSAT) suite, using phenological data output by the latter (Ritchie et al. 1998). Fernandes et al. 2004, linked process-based models to assess the potential impact of climate change in the epidemics of Fusarium Head Blight in wheat growing regions in southern Brazil, Uruguay and Argentina.

ACKNOWLEDGEMENTS

This research was supported in part by a grant from the Embrapa Macro Programa 2 and a grant from AIACC LA 27 project. The support received from CNPq is also acknowledged. Thanks also for the programming assistance received from the participants in the Simuplan project.

REFERENCES

Del Ponte EM, Fernandes JMC, Pavan W (2005) A risk infection simulation model for Fusarium head blight of wheat. Fitopatologia Brasileira 30(6): 634–642

Del Ponte EM, Fernandes JMC, Pierobom CR, Bergstrom GC (2004) Giberela do trigo – aspectos epidemiológicos e modelos de previsão. Fitopatologia Brasileira 29:587–605

Fernandes JM, Cunha GR, Del Ponte E, Pavan W, Pires JL, Baethgen W, Gimenez A, Magrin G, Travasso MI (2004) Modelling Fusarium Head Blight in wheat under climate change using linked process-based models. In: Canty SM, Boring T, Wardwell J, Ward RW (eds) 2nd international symposium on Fusarium head blight; incorporating the 8th European Fusarium Seminar; 2004, 11–15 December; Orlando, FL, USA. Michigan State University, East Lansing, MI, pp 441–444

McMullen M, Jones R, Gallenberg D (1997) Scab of wheat and barley: A re-emerging disease of devastating impact. Plant Dis 81:1340–1348

Picinini EC, Fernandes JMC (2001) Efeito da época de pulverização com fungicidas sobre o controle de *Gibberella zeae* em trigo. Comunicado Técnico, Embrapa Trigo, Passo Fundo, pp 8:

Reis EM (1986) Caracterização da população de *Fusarium graminearum* ocorrente no Sul do Brasil. Fitopatologia Brasileira 11:527–533

Ritchie JT, Singh U, Godwin DC, Bowen WT (1998) Cereal growth, development and yield. In: Tsuji GY, Hoogenboom G, Thornton PK (eds) Understanding options for agricultural production. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 79–98

Seem R (2001) Plant disease forecasting in the era of information technology. Plant disease forecast: Information technology in plant pathology, Kyongju, Republic of Korea, 25 October 2001

Xu X (2003) Effects of environmental conditions on the development of Fusarium ear blight. Eur J Plant Pathol 109:683–689

IMPACT OF CROP MANAGEMENT SYSTEMS ON DISEASES OF SPRING WHEAT ON THE CANADIAN PRAIRIES

M.R. FERNANDEZ[1,*], D. ULRICH[2], L. SPROULE[2], S.A. BRANDT[2], A.G. THOMAS[3], O. OLFERT[3], R.P. ZENTNER[1] AND B.G. McCONKEY[1]

[1]*Semiarid Prairie Agricultural Research Centre, Agriculture and Agri-Food Canada (AAFC), P.O. Box 1030, Swift Current, Saskatchewan, Canada S9H 3X2*

[2]*Scott Research Farm, AAFC, P.O. Box 10, Scott, Saskatchewan, Canada S0K 4A0*

[3]*Saskatoon Research Centre, AAFC, 107 Science Place, Saskatoon, Saskatchewan, Canada S7N 0X2*

[*]*E-mail: fernandezm@agr.gc.ca*

Abstract: *Fusarium* spp. (*F*spp.) associated with crown/root rot of wheat are also responsible for Fusarium head blight (FHB), a disease of increasing importance on the Canadian Prairies. This study showed that tillage/input level (organic [OI], reduced [RI] or high [HI]) had a greater impact on common root rot (CRR) than cropping diversity (low diversity, diversified with annual grains, or diversified with annual grains/forage). Levels of CRR and *Cochliobolus sativus* were highest for OI and HI, and lowest for RI. Higher disease levels were also observed under low cropping diversity where the frequency of cereals and fallow was highest. *F*spp. were more common in OI and RI than HI, and in the more diverse systems, although the nature of these associations varied with the species. *F. avenaceum* and *F. culmorum*, two of the main crown/root rot and FHB pathogens, were most associated with RI systems and/or continuous diversified grain rotations, and least associated with OI systems. Further work on crop management effects on *Fusarium* populations in wheat is warranted considering the increased development of important diseases caused by *F*spp. in western Canada

Keywords: *Fusarium*, *Cochliobolus sativus*

INTRODUCTION

Common root rot (CRR) is a widespread disease of cereal crops on the Canadian Prairies. The main root pathogen is *Cochliobolus sativus* (cs), followed by *Fusarium* spp. (Fspp.) (Fernandez and Jefferson 2004). Some of these Fspp. also cause

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 265–271

Fusarium head blight (FHB), a very damaging cereal disease which continues to spread in this region.

Past research has shown that the presence of cs and Fspp. in wheat roots was affected by tillage and crop rotation, although results have not always been consistent. Conversion to organic management has also been shown to affect the relative prevalence of Fspp.

Because current production trends in western Canada include increased incorporation of noncereal crops in cereal-based rotations, partly due to the increased adoption of conservation tillage practices, and because of continuing conversion to organic management practices, it is of interest to determine how these production factors might affect CRR levels and fungal populations, including Fspp. associated with FHB. This study was conducted in 2001–2004 in a cropping system experiment initiated in 1995 at the AAFC Research Farm at Scott in west-central Saskatchewan, which incorporates different tillage, chemical input and cropping diversity treatments representative of current production trends in western Canada.

MATERIALS AND METHODS

The experiment was a 4-replicate split plot design with main plot treatments consisting of 3 levels of tillage/input usage and sub-plots comprised of 3 levels of cropping diversity, each on a 6-year rotation cycle. Tillage/input levels were: Organic (OI) with intensive tillage and green manure fallow; Reduced (RI) with reduced tillage and input rates; and High (HI) with conventional-till and recommended rates of pesticides and fertilizers. Cropping diversity levels were: Low (LOW), a wheat-based rotation of fallow-wheat-wheat-fallow-oilseed-wheat, with legume green manure used as fallow substitute in OI, and green manure (year 1) and chemical fallow (year 4) used in RI; Diversified Annual Grains (DAG), a canola-winter cereal-pea-barley-flax-wheat rotation for HI and RI, and green manure fallow-wheat-pea-barley-green manure fallow-canola for OI; and Diversified Annual and Perennial (DAP), with oilseed-wheat-barley-alfalfa-alfalfa-alfalfa.

CRR index (CRRI) and severity (CRRS) were estimated from subcrown internode ratings at late milk-early dough stage. For each plot, percent isolation of each fungus was estimated based on the total number of isolates growing out of discoloured tissue plated on nutrient agar.

Data were analyzed with the PROC MIXED procedure of SAS along with the GLIMMIX macro (SAS Version 8.0, SAS Institute, Cary, NC). Raw and presence/absence data were analyzed with appropriate model specifications. Treatment effects were significant at $P \leq 0.10$. A multidimensional preference (multivariate) analysis was conducted to investigate the coincidence of disease/fungal attributes with tillage/input by diversity treatment combinations. The results were summarized in a biplot with each tillage/input by diversity system combination plotted as points in the ordination space and disease/fungal variables plotted as vectors.

RESULTS

On average, cs was present in 98% of plots, Fspp. in 94%, and *Microdochium bolleyi* (mb) in 73%. Among the most common Fspp. were *F. equiseti* (fe) (77% of all plots), *F. avenaceum* (fa) (27%), and *F. culmorum* (fc) (17%).

The multivariate analysis indicated a polarization of the treatments; the HI and OI systems tended toward the left/middle ordination space, whereas RI-DAG was positioned in the top-right space and the other RI systems in the bottom right space (Fig. 1). The CRR vectors pointed mainly towards HI and OI systems, particularly in the LOW diversity treatments. Mean differences confirmed the preceding relationships; CRRI and CRRS often were greatest for HI and OI, regardless of the crop diversity system (Table 1). Cs was most closely related to the CRR vectors, especially for LOW diversity. Mb was in the ordination space where the RI vectors resided, but average differences were only significant at $P \leq 0.10$ for the LOW treatments.

Fspp. appeared to be more common in the OI and RI than in the HI system (Fig. 1). For all Fspp. combined, means were lowest for HI (Table 1), although there were differences among these species in their orientation relative to the CRR

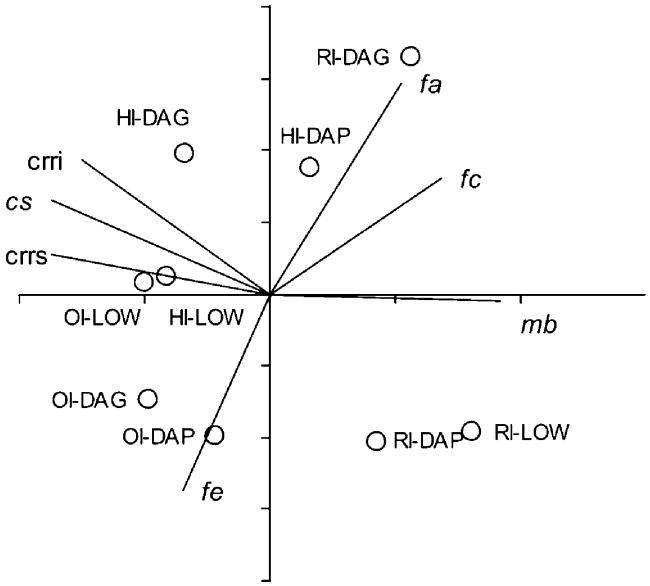


Figure 1. 1 Biplot for the first two principal components derived from the multidimensional preference analysis. The most prevalent isolates (see abbreviations below), CRR (common root rot) Index (CRRI), severity (CRRS) and different combinations of tillage/input system (organic, OI; reduced, RI; high, HI) with cropping diversity (low diversity, LOW; diversified annual grains, DAG; diversified annual and perennial, DAP) are plotted as vectors. The principal component scores (generally plotted as points) were not included. Abbreviations for fungal isolates = *cs*: *Cochliobolus sativus, fa*: *F. avenaceum*, *fc: F. culmorum*, *fe*: *F. equiseti*, *mb*: *Microdochium bolleyi*

Table 1. Analysis of variance and average disease and fungal responses to tillage/input and diversity treatments in west-central Saskatchewan, Canada (2000–2004)

	CRRI[a]	CRRS	Fungal species					
			fa	*fc*	*cs*	*fe*	*Fusarium spp.*	*mb*
			(P value)					
Input (I)	0,121	0,013	0,292	0,109	<0.001	<0.001	0,005	0,162
Diversity (D)	0,525	0,855	0,331	0,049	0,895	0,367	0,769	0,453
I x D	0,136	0,604	0,586	0,079	0,062	0,538	0,342	0,439
Organic[b]	0,062	0,294	0,590	0,205	0,880	0,790	0,696	0,422
Reduced	0,436	0,934	0,298	0,527	0,144	0,233	0,171	0,589
High	0,559	0,880	0,465	0,006	0,332	0,515	0,693	0,300
LOW	0,049	0,014	0,513	0,014	0,002	0,017	0,007	0,093
DAG	0,174	0,040	0,376	0,127	0,301	0,004	0,154	0,147
DAP	0,183	0,121	0,476	0,158	0,156	0,023	0,167	0,552
	(Mean values)		**(Prob. of occurrence)**[c]				**(Mean values)**	
Organic								
LOW	1,26	14,2	0,125	0,021	58,3	15,9	23,5	7,6
DAG	1,20	14,5	0,125	0,031	55,6	15,7	24,0	4,3
DAP	1,06	10,1	0,063	0,093	59,1	13,3	20,6	6,9

Reduced								
LOW	0,97	6,5	0,146	0,134	38,3	10,2	25,3	13,9
DAG	1,06	6,8	0,250	0,186	51,4	5,9	18,3	10,3
DAP	1,05	7,2	0,125	0,092	47,4	10,6	25,1	10,0
High								
LOW	1,23	12,0	0,094	0,031	65,0	6,7	13,3	6,4
DAG	1,31	12,6	0,156	0,062	60,9	5,1	15,1	5,6
DAP	1,27	13,3	0,156	0,250	54,9	4,5	16,5	10,4
$LSD_{0.10}$[d]	0,15	1,4	0,049	0,043	11,9	1,3	6,8	1,6

[a] CRRI: Common root rot (CRR) index; CRRS: CRR severity; *cs*: *Cochliobolus sativus*; *fa*: *F. avenaceum*; *fc*: *F. culmorum*; *fe*: *F. equiseti*; *mb*: *Microdochium bolleyi*.

[b] Diversity effect for each level of input, and then input effect for each level of diversity. Diversity levels = LOW: low diversity, DAG: diversified annual grains, DAP: diversified annual and perennial.

[c] Analysis was conducted across years and phases with presence data, binary distribution, and logit link specification. Probability of occurrence (converted to percentage basis) represents the chance of encountering an isolate in a treatment; a probability of 100% indicates that an isolate would be found in all plots.

[d] SE rather than $LSD_{0.10}$ is presented for CRRI, *fe*, and *Fusarium* spp.

vectors and the various treatments. Fa and fe occupied opposite regions of the ordination space. Percentage isolation of fe was highest in the OI and lowest in the HI system. Despite the opposite position of fa to the OI vectors, differences in the averages among treatments were relatively small with the exception of a greater average for RI-DAG. Fc was also positioned opposite to the OI systems and its averages were highest for RI for the LOW and DAG diversity, although the highest levels of this fungus were detected in the HI-DAP treatment. A combination of the multivariate plot and average responses for the different treatment combinations indicated that the individual Fspp. tended to prefer the diversified systems (DAG and DAP), despite the ANOVA not being able to clearly confirm that the diversified systems were different from the other systems.

DISCUSSION

Tillage/input level had a greater impact on CRR than cropping diversity, with those effects being most significant within the rotations most dependent on wheat and fallow (LOW). Our results that levels of Fspp., such as fa, increased, and levels of cs decreased, with a reduction in tillage intensity agree with results reported by Stevenson et al. (2005). In contrast, the high Fspp. levels in OI agree with Knudsen et al. (1995). Higher levels of the weak pathogen/saprophyte fe in organic fields were also observed by Elmholt (1996), whereas lower levels of pathogenic Fspp., such as fa and fc, in fields under organic management were also reported by Hannukkala and Tapio (1990) and Knudsen et al. (1995).

Fspp. were also most associated with the more diversified cropping sequences. The higher levels of Fspp., and lower levels of cs, in continuous cropping systems that included a higher proportion of noncereal crops than in sequences consisting mostly of wheat and fallow agree with Bailey et al. (2001) and Fernandez and Zentner (2005). In particular, an association of fa with continuously cropped cereals grown in rotation with pulses under zero-till was also observed recently in two Saskatchewan studies (Fernandez and McConkey, unpublished; Stevenson et al. 2005). The latter study also showed effects of previous glyphosate applications on levels of the most commonly isolated fungi, but not on total CRR severity. It is not known whether previous glyphosate use affected fungal communities in wheat roots in the present study.

CONCLUSIONS

Management systems that increase crop diversity and decrease the use of tillage and fallow will help control CRR in spring wheat, although continuously cropped diversified grain rotations (especially under OI or RI management) favour the development of Fspp. However, whereas diversified cropping under RI management might further increase fa levels, OI management will likely result in a reduction in populations of fa and fc, two of the most common crown/root rot and FHB pathogens. Further work to separate the effect of cropping sequence from that

of tillage/input system on Fusarium infections, and to elucidate the mechanisms responsible for the changes in populations of pathogenic Fspp. under different tillage/input systems is warranted considering the continuing adoption of conservation tillage practices and incorporation of noncereal crops in cereal-based systems by western Canadian producers, and the increased development of important wheat diseases caused by Fspp. in this region.

ACKNOWLEDGEMENTS

The technical assistance of M. Boire, W. Galecki, S. Walter and K. Brandt, and statistical analysis by Dr. F.C. Stevenson are greatly acknowledged.

REFERENCES

Bailey KL, Gossen BD, Lafond GP, Watson PR, Derksen DA (2001) Effect of tillage and crop rotation on root and foliar diseases of wheat and pea in Saskatchewan from 1991 to 1998: univariate and multivariate analyses. Can J Plant Sci 81:789–803

Elmholt S (1996) Microbial activity, fungal abundance, and distribution of *Penicillium* and *Fusarium* as bioindicators of a temporal development of organically cultivated soils. Biological Agriculture and Horticulture 13:123–140

Fernandez MR, Jefferson PG (2004) Fungal populations in roots and crowns of common and durum wheat in Saskatchewan. Can J Plant Pathol 26:325–334

Fernandez MR, Zentner RP (2005) The impact of crop rotation and N fertilizer on common root rot of spring wheat in the Brown soil zone of western Canada. Can J Plant Sci 85:569–575

Hannukkala AO, Tapio E (1990) Conventional and organic cropping systems at Sutia V: Cereal diseases. J Agr Sci Finland 62:339–347

Knudsen IMB, Elmholt S, Hockenhull J, Funck Jensen D (1995) Distribution of saprophytic fungi antagonistic to *Fusarium culmorum* in two differently cultivated field soils, with special emphasis on the genus Fusarium. Biological Agriculture and Horticulture 12:61–79

Stevenson FC, Fernandez MR, Selles F (2005) Response of leaf spots and common root rot to crop management: field survey of spring wheat in eastern Saskatchewan, Canada. ACTA of 7th International Wheat Conference, November 2005, Mar del Plata, Argentina

EFFECT OF POTASH DEFICIENCY ON HOST SUSCEPTIBILITY TO *COCHLIOBOLUS SATIVUS* CAUSING SPOT BLOTCH ON WHEAT

D. MERCADO VERGNES[1,*], M.E. RENARD[1], E. DUVEILLER[2] AND H. MARAITE[1]

[1] *Unité de Phytopathologie, Université catholique de Louvain, Croix du Sud 2 bte 3, B-1348 Louvain-la-Neuve, Belgium*

[2] *CIMMYT, South Asia Office, Singha Durbar Plaza Marg, P.O. Box 5186, Kathmandu, Nepal*

[*] *E-mail: mercadovergnes@fymy.ucl.ac.be*

Abstract: Helminthosporium leaf blight is the most important disease of bread wheat in the Indo-Gangetic plains and is caused by *Cochliobolus sativus* and *Pyrenophora tricirepentis*. Late planting and unbalanced fertility increase disease severity. A study on two genotypes BL2217 (moderately resistant) and Ciano 79 (susceptible) was conducted in a greenhouse under controlled-environment with 3 levels of potassium fertilisation. Treatments consisted of half-strength standard Hoagland solution pH 7.0 as control, and two levels of potassium deficient solutions containing only 36.8% and 13.5% potassium of the standard half-strength solution. Plants were inoculated with a *C. sativus* virulent isolate from Nepal. Disease severity (% DLA) and chlorophyll content measured as SPAD value (SPAD-502, Minolta Co., Ltd, Japan) at 3, 7, 10, 14, 17 and 21 days after inoculation were evaluated in inoculated and control plants, respectively. To assess the interaction of the pathogen with host cells, microscopic analysis and histochemical assays for H_2O_2 detection were performed 24-h and 48-h after inoculation. Potassium deficiency significantly increased disease severity in both genotypes. A significant ($P < 0.01$) negative linear correlation between AUDPC and chlorophyll content was observed for both genotypes. Microscopic analysis and histochemical assay for H_2O_2 detection showed that cell wall apposition formation and hypersensitive reaction were significantly reduced in BL 2217 under potassium deficiency, stressing the importance of the soil fertility as part of an integrated crop management of HLB

Keywords: potash deficiency, *Cochliobolus sativus*

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 273–279.

INTRODUCTION

Cochliobolus sativus (Ito & Kurib.) Drechsler ex Dastur (anamorph: *Bipolaris sorokiniana* (Sacc.) Shoem.) and *Pyrenophora tritici-repentis* (Died.) Drechs. are two non-specific hemi-biotrophs pathogens responsible of HLB, a serious disease of wheat (*Triticum aestivum* L.) in warm areas of South Asia. Grain yield reductions of up to 20% have been observed in farmers fields (Sharma and Duveiller 2004).

Unbalanced fertility and nutrient mining are some of the constraints. By affecting the growth pattern, the anatomy and morphology and particularly the chemical composition, a well balanced nutrition of plants may contribute to an increase of the resistance and/or tolerance to diseases (Krauss 2001). Potassium affects the reaction of a plant to diseases by: i) direct effects on the pathogen multiplication, development and survival, ii) direct effects on the internal metabolism of the plant, thus affecting food supply for the pathogen, and iii) effects on the establishment of the pathogen and its spread within the plant, through the influence of potassium on plant defence responses, cell-wall structures and function of stomata (Perrenoud 1990). Reactive oxygen species (ROS) play a number of critical roles, including modification of cell walls, activation of defence-response genes, and a direct antimicrobial effect (Li et al. 2005). Among ROS, H_2O_2 plays an important role in penetration resistance. Quantitative resistance or basal disease resistance is frequently based on efficient papillae formation as well as hypersensitive response (HR). Papillae are composed of phenolics, callose, peroxidases and cell wall material. In addition, ROS such as H_2O_2 have been shown to accumulate in papillae and adjacent areas, coincident with attempted fungal penetration (Ibeagha et al. 2005). This suggests that oxidative cross-linking reinforces the cell wall, thereby creating a structural barrier against fungal invasion. Plant defence responses and cell-wall structures can be modulated by potassium deficiency, which could favour the pathogen establishment and spread within the plant. Spot blotch and common root rot incidence caused by *C. sativus* on wheat were shown to decrease by application of high KCl level (Timm et al., 1986). Fixen et al. (1986) reported a dramatic and visually obvious tan spot suppression following the application of KCl. Sharma et al. (2005) demonstrated that potash application significantly reduced HLB field severity in the lowlands of Nepal. Nevertheless, variation in temperature and inoculum may affect evaluation of genotype response to potash under field conditions. Therefore, a comparative study was conducted under controlled conditions to evaluate the effect of potassium deficiency in adult plants of two genotypes differing in resistance to *C. sativus*.

MATERIALS AND METHODS

Host

Susceptible Ciano T-79 and moderately resistant BL 2217 were selected based on earlier field resistance studies. Plants were grown in plastic trays ($37 \times 57 \times 14$ cm) containing an autoclaved nutrient-poor 2–3 mm quartz gravel and kept in a greenhouse with a 15/10 °C day/night temperature and a 16-h photoperiod. Control plants

were fertilised with a half-strength standard Hoagland solution pH 7.0. Potassium deficiency was induced by replacing the standard nutrient solution (K_{comp}) with a solution containing only 36.8% ($K_{0.368}$) and 13.5% ($K_{0.135}$) KNO_3 and KH_2PO_4 (equimolecular amounts of NaNO3 and NaH_2PO_4 were used instead). The nutrient solution was aerated and replaced with fresh solution once a week.

Pathogen and Inoculation

A monoconidial strain of *C. sativus* from Nepal (MUCL 45264, UCL, Louvain-la-Neuve, Belgium) was grown for 7 days in Petri dishes containing V8 juice potato dextrose agar medium (V8-PDA) at room temperature under constant light. Conidia were collected by spraying the Petri dishes with 10 ml of sterile distilled water using an atomiser. Plants at heading stage (GS 59) were sprayed with a suspension containing 5000 spores per ml water plus 0.02% Tween 20 as adjuvant. 90 ml of conidial suspension were sprayed per tray. The inoculated plants were moved for 24 h to a mist chamber at 23/20°C day/night with a 16 h photoperiod before incubation in the greenhouse at the same temperature given above, using a randomised complete block design with two replicates.

Microscopic Analysis and Histochemical Assay for H_2O_2 Detection

Calcofluor-stained hyphae were observed with a Reichert-Jung (Vienna, Austria) Polyvar microscope equipped with epifluorescence optics (HBO 200 mercury lamp; Osram, Münich, Germany), by using the U1 filter combination (excitation filter BP 330-380, barrier filter LP 418 and dichroic mirror DS 420). Calcofluor has been used for cell and hyphal wall detection (Schäfer et al. 2004, Ibeagha et al. 2005) and facilitates the location of fungal penetration sites in leaf tissue. For better contrast of reactions, histochemical detection of H_2O_2 was carried out by an endogenous peroxidase-dependent in situ staining procedure with 3,3-diaminobenzidine (DAB) (Thordal Christensen et al. 1997). Two hours before sampling, 1 cm segments were cut from the tip of flag leaves. The cut ends of leaves were immersed in the DAB solution (1 mg L^{-1}). Inoculated leaves were sampled at 24 and 48 h after inoculation (hai). Calcoflour staining was done according to Schäfer et al. (2004). Forty interaction sites were determined per leaf segment. Four leaf segments were analysed in at least two independent experiments. Interaction sites were defined as locations where a fungal appresorium caused a visible plant response. Interactions were evaluated in all epidermal cell types except hair cells.

Disease Assessment

Percent of diseased leaf area (% DLA) was visually assessed on the flag (F) and penultimate (F-1) leaves at 3, 7, 10, 14, 17 and 21 days after inoculation (dai). After averaging F and F-1 scores, the area under disease progress curve (AUDPC) was calculated using the severity estimates corresponding to the six ratings. SAS

v.7.12 (SAS Institute Inc., Cary, NC) was used for data analyses; severity data are the means of two replicates of 10 plants. The AUDPC was analysed with the PROC analysis of variance procedure and Tukey's test ($P = 0.05$) was used for comparing treatments.

Chlorophyll Content Evaluation

A Soil Plant Analysis Development meter (SPAD-502, Minolta Co., Ltd, Japan) was used to measure non destructively the loss of chlorophyll on the flag leaf and to assess the plant nutrition status. Five readings (SPAD values) taken from the tip to the base of the leaf blade of each plant were recorded after flag leaf emergence (GS37). The average of the ten tagged flag leaves was used as observation. Two replicates were used per treatment. Six SPAD readings were taken during the experiment and used to calculate the area under the SPAD progress curve (AUSPAD), as described previously for the AUDPC. The AUSPAD was analysed with the PROC analysis of variance procedure and Tukey's test ($P = 0.05$) was used for comparing treatments.

Quantification of Fungal Development and Defense Responses

Papilla-like structures and the hypersentive reaction (HR) were detected as described by Schäfer et al. (2004). Papillae-like cell wall appositions with halo appear light blue under UV excitation or as reddish-brown coloration around fungal appresoria under bright-field. Whole-cell DAB staining was used to detect the HR. Autofluorescence correlated closely with subcellular and whole-cell accumulation of H_2O_2. In some cases, fungal growth was arrested only after penetration and infection hyphae formation within epidermal cells by a subsequent cell death reaction is termed post-penetration HR (PPHR). Fungal penetration into epidermal cells was termed successful penetration while invasion of the mesophyll tissue was termed successful infection (Ibeagha et al. 2005).

RESULTS AND DISCUSSION

Chlorophyll content evaluation SPAD values of flag leaves before inoculation showed a highly significant difference ($P < 0.001$) between potassium treatments, with plants fertilised with solution $K_{0.135}$ showing the lowest SPAD value. Before inoculation, no significant statistical difference in SPAD value was observed between plants grown under K_{comp} and $K_{0.368}$ solutions.

The main effect of potassium stress on AUSPAD was highly significant ($P<0.001$). Both genotypes showed lower SPAD values under potassium deficiency ($K_{0.135}$ and $K_{0.368}$) compared to the standard Hoagland fertilisation (Fig. 1A). The difference in AUSPAD between the two wheat genotypes was not significant. The genotype x fertilisation interaction was not significant for AUSPAD indicating no difference between genotypes.

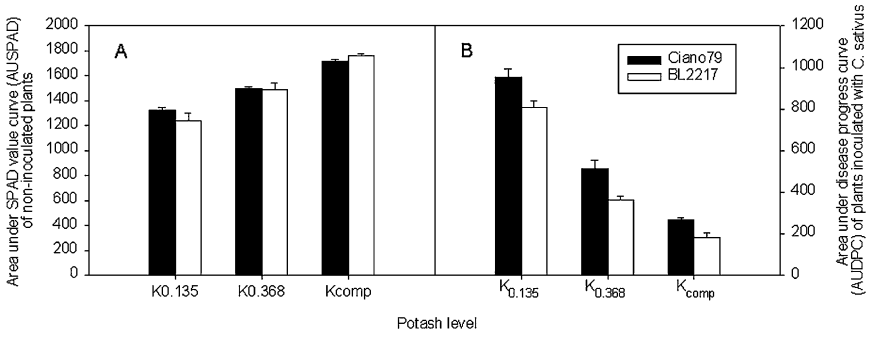


Figure 1. **A**, Area under the SPAD progress curve (AUSPAD) calculated with six SPAD readings taken on the flag leaves of non-inoculated plants, and **B**, disease severity measured as the area under disease progress curve (AUDPC) and calculated using the severity estimations corresponding to six ratings after inoculation with *C. sativus* MUCL 45264 of wheat genotypes Ciano T-79 and BL 2217 under different potassium treatments

Disease Assessment

The effect of potassium stress on AUDPC was highly significant ($P < 0.001$). Both genotypes showed higher disease severity under potassium deficiency compared to the standard fertilisation (Fig. 1B). Ciano T-79 always showed higher AUDPC than BL 2217 ($P < 0.001$). The interaction genotype x fertilisation was not significant for the AUDPC indicating that relative differences in spot blotch resistance among both genotypes were constant under different potassium treatments.

Correlation Between Chlorophyll Content and Disease Severity

The effects of increasing potassium deficiency on spot blotch severity are presented as regression of means of AUSPAD values of non-inoculated plants and AUDPC (Fig. 2). Disease severity increased linearly as AUSPAD of non-inoculated plants decreased in both genotypes. A negative linear correlation was observed between the AUDPC of inoculated and the AUSPAD of non-inoculated flag leaves for both genotypes. The coefficients of determination (r^2) were significant for both genotypes and ranged from 0.896 for Ciano T-79 to 0.806 for BL 2217.

Quantification of Resistance Response

Epidermal penetration resistance includes cell wall apposition (CWA) beneath sites of fungal attack, epidermal HR and postpenatration HR (PPHR). CWA and epidermal cells undergoing HR were found more frequently in BL 2217 than in Ciano T-79 under standard Hoagland fertilisation. CWA-associated defence mechanism was specially affected by potassium deficiency in both genotypes, with BL 2217 being relatively more affected than Ciano T-79. The differences between

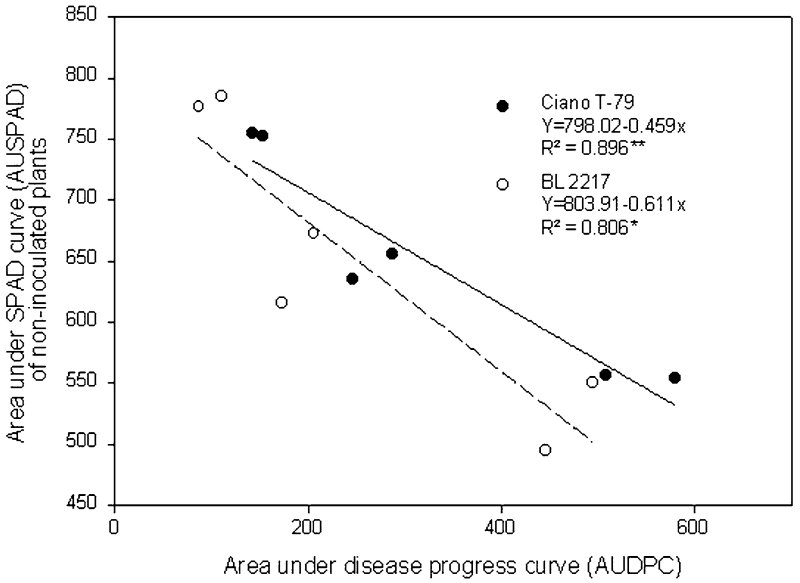


Figure 2. Linear regression of means of AUSPAD values of non-inoculated plants and disease severity measures as AUDPC of wheat genotypes Ciano T-79 and BL 2217

genotypes was more visible 48 hai. PPHR was also found more frequently in BL 2217 than in Ciano T-79 under standard solution. However, about equal numbers of PPHR in both genotypes were found at both times.

Potassium deficiency resulted in the increase of successful fungal infection of the mesophyll layer. The frequency of successful fungal infection of the mesophyll was always higher in Ciano T-79 compare to BL 2217 under the different potassium treatments

These findings are in agreement with field observations made by Sharma et al. (2004), who reported that potash application significantly reduced HLB severity in the lowlands of Nepal. Our data indicate that potassium deficiency increase disease susceptibility by affecting penetration resistance.

Resistance of wheat genotype BL 2217 is primary attributed to higher frequencies of CWA compare to susceptible Ciano T-79. Similar results were reported by Ibeagha et al. (2005) with resistant and susceptible wheat genotypes at seedling stage. HR and PPHR was also found more frequently in BL 2217 than in Ciano T-79, suggesting that these defence mechanisms could also explain the differences in resistance to *C. sativus* among genotypes at adult plant stages, as previously observed with genotypes Chirya 7 and Ciano T-79 (Ibeagha and Mercado, *unpublished*). However, our results contrast with previous findings reporting that HR occurs in all genotypes at a similar extent (Ibeagha et al. 2005). A possible explanation for this difference could be related with different plant growth stage at the

time of inoculation, since Ibeagha's study was based on first leaves of 10-day-old-seedlings, while this study was based on flag leaves of intact adult plants.

The higher rate of successful invasion under potassium stress could have been affected by the effect of potassium on other plant defence mechanisms as suggested by Ruan et al. (1999) who showed that an adequate potassium nutrition increase the synthesis and accumulation of polyphenols leading to anthracnose (*Gloeosporium theaesinensis*), brown blight (*Guignardia camelliae*) and grey blight (*Pestalotiopsis theae*) reduction in tea (*Camelia sinnesis*) seedlings.

ACKNOWLEDGEMENTS

This project study was supported by DGCD funding from the Belgium Government for the CIMMYT-UCL project on 'Non-specific Foliar Pathogens', Phase II.

REFERENCES

Fixen PE, Buchenau GW, Gelderman RH, Schumacher TE, Gerwing JR, Cholik FA, Farber BG (1986) Influence of soils and applied chloride on several wheat parameters. Agron J 78:736–740

Ibeagha AE, Hückelhoven R, Schäfer P, Singh DP, Kogel KH (2005) Model wheat genotypes as tools to uncover effective defense mechanisms against the hemibiotrophic fungus *Bipolaris sorokiniana*. Phytopathology 95528–532

Krauss A (2001) Potassium and biotic stress. Presented at the 1st FAUBA-FERTILIZAR-IPI Workshop on Potassium in Argentina's Agricultural Systems 20–21 November 2001, Buenos Aires, Argentina

Perrenoud S (1990) Potassium, Plant Health. 2nd edn. International Potash Institute, Bern, Switzerland (IPI Research Topics No. 3)

Ruan J, Wu X, Härdter R (1999) Effects of potassium and magnesium nutrition on the quality components of different types of tea. J Sci Food Agric 79:47–52

Schäfer P, Hückelhoven R, Kogel KH (2004) The white barley mutant *Albostrians* shows a super-susceptible but symptomless interaction phenotype with the hemibiotrophic fungus *Bipolaris sorokiniana*. Mol Plant Microbe Interact 17:366–373

Sharma S, Duveiller E, Basnet R, Karki CB, Sharma RC (2005) Effect of potash fertilization on Helminthosporium leaf blight severity in wheat, and associated increases in grain yield and kernel weight. Field Crop Res 93:142–150

Sharma RC, Duveiller E (2004) Effect of helminthosporium leaf blight on performance of timely and late-seeded wheat under optimal and stressed levels of soil fertility and moisture. Field Crop Res 89:205–218

Thordal CH, Zhang Z, Wei Y, Collinge DB (1997) Sub-cellular localization of H_2O_2 accumulation in papillae and hypersensitive response during the barley-powdery mildew interaction. Plant J 11: 1187–1194

Timm CA, Goos RJ, Johnson BE, Sobolik FJ, Stack RW (1986) Effect of potassium fertilizers on malting barley infected with common root rot. Agron J 78:197–200

IMPLICATIONS FOR FUSARIUM HEAD BLIGHT CONTROL FROM STUDY OF FACTORS DETERMINING PATHOGEN AND DON CONTENT IN GRAIN OF WHEAT CULTIVARS

V. SIP*, J. CHRPOVA, L. LEISOVA, S. SYKOROVA, L. KUCERA AND J. OVESNA

Research Institute of Crop Production, Prague-Ruzyne, Czech Republic, CZ-161 06
**E-mail: sip@vurv.cz*

Abstract: Experiments with nine winter wheat cultivars artificially infected with *Fusarium culmorum*, during four years, showed highly variable and often not proportional effects of fungicides (metconazole or tebuconazole based) on reduction of DON and pathogen DNA content. Results of these experiments were supplemented by a survey of DON content in naturally infected farm fields. This enabled to specify the impact of different studied factors and weather conditions and to bring conclusions for improvement of control measures. When combining cultivar resistance with effective fungicide treatment 89% reduction of DON content and 96% reduction of pathogen content were reached. It was found that disease control should be maintained, besides cultivar and fungicide specifications, particular effects of years, regions and preceding crops. Predictive models of disease development in a particular year (region) are highly desirable for decision making purposes related the use of fungicides

Keywords: DON content, *Fusarium*

INTRODUCTION

Fusarium head blight (FHB), predominantly caused in the examined conditions by *Fusarium graminearum* (Schwabe) and *Fusarium culmorum* (WG Smith) Sacc., is one of the most damaging diseases, particularly in years with intensive rainfall. Mycotoxin contamination of human food and animal feed became a more important feature than the direct yield losses that often occur irregularly. Deoxynivalenol (DON) is also in the conditions of Central Europe the most frequent toxin reaching the highest concentration levels.

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 281–287.

Four primary strategies for the control of FHB are biological, cultural, chemical, and genetic. Development of a biological agent to effectively control FHB (suppress the pathogen) is undoubtedly a difficult task and also cultural practices that rely on reduction of the amount of *Fusarium* inoculum residing in crop debris cannot guarantee sufficient protection. Fungicides based on tebuconazole and metconazole were reported to suppress FHB and accumulation of mycotoxins, however, the protection by this means is generally not yet sufficient under all conditions both in barley and wheat (Mesterházy et al. 2003, Sip et al. 2004). Though more effective prothioconazole-based fungicides are now available, it is evident that control of this complicated disease cannot rely only on a single control measure and should be respectful of different factors that influence progression of FHB and accumulation of mycotoxins. Genetically conditioned resistance could be reckoned as the most valuable and environmentally friendly means. Recent studies (Ramirez et al. 2004, Hope et al. 2005, Brennan et al. 2005) revealed different ecological requirements for growth and mycotoxin production by *Fusarium* species. This type of information is without any doubt essential for developing climate-based risk models for determining the potential for contamination of cereal grain with mycotoxins (Hope et al. 2005).

The aim of this contribution was to bring conclusions for FHB control from study of DON and pathogen DNA content under different environmental conditions and with winter wheat cultivars differing in resistance level.

MATERIALS AND METHODS

Survey of DON content in grain samples collected from different districts of Czech Republic. Collection of grain samples from winter wheat farm fields suspicious of FHB infection was systematically performed in 2003 and 2004 by the State Phytosanitary Administration. The obtained samples (in total 401) from different regions were analyzed for content of DON using ELISA method and sorting of data with respect to region, cultivar, preceding crop, tillage practice and plant protection by chemicals was performed.

Field infection tests. Response to artificial infection with *Fusarium culmorum* and fungicide treatment was studied in nine winter wheat cultivars with varying level of resistance to FHB. On the basis of long term studies the cultivars Arina and Petrus could be classified as resistant – medium resistant (R-MR), Nela, Bona, Ebi, Sarka and Saskia as medium resistant – medium susceptible (MR – MS) and Sepstra and Siria as susceptible (S) to FHB. Experiments at the location Prague – Ruzyne lasted four years (2001–2004) that highly differed in weather conditions. Each genotype was grown on 2.5 m^2 plots in three replicates of variants I (infection) and IF (infection and fungicide treatment). Highly pathogenic isolate (B) of *Fusarium culmorum* was used for inoculation by spraying in two terms (Sip et al. 2002). Fungal infection was promoted by mist irrigation of plots. In 2001 the fungicide Caramba (active ingredient Metconazole) and in the years 2002, 2003 and 2004 fungicide Horizon 250 EW (active ingredient Tebuconazole) were applied

following manufacturer instructions (1.0 liter ha^{-1}). Inoculation with *Fusarium* conidia suspension followed in IF variant after 24 hours, when positive occurrence of fungicide in plant tissue was assured.

For this study data on DON content and pathogen DNA content were exploited. The content of DON was determined by ELISA on RIDASCREEN^{R} FAST DON kits from R- Biopharm GmbH, Darmstadt, Germany (Sip et al. 2004). The description of real time (RT) quantitative PCR, used for determination of pathogen DNA content, is available in publication of Sip et al. (2004). Analysis is based on obtained CT (threshold cycle) values that were transformed in the following way: $CT_{\text{Fus transf}} = 10^7 * 2^{-Ct(Fus)}$, which allowed positive and linear relationships with examined traits.

RESULTS AND DISCUSSION

Two year systematic surveys of DON content in wheat grain samples collected in the Czech Republic (Table 1) indicate substantial threat to farm fields from these aspects. Tolerance limit, now set in cereals for direct consumption to 0.5 mg kg^{-1}, was exceeded in 137 out of 401 seed samples suspicious of infection. It can be implied from Table 1 that year, regional and cultivar effects on DON level were high. From cultivars occurring more frequently (n>15) especially Czech winter wheat cultivars Nela, Alana and Sulamit showed average DON content lower than 1 mg kg^{-1}, while Drifter and Clarus had more than 5 mg kg^{-1}. Significantly higher ($P < 0.05$) contamination of grain by mycotoxin DON was detected with the preceding crop maize. This effect was not significantly different from effect of forage crops clover or alfalfa, but the other preceding crops including cereals did not appear to have substantial deleterious effect. Application of reduced tillage indicated slight increase in DON content, however, not significantly different from conventional tillage. Average DON 1.5 mg kg^{-1} obtained after application of fungicides Horizon or Caramba in comparison with 2.9 mg kg^{-1} for not directed or absent control by chemicals may indicate approximately 50% efficacy of treatment with

Table 1. Estimates of effects of different factors on DON content ($mgkg^{-1}$) obtained from analysis of winter wheat seed samples suspicious of FHB infection collected from Czech farm fields (total number of samples from two years: 401; 94 in 2003 and 307 in 2004)

Year		Oil crop (winter rape)	1.902
2003	0.338	Potato/sugar beet	1.553
2004	2.901	Tillage practice	
Region (14 districts)	0.915–7.743	Conventional	2.545
Cultivar (n>15)	0.480–6.555	Reduced tillage	3.532
Preceding crop		Protection by fungicides	
Maize	5.719	Horizon/Caramba	1.546
Clover/alfalfa	3.304	Not directed against FHB	2.941
Peas	1.657	Not protected	2.876
Cereal crop	1.713	Total average	2.300–2.862

the mentioned fungicides. Main reason for presenting these data was to estimate risks involved with different situations in agricultural practice that undoubtedly have to be taken into consideration when deciding on control measurements.

Trials with artificial infection with *Fusarium culmorum* performed in four years enabled to study the effects of cultivar, fungicide treatment and weather conditions on DON and pathogen content in grain. Over environments and cultivars pathogen DNA content was significantly related to content of DON in both variants I ($r = 0.60$) and IF ($r = 0.54$; $P < 0.001$), but it could be deduced from the detailed study and implied from Fig. 1 that different levels of DON could be detected at similar pathogen content (as reported by Gosman et al. 2005). Average efficacy of fungicide treatment, calculated as percentage of the inoculated (I) variant, reached 46.2 % for DON and 35.1 % for pathogen DNA content. However, it is evident from the figure that efficacy of fungicide treatment for these traits was highly variable in years and cultivars. It was high for DON content (approaching 70 %) in the years 2003 and 2004 that could be characterized by relatively shorter and not so harmful development of the disease (in these years the respective average CT values were 4.18 and 1.61, while in 2001 and 2002 8.17 and 9.32). Fungicide efficacy for DON was particularly low under conditions of 2001 (21.7 %) that enabled long lasting development of the disease, leading to high accumulation of both DON and pathogen. While in 2003 and 2004 the application of fungicide with high immediate action could bring satisfactory results, in 2001 and obviously also in 2002 application of fungicide with high longevity or another spraying was evidently needed. Though efficacies for DON and pathogen content were positively related ($r = 0.44$; $P < 0.01$), reductions of both traits were often not proportional and in 2001 fungicide treatment resulted in significantly higher DON/CT ratio in IF variant ($I = 4.3/IF = 6.5$), similarly as did application of azoxystrobin in experiments of Simpson et al. (2001). Increase of DON content after application of fungicides did not occur, but increase in pathogen content was not exceptional (2002, 2003).

Under wet conditions (generated using a mist-generator apparatus) temperature during infection period evidently influenced accumulation of DON and pathogen and efficacy of fungicide treatment (Table 2). Relatively low air temperatures in the period 5 days after inoculation (heading) contributed to higher DON and pathogen content in mature grain and the used fungicides were found less effective in reducing DON. Also large studies on farm fields in Canada (Hooker et al. 2002) showed that relatively narrow time periods around heading (4–7 days before and 3–6 days after heading) were particularly decisive for prediction of DON content. Higher temperature in the period 11–20 days after inoculation promoted accumulation of fungal DNA. Low fungicide effect on reduction of pathogen content was connected with higher temperature in 30 day period of disease development. FHB disease was in terms of fungal DNA greater at the higher temperature of 20° C than at 16° C in experiments of Brennan et al. (2005). Control of the disease by fungicides may be more problematic particularly under conditions favorable for colonization of the grain by the fungus (2001 and 2002). Development of predictive models for

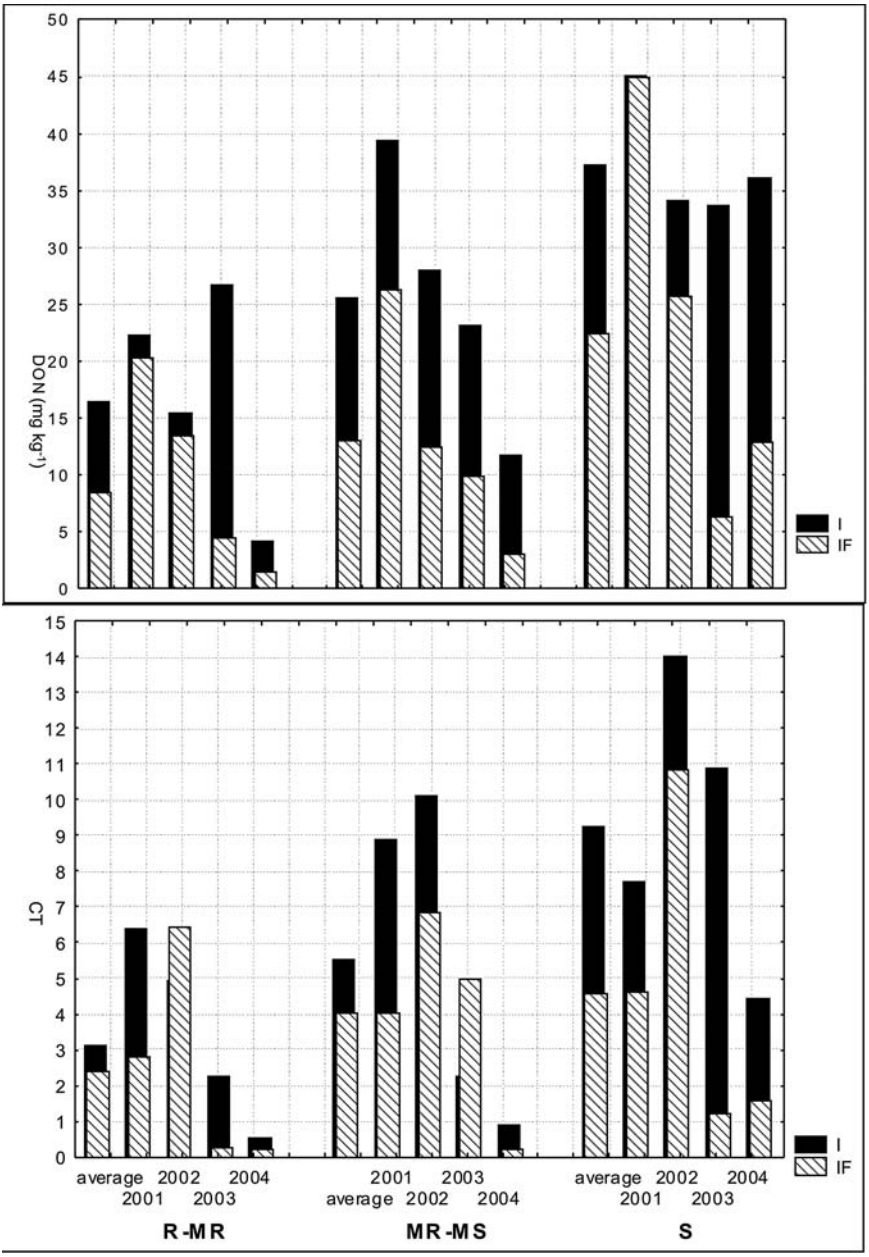


Figure 1. DON content and pathogen DNA content (CT) in four years (2001, 2002, 2003 and 2004) and three groups of winter wheat cultivars differing in resistance to FHB (R-MR, MR-MS and S) for variants I (infection) and IF (infection and treatment with fungicide)

Table 2. Evaluation of effects of heading date, plant height and sum of average day temperatures (DT) in periods of disease development on DON and pathogen DNA content (CT) - in I and IF variants and efficacies (EF) of fungicide treatment (coefficients of correlation)

	DON-I	DON-IF	EF DON	CT-I	CT-IF	EF CT
Heading date	0.30*	0.52***	−0.47**	0.11	0.02	0.18
Plant height	−0.29*	−0.08	−0.18	−0.04	0.16	−0.19
DT 5 days	−0.43**	−0.70***	0.63***	−0.49**	−0.34*	0.00
DT 6–10 days	−0.08	0.00	−0.16	0.01	0.41**	−0.63***
DT 10 days	−0.29*	−0.40**	0.28	−0.27	0.06	−0.40*
DT 11–15 days	0.28	0.14	−0.15	0.41**	0.60***	−0.52***
DT 15 days	−0.14	−0.26	0.15	−0.06	0.26	−0.49**
DT 16–20 days	0.36*	0.17	0.03	0.51***	0.27	0.13
DT 20 days	−0.03	−0.21	0.16	0.10	0.34*	−0.44**
DT 21–25 days	0.26	0.15	−0.03	−0.04	−0.06	−0.10
DT 25 days	0.01	−0.17	0.14	0.08	0.31*	−0.43**
DT 30 days	0.01	−0.11	0.04	0.07	0.36*	−0.54***

decision making purposes and the use of fungicides would be highly desirable to control the disease more effectively.

It can be implied from this study that disease control should take into consideration different risk factors, mainly cultivar resistance, regional effects and effects of preceding crops. Not only conditions for longer lasting disease development, but also genotype lateness might promote higher accumulation of DON and result in lower fungicide efficacy for DON (Table 2). Highly beneficial supporting effect had cultivar resistance. In these experiments the exploitation of moderate resistance (cultivars Arina and Petrus) resulted when combined with fungicide treatment in 68% reduction of DON and in the years of high fungicide effectiveness (2003 and 2004) even in 89% DON reduction and 96% reduction of pathogen content (Fig. 1).

ACKNOWLEDGEMENTS

The work was supported by the Ministry of Agriculture of the Czech Republic, project QG 50076.

REFERENCES

Brennan JM, Egan D, Cooke BM, Doohan FM (2005) Effect of temperature on head blight of wheat caused by *Fusarium culmorum* and *F. graminearum*. Plant Pathol 54:156–160

Gosman N, Chandler E, Thomsett M, Draeger R and Nicholson P (2005) Analysis of the relationship between parameters of resistance to *Fusarium* head blight and *in vitro* tolerance to deoxynivalenol of the winter wheat cultivar WEK0609. Eur J Plant Pathol 111:57–66

Hooker DC, Schaafsma AW, Tamburic Ilincic L (2002) Using weather variables pre- and post- heading to predict deoxynivalenol content in winter wheat. Plant Dis 86:611–619

Hope R, Aldred D, Magan N (2005) Comparison of environmental profiles for growth and deoxynivalenol production by *Fusarium culmorum* and *F.graminearum* on wheat grain. Lett Appl Microbiol 40:295–300

Mesterházy A, Bartók T, Lamper C (2003) Influence of wheat cultivar, species of *Fusarium*, and isolate aggressiveness on the efficacy of fungicides for control of Fusarium head blight. Plant Dis 87:1107–1115

Ramirez ML, Chulze S, Magan N 2004. Impact of environmental factors and fungicides on growth and deoxynivalenol production by *Fusarium graminearum* isolates from Argentinian wheat. Crop Protect 23:117–125

Simpson DR, Weston GE, Turner JA, Jennings P and Nicholson P (2001) Differential control of head blight pathogens of wheat by fungicides and consequences for mycotoxin contamination of grain. Eur J Plant Pathol 107:421–431

Sip V, Sykorova S, Stuchlikova E, Chrpova J (2002) The effect of infection with *Fusarium culmorum* L on deoxynivalenol content in grain of selected winter wheat varieties. J Appl Genet 43A: 319–332

Sip V, Tvaruzek L, Chrpova J, Sykorova S, Leisova L, Kucera L, Ovesna J (2004) Effect of Fusarium head blight on mycotoxin content in grain of spring barley cultivars. Czech Journal of Genetics and Plant Breeding 40:91–101

DROUGHT RESISTANCE: GENETIC APPROACHES FOR IMPROVING PRODUCTIVITY UNDER STRESS

R.M. TRETHOWAN* AND M. REYNOLDS

Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT) Apdo. Postal 6-641, 06600 México, D.F., México

**E-mail: r.trethowan@cgiar.org*

Abstract: Drought and scarcity of water for irrigation severely limit wheat productivity in many different environments around the world. Wheat breeders have made significant progress in adapting cultivars to water limited conditions, even though the genetic control of drought tolerance and water-use-efficiency (WUE), the two primary mechanisms of adaptation to moisture deficit, are not well understood.

There are a number of options available to plant breeders for improving the productivity of wheat under moisture stress. These include: (1) analysis of genotype x environment interaction to improve parental selection and identify key evaluation sites; (2) physiological characterization of germplasm to identify parents with complementary traits and to identify tools that will improve the heritability of selection; (3) development of reliable and repeatable drought screening methods; (4) broadened genetic variation for drought adaptive traits (5) improved water harvesting via improved root health; (6) enhanced cultivar adaptation to moisture conserving crop management practices; and (7) identification and conservation of genomic regions that are associated with performance under moisture stress across environments and time. These options are examined in the context of a wheat breeding program and their application to wheat improvement in water limited environments is discussed

Keywords: stress, drought resistance

INTRODUCTION

Drought severely limits wheat productivity in many environments around the world. Some estimates indicate that 50% of the approximately 230 M ha sown to wheat annually in the world is regularly affected by drought (Pfeiffer et al. 2005). Wheat breeders have made significant progress in developing cultivars better adapted to moisture limited conditions. Improvements in grain yield of between 0.4 and 1.3% per annum have been reported for many of the drier wheat producing areas of

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 289–299.

the world (Byerlee and Traxler 1995). It is recognized that improved agronomic techniques account for a considerable portion of this variation (Bell et al. 1995). However, genetic improvement has also contributed significantly to improvements in yield stability and productivity, and in many instances, realizing the benefits of improved farming practices is dependent upon the availability of suitable or responsive cultivars. This paper examines the various genetic options available to wheat breeders to improve productivity under stress. A number of examples are drawn from the experience gained by wheat breeders at the International Maize and Wheat Improvement Center (CIMMYT) located in Mexico. We emphasize productivity under stress, or water use efficiency defined in this case as yield per mm water applied, rather than survival under stress or drought tolerance as this character is of greater economic importance to farmers.

Exploiting Genotype X Environment Interactions to Identify Parents and Key Selection Environments

The yield and yield stability of genotypes across many stress environments has always been an important criterion used by plant breeders to select candidates for release to farmers and to identify parents for crossing. At CIMMYT, wheat germplasm is developed by shuttling segregating materials between two contrasting environments, one located near Ciudad Obregon in northwestern Mexico (27 °N, 60 masl) and the other in the central Mexican highlands near Toluca (19 °N, 2600 masl). This germplasm shuttle allows two generations to be grown each year and has been described in detail by Rajaram et al. (1994). The site near Ciudad Obregon is an arid, irrigated environment and drought and heat stress can be reliably generated by controlled irrigation and delayed planting date. The lines developed in this way are then distributed internationally through CIMMYT's international wheat network and collaborators from many countries grow the trials and return data for analysis. These data are then used by both CIMMYT and regional breeders to identify key discriminating locations and to select parents for crossing.

Twenty years of data from the Elite Spring Wheat Yield Trial (ESWYT) were analyzed to examine associations among international locations with the aim of identifying those locations that discriminate germplasm in a similar way to CIMMYT's primary yield testing location located in northwestern Mexico (Trethowan et al. 2003b). Fig. 1 summarizes the findings derived from cluster analysis of locations; these results have been used by CIMMYT breeders to source and exchange germplasm, particularly from regions that do not cluster with sites in Mexico. The international performance of genotypes is also used to examine both broad and specific adaptation. Fig. 2 summarizes the findings of an analysis of CIMMYT's High Temperature Wheat Yield Trial (HTWYT) (Lillemo et al. 2005). In this instance, genotypes were identified that performed similarly under different environmental conditions. These different genotype groupings represent different adaptive gene pools and breeders use this information to better target germplasm to specific environments and to design crosses among gene pools to broaden the adaptation of wheat germplasm.

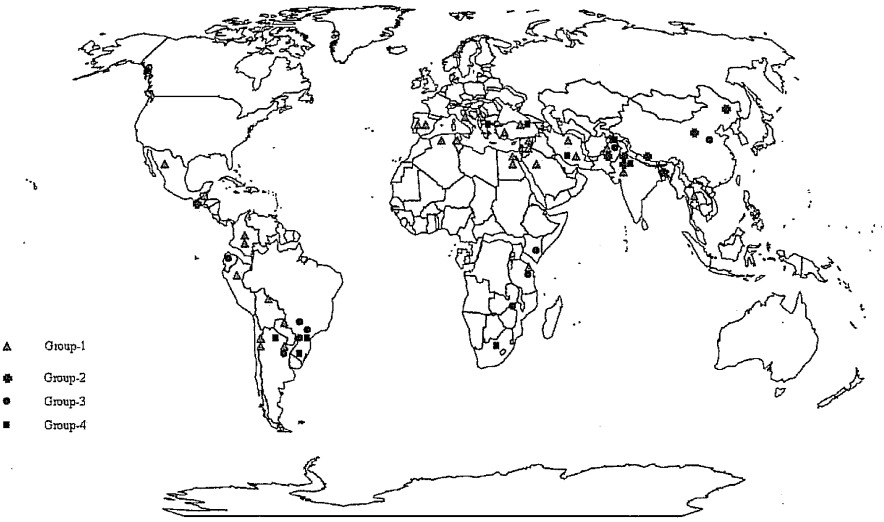
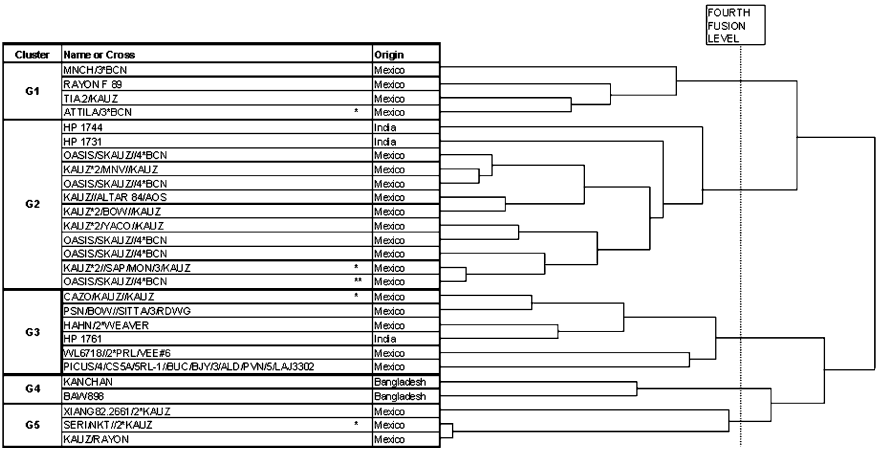


Figure 1. Summary of cluster analysis of 20 years of the Elite Spring Wheat Yield Trial. Sites are grouped based on non-significant cross-over interaction. Source: Trethowan et al (2003)



Cluster	Name or Cross		Origin
G1	MNCH/3*BCN		Mexico
	RAYON F 89		Mexico
	TIA2/KAUZ		Mexico
	ATTILA/3*BCN	*	Mexico
G2	HP 1744		India
	HP 1731		India
	OASIS/SKAUZ//4*BCN		Mexico
	KAUZ*2/MNV//KAUZ		Mexico
	OASIS/SKAUZ//4*BCN		Mexico
	KAUZ//ALTAR 84/AOS		Mexico
	KAUZ*2/BOW//KAUZ		Mexico
	KAUZ*2/YACO//KAUZ		Mexico
	OASIS/SKAUZ//4*BCN		Mexico
	OASIS/SKAUZ//4*BCN		Mexico
	KAUZ*2//SAP/MON//3/KAUZ	*	Mexico
	OASIS/SKAUZ//4*BCN	**	Mexico
G3	CAZO/KAUZ//KAUZ	*	Mexico
	PSN/BOW//SITTA/3/RDWG		Mexico
	HAHN/2*/WEAVER		Mexico
	HP 1761		India
	WL6718//2*PRL/VEE#6		Mexico
	PICUS/4/CSSA/5RL-1//BUC/BJY/3/ALD/PVN/5/LAJ3302		Mexico
G4	KANCHAN		Bangladesh
	BAW898		Bangladesh
G5	XIANG82.2661/2*KAUZ		Mexico
	SERI.1/KT//2*KAUZ	*	Mexico
	KAUZ/RAYON		Mexico

Figure 2. Dendrogram of genotype associations from cluster analysis of 10 years of the Heat Wheat Yield Trial. Source: Lillemo et al (2005)

Use of Physiological Tools to Identify Parents and Improve the Heritability of Selection

While the predominance of an upstream focus in plant stress research has led to a greater emphasis on traits associated with survival under extreme stress than those associated with agronomic productivity under resource-limited conditions, crop physiologists

Table 1. Physiological traits measured on parental materials at Ciudad Obregon 2003–2004

Pedigree	Yield	Biomass anthesis	CT[1] Vegetative	CT Grainfill	Carbon isotope Discrim.	Stem CHO[2] at anthesis	Water extraction by roots
	g m^{-2}	g m^{-2}	*C*°	*C*°		% stem dry weight	(% available water)
Jun/Gen	338	424	19.2	21.8	–23.1	13.3	84
Weebill 1	348	513	19.3	21.7	–22.5	17.5	83
Synthetic	278	510	19.8	22.6	–22.5	19.1	79
Frame	213	503	20.5	23.2	–21.7	6.8	79
Klein Cacique	247	638	20.1	23.3	–22.6	3.4	82
Prointa Federal	223	572	20.0	22.9	–22.4	11.2	79

[1] canopy temperature;
[2] carbohydrate

have nonetheless identified many traits that are associated with adaptation of wheat to dry environments, albeit that understanding remains incomplete (Reynolds et al. 2006). However, until recently, few wheat breeding programs have actively selected for physiological traits. This lack of application to some extent reflects the expense and time consuming nature of many physiological applications, making it difficult to select for physiological traits in segregating generations. Work at CIMMYT has shown that physiological tools can be effectively used to characterize parents for the presence of complementary physiological traits, therefore allowing plant breeders to combine these traits in crosses (Reynolds et al. 2005). Values for various physiological traits measured on key parental materials grown under drought stress in Mexico show significant variation among lines (Table 1). These data were collected on lines grown near Ciudad Obregon using a managed drought stress regime described in Trethowan et al. (2001a). Initially there was some doubt as to whether it would be possible to combine these traits as increases in one trait may be matched by decreases in another. However, evidence indicates that it is possible to combine different physiological traits through crossing as some parental lines with good yield performance under drought stress, such as Weebill 1 and Jun/Gen, already combine the expression of combined traits. Weebill 1 combines cooler canopy temperature with greater stem carbohydrate translocation and Jun/Gen combines cooler canopies, improved C isotope discrimination and better water extraction by roots with intermediate stem carbohydrate translocation. Furthermore, quantitative analysis of physiological traits in a broad range of genetic backgrounds (including materials derived from interspecific hybridization and selected landraces) suggest that traits like WUE, stem carbohydrates and access to water at depth in the soil, if combined into modern varieties could increase yields under drought by 30% or more over current elite checks (Reynolds and Condon 2006).

During selection, easy to measure tools such as canopy temperature depression (CTD) can be used to select superior lines or bulks. The only limitation to the use of CTD is the presence of a canopy and clear sunny conditions. Reynolds et al. (2005) demonstrated a significant association between yield and CTD in wheat bulks grown under drought stress in northwestern Mexico. CTD is now used routinely by CIMMYT's wheat breeding program to complement visual selection under drought stress in the early generations of the wheat improvement program.

Development of Reliable and Repeatable Drought Screening Methods

Determining the drought phenotype in any crop species is difficult because of the variable nature of drought in the field, the lack of correlation between field and green house results and the confounding effects of constraints other than drought on phenotype. At CIMMYT, wheat germplasm is screened for adaptation to moisture stress in carefully managed stress scenarios in the field in northwestern Mexico. A combination of drip and gravity fed irrigation in an arid environment allows these drought stress scenarios to be generated each year. A description of the irrigation methods employed by CIMMYT can be found in Trethowan et al. (2004).

Germplasm is developed by growing the F2, F3 and F6 generations under optimal moisture and foliar disease pressure, and the F4 and F5 generations under one dominant drought stress scenario (generated historically using gravity fed irrigation, but now managed using a drip fed system). The derived fixed lines are first yield tested under well watered conditions to identify those with yield potential, followed by testing under the same primary drought stress scenario. Selected lines are then tested under 3 different scenarios; optimal irrigation; pre-anthesis drought stress and post-anthesis drought stress. Those performing well under all three scenarios are sent globally for further testing in CIMMYT's international wheat network and the best become parents.

Clearly, this methodology is effective only if the drought screening conducted in Mexico is relevant to the drought patterns found in target regions around the world. Whilst analysis of historical data shows that lines selected using gravity fed irrigation do associate well with many drier locations, particularly in South Asia, there are areas of the world that consistently differentiate wheat germplasm differently (Trethowan et al. 2001a). To examine if drought screening in Mexico could be managed more effectively to mimic the long-term drought patterns of locations that do not associate well with drought screening in Mexico, a series of different managed stress environments were generated over a four year period in Mexico. A tester set of genotypes that had already been extensively tested internationally were planted in these managed stress environments in Mexico to examine the relationships among Mexican and international trial locations. The results are summarized in Trethowan et al. (2005) and clearly demonstrate that it is possible to tailor drought stress in Mexico to develop more relevant germplasm for a broader range of geographical areas.

Broadened Genetic Variation for Drought Tolerance and WUE

The wheat breeding program in Mexico has made good progress in developing bread wheats adapted to drier environments (Trethowan et al. 2002). However, it was recognized some years ago that if productivity under moisture deficit was to continue to be improved, new sources of genetic variability would have to be found and introgressed. Synthetic wheat, developed by crossing tetraploid durum wheat with *Aegilops tauschii*, the ancestral donor of the D genome in hexaploid wheat, has been a rich source of diversity for many characters including tolerance to drought stress (Villareal et al. 1998, Trethowan et al. 2003a). Data generated at CIMMYT shows that when these synthetic wheats are combined in crosses, the yield of the derived synthetic is considerably higher than the adapted recurrent parent under drought stress (Table 2). However, whilst improved productivity under drought stress has clearly been achieved in Mexico, the real test of the potential of these materials is performance in the regions targeted by the CIMMYT breeding program and partners. Preliminary evidence obtained from CIMMYT's 11th Semi-Arid Wheat Yield Trial (11th SAWYT) indicates that the synthetic materials, developed specifically for adaptation to drought stress, perform well cross a range of different locations

Table 2. Yield of synthetic derivatives under drought stress expressed as a % of the recurrent or adapted parent in 2003/2004 and 2004/2005 from managed drought stress trials conducted at Ciudad Obregon

Pedigree	2003–2004	2004–2005
	Yield as % of recurrent parent	Yield as % of recurrent parent
SCA/AE.SQUARROSA (409)//2* PASTOR	119.1*	113.7*
SCA/AE.SQUARROSA (409)//2* PASTOR	124.9*	115.1*
CHEN/AE.SQ//OPATA/3/2* PASTOR	117.8 *	103.8
D67.2/P66.270//AE.SQUARROSA (320)/3/CUNNINGHAM	113.4[1]*	119.4*
D67.2/P66.270//AE.SQUARROSA (320)/3/CUNNINGHAM	115.5[1]*	123.0*
CROC_1/AE.SQUARROSA (224)//2*KULIN	104.6[1]*	129.0*
CROC_1/AE.SQUARROSA (224)//2 *KULIN	104.3[1]*	124.1*

[1] Yields from reduced (2 irrigation) irrigation trials, all other trials conducted with 1 irrigation;
* Significantly different from the recurrent or adapted parent at $P < 0.05$

(Fig. 3). Fig. 3 compares one such synthetic derivative, Vorobey, with the best locally adapted cultivar at each of 30 locations internationally. The performance of this line is either equivalent to or better than the local cultivar across many

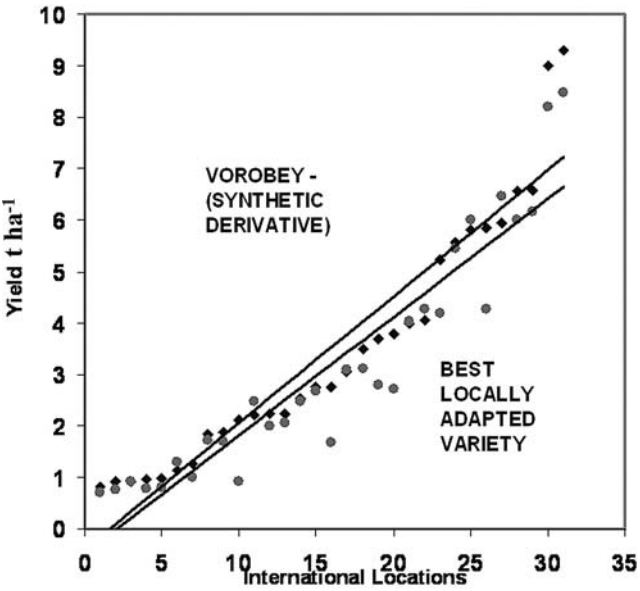


Figure 3. Yield of the synthetic derivative Vorobey in relation to the yield of the best locally adapted cultivar at 30 international trial locations: data collected from the 11th SAWYT

locations, including the more productive environments. Clearly these types of wheat, developed using the strategy outlined in this paper, have improved yield stability and productivity across a wide range of growing conditions.

Improved WUE Via Improved Root Health

In some dry environments much of the improvement in yield over time can be attributable to better root health (Trethowan et al. 2004), the inference being that healthier roots make better use of the available soil moisture. As there is genetic variability for nematode and root rot resistance and tolerance to micronutrient toxicities such as boron, and the mode of inheritance of these genes is relatively simple, wheat breeders in areas prone to these problems have found it easier to manipulate these gene systems rather than the complex character of drought tolerance *per se*. Molecular markers are now available for many of these simply inherited characters, and as marker assays are more cost effective than bioassays for root diseases or constraints, and have a higher heritability, they are increasingly being used by wheat breeders.

At CIMMYT, markers for genes conferring resistance to cereal cyst nematode, root lesion nematode, crown rot and tolerance to boron have been routinely used for many years (William et al. 2003). Advanced lines with improved root health developed using marker assisted selection have been developed at CIMMYT and distributed globally in the Semi-Arid Wheat Screening Nursery. Interestingly, many

Table 3. Yield of improved germplasm selected using molecular markers for various root constraints under reduce irrigation and drought at Ciudad Obregon in 2004/2005

Pedigree	Target gene[1]	Yield in reduced irrigation as % of source parent[2]	Yield in drought as % of the source parent[2]
CROC_1/AE.SQUARROSA (205)//KAUZ/3/SILVERSTAR	*Cre 1*	107.0	125.3*
CROC_1/AE.SQUARROSA (205)//KAUZ/3/SILVERSTAR	*Cre1*	118.6*	116.3*
CNDO/R143//ENTE/MEXI_2/3/ AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/2*FRAME	*Bo1*	114.1*	167.6*
CNDO/R143//ENTE/MEXI_2/3/ AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/2*FRAME	*Bo1*	113.4*	166.3*
KRICHAUFF/2*PASTOR	*Rln 1*	120.1*	133.2*
KRICHAUFF/2*PASTOR	*Rln 1*	127.4*	125.7*

[1] *Cre 1*, *Bo1* and *Rln 1* confer resistance to cereal cyst nematode, tolerance to boron toxicity and resistance to root lesion nematode, respectively.

[2] The source parents are Sliverstar, Frame and Krichauff. Reduced irrigation represents two applied irrigations and drought one applied irrigation.

* Significantly different from the recurrent or adapted parent at $P < 0.05$

of these lines are significantly higher yielding than their recurrent parents even in the absence of root disease (Table 3).

Enhanced Cultivar Adaptation to Moisture Conserving Crop Management Practices

In many parts of the world farmers have adopted conservation tillage. These changes to the farming system, characterized by no tillage or reduced tillage and the retention of stubble from the previous crop, have improved water infiltration, reduced water loss and reduced soil erosion. Initially, most studies found no interaction between genotype and tillage practice (Dao and Nguyen 1989; Ditsch and Grove, 1991). However, this lack of interaction is not surprising as small numbers of genotypes were tested, all of which had been developed under conventional tillage. Recently, the existence of significant tillage x genotype interactions among more diverse genotypes has offered plant breeders the opportunity to tailor cultivars to the farming system (Klein 2003). At CIMMYT, breeders carefully select parental materials on the basis of their performance on zero-tillage and the subsequent segregating generations are planted and selected under zero-tillage. This process has developed advanced lines with significantly better performance under zero-tillage (Sayre and Trethowan, unpublished data). Improved emergence and establishment, particularly from deep planting, and enhanced resistance to foliar blights are also important selection criteria. Improved emergence is to some extent linked to removal of the GA-insensitive dwarfing genes *Rht1* and *Rht2*. However, experience at CIMMYT shows that there is significant residual variation for coleoptile length that can be exploited (Trethowan et al. 2001b). Rebetzke and Richards (2000) have also characterized a number of GA-sensitive dwarfing genes and some these and other sources of variation are being used in crosses at CIMMYT.

Identification and Conservation of Genomic Regions that are Associated With Performance Under Moisture Stress

It is difficult to see functional genomics playing a significant role in the short term in the development of drought tolerance or WUE cultivars. Large numbers of up and down regulated genes and the confounding effects of genotype x environment interaction make it very difficult to use these data. However, DNA fingerprinting is being used by many breeders to select parental materials and to calculate more realistic coefficients of parentage.

The CIMMYT wheat program and international partners have generated an extensive data set of yield and disease performance collected from CIMMYT yield and screening nurseries over the past 30 years. There is scope to use these data and fingerprints of the key germplasm representing this 30 year period to identify genomic regions linked to performance under defined sets of environmental conditions. It may be possible in the near future, to link drought performance with specific genomic regions always present in materials performing well under drought

stress. Wheat breeders could then ensure these regions are present in their parental materials and could actively select for them in segregating populations. This work continues.

CONCLUSION

Clearly there is no optimum strategy for developing wheat cultivars better adapted to drought conditions. The existence of large genotype x environment interactions makes it difficult to pin point the underlying genetic control of adaptation. To make progress, the wheat breeder must try and separate performance under drought into manageable sub-objectives each with measurable genetic variation. The seven strategies outlined in this paper each address a different component of adaptation to drought stress. However, the challenge is to combine these different strategies most effectively to produce well adapted wheat germplasm.

REFERENCES

Bell MA, Fischer RA, Byerlee D, Sayre K (1995) Genetic and agronomic contributions to yield gains: a case study for wheat. Field Crop Res 44:55–56

Byerlee D, Traxler G (1995) National and international wheat improvement research in the post green revolution period. Evolution and impacts. Am J Agr Econ 77:268–278

Dao TH, Nguyen HT (1989) Growth response of cultivars to conservation tillage in a continuous wheat cropping system. Agron J 81:923–929

Ditsch DC, Grove JH (1991) Influence of tillage on plant populations, disease incidence, and grain yield of two soft red winter wheat cultivars. J Prod Agr 4:360–365

Klein OA (2003) Trigos adaptados a siembra directa: conocimientosy desconocimientos. In: Kohli MM, Diaz M, Castro M (eds.) 'Estrategias y Metodologías Utilizados en el Mejoramiento de Trigo. Seminario Internacional, La Estancuela, Uruguay. CIMMYT-INIA pp 37–42

Lillemo M, van Ginkel M, Trethowan RM, Hernandez E, Crossa J (2005) Differential adaptation of CIMMYT bread wheat to global high temperature environments. Crop Sci 45:2443–2453

Pfeiffer WH, Trethowan RM, van Ginkel M, Ortiz MI, Rajaram S (2005) Breeding for abiotic stress tolerance in wheat. In: Ashraf M, Harris PJC (eds) Abiotic Stresses: Plant Resistance through Breeding and Molecular Approaches. The Haworth Press, Inc. NY pp 401–489

Rajaram S, van Ginkel M, Fischer RA (1994) CIMMYT's wheat breeding mega-environments (ME). In: Proceedings of the 8th International Wheat Genetics Symposium, Beijing, China. pp 1101–1106

Rebetzke GJ, Richards RA (2000) Gibberellic acid sensitive dwarfing genes reduce plant height to increase kernel number and grain yield of wheat. Aust J Agr Res 51:235–245

Reynolds MP, Condon AG (2006) Quantifying potential genetic gains in wheat using a conceptual model of drought adaptation (same as current volume)

Reynolds MP, Mujeeb KA, Sawkins M (2005) Prospects for utilizing plant-adaptive mechanisms to improve wheat and other crops in drought & salinity-prone environments. Ann Appl Biol 146:239–59

Reynolds MP, Rebetzke G, Pellegrinesci A, Trethowan R (2006) Drought Adaptation in Wheat. In: Ribaut JM (ed.) Drought Tolerance in Cereals. Haworth's Food Products Press, New York, Chapter 11, pp 402–436

Trethowan RM, Borja J, Mujeeb KA (2003a) The impact of synthetic wheat on breeding for stress tolerance at CIMMYT. Annual Wheat Newsletter, 49:67–69

Trethowan RM, Crossa J, van Ginkel M, Rajaram S (2001a) Relationships among Bread Wheat International Yield Testing Locations in Dry Areas. Crop Sci 41(5):1461–1469

Trethowan RM, Reynolds MP, Pfeiffer WH, Ammar K, van Ginkel M, Crossa J (2004) Managed drought stress environments in Mexico and their association with global wheat growing environments.

Resilient crops for water-limited environments – The Rockefeller Foundation's Food Security Theme Workshop. May 24–28, 2004 Cuernavaca, Mexico. Extended Abstracts, México D.F. CIMMYT. pp 289–291

Trethowan RM, Reynolds MP, Sayre KD, Ortiz-Monasterio I (2005) Adapting wheat cultivars to resource conserving farming practices and human nutritional needs. Ann Appl Biol 146:404–413

Trethowan RM, Singh RP, Huerta EJ, Crossa J, van Ginkel M (2001b) Coleoptile length variation of near isogenic Rht lines of modern CIMMYT bread and durum wheat. Field Crop Res 70:167–176

Trethowan RM, van Ginkel M, Rajaram S (2002) Progress in breeding for yield and adaptation in global drought affected environments. Crop Sci 42(5):1441–1446

Trethowan RM, van Ginkel M, Ammar K, Crossa J, Payne TS, Cukadar B, Rajaram S, Hernandez E (2003b) Associations among Twenty Years of Bread Wheat Yield Evaluation Environments. Crop Sci 43:1698–1711

Villareal RL, Banuelos O, Borja J, Mujeeb KA (1998) Drought tolerante of synthetic wheats (*Triticum turgidum* x *Aegilops tauschii*) Annual Wheat Newsletter 44:142–144

William HM, Crosby M, Trethowan R, van Ginkel M, Mujeeb KA, Pfeiffer W, Khairallah M, Hoisington D (2003) Molecular Marker Service Laboratory – An Interface between the Laboratory and the Field. Proceedings of the 10th International Wheat Genetics Symposium, September 1–6, Paestrum, Italy, 2003. pp 852–854

PROGRESS IN BREEDING WHEAT WITH TOLERANCE TO LOW TEMPERATURE IN DIFFERENT PHENOLOGICAL DEVELOPMENTAL STAGES

D.B. FOWLER* AND A.E. LIMIN

Department of Plant Sciences, University of Saskatchewan, 51 Campus Dr., Saskatoon, Saskatchewan, Canada, S7N 5A8
**E-mail: Brian.Fowler@usask.ca*

Abstract: Low-temperature (LT) tolerance is a complex quantitative character that is expressed in anticipation of and during exposure of plants to temperatures that approach freezing. This environmentally induced character is determined by a highly integrated system of structural and developmental genes that are regulated by environmentally responsive, complex pathways. Genetic analyses at the whole plant level have shown there is developmental regulation of LT-tolerance gene response and transition from the vegetative to the reproductive growth stage is a critical switch that initiates the down regulation of LT-tolerance genes. Consequently, full expression of cold-hardiness genes only occurs in the vegetative stage and plants in the reproductive phase have a limited ability to cold acclimate. Our ability to manipulate the differences in genetic and environmental response has allowed for separation of the genetic factors that determine the *rate* acclimation from those responsible for the *duration* of LT acclimation. The developmental genes (vernalization, photoperiod, etc) determine the duration of expression of LT-tolerance conferring genes while the rate of LT acclimation is determined by genotype dependent expression levels of these genes. An understanding of these relationships has permitted the successful transfer of the superior LT-tolerance genes from a winter wheat cultivar into spring wheat (*Triticum aestivum* L.)

In the last decade, a virtual flood of genetic and genomic information has arisen from investigations using model plant systems and tools with an unprecedented level of sophistication for analyses. The superior LT-tolerance genes have been tagged using molecular markers that allow plant breeders to select hardy genotypes without having to wait for a test frost in the field. A greater appreciation of the interactions between growth stage and LT-tolerance gene expression has provided us with the ability to design strategies to minimize the risk of LT damage in different stages of phenological development. However, even with the opportunities offered by advances in technology, we have been unable to produce super-hardy cultivars. For example, while the structural genes within the Triticeae have a high degree of homology and the regulation of LT

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 301–314.

tolerance is operational across genomes, we have not been able to successfully exploit the superior LT tolerance of rye (*Secale cereale* L.) for improvement of related cereal species. Progress in this area will have to wait for a clearer understanding of LT signal transduction and the genetic cascade controlling LT-gene expression

Keywords: low temperature, tolerance gene

INTRODUCTION

To be successful in cool season and high winter stress climates, plants must be programmed to recognise and respond to temperatures that are favourable for growth and the environmental cues that signal seasonal changes typical of the regional environment for which they were selected or in which they evolved. In regions with cold winters, vernalization requirement is an important adaptive feature that delays heading by postponing the transition from the vegetative to the reproductive phase. Similarly, photoperiod requirement is an adaptation that allows the plant to flower at the optimum time. Wheat and its relatives also have the ability to LT acclimate. Phenotypic studies have shown that the LT-induced protective mechanisms responsible for acclimation are developmentally regulated (Fig. 1) and involve processes that can be stopped, reversed and restarted (Fowler et al. 1999). As a result, full expression of LT induced genetic systems is revealed only under genotypically dependent optimum combinations of time and temperature (Fowler et al. 2004).

LT acclimation is a cumulative process that is initiated once temperatures drop below 10 to 15 °C. There is an inverse relationship between temperature and acclimation rate and, when plants are grown at constant temperatures in the acclimation range, the most rapid changes in LT tolerance occur during the initial stages of acclimation. Exposure of hardened plants to higher temperature results in de-acclimation,

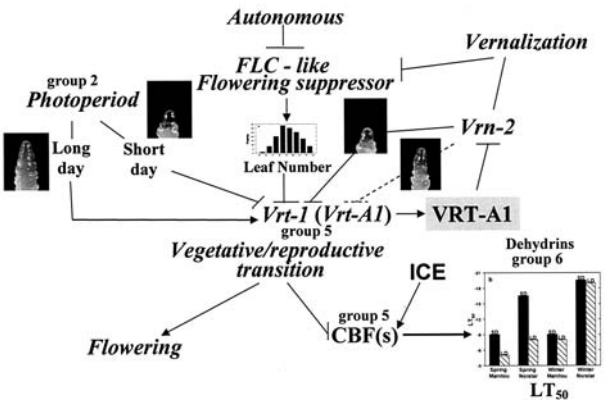


Figure 1. Flowering pathway (17) and regulation of LT tolerance gene expression (25) in common wheat (*T. aestivum*). *Vrt-1* = *Vrn-1* = Vegetative reproductive transition genes = Meristem identity

but the process of LT acclimation can be re-initiated by exposing plants that are still in the vegetative stage to inducing temperatures. Because LT response is determined by a highly integrated system of structural and developmental genes regulated by environmentally responsive, complex pathways that allow full expression of LT induced genes only when they are required in the life cycle, it has been difficult to separate cause and effect adjustments to LT and other environmental cues that signal seasonal changes (Fowler et al. 1999).

According to the developmental theory of LT gene regulation (Fowler et al. 1999), *duration* and *rate* of gene expression determine the degree of LT tolerance. The developmental genes act as the switches controlling the *duration* of expression of LT-induced structural genes (Fowler et al. 1996a, Fowler et al. 1996b) while the *rate* component determines the degree that the structural genes are up regulated (Fig. 1). In this system, full expression of cold hardiness genes only occurs in the vegetative stage and plants in the reproductive phase have a limited ability to cold acclimate. Plants that are still in the vegetative stage also have the ability to re-acclimate following periods of exposure to warm temperatures while plants in the reproductive phase have only a limited ability to re-acclimate (Fowler et al. 1999, Fowler et al. 1996b). Vernalization requirements allow LT-tolerance genes to be expressed for a longer period of time at temperatures in the acclimation range (Fowler et al. 1996a, Fowler et al. 1996b). Similarly, photoperiod sensitivity allows plants to maintain LT-tolerance genes in an up-regulated state for a longer period of time under short day (SD) compared to long day (LD) environments (Limin and Fowler 2006, Fig. 1). In both instances, the delay in the transition from the vegetative to the reproductive stage produces increased LT tolerance that is sustained for a longer period of time. These observations explain why winter habit genotypes eventually lose their ability to maintain a high level of LT-tolerance under acclimating temperatures and only limited levels of LT tolerance have been observed in spring habit cultivars.

Low-Temperature Induced Structural Genes

Winter cereals produce many cold regulated (COR) proteins in response to LT stress. Among these LT induced proteins, the dehydrin families (e.g. *Wcs120, Dhn5*, and others) have received the most attention in this area of LT research. As a group, dehydrins have a wide size range, have no similarity with any enzymes or proteins of known function, are largely hydrophilic, and accumulate to high levels during the late stages of embryogenesis or in response to ABA application, LT, or any environmentally imposed dehydrative force such as drought, cold or salinity (Close 1996). The LT-induced dehydrin gene families have been studied extensively in wheat where they have been mapped to the group 6 chromosomes (Limin et al. 1997, Danyluk et al. 1998). This presents a complication in that conventional, non-molecular genetic studies have associated as many as 15 chromosomes with the genetic control of LT tolerance in wheat but these chromosomes do not include the sixth group.

Studies with the LT-induced *Wcs120* and *Wcor410* dehydrin gene families indicate that similar proteins are expressed by spring and winter-habit cultivars within species despite large differences in LT tolerance (Fowler et al. 1996a, Limin et al. 1997). Cold hardy genotypes just produce more of the same dehydrins than less hardy genotypes. The important observations in these studies are that the proteins of these LT-induced gene families increase and decrease in unison in response to LT indicating that differences in their level of expression in hardy and nonhardy genotypes is regulated by a few major genes (Fowler et al. 1996a, Limin et al. 1995). The amount of WCS120 LT-tolerance associated protein has been shown to be directly related to LT tolerance and changes in the plant's ability to accumulate these proteins is determined at both the transcriptional (Fowler et al. 1996a, Danyluk et al. 2003) and translational (accumulation) levels (Fowler et al. 1996a) through changes in the level of repression of the LT-tolerance pathway (Fig. 11). Observations made on a large number of wheat and rye cultivars show a very close association between the point of vernalization saturation and a decline in LT tolerance (Fowler et al. 1996b). A decline in *Wcs120* mRNA levels has also been associated with a decline in WCS120 protein product and the point of vernalization saturation (Fowler et al. 1996a).

Duration of Low-Temperature Gene Expression

Vernalization requirement is the key difference between spring and winter habit genotypes in cereals and the phenotypic expression of this character has been studied in detail. Within the Triticeae, phenotypic analyses have determined that the genes for vernalization are found on the 4th, 5th, and 1st group chromosomes (McIntosh et al.1998). *Vrn1* in wheat is homoeoallelic to locus *Sh2* in barley (Hayes et al. 1993) and *Sp1* in rye (Plaschke et al.1993), both of which have been shown to be linked to genetic differences in LT tolerance (Laurie et al. 1995, Brule Babel and Fowler 1989). In *Triticum aestivum* (ABD genome), the three major vernalization determining loci have been mapped to the long arms of chromosomes 5A (*Vrn-A1*) 5B (*Vrn-B1*) and 5D (*Vrn-D1*) (11). *Vrn-A1* does not require vernalization treatment while *Vrn-B1* and *Vrn-D1* have short vernalization requirements and winter habit genotypes are recessive for all three genes. The main developmental genes in the diploid species *Triticum monococcum* (A genome) are *Vrn-A^{m}1* and *Vrn-A^{m}2*. The dominant *Vrn-A^{m}1* is responsible for spring habit while *Vrn-A^{m}2* is dominant for winter habit (Dubcovsky et al. 1998).

Positional cloning studies (Yan et al. 2003) have shown that *Vrn-A^{m}1* is completely linked to MADS-box gene *AP1* (the apparent *Triticum* orthologue of the *Arabidopsis* meristem identity gene *APETALA1*). It has been suggested that a mutation in the promoter region or a deletion in the first intron could result in the lack of recognition of *Vrn-1* by *Vrn-2*, essentially short-circuiting the vernalization pathway thereby bringing about a dominant spring growth habit (Fig. 11). This mechanism would provide a simple explanation for the 3: 1 ratio normally reported in spring by winter crosses in hexaploid wheat. Concurrently, a gene

designated *TaVRT-1* (Danyluk et al. 2003) was cloned, characterized and localized to the *Vrn-1* regions on the long arms of homoeologous group 5 chromosomes, regions that are associated with vernalization and freezing tolerance in wheat. The level of expression of *TaVRT-1* was associated with the vernalization response and transition from the vegetative to reproductive phase, a finding supported by the results of Murai et al. (2003) for the *WAP1* gene. *TaVRT-1* has very close sequence homology and similar expression patterns to *Vrn-A*^{m}*1* and to the barley (*Hordeum vulgare* L.) homolog *HvBM5* (Danyluk et al. 2003, Trevaskis et al. 2003). Since these 'vernalization' genes are orthologs of *Arabidopsis AP1*, which plays a central role in the flower induction pathway, very early up-regulation of the *Vrn-1* genes in spring wheat and their up-regulated expression coincident with vernalization saturation in winter habit genotypes (Danyluk et al. 2003) make these genes key candidates for the LT-tolerance repression pathway (Fig. 1). However, the mechanism of repression likely also involves members of the flowering pathway other than *Vrn-1* itself.

Molecular studies have demonstrated that the *TaVRT-1* and *HvBM5* genes associated with the vernalization response and reproductive transition in wheat and barley are both regulated by photoperiod and cumulative low temperatures and that the accumulation of their encoded products is associated with the progressive repression of cold-induced genes and a decrease in LT tolerance (Danyluk et al. 2003). Response to photoperiod has been shown to affect accumulation of LT tolerance in spring and winter habit cereals particularly after, or shortly before, vernalization saturation (Fowler et al. 2001). Both photoperiod sensitivity and vernalization are responses to environmental stimuli affecting the developmental pathway (flowering pathway) in plants ultimately affecting timing of the reproductive transition and thereby the duration of expression of LT-tolerance genes (Mahfoozi et al. 2001). The photoperiod and vernalization genes influence the expression of LT-induced genes in cereals through separate pathways (Fowler et al. 2001) that eventually converge to activate genes controlling plant development (Fig. 1). In both instances, delay in the transition from the vegetative to the reproductive stage causes increased LT tolerance that is sustained for longer in plants that have a vernalization or photoperiod requirement. This indicates that the developmental genes determine the *duration* of expression of LT-induced structural genes. In winter-habit genotypes, photoperiod sensitivity influences LT-tolerance gene expression even before vernalization saturation (Mahfoozi et al. 2001), implying that vernalization is progressive and that plant development can be influenced by photoperiod during the vernalization process.

An inability to completely recover what is supposedly a single growth habit gene after 10 backcrosses in a program that produced *Vrn-A1* reciprocal near isogenic lines (NILs) using the non-hardy spring habit (*Vrn-A1*) cultivar 'Manitou' and the very cold-hardy winter habit (*vrn-A1*) cultivar 'Norstar' (Limin and Fowler 2002) raises a number of questions with regard to the complexity of interactions among genes in the flowering pathway. Detailed analyses of flowering pathways using the *Arabidopsis* model indicates that multiple developmental and environmental

cues and pathways regulate the vegetative/reproductive transition in plants. There are at least 80 genes and loci and a number of genetic pathways known to affect flowering time in *Arabidopsis* (Simpson et al. 1999, Simpson and Dean 2002) and as complex, or more complex, regulatory system likely exist in wheat (Loukoianov et al. 2005). Using the *Arabidopsis* model as a basis for discussion, it can be seen that a comparable picture is unfolding in wheat (Fowler and Limin 2003, Fig. 1). The inability to fully recover the spring or winter phenotypes in the NILs for the major growth habit gene (Limin and Fowler 2002) *Vrn-A1*, leaves open the possibility that clusters of flowering promoting genes are tightly linked to the *Vrn-A1* locus. Tightly linked genes would be expected to segregate as a group in spring x winter crosses suggesting the existence of a single gene that was dominant for spring habit when a broad classification of spring and winter habit is used. At the same time, a gradual erosion or addition of tightly linked flowering pathway alleles, which promote or delay the vegetative/reproductive transition, would be expected to give rise to variation within the spring and winter habit groupings. Examination of doubled haploid lines in a specially constructed mapping population isogenic in the *Vrn-A1* region for the winter habit allele *vrn-A1* also shows a wide range of variation in final leaf number (indicator of the vegetative/reproductive transition) that is responsive to vernalization temperatures. This demonstrates that there is additional genetic variation in the flowering pathway upstream from *Vrn-A1*, which directly influences the vegetative/reproductive transition (Fig. 2a).

Early indications are that there are differences in the evolution of mechanisms regulating the flowering pathways between dicots and monocots as well as within the *Triticeae* group and it is likely that a much more complex system will eventually be revealed in hexaploid wheat. In addition, it appears that the descriptive name associating vernalization directly with the function of *vrn-A1* is misleading and should be corrected. The *vrn-A1* locus functions as part of the vegetative/reproductive complex

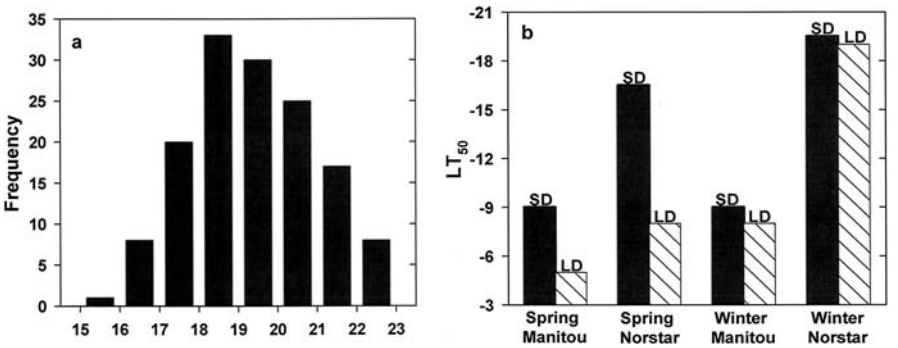


Figure 2. a) Final Leaf Number frequency distribution for Norstar x Winter Manitou mapping population grown at 20 °C. b) LT tolerance (LT_{50}) of Norstar x Manitou NILs: winter Norstar and Spring Norstar, spring Manitou and Winter Manitou, following 28 days acclimation at 4 °C. Short-day (SD) = 8 h, long-day (LD) = 20 h

that is suppressed by the action of upstream vernalization responsive genes. Current theory has spring habit genotypes arising from a mutation(s) resulting in the loss of recognition of a suppressor of flowering and, as such, it would be more appropriate if *vrn-1* was designated *vrt-1* (vegetative-reproductive transition - 1) to reflect its true function at this location in the flowering pathway (Fig. 1). The accumulated evidence indicates that the *vrn-A1* region is a convergence point, or master switch, for pathways that determine the vegetative/reproductive transition thereby giving it a direct influence on the duration of expression of the *rate* determining LT-tolerance genes. These distinctions become important as a clear understanding of the gene networks and complex interactions that determine LT tolerance is required before effective strategies can be designed for the identification and selection of the factors influencing this character of major economic importance in wheat.

CBFs – Transcriptional Activators of Low-Temperature Tolerance

A large number of chromosomes have been shown to influence LT tolerance in conventional, non-molecular genetic studies and, for this reason, it has been generally assumed that a large number of genes with small effects and complex interactions determine the phenotypic expression of LT tolerance. However, molecular mapping studies have only succeeded in locating LT-tolerance genes that are linked to the vernalization genes of the fifth group chromosomes in wheat (Galiba et al. 1995, Storlie et al. 1998) and the homoeologous chromosome 5H in barley (Hayes et al. 1993, Pan et al. 1994). Earlier genetic studies had identified a gene on 5A with a dominant effect for LT tolerance that was normally expressed in association with the recessive *vrn1* allele for winter growth habit in wheat (Brule Babel and Fowler 1988) and the homoeoallelic locus *Sh2* in barley (Laurie et al. 1995, Hayes et al. 1993) and *Sp1* in rye (Brule Babel and Fowler 1989). This region of chromosome 5A and homoeologous loci in wheat and other cereals appear to play an especially important role in determining plant responses to stress.

Evaluation of the NILs for the non-hardy spring habit (*Vrn-A1*) cultivar 'Manitou' and the very cold-hardy winter habit (*vrn-A1*) cultivar 'Norstar' (Limin and Fowler 2002) has shown that both *duration* and *rate* of acclimation contributed significantly to the 13.8 °C difference in minimum survival temperature between Norstar and Manitou (Fowler and Limin 2004). These studies established that genes responsible for the *duration* of expression of LT-tolerance genes can be separated from genes determining the *rate* of acclimation. In these studies, *duration* of LT acclimation was found to be dependent upon the rate of phenological development, which in turn was determined by acclimation temperatures, vernalization and photoperiod requirements. *Rate* of acclimation was faster for genotypes with the Norstar genetic background but the ability to sustain a high rate of acclimation was dependent upon the length of the vegetative stage. In addition to these complex time/temperature relationships, these studies also indicated that there were significant unexplained genetic interactions.

Several reputed homoeologous LT-tolerance genes have been mapped to the homoeologous group-5 chromosomes of *T. aestivum*. A LT-tolerance gene (designated *Fr* (Stutka and Snape 1989)), tightly linked to *Vrn-A1* of chromosome 5A was first mapped (Galiba et al. 1995) followed by reports of a homoeologous series of frost resistance genes that were closely linked to the *Vrn-1* genes on the group 5 chromosomes in hexaploid wheat (Toth et al. 2003) and barley (Hayes et al. 1993). However, most of the mapping studies utilized spring by winter comparisons that include the confounding effects of duration of LT gene expression due to differences at the *Vrn* region and winter by winter comparisons only accounted for about 0.5 °C of the differences in LT tolerance (Storlie et al. 1998). The first group of homoeologous LT-tolerance QTLs (designated *Fr-1* for frost resistance 1) mapped to positions 2 (*Fr-A1*), 10 (*Fr-D1*), and 40 (*Fr-B1*) cM from the homoeologous *Vrn-1* (spring/winter determining) loci (Galiba et al. 1995, Toth et al. 2003) on the group-5 chromosomes of *T. aestivum*. A second frost resistance locus designated *Fr-A2* mapped to the long arm of *T. monococcum* chromosome 5 (Vagujfalvi et al. 2003), 30 cM proximal to the RFLP marker *Xwg644* that is known to be tightly linked to *Vrn-A1* and the *Fr-A1* locus (Galiba et al. 1995). The homoeologous *Fr-1* QTLs were mapped to varying distances from the *Vrn-1* vernalization loci before the existence of *Fr-A2* was known. Marker *Xgwm639-5B*, which mapped near the peak of the *Fr-B1* QTL (Toth et al. 2003), is closely linked to *Xbcd508,* which is located at the peak of *Fr-A2* (Vagujfalvi et al. 2003) indicating that *Fr-B1* is an ortholog of *Fr-A2* not of *Fr-A1*. Given that vernalization has a major affect on the expression of LT tolerance and *Fr-A1* has been mapped to several locations both proximal and distal to *Vrn-A1* (Galiba et al. 1995, Toth et al. 2003) and has never been isolated or sequenced, its existence as a separate locus from *Vrn-1* must be considered inconclusive for the moment (Limin and Fowler 2006).

The group 5 chromosomes, 5A in particular, have been shown to affect LT_{50} levels and regulate the LT-tolerance associated *Wcs120* gene family located on the group 6 chromosomes of all three hexaploid wheat genomes (Limin et al. 1997). Molecular studies designed to investigate these interactions have demonstrated that the regulatory influence exerted by the *vrn* complex over LT- induced structural gene (*Wcs120* and *Wcor410*) expression occurs at the transcriptional level in winter cereals (Fowler et al. 1996a). These factors on wheat group 5 chromosomes (particularly 5A) can induce higher levels of expression in many LT-induced genes *dispersed across all 3 wheat genomes* (Limin et al. 1997, Danyluk et al. 1998) indicating that one or more transcriptional activator(s) on chromosome 5A are able to target the LT-induced genes. In *Arabidopsis, Cbf* genes are transcriptional factors that are rapidly up-regulated in response to LT treatment and are activators of cold regulated COR genes (Thomashow et al. 2001). The *Cbf* genes are induced in turn by *ICE* transcriptional activators, which have a complex, wide ranging transcriptome. Transcripts encoding CBF-like proteins have been shown to accumulate rapidly in response to LT in 'Puma' rye and Norstar wheat (Jaglo et al. 2001) suggesting that a similar mechanism operates in cereals. Two-tandem clusters of eleven CBF genes map to the *Fr-H2* QTL in barley (Skinner et al. 2005)

and *Rcg1* (regulator for *Cor 14b*) maps to the *Fr-A2* QTL in wheat indicating that CBF-like genes are primary candidates for the *Fr-2* frost tolerance genes (Vagujfalvi et al. 2005). As transcriptional activators for LT tolerance associated genes, the CBF-like genes are also prime candidates for the *rate* genes (Fig. 1).

Pathway Interactions

Detailed evaluations of NILs for the *Vrn-A1* locus have shown the 'Norstar' genetic background achieves greater LT tolerance than that of 'Manitou' due to a faster *rate* of acclimation (Fowler et al. 1999). A vernalization requirement allows virtually full expression of the LT tolerance potential of both Norstar and Manitou genetic backgrounds under a 20-h day length (Fig. 2b). Similarly, almost full expression of LT tolerance potential can be achieved by photoperiod responsive genotypes in the absence of a vernalization requirement when grown under an 8-h day length (Limin and Fowler 2006). The critical observation in these studies is that plant development toward flowering progressively reduces LT acclimation ability and the *duration* of time in early developmental stages determines the degree to which the LT-tolerance genetic potential is expressed. This makes the expression of LT-tolerance genes pathway-dependent rather than a result of the action of single genes operating in isolation. These interactions also make LT-tolerance-related characters appear to be associated with genes that determine flowering time, explaining the pleiotropic effect (growth habit and LT tolerance) attributed to genes like *vrn-A1* (Brule Babel and Fowler 1988).

A plant must be programmed to recognize and respond to temperatures that are favorable for growth and to the environmental cues that signal seasonal changes. In this environmentally responsive system there is also a need for the plant to record the progress of seasons so that it can properly anticipate the normal periods of LT stress and commit fully to growth and reproduction once the weather is favorable. The fact that both LT acclimation and vernalization have similar above freezing activation ranges also suggests the likelihood of an extensive integration of LT-sensing mechanisms. These complicated time/temperature relationships (Fig 2b) and unexplained genetic interactions indicate that detailed functional genomic or phenomic analyses of natural allelic variation will be required to identify the critical genetic components of the highly integrated systems for LT adaptation that are regulated by environmentally-induced complex pathways. Consequently, the linkage of genes to function is required if we are going to make use of genomic information to elucidate the underlying mechanisms of LT response in wheat.

Phenotypic and molecular studies have shown that the *duration* of LT tolerance is determined by the rate of phenological development and the time to vegetative/reproductive transition, which in turn is a function of a) vernalization requirements, b) photoperiod requirements, c) leaf number, d) length of phyllochron (Limin and Fowler 2002) and e) low temperatures that delay reproductive transition in plants that have reached the stage of competence to flower (Fowler et al. 1999). Related studies have also shown that the mechanism regulating the level

of expression of LT-induced genes is associated with a gene(s) integrated into the developmental pathway and the *rate* of acclimation is determined by a) acclimation temperature and b) LT tolerance genetic potential (Fowler et al. 1999, Fowler and Limin 2004). Consequently, given the right combinations of time, temperature and day length, LT-tolerance QTLs will locate to molecular map positions for the genes associated with variability in phenological development. Conversely, LT-tolerance QTLs associated with variation in phenological development will only be revealed in mapping populations under the appropriate conditions of time, temperature and day length.

Progress in Breeding Wheat with Tolerance to Low Temperature in different Phenological Developmental Stages

In a 1929 publication, Quisenberry and Clarke (1929) noted that "The possibility of developing hardier varieties through breeding has been recognized for years". However, the reality is that the maximum cold hardiness potential of most cereal crops has reached a stubborn plateau that has not been breached for decades. In fact, all the efforts of modern science have been unable to produce the super hardy cultivars needed to expand winter crop production into regions requiring a level of cultivar LT tolerance superior to that found in the land races selected by early farmers indicating that improvements in LT tolerance do not come easily. In contrast, the last 80 or more years have seen improvements in agronomic practices within most established production areas that have allowed plant breeders to reduce their selection pressure for LT tolerance. Consequently, while plant breeding efforts over the years have created cultivars with a high level of adaptation, there is still considerable potential for improvement in LT tolerance of cultivars available for most of the current winter wheat production areas.

The possibility that genes can be transferred between species to increase the genetic variability available to wheat breeding programs has been explored (Limin and Fowler 1989). However, these attempts have done little more than demonstrate the difficulties that must be overcome before the full potential of superior species-specific cold-tolerance gene expression can be captured through interspecific gene transfers. The superior LT tolerance of rye was not expressed when combined in tetraploid and hexaploid wheat backgrounds. Artificially synthesized ABD genome hexaploid wheat also demonstrated the nonadditivity of closely related genomic systems. Further investigation of LT gene expression in hybrids among Triticeae species has lead to the conclusion that chromosome dosage or ratios influence LT tolerance by shifting competitively balanced systems toward the parent with the greatest chromosome number. Molecular investigations of these hybrids have subsequently revealed that highly conserved and coordinately regulated LT-induced gene families of both species are expressed in interspecific crosses (Limin et al. 1995). However, these genes were not expressed independently and the degree of LT gene expression in interspecific crosses was regulated at the transcriptional level by the higher ploidy parent. These observations indicate that before we can

successfully exploit alien genetic variability for LT tolerance using cytogenetic or transformation techniques, we must first acquire a greater understanding of the complex genetic mechanisms that plants have evolved for the efficient integration of LT responses into the daily processes of survival, growth, and reproduction. These investigations have started with a search for the principle mechanisms used to regulate LT gene expression.

The linkage of LT tolerance expression to phenological development adapts the plant to the environment for which it was selected or in which it evolved. For example, a high level of LT tolerance is no longer required after the onset of warm conditions in the spring when rapid growth and reproduction begin. Consequently, satisfaction of vernalization and photoperiod requirement results in a decline in LT tolerance of over-wintering plants. In fact, for cultivars adapted to regions with long, mild winters, a high level of freezing tolerance is often less important than a long vegetative phase that prevents plants from entering the extremely cold-sensitive reproductive growth stage until the risk of LT damage has passed. This results in complicated phenological development by LT tolerance interactions that must be optimized for each production area if cultivars are to be successful. Consequently, because individual genes are part of a complex system, a better understanding of the LT response mechanisms will greatly assist plant breeders in designing strategies to significantly improve the LT adaptation of important economic crops. For example, we have been able to successfully transfer the superior frost tolerance genes from a hardy winter wheat cultivar (Norstar) into a spring wheat line (Spring Norstar) demonstrating that the LT tolerance of spring habit wheat genotypes can be significantly improved by the inclusion of LT tolerance *rate* determining gene(s) from Norstar (Fowler and Limin 2004). When the superior *rate* determining gene(s) were combined with a rigorous photoperiod requirement (Limin and Fowler 2006), Spring Norstar was able to achieve a winter hardiness level approaching that of winter Norstar (Fig. 2b) and survive the high stress winters in western Canada when sown in the fall at the recommended seeding date for winter wheat. While over-winter LT damage in the seedling stage is primarily a concern in temperate climates, frost damage during the reproductive stage can cause severe economic losses in most wheat producing regions of the world. Widely fluctuating late afternoon and early morning temperatures make the timing and severity of LT stress important considerations during the active growing season. Both spring and winter habit genotypes can cold acclimate after reproductive transition and before heading demonstrating that the vegetative/reproductive transition does not act as an off switch for LT-tolerance genes (Fowler et al. 1996). However, plants have only a limited ability to cold acclimate during this period and they reach their maximum level of LT tolerance very quickly once they are exposed to temperatures in the acclimation range indicating that a short, rapid LT response mechanism is functional up to the time of heading. The lack of progress in selecting for frost resistance after head (ear) emergence suggests that LT tolerance expression is minimal at these stages. Selection for resistance after head emergence becomes a much more complex problem because avoidance mechanisms like supercooling play a greater role when

plants are exposed to temperatures just below freezing during this period. Also, LT acclimation is a cumulative process and we do not have a clear understanding of how responsive plants are after head emergence indicating that more detailed studies are required to establish if the limited LT tolerance after heading is due to insufficient induction time or an inability to respond to temperatures in the acclimation range.

In the last decade, a virtual flood of genetic and genomic information has arisen from investigations using model plant systems and tools with an unprecedented level of sophistication for the analysis of the transcriptome, proteome and metabolome. However, a large gap exists between these basic scientific developments and the utilization of this knowledge in crop improvement programs that focus on breeding for complex traits like LT tolerance. Progress has been made in the mapping, isolation, and characterization of the major LT adaptation genes that will allow for the more rapid and directed incorporation of LT tolerance genes using marker assisted backcrossing and other molecular techniques. The tagging of superior frost-tolerance genes using molecular markers will allow plant breeders to select critical genes without having to wait for a test frost in the field. This will speed up the selection process for LT tolerance in both spring and winter wheat breeding programs and significantly increase the chance of having the correct lines in the field for evaluation when a damaging frost that permits field evaluation occurs. Advances in biotechnology have provided even greater opportunities for plant breeders to expand their attack on the winter-hardiness barrier that has frustrated them for so long. However, exploitation of this new technology to produce adapted, super-hardy cultivars will require close co-operation between plant breeders and biotechnologists. This interdisciplinary effort will be expensive and immediate breakthroughs should not be expected, but progress to date suggests that we now have the tools to identify the pieces of the LT-tolerance puzzle.

ACKNOWLEDGEMENTS

This work was supported by Genome Prairie/Genome Canada, the Natural Sciences and Engineering Research Council of Canada, and Ducks Unlimited Canada.

REFERENCES

Brule BAL, Fowler DB (1988) Genetic control of cold hardiness and vernalization requirement in winter wheat. Crop Sci 28:879–884

Brule Bl AL, Fowler DB (1989) Genetic control of cold hardiness and vernalization requirement in rye. Genome 32:19–23

Close TJ (1996) Dehydrins: Emergence of a biochemical role of a family of plant dehydration proteins. Physiol Plantarum 97:795–803

Danyluk J, Kane NA, Breton G, Limin AE, Fowler DB, Sarhan F (2003) *TaVRT-1*, a putative transcription factor associated with vegetative to reproductive transition in cereals. Plant Physiol 132:1–11

Danyluk J, Perron A, Houde M, Limin A, Fowler B, Benhamou N, Sarhan F (1998) Accumulation of an acidic dehydrin in the vicinity of the plasma membrane during cold acclimation in wheat. Plant Cell 10:623–638

Dubcovsky J, Lijavetzky D, Appendino L, Tranquilli G. (1998) Comparative RFLP mapping of *Triticum monococcum* genes controlling vernalization requirement. Theor Appl Genet 97:968–975

Fowler DB, Chauvin LP, Limin AE, Sarhan F (1996a) The regulatory role of vernalization in the expression of low-temperature-induced genes in wheat and rye. Theor Appl Genet 93:554–559

Fowler DB, Limin AE, Wang SY, Ward RW (1996b) Relationship between low-temperature tolerance and vernalization in wheat and rye. Can J Plant Sci 76:37–42

Fowler DB, Limin AE, Ritchie JT (1999). Low-temperature tolerance in cereals: Model and genetic interpretation. Crop Sci 39:626–633

Fowler DB, Breton G, Limin AE, Mahfoozi S, Sarhan F (2001) Photoperiod and temperature interactions regulate low-temperature-induced gene expression in barley. Plant Physiol 127:1676–1681

Fowler DB, Limin AE (2003) Functional genomics of low-temperature stress. Proc. 10th International Wheat Genetics Symp. Paestum, Italy pp 949–951

Fowler DB, Limin AE (2004) Interactions among factors regulating phenological development and acclimation rate determine low-temperature tolerance in wheat. Ann Bot 94:717–724

Galiba G, Quarrie SA, Sutka J, Morgounov A, Snape JW (1995) RFLP mapping of the vernalization (*Vrn1*) and frost resistance (*Fr1*) genes on chromosome 5A of wheat. Theor Appl Genet 90:1174–1179

Hayes PM, Blake T, Chen THH, Tragoonrung S, Chen F, Pan A, Liu B (1993) Quantitative trait loci on barley (*Hordeum vulgare* L.) chromosome 7 associated with components of winter hardiness. Genome 36:66–71

Jaglo KR, Kleff S, Amundsen KL, Zhang X, Haake V, Zhang JZ, Deits T, Thomashow MF (2001) Components of the Arabidopsis C-repeat/dehydration-responsive element binding factor cold-response pathway are conserved in *Brassica napus* and other plant species. Plant Physiol 127:910–917

Laurie DA, Pratchett N, Bezant JH, Snape JW (1995) RFLP mapping of five major genes and eight quantitative trait loci controlling flowering time in a winter x spring barley (*Hordeum vulgare* L.) cross. Genome 38:575–585

Limin AE, Fowler DB (1989) The influence of cell size and chromosome dosage on cold hardiness expression in the *Triticeae*. Genome 32:667–671

Limin AE, Fowler DB (2002) Developmental traits affecting low-temperature tolerance response in near-isogenic lines for the vernalization locus *Vrn-A1* in wheat (*Triticum aestivum* L. em Thell). Ann Bot 89:579–585

Limin AE, Fowler DB (2006) Low-temperature tolerance genetic potential in wheat (*Triticum aestivum* L.): Response to photoperiod, vernalization, and plant development. Planta 224:360–366

Limin AE, Houde M, Chauvin LP, Fowler DB, Sarhan F (1995) Expression of the cold-induced wheat gene *Wcs120* and its homologs in related species and interspecific combinations. Genome 38:1023–1031

Limin AE, Danyluk J, Chauvin LP, Fowler DB, Sarhan F (1997) Chromosome mapping of low-temperature induced *Wcs 120* family genes and regulation of cold-tolerance expression in wheat. Mol Genet Genom 253:720–727

Loukoianov A, Yan L, Blechl A, Sanchez A, Dubcovsky J (2005) Regulation of *VRN-1* Vernalization Genes in Normal and Transgenic Polyploid Wheat. Plant Physiol 138:2364–2373

Mahfoozi S, Limin AE, Fowler DB (2001) Influence of vernalization and photoperiod responses on cold hardiness in winter cereals. Crop Sci 41:1006–1011

McIntosh RA, Hart GE, Gale MD (1998) Catalogue of gene symbols for wheat. In: Li ZS, Xin ZY (eds) Proc. 8th Inter. Wheat Gen. Symp. Agric. Scientech Press, Beijing, pp 1333–1500

Murai K, Miyamae M, Kato H, Takumi S, Ogihara Y (2003) WAP1, a Wheat APETALA1 Homolog, Plays a Central Role in the Phase Transition from Vegetative to Reproductive Growth. Plant Cell Physiol 44:1255–1265

Pan A, Hayes PM, Chen F, Chen THH, Blake T, Wright S, Karsai I, Bedo Z (1994) Genetic analysis of the components of winter hardiness in barley (*Hordeum vulgare* L.). Theor Appl Genet 89:900–910

Plaschke J, Borner A, Xie DX, Koebner RMD, Schlegel R, Gale MD (1993) RFLP mapping of genes affecting plant height and growth habit in rye. Theor Appl Genet 85: 1049–1054

Quisenberry KS, Clarke JA (1929) Breeding hard red winter wheats for winter hardiness and yield. USDA Tech Bull No. 136

Simpson GG, Dean C (2002) Arabidopsis, the Rosetta stone of flowering time? Science 296:285–289

Simpson GG, Gendall AR, Dean C (1999) When to switch to flowering. Ann. Rev Cell Develop Biol 15:519–550

Skinner JS, Szücs P, von Zitzewitz J, Marquez CL, Filichkin T, Thomashow MF, Stockinger EJ, Chen THH, Hayes PM (2005) Mapping of barley homologs to genes that regulate low temperature tolerance in Arabidopsis. Theor Appl Genet 112:832–842

Storlie EW, Allan RE, Walker SMK (1998) Effect of the *Vrn1-Fr1* interval on cold hardiness levels in near-isogenic wheat lines. Crop Sci 38:483–488

Sutka J, Snape JW (1989) Location of a gene for frost resistance on chromosome 5A of wheat. Euphytica 42:41–44

Thomashow MF, Gilmour SJ, Stockinger EJ, Jaglo OKR, Zarka DG (2001) Role of *the Arabidopsis* CBF transcriptional activators in cold acclimation. Phys Planta 112:171–175

Toth B, Galiba G, Feher E, Sutka J, Snape JW (2003) Mapping genes affecting flowering time and frost resistance on chromosome 5B in wheat. Theor Appl Genet 107:509–514

Trevaskis B, Bagnall DJ, Ellis MH, Peacock WJ, Dennis ES (2003) MADS box genes control vernalization-induced flowering in cereals. PNAS USA 100:13099–13104

Vagujfalvi A, Galiba G, Cattivelli L, Dubcovsky J (2003) The cold-regulated transcriptional activator *CBF3* is linked to the frost-tolerance locus *Fr-A2* on wheat chromosome 5A Mol Genet Genom 269:60–67

Vagujfalvi A, Aprile A, Miller A, Dubcovsky J, Delugu G, Galiba G, Cattivelli L (2005) The expression of several *Cbf* genes at the *Fr-A2* locus is linked to frost resistance in wheat. Mol Genet Genom 274:506–514

Yan L, Loukoianov A, Tranquilli G, Helguera M, Fahima T, Dubcovsky J (2003) Positional cloning of the wheat vernalization gene *VRN1*. PNAS USA 100:6263–6268

IDENTIFICATION OF WHEAT GENOTYPES ADAPTED TO MEDITERRANEAN RAINFED CONDITION WITH RESPONSIVENESS TO SUPPLEMENTARY IRRIGATION

M. MOSAAD[1,*], M. SINGH[1], M. ROUSTAII[2], H. KETATA[3] AND S. RAJARAM[1]

[1] *ICARDA, Aleppo, Syria*
[2] *DARI, Maraghe, Iran*
[3] *ICARDA, Tehran, Iran*
[*] *E-mail: m.mosaad@cgiar.org*

Abstract: Water availability is a major factor limiting cereals production in Mediterranean environments. Cultivars with drought tolerance and built-in responsiveness to supplementary irrigation would enhance wheat production in this region. The objective of this study was to assess variability in grain yield among 25 diverse winter and facultative bread wheat genotypes (*T. aestivum*) which include landrace, released cultivars and advanced lines. These varieties were grown in field for 3 years under nine moisture regimes in Aleppo, Syria. The mean grain yield decreased with increasing water stress. The mean grain yield under lowest moisture regime of 302 mm was 55% lower compared to the highest moisture regime of 583 mm. The genotypes varied significantly for grain yield under all moisture regimes

The data supported the hypothesis that some of the highest yielding genotypes under supplementary irrigation conditions can also be among the highest yielding under rainfed condition. However, evaluation under rainfed condition appeared to be necessary to preserve genotypes possessing alleles for drought tolerance. The data support the assumption that the yield potential (responsiveness under supplementary irrigation) and traits for drought tolerance (yield under rainfed condition) can be combined in one single genotype

Keywords: drought tolerance, yield potential, winter and facultative wheat

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 315–320

INTRODUCTION

A major impact of drought in rainfed agriculture is the reduction of crop productivity. The effect of drought is dependent on the amount and distribution of rainfall and soil characteristics. It is further complicated by the prevailing temperatures. Therefore, development of wheat cultivars that are able to tolerate drought and response well to supplementary irrigations are necessary to increase productivity in drought prone environments. Based on long-term average, the rainfall pattern in the Mediterranean region is described as a typical winter rainy season where water availability through precipitation only is less than 400 mm (Rajaram et al. 1994).

Two strategies have been suggested for identifying genotypes that will be high yielding under drought: 1) genotypes adapted to low input condition which exhibit low genotype x environment ($G \times E$) interaction (Ceccarelli 1989) and 2) genotypes adapted to both favorable and low input environments (Braun et al. 1992, Rajaram 2000). Bread wheat is an important cereal in the rainfed agriculture of Mediterranean regions of West Asia and North Africa. The objective of this research was to compare genotypes of bread wheat for drought tolerance and responsiveness under variable moisture regimes.

MATERIALS AND METHODS

The experiments reported here were conducted at Tel Hadya, ICARDA situated at 36° 10′N, 36° 56′E near Aleppo, Syria during 2002/03, 2003/04 and 2004/05 crop seasons. Twenty five winter and facultative bread wheat genotypes of diverse origins were included in this study (Table 1). The 14 advanced lines were from different breeding programs in USA, Turkey and Mexico, with some final selection done by Turkey/CIMMYT- ICARDA program in Turkey and Syria. Oweis et al. (2001) showed that a supplementary application of 33 to 66% of full irrigation achieved the best yield and highest water use efficiency. Based on this, we chose the treatment of 50 and 100 mm through supplementary irrigations which was split into 2 applications of 25 mm and 50 mm respectively. The three moisture treatments created were as follows: 1) rainfed, designated RF with a total rainfall of 483 mm, 402 mm and 302 mm for 2002/03, 2003/04 and 2004/05 respectively and with an average of 395 mm for the three seasons; 2) supplementary irrigation with 25 mm at pre-booting and 25 mm at early grain filling, designated as RF +50 mm; and 3) supplemental irrigation with 50 mm at pre-booting and 50 mm at early grain filling, designated as RF +100mm.

The experimental design was a split-plot in randomized complete blocks with two replications. The main-plots were allocated to the moisture regimes and subplots to the genotypes. Each plot comprised 8 rows of 7.0 m long with row-to-row distance of 20 cm. Seeds were drilled at 120 kg ha^{-1}. Prior to planting, 20 kg N ha^{-1} through urea and 60 kg P_2O_5 ha^{-1} through super-phosphate were applied into the soil. Additional urea providing 40 kg N ha^{-1} was added as a top dress at stem elongation stage. The sowing date was 22, 18 and 29 November in 2002, 2003 and 2004 respectively. The plots were kept free from diseases, weeds and there was no major

Table 1. Genotypes, their grain yield (t/ha) under dry and wet moisture regimes, response type and classification based on drought tolerance and responsiveness to supplemental irrigation at Tel Hadya, Syria

Entry No	Cross/Name	Origin	Diversity groups	Yield under (302 mm)	Yield under (583mm)	Response/ Drought category[@]
1	Azar-2	Iran	NR	3.18	3.12	G_{NR}
2	Sardari	Iran	LR Ir	2.73	3.33	G_{NDR}
3	Son64/4/wr51//mayall /n.th/3/k117	Iran	AL Ir	2.87	3.20	G_{NR}
4	Fen kang15/sefid	Iran	AL Ir	2.68	3.49	G_{NR}
5	Sbn/1–64–199	Iran	AL Ir	2.75	4.77	G_{DRL}
6	Tjb368.251/buc//anb/buc	Mexico	AL	3.00	6.55	G_{NDR}
7	Asv/parrot//tam200	Mexico	AL	2.95	6.80	G_{DRL}
8	Sdy/ald/3/nai60/hn7//buc/ 4/alucan	Mexico/USA	AL	2.19	5.99	G_{NDR}
9	Tam200/mo88//sdy*3/ami	Mexico	AL	3.12	6.39	G_{DRL}
10	Vorona/hd2402	Mexico	AL	2.80	5.78	G_{DRL}
11	Co72.3839/ti-r//fasan/3/co72.3839/ti-r	Mexico	AL	2.43	6.44	G_{NDR}
12	Saulesku32/weaver//f41 05w2.12	Turkey	AL	2.65	6.06	G_{NDR}
13	Ks82w409/spn	USA	AL	2.15	4.94	G_{NDR}
14	Agri/nac//Attila	Mexico	AL	2.83	3.88	G_{NDR}
15	Atay/galvez87	Mexico	AL	367	6.05	G_{DRL}
16	Vorona/tr810200	Mexico	AL	2.65	6.05	G_{NDR}
17	Id800994w/vee//f900k/3/ pony/opata	Turkey	AL	3.24	5.14	G_{DRQ}
18	Agri/nac//kauz P8-8/	Mexico	AL	2.78	8.16	G_{DRL}
19	llkofen/3/bez/nad//kz m/4/bb//cc/cno *2/3/tob156/bb/5/ning8675	Mexico	AL	1.67	6.46	G_{NDR}
20	Kinaci	Turkey	NR	2.55	5.56	G_{NDR}
21	Katia	Bulgaria	NR	3.98	5.38	G_{DRL}
22	Bezostaya	Russia	WA	3.05	5.07	G_{DRL}
23	Gerek	Turkey	WA	2.60	3.79	G_{NR}
24	Cham 6	Syria	WA	3.26	6.76	G_{DRQ}
25	Sultan	Turkey	NR	164	5.47	G_{NDR}
S.E.				±0.23		
Mean				2.78	5.38	

NR = Newly released. LRIr = Iranian landrace. AL Ir = Iranian Advanced lines. WA = Widely adapted. AL = Advanced lines from diverse sources with some selection done by Turkey/CIMMYT-ICARDA program.

incidence of insect. For each individual year, analysis of variance (ANOVA) was carried out according to split-plot design in complete block. On finding that the error variances for assessing genotypic response and interaction with irrigation regime were homogeneous over years, ANOVA was carried out on the combined data.

Definitions of Drought Tolerance and Input Responsiveness

The mean yields of each genotype over replications were used to model the response to the moisture applied as the sum of seasonal rainfall and the supplemental irrigation. Linear and quadratic response function of the moisture regimes using weighted regression were fitted where the weights were computed as inverse of the squared standard error of the means. If the deviation from the linear regression is non-significant, then the response function would be evaluated as linear. If the deviation from linear response and the linear regression *per se* are non-significant, then the genotype would be considered non-responsive. If the deviation from linear regression is significant, then we examined the quadratic function as well. Adjusted R-square values, also known as percentage variance accounted for, were computed for linear and quadratic regressions. The higher adjusted R-square value determined whether the response was linear or quadratic. For a variety belonging to linear or quadratic response group, the drought tolerance was calculated as predicted value from its regression equation at the minimum moisture level. For a variety from non-responsive group, the drought tolerance was calculated as mean response under rainfed condition, denoted by $\overline{Y}_D$. A genotype is defined as drought tolerant if its response under low moisture regime is more than $\overline{Y}_D$. Using the response pattern and the response under drought, a variety was classified as non-responsive, denoted as G_{NR}, if its response does not change with input. The second category of varieties denoted as G_{NDR} is based on their poor performance under low moisture condition while these responded to supplemental irrigations. The third categories of varieties denoted as G_{DRQ} were grouped together based on their high performance under low moisture conditions and quadratic response relationship under high input condition. The fourth category denoted as G_{DRL} comprised those lines with drought tolerance and linear response relationship under supplemental irrigation condition.

RESULTS AND DISCUSSION

Genotypic differences, genotype × irrigation and genotype × irrigation × year interactions were significant ($P < 0.001$) for grain yield. The mean grain yield of the genotypes in the nine moisture regimes was used to evaluate genotypes' drought tolerance and responsiveness to the input. Table 1 presents the genotypes details, diversity groups, mean grain yield under driest and wettest moisture regimes, the response type and the classification into the drought tolerance and responsiveness categories. The 25 genotypes are classified into the four categories based on the yield response and drought classification criteria. The linear responsive group G_{DRL} comprised of genotypes 5, 7, 9, 10, 15, 18, 21 and 22. The quadratic responsive group G_{DRQ} is represented by varieties 17 and 24. The non-responsive group G_{NR} varieties are 1, 3, 4 and 23. The largest number of remaining entries was designated responsive. These genotypes were the poorest performer under lowest moisture regime giving lower yields than $\overline{Y}_D$. These are listed in Table 1 and their mean performance is plotted in Fig. 1. Of the 25 genotypes, 10 genotypes showed drought

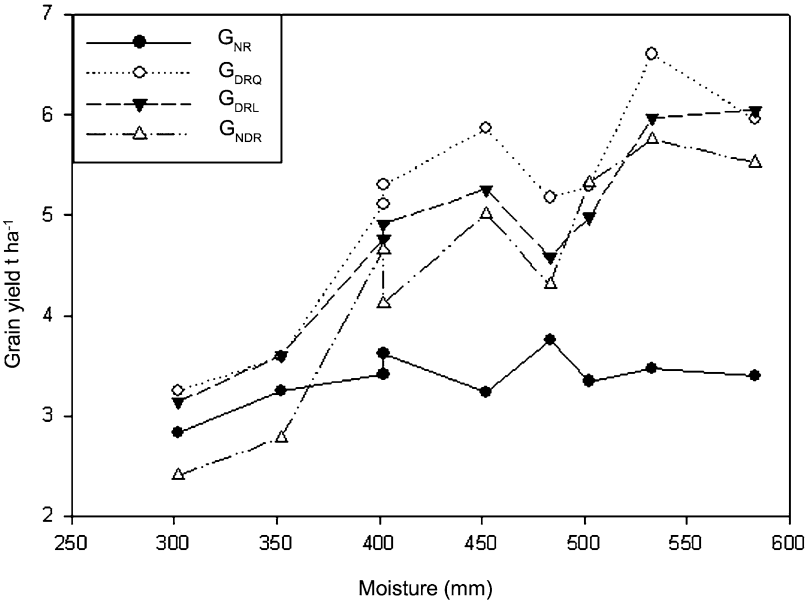


Figure 1. Mean grain yields of genotypes as response to 9 moisture regimes over 3 years 2002/03, 2003/04 and 2004/05 cropping seasons at ICARDA, Aleppo, Syria [@]G_{NR} = Non-responsive genotype. G_{NDR} = Responsive genotype without drought tolerance. G_{DRL} = Genotype with drought tolerance and linear response combined. G_{DRQ} = Genotype with drought tolerance and quadratic response combined

tolerance (yield $> \overline{Y}_D$) as well as responsiveness to the supplementary irrigation. Of these genotypes, six were advanced lines; two were widely grown varieties, one Iranian advanced line, and a newly released variety Katia.

Partitioning of the genotypes into between and within drought tolerance and responsiveness groups revealed significant differences at $P < 0.01$ between the categories and interaction between the category, irrigation and year. Further comparison of category mean at a given moisture regime (Fig. 1) showed that, at the wet end, each of the three responsive groups give significantly higher grain yield compared to the non-responsive group, while at the dry end, their differences with non-responsive group is not significant. Further, the linear-response group G_{DRL} gives significantly higher yield than the non-drought responsive group G_{NDR} at both ends. However, the quadratic-response group G_{DRQ} gives significantly higher yield compared to non-drought tolerant responsive G_{NDR} group at dry end but not at the wet end. While there is variation of various degrees at the moisture regimes in the middle, the two drought tolerant categories with linear and quadratic response did not differ at the either end.

The data supported the hypothesis that the highest performing genotypes under supplementary irrigation conditions can also be amongst the highest yielding under less favorable conditions. However, evaluation under drought stress appeared to

be necessary to preserve genotypes possessing alleles for drought tolerance. Such observation has been made earlier in case of wheat. Rajaram et al. (1994) made a very strong hypothetical case of combining high yield potential and drought tolerance in one single genotype. The data presented here fully support such possibility.

The issue of yield potential and drought tolerant combination for rainfed situation is important because of variable amount of precipitation in marginal rainfed environments. It is prudent to construct a genetic system in which plant responsiveness provides a bonus whenever higher rainfall improves the production environment. With such a system, improved moisture is immediately translated into greater yield gains for farmers. This phenomenon clearly suggests that to obtain a suitable widely adapted genotype, we need to combine the traits of yield potential and traits of adaptation to drought tolerance in one single genotype. Such results have been obtained earlier (Rajaram 2000) and obtained in this study (the genotypes no. 9, 10, 15, 17, 18, 21 and 22). The breeding methodology to combine such trait in single genotypes has been discussed (Calhoun et al. 1994 and Rajaram 2000). Rajaram (2000) proposed such a methodology based on his experimentation in Mexico. Although this experiment was done in Syria under Mediterranean climatic condition, but still supports the results obtained in Mexico.

REFERENCES

Braun H, Pfeiffer WH, Pollmer WG (1992) Environments for selecting widely adapted spring wheat. Crop Sci 32:1420–1427

Calhoun DS, Gebeyehu G, Miranda A, Rajaram S, van Ginkel M (1994) Choosing evaluation environment to increase wheat grain yield under drought conditions. Crop Sci 34:673–678

Ceccarelli S (1989) Wide adapted: how wide? Euphytica 40:197–205

Oweis T, Zhang H, Pala M (2001) Water use efficiency of rainfed and irrigated bread wheat in a Mediterranean environment. Agron J 92:231–238

Rajaram S (2000) Prospects and promise of wheat breeding in the 21st century. In: Bedo Z (ed) Wheat in a Global Environment. 6th International Wheat Conference; Budapest, 5–9 Jun 2000

Rajaram S, van Ginkel M, Fischer RA (1994) CIMMYT's wheat breeding mega environment (ME). In Proceedings of the 8th International Wheat Genetics Symposium. China Agricultural Scintech, Beijing, China. pp 1101–1106

GENETIC ACHIEVEMENTS UNDER RAINFED CONDITIONS

R. MAICH*, D. ORTEGA, A. MASGRAU AND G. MANERA

Genética, Industrias Agrícolas, Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, C.C. 509, 5000 Córdoba, Argentina
**E-mail: rimaich@agro.uncor.edu*

Abstract: Plant breeding program under rainfed conditions is not an easy enterprise. The slow genetic progress is achieved here results from presence of genotype x environment interaction, grain yield components compensation and the enigmatic origin of drought tolerance. However, it is necessary to increase breeding efficiency in order to meet world food requirements. With an objective to evaluate agronomic differences after six cycles of a recurrent selection, twenty eight late flowering S_1 derived families (four per cycle) and four commercial cultivars were grown under rainfed conditions in the central semiarid region of Argentina. Significant increase was observed for spikelet number per spike (13.3%) and spike biomass (8.1%), while spike number m^{-2} was reduced by 20.2%. Positive tendencies for spikelet number per spike ($b = 0.346$), spike biomass ($b = 0.0357$) and spike grain yield ($b = 0.0175$) were observed; while the tendency for grain number m^{-2} was negative ($b = -144.6$). Independent of cycles, the direct effect of grain number m^{-2} on grain yield was highest. Overall the yield components tended to balance out their individual contributions. The results show a negative overcompensation of the spike number m^{-2}, in response to increased spike reproductive structures. Considering that both aspects were used as selection criteria, the unproductive tiller mortality was more sensitive to environmental changes than those characters related to the spike morphology. The direct effects of grain yield components on grain yield depends on the genetic progress in each component and their compensation. At the end of the sixth cycle of selection, no increase in grain yield was observed. It can be due to: a) the agronomic performance of the fifth and sixth cycles derived families during the driest year of evaluation was similar to the commercial varieties (selected in low-stress environments), indicating that a lower flexibility and stability were fixed; b) the selection criteria (index) was not appropriate due to wrong weights assigned to its components and c) the phenological characteristic (later flowering) of the materials resulted inappropriate to measure the genetic progress under drought stress conditions

Keywords: rainfed, recurrent selection

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 321–329.

INTRODUCTION

Developing a plant breeding program under rainfed conditions in a semiarid environment, where the crop, for the most part, develops on stored moisture in the soil is very difficult. The presence of the genotype x environment (G x E) interactions create a dilemma of selection criteria where production and adaptability are generally opposed. Positive trends for grain yield were observed in several studies using recurrent selection (RS) in autogamous species. (Bravant et al. 1991, Olmedo Arceaga et al. 1995, Dubois et al. 1998, Maich et al. 2003). However, there is little information available from fourth or fifth RS cycles on. Ortega et al. (2004) observed advances after six cycles of RS for grain yield (C_0 to C_3), several grain yield components (C_4 to C_6) and an increase in the number of reproductive structures of the spike in the more advanced cycles; yet no increase in the grain yield was observed. The use of several traits as selection criteria allows a plausible route to moderate the negative effects of G x E interaction. In this sense, Richards et al. (2001) affirm that a trait may be important as selection criteria one year but not in the next year. In practice, the preferred method of selection involves a combination of direct and indirect selection using a selection index of desirable secondary traits and grain yield under stressed and unstressed conditions (Fischer et al. 1983). Two factors important in trait selection to be discussed here are the environment, where the crop is grown and its timing and secondly, the biological hierarchy and the milieu of processes and interactions that may be important in the expression of the trait (Richards 1989). In this approach, selection is based on yield components rather than on yield itself; however, major difficulties are the yield component compensation and the allometric relationships of size of organs of the plant (Smith 1987). Synthetically, the selection criteria could be constituted by those traits related to higher grain yield potential and adaptability; however, it is well known that the plant survival is not connected necessarily to the economical grain production. In order to achieve the just mentioned goal, it is necessary to establish an equal relationship between the grain yield components, substituting the compensatory effects by the additive ones. Probably, under rainfed conditions of cultivation the genetic progress will be of scarce magnitude due to the fact that drought stress enhances the compensatory effects. A clear picture of the importance of each grain yield component is provided by the path coefficient analysis (Li 1956). Dencic et al. (2000) observed that under near optimum conditions, none of the analyzed characters showed a significant direct association with yield, while under drought stress conditions a significant positive effect was assigned to the number of grains per spike. The existence of compensatory effects between wheat grain yield components under drought stress, but not under favorable conditions, were observed by Garcia del Moral et al. (2003). The present investigation makes an effort to measure genetic progress in wheat after six cycles of recurrent selection under rainfed conditions, in a semiarid environment.

MATERIALS AND METHODS

Sixteen commercial varieties of bread wheat (*Triticum aestivum L.*) were crossed to obtain eighty three F_1 hybrid combinations used as initial population (C_0) and starting point for a RS scheme with two years per cycle (evaluation and recombination). This work was carried out at the Experimental Farm of the College of Agriculture (Cordoba National University), Cordoba (31° 29′ S; 64° 00′ W) in central semiarid region of Argentina. On all occasions the materials were grown under rainfed conditions. S_0 progenies (full sib families) were used as selection units, grown in single row plots of 1.3 m long and 0.2 m apart, at seeding rate of 100 grains per m^2, without replications and with regular controls. During the earlier cycles of RS (C_0 to C_3) grain yield was used as the main selection criteria. Later (C_4) aerial biomass and the harvest index were added and finally (C_5 and C_6) a selection index constituted by eighteen traits was used.

Seed bulks of the selected families were created for each population (C_0 to C_6). Plants from these seed mixtures were grown at regular spaces. Seeds were harvested only from a sample of thirty S_1 plants per population and reduced to twelve with satisfactory grain production for plot evaluation. The eighty four S_1 derived families were characterized for flowering date during 2001. Four late flowering S_1 derived families per population and four commercial varieties were grown during 2002, 2003 and 2004. A complete randomized design in each of the three years of evaluation was employed using the families of the same cycle as replications. One row plots 5 m long and 0.2 m apart with a seeding rate of 250 grains per m^2 were used. At plot level, grain and biomass yield (g m^{-2}), grain and spike number per m^2, harvest index (%) and thousand grain weight (g) were estimated. From a random sample of ten tillers per plot the following traits were measured: spikelets per spike, grains per spikelet and per spike, grain and biomass yield per spike (g) and spike harvest index (%). Analyses of variance were computed for all characters, considering selection cycles and years as variation sources. Significant differences between mean values were determined by the DGC test (Di Rienzo et al. 2001). Linear regressions of the traits on cycles of RS were calculated. The path coefficient analysis was performed in order to separate the direct influence of each yield component on yield from indirect influences caused by mutual relationship among yield components.

RESULTS

Significant differences between mean values corresponding to the recurrent selection cycles were observed for spikelets per spike, spikes per m^2, thousand grain weight, biomass and harvest index per spike (Table 1). Increases of 13.3 % and 8.1 %, respectively, were observed for spikelet number and spike biomass. On the other hand, the spikes per m^2 were subjected to a diminution of 20.2 % at the end of the sixth cycle of RS. Independent of the RS cycles, significantly superior mean value differences with respect to the check cultivars were observed for grain and biomass yield per spike.

Table 1. Mean values of twelve traits measured during six cycles of recurrent selection in bread wheat

Cycle	SPN	GN-S	GN-SP	SN	GY	BY	HI	1000 GW	GN	SGY	SBY	SHI
C_0	17.55 b	24.58 a	1.37 a	293.42 a	166.33 a	778.50 a	20.29 a	32.17 a	5321.58 a	0.85 a	1.35 b	62.92 b
C_1	18.78 b	27.92 a	1.44 a	274.83 a	146.17 a	656.42 a	20.50 a	30.21 b	5299.17 a	0.84 a	1.33 b	62.23 b
C_2	18.13 b	23.80 a	1.29 a	320.17 a	160.92 a	805.83 a	18.68 a	34.05 a	4973.83 a	0.84 a	1.31 b	64.37 a
C_3	17.87 b	24.38 a	1.32 a	273.33 a	158.50 a	716.83 a	20.78 a	34.44 a	4770.50 a	0.88 a	1.40 b	62.13 b
C_4	18.42 b	26.28 a	1.41 a	266.75 a	161.50 a	754.75 a	19.95 a	33.29 a	5113.83 a	0.92 a	1.50 a	60.93 b
C_5	19.98 a	28.08 a	1.37 a	265.83 a	151.58 a	730.42 a	20.03 a	32.77 a	4964.75 a	0.97 a	1.57 a	60.91 b
C_6	19.88 a	26.05 a	1.28 a	234.08 b	130.33 a	639.58 a	18.98 a	32.80 a	4150.50 a	0.90 a	1.46 a	61.30 b
Checks	18.83 b	25.33 a	1.32 a	300.12 a	162.50 a	693.42 a	22.63 a	27.98 b	6070.92 a	0.74 b	1.19 c	61.69 b

SPN: spikelets number spike^{-1}; GN-S: grains number spike^{-1}; GN-SP: grains number spikelet^{-1}; SN: spikes number m^{-2}; GY: grain yield (gm^{-2}); BY: biological yield (gm^{-2}); HI: harvest index (%); 1000 GW: thousand grain weight (g); GN grains number m^{-2}; SGY: spike grain yield (g); SBY: spike biological yield (g); SHI: spike harvest index (%).

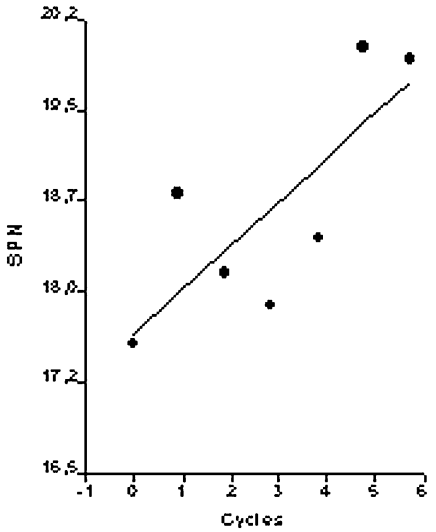


Figure 1a. Relationship between spikelets number per spike (SPN) and cycles of recurrent selection

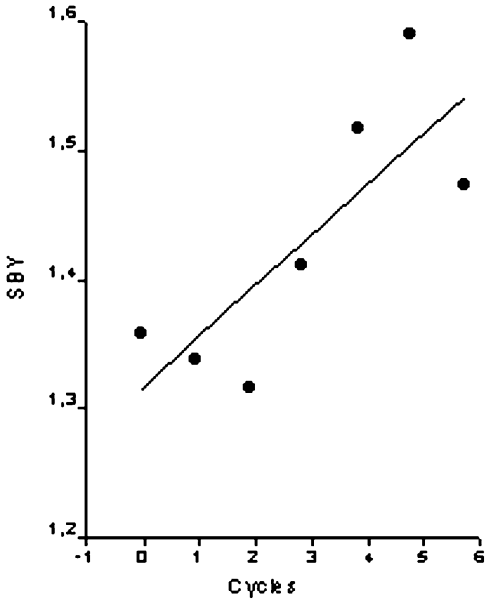


Figure 1b. Relationship between spikelets biological gram yield (SBY) and cycles of recurrent selection

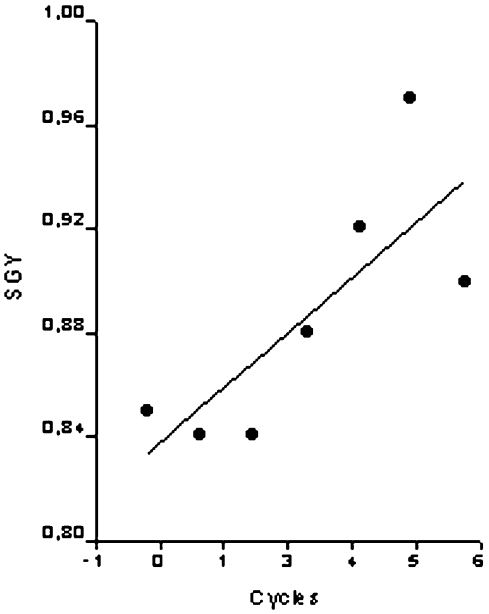


Figure 1c. Relationship between spike grain gram yield (SGY) and cycles of recurrent selection

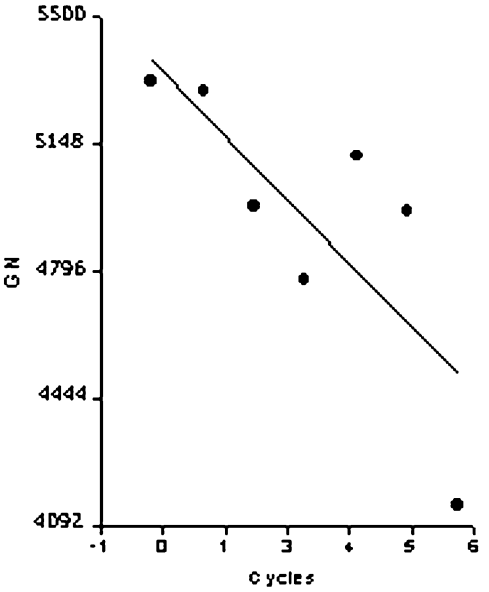


Figure 1d. Relationship between grain number per spike m^{-2} (GN) and cycles of recurrent selection

Positive and significant tendencies were observed for spikelet number ($b = 0.346$), spike biomass ($b = 0.0357$) and spike grain yield ($b = 0.0175$) (Fig. 1 a, b, c). A negative tendency was observed for grains per m^2 ($b = -144.6$) (Fig. 1d).

Table 2. Direct effect of yield components on grain yield

Cycle	SN	GN	1000 GW	SPN	GN-S	GN-SP	SGY
0	−0.16	1.08	0.16	0.48	−0.96	0.14	0.48
1	−0.03	0.94	0.06	0.69	−1.29	0.54	0.28
2	0.60	0.58	0.09	−0.44	0.15	−0.07	0.20
3	0.06	0.91	0.21	0.21	−0.15	0.09	0.01
4	0.24	0.77	0.02	0.33	−0.88	0.23	0.44
5	0.11	1.11	0.37	0.16	−0.25	0.09	0.13
6	0.06	1.03	0.19	0.17	−0.27	0.22	−0.01
Checks	−0.04	1.18	0.17	−0.05	−0.17	0.00	0.23

SN: spikes number m^{-2}; GN: grains number m^{-2}; 1000 GW: thousand grain weight; SPN: spikelets number $spike^{-1}$; GN-S: grains number $spike^{-1}$; GN-SP: grains number $spikelet^{-1}$; SGY: spike grain yield.

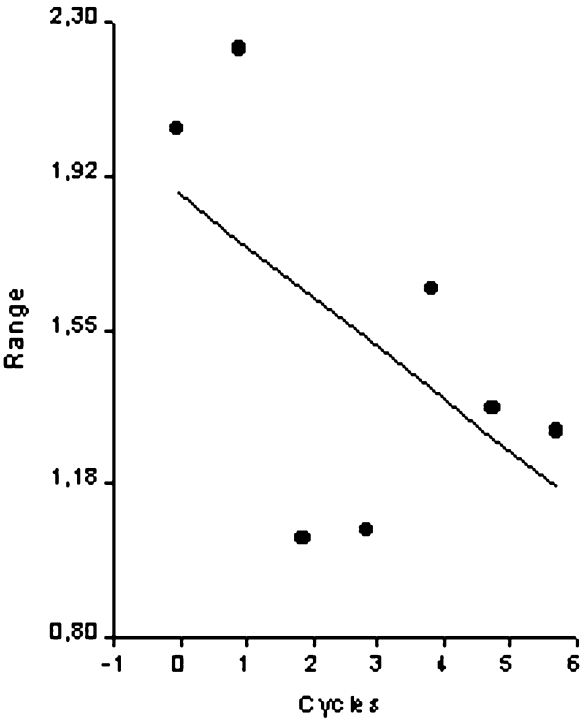


Figure 2. The relationship between the direct effects of the range of grain yield components and cycles of recurrent selection

Only the grains per m^2 maintained an increasing trend through the RS cycles impacting directly on grain yield (Table 2). Fig. 2 shows the range of decrease in the direct effects of grain yield components as the genetic pool evolves.

Means in a column with different letters are significantly different at 0,05 probability level (DGC test).

DISCUSSION

The results show that an increase in the biomass and spikelets per spike was associated with an increase in grain yield per spike. However, grain yield per m^2 did not increased, due to a loss in grains number per m^2 caused by a lower spike number in the more advanced cycles of RS. Taking into account that the number of non productive tillers was higher at the sixth RS cycle (unpublished data) and that the spikelets per spike were fixed in the early stages of spike development (Slafer and Rawson 1994), higher drought stress caused an interference (compensation effects) between both grain yield related traits. The terminal drought stress, characteristic of the region, determined a negative compensation of the spikes per m^2 in response to the higher spike reproductive structures. Both, the spikelet and spike numbers were components of the index used as selection criteria. However, only the former trait shows a significant response to selection; the bigger spikes of the more evolved cycles of RS increased the tiller mortality.

The path coefficient analysis shows that the grain number per m^2 has direct effect on grain yield which impacts the grain production in the semiarid regions. On the other hand, a retrospective analysis shows a progressive compensation of other grain yield components. Independent of the level of significance, Table 2 of Dencic et al. (2000), these results show a higher compensation among the component of grain yield in the cultivars than the local landraces. Taking into account that the latter are less evolved than the commercial cultivars, a similar comparison may be made between original population (C_0) and more evolved ones (C_6). In this case, though plant breeding helps reduce the direct effects of individual components on grain yield, no changes for grain yield were observed. This situation may be explained by: (1) The agronomic performance of C_5 and C_6 populations during the driest years of evaluation was similar to the check cultivars, developed under favorable conditions (2) An error in the selection and weightage of the component traits of the index. (3) The late flowering phenological characteristic of the genotypes was not appropiate to measure the genetic progress under a terminal drought stress. Although all analyzed genotypes had a similar date of flowering, those pertaining to the fifth and sixth cycles of RS showed a longer grain filling period. This observation is another proof of correct course of our plant breeding program.

REFERENCES

Bravant P, Kervella J, Doussnault G, Piccard E, Rousset M (1991) Effect of the first cycle of recurrent selection on a winter wheat population. Agronomie 11:473–482

Dencic S, Kastori R, Kobiljski, B, Duggam B (2000) Evaluation of grain yield and its components in wheat cultivars and landraces under near optimal and drought conditions. Euphytica 113:43–52

Di Rienzo J, Guzmán WY, Casanoves F (2001) DGC: Prueba de comparaciones de medias. InfoStat Versión 1.1/Profesional. Grupo Infostat. Facultad de Ciencias Agropecuarias. Universidad Nacional de Córdoba. Argentina

Dubois ME, Gaido ZA, Manera GA, Maich RH (1998) Evaluación del efecto de dos ciclos de selección recurrente por rendimiento en semilla sobre la calidad panadera en trigos (*Triticum aestivum L.*) aptos para la región semiarida del centro de Argentina. Phyton 62(1–2):167–174

Fischer KS, Johnson EC, Edmeades GO (1983) Breeding and selection for drought resistance in tropical maize. CIMMYT, El Batan, Mexico

Garcia del Moral LF, Rharrabti Y, Villegas D, Royo C (2003) Evaluation of grain yield and its components in durum wheat under mediterranean conditions: An ontogenyc approach. Agron J 95(2):266–274

Li CC (1956) The concept of path coefficient and its impact on population genetics. Biometrics 12:190–210

Maich RH, Torres LE, Manera GA, Dubois ME (2003) Grain yield improvement in bread wheat (T*riticum aestivum L.*) after three cycles of recurrent selection. J Genet Breed 57:201–206

Olmedo Arceaga OB, Elias EM, Cantrell RG (1995) Recurrent selection for grain yield in durum wheat. Crop Sci 35:714–719

Ortega D, Manera G, Argenti RY, Maich R (2004) Progreso genético en trigo cultivado en secano. Agriscientia XXI(2):89–92

Richards RA (1989) Breeding for drought resistance in cereals. Physiological approaches. In: Baker FWC (ed) Drought resistance in cereals. CAB International pp 65–79

Richards RA, Condon AG, Rebettzke GJ (2001) Traits to improve yield in dry environments. In: Reynolds MP, Ortiz Monasterio JI, Mc Nab A (eds) Application of physiology in wheat breeding. Mexico, DF, CIMMYT chap VII pp 88–100

Slafer GA, Rawson FH (1994) Sensitivity of wheat phasic development major environmental factors: A re-examination of some assomptions made by physiologist and modelers. Aust J Plant Physiol 21:393–426

Smith EL (1987) A review of plant-breeding strategies for rainfed areas. In: Srivastava et al. (eds) Drought tolerance in winter cereals. Wiley J Ltd. pp 79–87

QUANTIFYING POTENTIAL GENETIC GAINS IN WHEAT YIELD USING A CONCEPTUAL MODEL OF DROUGHT ADAPTATION

M.P. REYNOLDS[1,*] AND A.G. CONDON[2]

[1]*CIMMYT AP 6-641, 06600 Mexico, D.F. Mexico,*
[2]*CSIRO, Plant Industry, Canberra. Australia*
[*]*E-mail: m.reynolds@cgiar.org*

Abstract: Many candidate physiological traits have been suggested for wheat improvement under moisture stress and genetic diversity is present in the wheat gene pool for most of them. The objective of this study was to quantify the probable yield effect associated with variation in the expression of such traits within two sets of germplasm; *Sisters* (from an elite x elite cross) showing a range of drought adaptation, and *Diverse* lines (representing synthetic-derived wheat as well as selected landraces) assembled for favourable expression of one or more of the following traits under moisture stress: final biomass, ability to extract water at depth from the soil, remobilization of stem soluble carbohydrates, and transpiration & water-use efficiency (WUE). Agronomic and physiological traits were measured in NW Mexico in 2005 under post-anthesis moisture stress that resulted in yield reductions of up to 65% compared with irrigated plots, depending on the genotype. There was a significant range of expression for all traits measured and calculations were performed to gain an idea of the relative potential contribution to crop performance if trait expression were maximized in the highest-yielding backgrounds. Theoretically, remobilization of stem carbohydrates would be associated with 7% yield gains for *Sister* and 20% for *Diverse Lines*. Maximizing WUE could achieve 16% and 4% gains in yield in *Sister* and *Diverse Lines* respectively, despite the already strong association of the trait with yield, and similar results were found for transpiration efficiency. In both sets of germplasm, putting the best expression for water extraction (to 120cm) in the highest yielding background was associated with yield gains of 13%. Taken together these results suggest substantial yield gains under moisture stress are achievable if the genes representing these traits were to be combined using a complementary-trait based approach to breeding

Keywords: drought adaptation, yield, model

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 331–340

INTRODUCTION

Research into the physiological basis of drought adaptation has been conducted for some time and while some of the research has been applied to wheat improvement (Condon et al. 2002, Richards et al. 2002), much has not. Since a large number of drought-adaptive responses exist it can be challenging to prioritize their application. For this reason a conceptual model for wheat was developed in which traits are divided into groups whose genes and/or physiological effects are likely to be independent such that when parents with contrasting traits are crossed, drought-adaptive genes would be combined (Reynolds et al. 2005). The model describes four main groups of traits relating to: (I) Pre-anthesis growth: rapid ground cover to shade the soil from evaporation (Richards et al. 2002), and strong assimilation capacity between jointing and lag-phase to permit accumulation of stem carbohydrates (Blum 1998). (II) Access to water as a result of root depth or intensity that would be indicated by a relatively cool canopy (Reynolds et al. 2005) or favourable expression of water relations traits (Blum et al. 1989). (III) Water use efficiency (WUE) of canopy growth as indicated by relatively higher biomass per mm of water extracted from the soil, transpiration efficiency of growth (TE = biomass per mm water transpired) indicated by C-isotope discrimination ($\Delta^{13}C$) of leaves (Condon et al. 2002), and WUE of spike photosynthesis associated with refixation of respiratory CO_2 (Bort et al. 1996). (IV) photo-protection including anti-oxidant systems (Niyogi et al. 1999), and anatomical traits such as leaf wax (Richards et al. 2002).

The objectives of this analysis was to estimate the potential contribution to yield of a number of these traits using quantitative trait information from genetically diverse cultivars grown at a drought stressed field location.

MATERIALS AND METHODS

Experimental Environments and Germplasm

Experiments were conducted in NW Mexico (27°20′N and elevation 38 m above sea level) in 2005. Agronomic and physiological traits were estimated on $8m^2$ plots seeded at 12g m^{-2}. The design was a randomised lattice with 2 reps. Water available to the crop from drip irrigation and rains amounted to 295 mm resulting in post-anthesis moisture stress; otherwise the trials were managed optimally.

Germplasm

Germplasm included: (i) a set of 14 F7-derived sisters from the bread wheat cross Seri/Babax with known contrasts in drought response plus the two parents. (ii) a set of 30 bread wheat lines from diverse genetic backgrounds (among them Mexican landraces and lines deriving from inter-specific hybridization of AB and D genomes) chosen for high expression of a number of drought-adaptive traits including: cool canopies and ability to extract water at depth from the soil profile, stem soluble carbohydrates shortly after anthesis and remobilization, and final biomass.

Physiological and Agronomic Measurements

Water use was determined by measuring gravimetric water content at the following profile depths: 0–30cm, 30–60cm, 60–90cm, and 90–120cm. This was determined on six random locations at crop emergence, and on two spots of every plot after harvest. When calculating available water it was assumed that in addition to the water applied as rain and irrigation water, additional fluxes associated with evaporation of water from the soil surface after crop emergence and gains associated with heavy dew (an almost daily phenomenon in this environment) cancelled each other out. Values of permanent wilting point of 20% and a soil bulk density of 1.3 were assumed, based on previous samplings at the same locality. Water use efficiency was calculated using total water extracted for each genotype and final above ground biomass. Remobilization of stem carbohydrates was estimated from the dry weight (g m^{-2}) of stems and their relative content of soluble carbohydrates 7 days after anthesis and at physiological maturity. Leaf $\Delta^{13}C$ values were measured on pre-flag leaf samples collected 30 days after emergence from irrigated plots (Condon et al. 2002). Canopy temperature was measured with an infra-red thermometer. Yield and yield components were calculated using standard procedures.

RESULTS AND DISCUSSION

Sister Lines

Moisture stress reduced line-mean yield by between 27 and 50% compared with the irrigated treatment. There was a significant range of values for all traits (Table 1). These values were used to calculate the theoretical range in yield associated with variation for each trait, to gain an idea of the relative potential contribution to crop performance encompassed by the genetic diversity. This was done by ranking the values for each trait and calculating the average expression for the two lines showing the largest and smallest value for each trait, respectively (Table 2). For biomass-WUE, the maximum and minimum values were 4.56 and 3.3 g $m^{-2}mm^{-1}$, respectively, representing a range of 1.26 units. Using average values for water used and harvest index, 254mm and 0.42, respectively, this translated to a yield range of approximately 140 g m^{-2}. Similarly, the range of values was calculated for remobilization of stem reserves and these represented 70 g m^{-2} of yield, assuming no losses from respiration associated with translocation and conversion to starch. This is theoretically possible as spikes have been shown to re-fix effectively all respiratory CO_2 (Bort et al. 1996). When comparing differences in water extracted from the soil profile down to 120cm, the full genotypic range was 40mm. Using average values for yield, WUE and HI, this was calculated to represent approximately 65g m^{-2} of yield. Relative differences in TE were estimated from $\Delta^{13}C$ values. The difference in $\Delta^{13}C$ between lines with the highest and lowest values equated to TE being 18% greater, at the plot scale, in the low- $\Delta^{13}C$ lines (Condon et al. 2002). Assuming average total water use and HI, this difference in TE represented 72 g m^{-2} of yield, assuming also that T averaged 75% of total water use. This

Table 1. Agronomic and physiological traits for Seri/Babax sisters lines and parents, NW Mexico, 2005

Seri/Babax sisters and parents	Yield irrigated	Yield drought	Stem CHO remobilization	Available H_2O at maturity (0–120 cm)	WUE of final above-ground biomass	$\Delta^{13}C$	Canopy temp grainfill (pm)	Harvest Index	Final above-ground biomass
	$g\ m^{-2}$	$g\ m^{-2}$	$g\ m^{-2}$	*mm*	$g\ m^{-2}\ mm^{-1}$	‰	*C*	%	g m^{-2}
1	736	369	45,4	42,7	3,385	20,2	26,3	0,425	871
2	746	468	84,5	43,6	3,833	20,9	26,5	0,475	983
3	716	470	55,2	37,3	3,893	20,9	26,6	0,460	1023
4	655	410	69,2	50,5	3,500	19,6	26,4	0,470	873
5	624	416	34,0	16,4	3,782	20,4	26,1	0,395	1073
6	687	449	25,3	48,0	4,555	20,6	26,3	0,395	1148
7	697	444	77,1	41,5	4,575	20,8	26,6	0,375	1183
8	584	352	70,0	48,2	3,859	18,9	26,5	0,365	972
9	576	349	61,1	51,9	3,716	18,3	26,6	0,380	922
10	649	409	53,4	44,0	4,002	19,6	26,6	0,400	1025
11	654	344	60,6	62,1	3,217	18,8	26,5	0,450	765
12	744	451	43,7	36,3	3,467	19,9	26,4	0,490	914
13	673	387	57,2	18,2	3,845	19,8	26,3	0,370	1083
14	630	461	49,4	45,5	4,193	20,1	26,5	0,435	1067
Babax	767	407	60,7	36,5	3,503	19,5	26,3	0,441	923
Seri	770	418	117	39,4	4,170	20,0	26,5	0,384	1087
Mean	682	413	60,2	41,4	3,84	19,9	26,4	0,420	994
correlation with yield	0,46	1,00	–0,04	–0,26	0,51	0,83	0,03	0,41	0,54
LSD (5%)	118	26,9	37,2	22,9	0,28	1,09	0,26	0,080	213

Table 2. Comparison of expression of agronomic and physiological traits for Seri, Babax and progeny, considering (i) top and lowest yielding lines, and (ii) lines showing the greatest contrast of the trait *per se*, NW Mexico, 2005 (see text for assumptions)

Seri/ Babax sisters and parents	Yield Drought g m^{-2}	Stem CHO remobi-lization g m^{-2}	Available H_2O at maturity (0–120cm) *mm*	Available H_2O at maturity (0–120cm)	WUE of final above-ground biomass g m^{-2} mm^{-1}	$\Delta^{13}C$ ‰	Transpiration Efficiency g $m^{-2} mm^{-1}$	Canopy temp grainfill (pm) *C*	Harvest Index	Final above-ground biomass g m^{-2}
(i)										
mean top 2 for yield (BM)	469	70	40,4	(44.7)	3,86	20,9	4,8	26,56	0,47	1003
mean last 2 for yield (BM)	347	61	57,0	(52.4)	3,47	18,6	5,7	26,58	0,42	844
(ii)										
mean top 2 for trait	469	101	17,3	–	4,56	18,6	5,7	26,20	0,49	1165
mean last 2 for trait	347	30	57,0	–	3,30	20,9	4,8	26,60	0,36	818
Trait range	122	71	39,7	–	1,26	2,3	0,9	–0,40	0,13	347
Yield range (g m^{-2})	–	71	64,0	–	137	–	72,0	–	124	146
dTrait (best–best yield line)	–	31,15	23,13	(27.4)	0,70	–2,3	0,9	–0,36	0,02	162,19
Extra yield (g m^{-2})	–	31,2	37,1	60,40	75,2	–	89,0	–	17	93,6

average value of 75% is reasonable, given the relatively infrequent rainfall. There was a positive association between yield and $\Delta^{13}C$, whereas a negative association between TE and $\Delta^{13}C$ is expected (Condon et al. 2002). This apparent anomaly could occur if there was large variation in T as a proportion of total water use (%T).

Transpiration was calculated to be 82% of total water use for the highest-yielding lines but only 62% for the lowest-yielding lines.

Additional calculations were performed to estimate the theoretical genetic gains that could be achieved if trait expression were maximized for each trait in the highest-yielding lines. Thus the highest average ($n = 2$) value for each trait was compared with its actual expression in the two highest-yielding lines and the difference was calculated. In all cases the difference was appreciable (Table 2). For biomass-WUE the average value for the two highest-yielding lines was 3.86 g m^{-2} mm^{-1}, 0.70 units less than the maximum value of 4.56. Using average values for water used and the HI of the highest-yielding lines, 260mm and 0.47, respectively, this translated to additional potential yield of 75 g m^{-2}. Similarly, the difference in remobilization of stem reserves between the best value for trait expression and that of the highest-yielding lines represented an extra 30 g m^{-2} of yield, again assuming no losses from respiration. When comparing differences in water extracted from the soil profile between 30 and 120cm, the best lines in terms of extraction left only 17.3mm of available water while the best-yielding lines left 40.4mm, representing a difference of 23.1 mm. Using the WUE and HI of the best-yielding lines, this represented approximately 37 g m^{-2} of untapped yield. An additional calculation was made comparing water extraction of the best lines in terms of extraction with that of those achieving the highest biomass. The difference was 27.4mm which translated into a yield of 60 g m^{-2} using the best values for WUE and HI, respectively. It was calculated that an extra 89 g m^{-2} of yield could be gained by expressing the highest TE (lowest $\Delta^{13}C$) in the highest-yielding lines. This theoretical gain was obtained assuming average soil water extraction but the high values for HI (0.47) and %T (82%) calculated for the highest-yielding lines.

Diverse Lines

The effect of moisture stress was to reduce yield by between 15 and 65% compared with the irrigated treatment. There was a significant range of values for all traits and these were used to calculate the theoretical range in yield associated with variation for each trait and the theoretical genetic gains associated with maximization for each trait using the same assumptions as previously (Table 3).

In both sets of germplasm, yield was most strongly associated with WUE, mainly through biomass production, while the ability to access water was not strongly associated with yield, except indirectly in *Diverse Lines* as indicated by the association of both yield and biomass with cooler canopies. Remobilization of stem reserves were not associated with yield in either set of germplasm. No direct data was collected estimating differences in early ground cover. However, large genotypic differences in non-productive evaporation of water were calculated

Table 3. Comparison of expression of agronomic and physiological traits for Diverse lines considering (i) top and lowest yielding lines, and (ii) lines showing the greatest contrast of the trait *per se*, NW Mexico, 2005 (see text for assumptions)

Diverse Lines	Yield drought g m^{-2}	Stem CHO remobilization g m^{-2}	Available H_2O at maturity (0–120cm) *mm*	Available H_2O at maturity (0–120cm)	WUE of final above-ground biomass g m^{-2} mm^{-1}	$\Delta^{13}C$ ‰	Transpiration Efficiency g $m^{-2} mm^{-1}$	Canopy temp grainfill (pm) *C*	Harvest Index	Final above-ground biomass g m^{-2}
Mean	306	77,4	44,6	–	3,28	20,0	5,2	25,2	0,380	795
SLD(5%)	50,8	73,1	0,95	–	0,45	0,50	0,15	0,58	0,079	193
(i)										
mean top 3 for yield (or biom)	483	58	(45.43)	48,4	5,42	19,90	5,25	26,31	0,35	1380
mean last 3 for yield (or biom)	147	100	(43.78)	45,4	1,57	20,30	5,16	26,98	0,41	400
(ii)										
mean top 3 for trait	483	142	27,0	27,0	5,65	19,40	5,50	26,10	0,50	1421
mean last 3 for trait	147	28	72,0	72,0	1,34	20,70	5,07	27,60	0,28	348
Trait range	336	114	45,0	–	4,31	1,3	0,43	−1, 50	0,22	1073
Yield range (g m^{-2})		114	56,6	–	413	–	31	–	184	411
dTrait (best-best yielding line)		83,87	18,43	21,43	0,23	−0, 5	0,250	−0, 21	0,15	40,73
Extra yield (g m^{-2})		98,4	35,0	60,54	19,9	–	13	–	206	14,3

in order to account for the positive association between $\Delta^{13}C$ and yield in the *Sister Lines*. The very low BM achieved by some of the *Diverse Lines* (350 g m^{-2} compared with 1420g m^{-2} for the most productive), for relatively little apparent difference in soil water extraction, indicates very large non-productive evaporation of water for the lowest-BM entries in this trial. Variation in $\Delta^{13}C$ was sufficient to account for variation in TE of up to 1.2-fold in each trial, although this was more than neutralized by very low total T by the highest-TE *Sister Lines*.

The results were used to estimate the range in yield associated with the full range of expression of physiological traits as a way of standardizing the genetic diversity in terms of its potential to affect yield within each group of germplasm. The diversity was considerably larger for the *Diverse Lines* than *Sister Lines*, with the exception of water extraction from the soil profile and TE (Tables 2 and 3). For both sets of germplasm water use efficiency represented the largest potential yield range of any trait, which may help explain the strong association of the trait with yield. Nonetheless, variation in remobilization of stem reserves and water availability both represented a yield range of over 50 g m^{-2}, while there was no association with yield, suggesting strong potential for genetic improvement in yield using these attributes.

The calculated theoretical values of maximizing drought-adaptive traits in high-yielding backgrounds suggested substantial gains in yield could be achieved (Fig. 1). Maximizing WUE could achieve 16% and 4% gains in yield in *Sister* and *Diverse Lines* respectively, despite the already strong association of the trait with yield. For the *Sister Lines*, the highest-yielding lines had the lowest TE. Combining high TE with high %T and HI could achieve a 19% gain in yield. Among the *Diverse Lines*, the highest-yielding entries already had relatively high TE, so potential further yield gains from this trait were small. Improved access to water at depth in the soil profile was associated with potential gains in yield of approximately 7.5 % and 13% for both sets of germplasm using the best yield and best biomass scenarios for calculating yield gains, respectively. Remobilization of stem carbohydrates was associated with 7% yield gains for *Sister* and 20% for *Diverse Lines*. The larger value for the latter may be an overestimate since there is a weak negative correlation (−0.40) between % stem carbohydrates and final biomass in this material, implying that high values of stem carbohydrates may be linked to low biomass. Potential genetic gains could be even higher with germplasm sources showing still higher values of trait expression, for example value for stem carbohydrates of over 25% have been observed in this environment. However, the possibility that remobilized stem carbohydrates may be supporting root function should also be considered. Similar analyses in drier years (where average drought yields are in the region of 200 g m^{-2}) will be necessary if these results are to be extrapolated to more severe environments.

With reference to the conceptual model for drought adaptation (Reynolds et al. 2005), the data suggest that for the three groups of traits evaluated, early growth as expressed by stem carbohydrate remobilization and greater %T, access to water, and greater TE and WUE can all still contribute significantly to increasing adaptation to

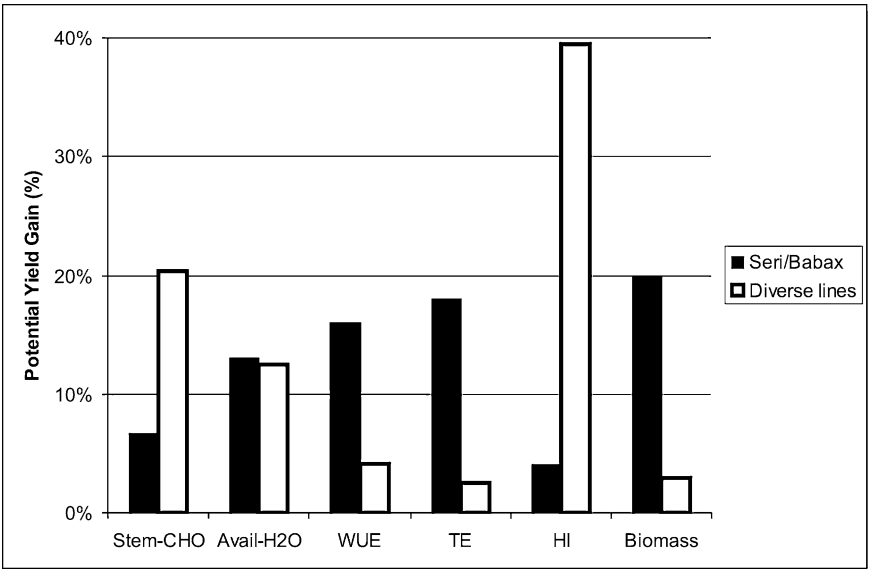


Figure 1. Potential yield gains associated with genetic variation in two sets of germplasm -Seri/Babax sisters & parents (n = 16) and Diverse Lines (n = 30)- for remobilization of stem carbohydrates at anthesis (stem-CHO), use of available water down to 120cm (avail-H20), water use efficiency (WUE), transpiration efficiency (TE), harvest index (HI), and final above-ground biomass (Biomass), NW Mexico, 2005

post-anthesis drought stress. In terms of the fourth group of traits, photo-protection, the potential for cooler canopies in both sets of germplasm (Tables 2 and 3) may also be indicative of further potential genetic gains since cooler temperatures would be associated with less photo-inhibition and photorespiration. These results support the idea that substantial yield gains under moisture stress are achievable if the genes representing different groups of traits are combined using a complementary-trait based approach to breeding.

ACKNOWLEDGEMENTS

The authors acknowledge the Australian Grains Research and Development Corporation for supporting this research in Mexico.

REFERENCES

Blum A (1998) Improving wheat grainfilling under stress by stem reserve mobilization. Euphytica 100:77–83

Blum A, Shpiler L, Gozlan G, Mayer J (1989) Yield stability and canopy temperature of wheat genotypes under drought stress. Field Crop Res 22:289–296

Bort J, Brown RH, Araus JL (1996) Refixation of respiratory CO_2 in the ears of C_3 cereals. J Exp Bot 47:1567–1575

Condon AG, Richards RA, Rebetzke GJ, Farquhar GD (2002) Improving intrinsic water-use efficiency and crop yield. Crop Sci 42:122–131

Niyogi KK (1999) Photoprotection revisited: genetic and molecular approaches. Annu Rev Plant Physiol Plant Mol Biol 50:333–359

Reynolds MP, Mujeeb Kazi A, Sawkins M (2005) Prospects for utilizing plant adaptive mechanisms to improve wheat and other crops in drought and salinity prone environments. Ann Appl Biol 146: 239–259

Richards RA, Rebetzke GJ, Condon AG, van Herwaarden AF (2002) Breeding opportunities for increasing the efficiency of water use and crop yield in temperate cereals. Crop Sci 42:111–121

CHANGES IN THE ABIOTIC STRESS TOLERANCE OF WHEAT AS A RESULT OF AN INCREASED ATMOSPHERIC CO_2 CONCENTRATION

O. VEISZ*, S. BENCZE AND G. VIDA

Agricultural Research Institute of the Hungarian Academy of Sciences Brunszvik u. 2., Martonvásár, Hungary, H-2462

**E-mail: veiszo@mail.mgki.hu*

Abstract: Climatic change, a global environmental problem particularly affecting agriculture and the natural environment, is now seen to be associated with an increase in the frequency and intensity of climatic anomalies. Weather extremes in Hungary have included droughts in the 1980s, hot, dry summers in 2001–2003, and excessive rainfall in 2004 and 2005

Research on the effect of climate extremes on the biomass, yield and abiotic stress tolerance of wheat has been done in the phytotron of the Agricultural Research Institute in Martonvásár. Studies on the frost resistance, heat tolerance and drought tolerance of wheat varieties with various genetic backgrounds were made when grown under normal and increased atmospheric CO_2 concentrations and at various nutrient supply levels. The following conclusions were drawn from the results

- The frost resistance of wheat grown and cold-hardened at double the current level of atmospheric CO_2 concentration improved to various extents; this effect was more pronounced in frost-sensitive genotypes
- Heat stress had severe effects: it decreased biomass accumulation and the thousand kernel weight, resulting in a yield loss of between 27% and 37%. Increased CO_2 concentration was able to counteract the deleterious effects of the heat stress
- When plants were exposed to heat stress, the yield loss was lower at low nitrogen levels
- Depending on the genotype the yield quality deteriorated to various extents in heat-stressed plants at doubled CO_2 concentration
- The doubled CO_2 concentration was able to reduce the deleterious effects of drought stress on yield components

It can be seen from the results that genetic differences among winter wheat varieties can allow breeders to select genotypes with better adaptability, enabling them to be grown reliably even under altered environmental conditions

Keywords: frost, heat stress, drought

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 341–347.

INTRODUCTION

The atmospheric concentration of CO_2 has been steadily increasing since the beginning of the industrial era. Climatic anomalies arising from the climate change, such as deficient or excessive rainfall, a higher frequency of hot days, drought, and winter or early spring frosts, are already causing problems for crop production each year.

Increased CO_2 levels during the growth of cereals result in higher rates of biomass accumulation, higher yields and better resistance to frost (Veisz 1997, Harnos et al. 1998). High temperature during anthesis and grain filling causes reductions in kernel number and size, kernels per spikelet, grain yield and harvest index (Blumenthal et al. 1995), but the effects of heat stress can be reduced by elevated CO_2 (Taub et al. 2000, Bencze et al. 2004). High temperature may have negative effects on flour quality. Blumenthal et al. (1995) reported that despite the higher protein content, there was a decrease in the glutenin-gliadin ratio and in the percentage of very large glutenin polymers following heat stress.

At higher CO_2 levels there is a reduction in stomatal conductance and an improvement in water use efficiency, due partly to a substantial decline in transpiration and partly to a simultaneous rise in the net photosynthesis level (Tuba et al. 1994). The net photosynthesis and water use efficiency of C_3 plants were usually better at elevated CO_2 in water-limited environments, too (Tuba et al. 1996). In durum wheat Kaddour and Fuller (2004) reported that water-deficient plants produced surplus yields at increased atmospheric CO_2 concentrations compared with those raised at normal CO_2 levels.

The present paper discusses the effect of doubled CO_2 level on the resistance of wheat varieties to frost, heat stress and drought, based on the results of phytotron tests.

MATERIALS AND METHODS

Cold Stress

A freezing test was carried out to examine the frost resistance of *Triticum aestivum* genotypes (Alba, Apollo, Bánkúti 1201, Bezostaya 1 and Martonvásári 15). Germinated wheat seeds were sown in boxes, in 9 rows with 20 seeds to a row, in a random design with four replications. The plants were grown for six weeks on the M29 climatic programme (Tischner et al. 1997), either at ambient (375 μmol mol^{-1}) CO_2 level or at double this rate (750 μmol mol^{-1}). Other growth conditions (temperature, light intensity, duration of illumination, water supplies, etc.) were the same in all the chambers. Freezing was carried out in the frost resistance testing chamber, where the temperature was gradually reduced to $-15\,^{\circ}C$, which was maintained for 24 hours. The plants were then grown for a further three weeks, after which plants that had survived freezing and had started to develop could be clearly distinguished from those that had died. In order to evaluate the frost resistance of different varieties,

the number of plants that had survived freezing was counted, and expressed as a percentage of the plant number prior to freezing.

Heat Stress

Three winter wheat (*T. aestivum* L.) varieties commonly cultivated in Hungary: Mv Martina, Mv Emma and Mv Mezőföld, all of which have different agronomic characteristics, were chosen for the tests. The experiment was carried out under controlled environmental conditions in Conviron PGV/36 growth chambers in the phytotron. Vernalized seedlings were planted four to a pot ($21 \times 21 \times 17$ cm) in a 4:1 mixture of garden soil and sand (corresponding to 3.82 kg dry weight) containing 0.1561 % total nitrogen (NH_4-N: 6.36; NO_3-N: 75.6 mg kg^{-1} soil dry weight). The pots were placed randomly in the growth chambers and rearranged regularly. The plants were watered daily with tap water and provided with macro- and microelements weekly in 150 ml nutrient solution (for details see Bencze et al. 2005) from the 4th until the 10th week with the exception of nitrogen. The plants received either no nitrogen (0N treatment) or a total of 400 mg N per kg soil dry weight (400 N) in the form of ammonium nitrate dissolved in tap water, in ten instalments from tillering till heading. The atmospheric CO_2 level in the growth chambers was either ambient (375 μmol mol^{-1}) or doubled (750 μmol mol^{-1}). The temperature regime changed weekly, beginning with a min/max/mean of 10/12/10.7 °C during the first week and increasing until it reached 20/24/22.7 °C in the 11th week. In the control and after the heat stress treatment it remained at this level till the end of the experiment. Heat stress began 12 days after the average heading date of each group (Zadoks 59). In the heat stress treatment the temperature was 20/35/25.2 °C, the maximum temperature being maintained for 8 hours a day for 15 days. There were 28 plants in each treatment. After harvest the aboveground biomass (g), grain number per plant, thousand kernel weight (TKW, g) and grain weight per plant (g) were determined. The protein content of the wholemeal (per g dry weight) was measured using a Kjeltec 1035 Analyzer, applying a factor of 5.7 (ICC105/2), and the gluten quality parameters of the flour were characterized with a Glutomatic 2200 instrument (ICC 137/1, ICC 155).

Drought Stress

Two winter wheat varieties, Alba, cultivated in Poland, and Martonvásári 15 (Mv 15), bred in Hungary, were used in the tests. All the growing conditions (including the CO_2 levels) were set as in the control treatment in the heat stress experiment, with the exception of the soil water content, which was adjusted to 65% of total soil water capacity in the control treatment, with two levels of water stress: 45% and 25% soil water capacity, in the other two treatments. The plants were grown at near-optimum nutrient supply level.

The statistical analyses in all the experiments were carried out on the collected data using two-way ANOVA to study the effects of the treatments on the varieties, one factor being the treatment and the other the variety.

RESULTS AND DISCUSSION

Cold Tolerance and Elevated CO_2 Level

In all but one variety high CO_2 level increased the survival rate of cereals to an extent (Fig. 1). This effect was more pronounced in frost-sensitive genotypes, where the increase ranged from 47–240%. In Bezostaya 1, a variety with high frost resistance, there was no increase in the survival rate due to high CO_2, while Martonvásári 15, though being as tolerant as Bezostaya 1, had an even better survival rate at the doubled CO_2 level.

Heat Stress and Elevated CO_2 Level

Heat stress had severe effects, decreasing biomass accumulation and grain size and resulting in a yield loss of up to 27% in the 0N treatment and 37% at 400N (Table 1). Plants grown at the doubled CO_2 level had higher aboveground biomass and number of grains per plant in Mv Martina and Mv Emma, leading to a significantly higher yield (20–22 % at 400N). In Mv Mezőföld there was also a slight, 12 %, increase, but this was due to larger grain size. When grown at high CO_2, the plants often had better tolerance to high temperature during grain filling. Depending on the combination of variety and nitrogen supply, the thousand kernel weight and the grain yield were significantly higher at doubled CO_2 than at the ambient level, though the values were still lower compared to the control. The grain number

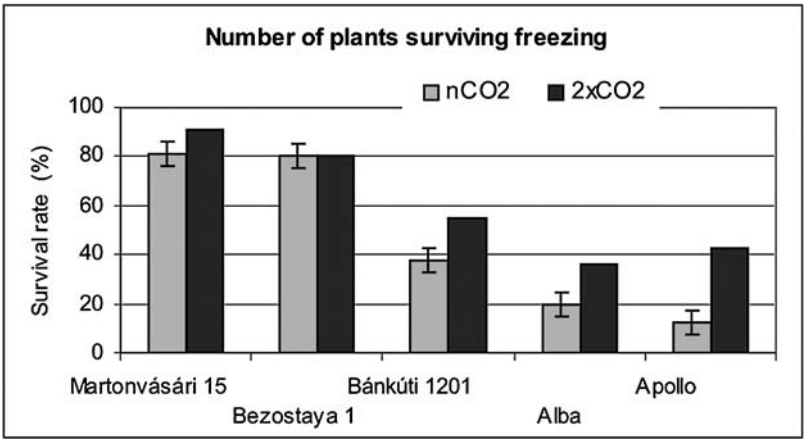


Figure 1. Effect of elevated CO_2 level on the survival of winter wheat varieties during freezing

Table 1. Effect of heat stress and elevated CO2 level

N	Temperature	Mv Martina		Mv Emma		Mv Mezőföld		$LSD_{5\%}$
		nCO_2	$2XCO_2$	nCO_2	$2XCO_2$	nCO_2	$2XCO_2$	
Above-ground biomass (g)								
0	Normal	9,5	10,3	10,5	11,2	8,1	9,5	1,4
400	Normal	9,9	12,8	10,5	12,8	10,2	10,9	
0	Heat stress	8,1	10,4	8,5	9,7	8,1	7,7	
400	Heat stress	8,0	10,0	8,9	10,5	8,1	9,8	
Grain weight per plant (g)								
0	Normal	3,70	3,84	3,63	4,31	3,27	3,80	0,54
400	Normal	3,79	4,54	3,60	4,40	3,49	3,91	
0	Heat stress	2,70	3,54	2,73	3,15	2,81	2,76	
400	Heat stress	2,40	3,27	2,28	3,10	2,27	2,61	
Grain number per plant								
0	Normal	102,8	108,0	109,4	121,3	101,2	112,1	17,0
400	Normal	113,6	132,5	107,5	129,7	116,4	114,2	
0	Heat stress	102,5	121,9	95,1	111,6	113,3	99,8	
400	Heat stress	105,0	117,6	101,6	112,1	104,3	119,7	
TKW (g)								
0	Normal	36,02	35,85	33,43	35,42	32,58	34,24	2,48
400	Normal	33,62	34,79	33,35	34,17	30,15	34,37	
0	Heat stress	26,74	29,24	28,92	28,63	24,71	28,22	
400	Heat stress	23,12	28,22	22,82	27,84	21,99	22,24	
Protein content % (d.w.)								
0	Normal	16,26	16,15	17,18	16,20	16,52	16,16	0,54
400	Normal	17,16	17,57	16,72	16,99	17,59	17,71	
0	Heat stress	17,36	16,90	18,79	18,38	18,23	17,34	
400	Heat stress	20,35	19,13	19,31	19,19	22,53	19,18	
Wet gluten content %								
0	Normal	29,80	30,50	38,45	37,50	40,45	39,90	1,95
400	Normal	33,20	32,85	38,70	37,35	44,15	41,95	
0	Heat stress	35,70	32,55	40,85	38,60	46,30	41,40	
400	Heat stress	36,80	33,40	45,40	43,35	48,20	49,95	
Gluten index								
0	Normal	96,8	91,9	99,0	99,2	76,0	78,5	7,0
400	Normal	96,8	97,1	99,1	99,9	74,2	81,0	
0	Heat stress	86,0	87,0	94,4	95,1	61,4	72,2	
400	Heat stress	95,1	91,3	88,5	86,5	56,6	62,5	

responded the least to CO_2 elevation under heat stress. At the normal (400N) nitrogen level, however, CO_2 enrichment was able to counteract the yield-reducing affect of heat stress in two genotypes. In Mv Mezőföld, elevated CO_2 level was unable to compensate for the negative effects of heat stress on the yield due to the low value of thousand kernel weight, although the biomass and grain number were similar to the control values, as in the other varieties.

Despite the higher protein and gluten contents, yield quality deteriorated to a significant or lesser extent due to high temperature, as can be seen from most of the gluten

index values at both nitrogen levels (Table 1), indicating that protein traits determining spatial structure were negatively affected, leading to less elastic dough.

Elevated CO_2 had very little effect on the protein and gluten contents in all varieties; a decrease in these values only being observed in one case. The gluten index did not change significantly by the high CO_2 level.

The increase in the protein and gluten contents as the result of heat stress was generally counteracted to some extent by doubled CO_2. In most cases the poorest quality was recorded in this treatment.

Drought Stress and Elevated CO_2 Level

Drought stress affected the plants dramatically, reducing the number of grains and the grain weight (TKW) and resulting in yield losses of 33% and 64% in Alba and 10% and 69% in Mv 15 at soil water levels of 45% and 25%, respectively (Fig. 2, Fig. 3). Doubled CO_2 level, however, affected the plants positively. While the thousand kernel weight did not change in Alba, the grains were larger in Mv 15 compared to the values at the ambient CO_2 level, and the number of grains increased in both varieties. These effects led to a yield increases of 58–89% in Mv 15 and 33–77% in Alba, depending on the treatment (though the increase was not significant in the 25 % soil moisture treatment).

As shown above, higher levels of atmospheric CO_2 could play an important role in reducing the deleterious effects of climatic anomalies in a possible future environment. The existing differences between wheat genotypes enable breeders to produce new varieties more tolerant to environmental stresses and responding more positively to higher levels of CO_2.

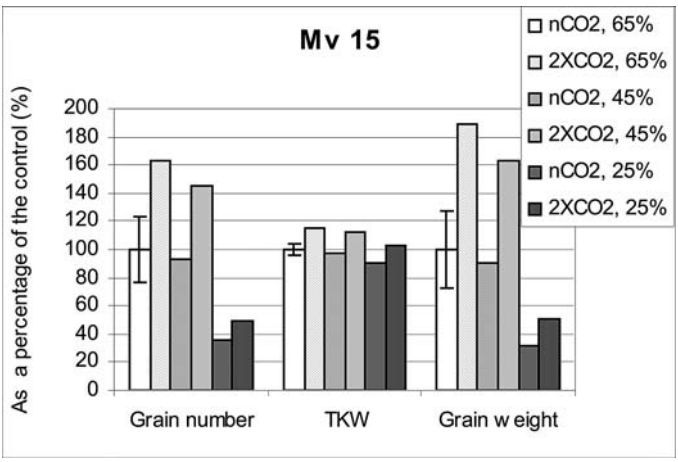


Figure 2. Effect of elevated CO2 level on the yield of the variety Mv 15 under water stress

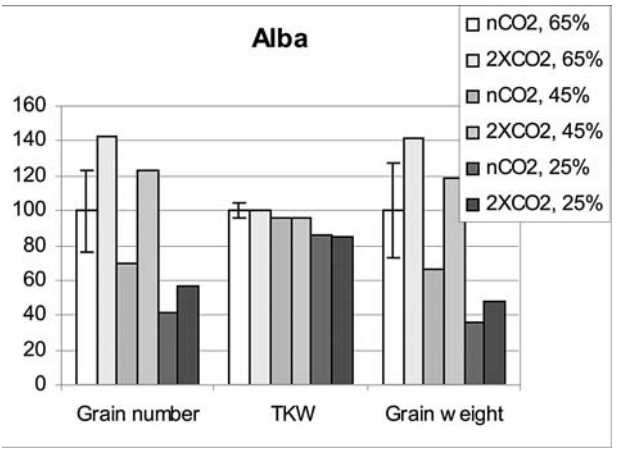


Figure 3. Effect of elevated CO2 level on the yield of the variety Alba under water stress

ACKNOWLEDGEMENTS

The experiments were carried out with the support of NKFP within the framework of Agro-ecological Research Project No. 3B/057/2002 and NKFP6-00079/2005.

REFERENCES

Bencze S, Veisz O, Bedő Z (2004) Effects of high atmospheric CO_2 and heat stress on phytomass, yield and grain quality of winter wheat. Cereal Res Comm 32:75–82

Bencze S, Veisz O, Bedő Z (2005) Effect of elevated CO_2 and high temperature on the photosynthesis and yield of wheat. Cereal Res Comm 33:385–388

Blumenthal C, Bekes F, Gras PW, Barlow EWR, Wrigley CW (1995) Identification of wheat genotypes tolerant to the effects of heat stress on grain quality. Cereal Chem 72:539–544

Harnos N, Veisz O, Tischner T (1998) Effects of elevated CO_2 concentration on the development and yield components of cereals. Acta Agronomica Hungarica 46:15–24

Kaddour AA, Fuller MP (2004) The effect of elevated CO_2 and drought on the vegetative growth and development of durum weat (*Triticum durum* Desf.) cultivars. Cereal Res Comm 32:225–232

Taub DR, Seeman JR, Coleman JS (2000) Growth in elevated CO_2 protects photosynthesis against high-temperature damage. Plant Cell Environ 23:649–656

Tischner T, Kőszegi B, Veisz O (1997) Climatic programmes used in the Martonvásár phytotron most frequently in recent years. Acta Agronomica Hungarica 45:85–104

Tuba Z, Szente K, Koch J (1994) Response of photosynthesis, stomatal conductance, water use efficiency and production to long-term elevated CO_2 in winter wheat. J Plant Physiol 144:661–668

Tuba Z, Szente K, Nagy Z, Csintalan Z, Koch J (1996) Responses of CO_2 assimilation, transpiration and water use efficiency to long term elevated CO_2 in perennial C_3 xeric loess steppe species. J Plant Physiol 148:356–361

Veisz O (1997) Effect of abiotic and biotic environmental factors on the frost resistance of winter cereals. Acta Agronomica Hungarica 45:247–255

GENETIC CONTROL OF WATER-SOLUBLE CARBOHYDRATE RESERVES IN BREAD WHEAT

G.J. REBETZKE[1,3,*], A.F. VAN HERWAARDEN[2], C. JENKINS[1,3], S. RUUSKA[1,3], L. TABE[1,3], N. FETTELL[4], D. LEWIS[3], M. WEISS[1] AND R.A. RICHARDS[1,3]

[1]*CSIRO Plant Industry, PO Box 1600, Canberra, ACT 2601 Australia*
[2]*CSIRO Plant Industry, 306 Carmody Rd., St. Lucia, QLD 4000 Australia*
[3]*GrainGene, PO Box 1600, Canberra ACT 2601 Australia*
[4]*NSW Department of Primary Industries, Condobolin NSW 2877 Australia*
[*]*E-mail: Geg.Rebetzk@csiro.au*

Abstract: The combination of high temperatures and lack of water decrease leaf area and reduce carbon assimilation in a terminal drought. It has been suggested that selection for greater stem storage of water soluble carbohydrate (WSC) would result in improved grain-filling and increased yields in drought-prone environments. No study has reported the extent or nature of genotypic variation for WSC in wheat. Progeny from three populations were genotyped with between 450 and 950 polymorphic molecular markers and phenotyped for stem WSC in well-watered environments. The range of WSC among progeny was large contributing to moderate to high narrow-sense heritabilities within environments ($h^2 = 0.58$ to 0.77); while relatively small genotype $\times$ environment interactions increased heritability on a genotype-mean basis (0.67 to 0.83). Large transgressive segregation suggested that stem WSC is controlled by multiple loci. Genetic control was complex with between 7 and 10 significant QTL (e.g. chromosomes 1A, 1B, 2B, 2D, 4B, 7A and 7B) identified for WSC across populations and environments. QTL were generally of small effect, each accounting for between 3 and 28% of the genotypic variance. Progeny with high WSC produced higher grain weight and larger diameter, significantly reducing grain shrivelling. High heritability indicates potential for phenotypic selection of WSC among families in breeding programs that target adaptation to terminal droughts

Keywords: carbohydrate, QTL, mapping populations

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 349–356

INTRODUCTION

Wheat crops growing in rainfed environments commonly experience water deficit during some stage of the crop growth cycle. In Australia, drought mostly occurs after anthesis. A lack of available water during grain-filling can inhibit leaf photosynthesis, interrupting carbon supply to developing seeds. This can result in lower grain yields and a greater proportion of shrivelled seed ('screenings') to further devalue the crop and returns to growers. Carbon remobilised from water-soluble carbohydrate (WSC) stored in the stem as fructans can make an important contribution to grain yield (Schnyder 1993, van Herwaarden et al. 1998). Modelling studies (Asseng and van Herwaarden 2003) indicate the maximum benefit of WSC occurs in seasons with a terminal drought.

An understanding of inheritance is critical in designing effective breeding strategies aimed at efficient genetic improvement and variety development. Yet there is little information regarding the amount and nature of genetic variation for WSC concentration in wheat. In a previous study, Ruuska et al. (2004) reported large and repeatable genetic variation for WSC concentration and content for a random set of wheat varieties evaluated throughout eastern Australia. This study extends this previous work to understand gene action and identify genomic regions associated with variation in WSC in three wheat mapping populations. Lines were phenotyped across multiple, well-watered environments to establish repeatability of genetic effects and their association with agronomic performance.

MATERIALS AND METHODS

Three populations containing between 148 and 190 doubled-haploid (DH) lines were derived from crosses between Cranbrook and Halberd, Sunco and Tasman, and CD87 and Katepwa. Development of each population is described in Kammholz et al. (2001). Doubled-haploids and parents of each population were sown into 6-m long, 5-row plots in a multiple-augmented design at Ginninderra Experiment Station, ACT in 2002 and 2003 (Katepwa was genotypically-mixed in 2003 and so wasn't harvested). Crops were managed with adequate nutrition and spraying of pesticides to control weeds and leaf diseases. At approximately 180 day-degrees past anthesis, grabs of 50 stems were made for each plot before drying in a fan-forced dehydrator set at 70 °C. Whole, dried samples were then ground to pass through a 1mm sieve and then scanned using near-infrared reflectance spectroscopy. A subset of these samples representing the variation in spectral reflectance was selected. Stepwise multiple-linear regression was used to predict laboratory-determined values for N (by semi-micro Kjeldahl) and WSC (by anthrone method after Ruuska et al. 2004) in the subset. These regressions were then used to predict N and WSC concentration in remaining samples. WSC content was then calculated from anthesis biomass and WSC concentration as the amount of WSC per unit area. At maturity, whole stems were hand-cut at ground level for weighing, counting of tillers and threshing of grain. 200-grain weight and diameter was determined for each line using the Single Kernel Characterisation System, and percentage of screenings for each plot

obtained as the weight of grain falling through a 2mm-slotted screen after 40 shakes of a 300g-sample on a modified shaker.

Narrow-sense heritability (h^2) was then calculated for each population on a genotype-mean basis assuming the covariance among F_1-derived doubled-haploids in each population equated to $1\sigma^2_A + 1\sigma^2_{AA}$ where, σ^2_A and σ^2_{AA}, refers to additive and additive × additive genetic variances, respectively. Genetic influence of WSC on agronomic traits was assessed via genetic correlation and correlated response following retrospective, divergent selection. Selection intensity was set at 20%, and differences in the means of selected low- and high-WSC sub-populations determined statistically with a 2-tailed *t*-test. The genetic maps and the development of the three populations were first described in Lehmensiek et al. (2005). QTL analysis was performed on spatially-adjusted, line means predicted using mixed linear models. The QTL detection was undertaken iteratively initially using composite interval mapping to obtain starting estimates for subsequent multiple interval mapping. Models were developed iteratively using changes in the bayesian information criterion (Kao et al. 1999) to identify main and interacting QTL.

RESULTS AND DISCUSSION

With the exception of Cranbrook and Halberd, parents of each population were similar for WSC concentration (Fig. 1). Previous studies across 7 to 12 environments demonstrated significant differences averaging +32% for Cranbrook over Halberd and smaller differences of 10% for Tasman over Sunco (data not shown). No previous data was available for CD87 and Katepwa. Progeny differences for WSC were large and significant with ranges extending beyond one standard deviation of the parental means indicating transgressive segregation. Transgressive segregation reflects the accumulation of positive or negative alleles from either parent to extend the trait range beyond one or both parents. Distributions of genotypic means were continuous and approximately normal for most traits although there was some evidence for bimodality with overlap in the distributions of WSC for C/H in 2003 (Fig. 1). The midparent-DH mean deviation was not significant ($P > 0.05$) providing little evidence for additive × additive epistasis for WSC. Given the approximately normal distribution and high narrow-sense heritabilities for WSC ($h^2 = 0.58$ to 0.73), WSC appears under genetic control of a number of independent alleles of small to large genetic effect. Indeed, Castle-Wright estimation suggests a minimum of three to four independent loci contribute to genotypic variation for WSC in each of the three populations (data not shown).

The influence of years on WSC was small for the C/H (compare 235 and 210 mg g^{-1} DW) but large for both the S/T (*cf.* 239 and 176 mg g^{-1} DW) and C/K (*cf.* 237 and 172 mg g^{-1} DW) populations. Genotype × environment effects were relatively small for WSC concentration resulting in moderate-to-large narrow-sense heritabilities for all three populations (Table 1). The magnitude of residual variances was relatively large for all three populations (data not shown) highlighting the importance of adequate replication and suitable experimental designs for assessing WSC concentration. In contrast to WSC concentration, large

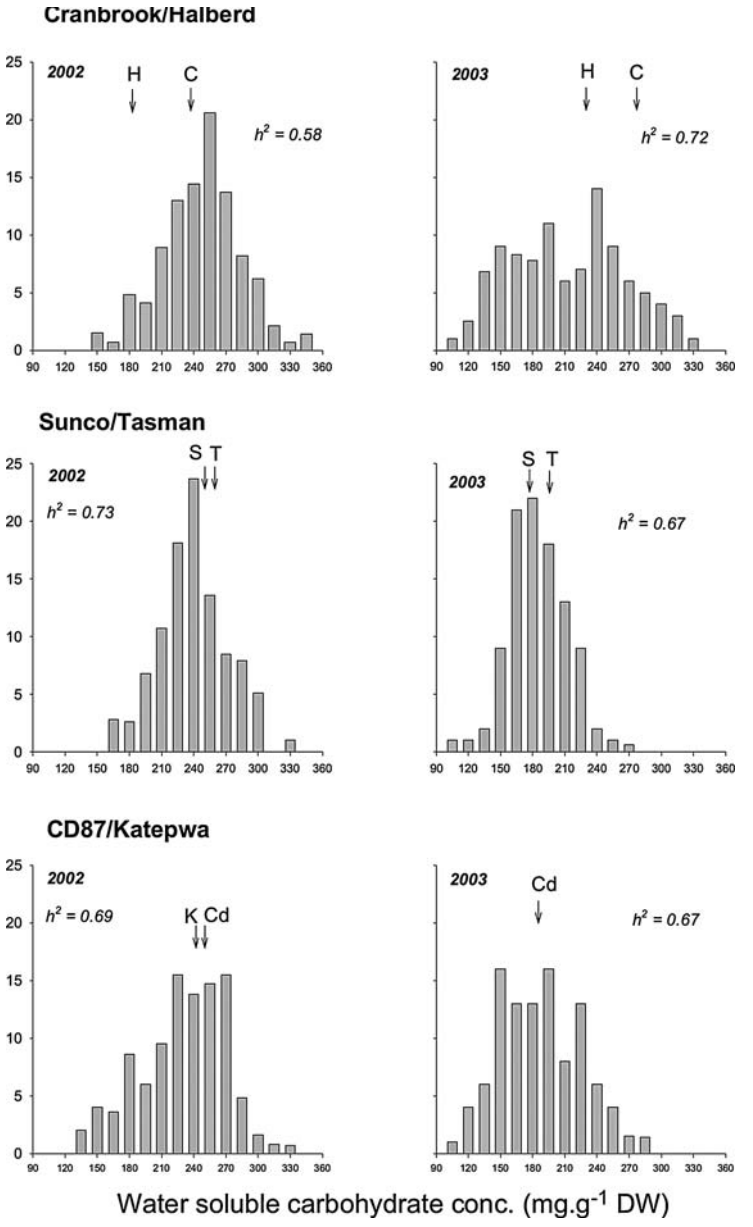


Figure 1. Frequency distributions for water soluble carbohydrate concentration measured on random lines from the Cranbrook/Halberd, Sunco/Tasman, and CD87/Katepwa mapping populations. C, H, S, T, CD and K are parental means for Cranbrook, Halberd, Sunco, Tasman, CD87 and Katepwa, respectively

Table 1. Genetic (σ^2_G) and genotype × environment interaction (σ^2_{GE}) variances (σ^2) (± standard errors), narrow-sense heritability estimates on a genotype-mean basis, and genotypic coefficients of variation (GCV) for WSC concentration (mg g^{-1} DW) and content (gm^{-2}) measured on random DH progeny in three wheat populations Cranbrook/Halberd (C/H), Sunco/Tasman (S/T) and CD87/Katepwa (C/K). * and ** indicate estimate is significantly different from zero at P = 0.05 and 0.01, respectively

Parameter	C/H WSC conc.	S/T WSC conc.	C/K WSC conc.	C/H WSC content	S/T WSC content	C/K WSC content
σ^2_G	$1347 \pm 206^{**}$	$376 \pm 69^{**}$	$821 \pm 139^{**}$	0	$509 \pm 284^{*}$	0
σ^2_{GE}	$234 \pm 179^{*,}$	83 ± 75ns	23 ± 96ns	$2650 \pm 691^{**,}$	$649 \pm 296^{**}$	$1430 \pm 517^{**,}$
h^2	0.83	0.67	0.78	ne	0.27	ne
GCV (%)	16.8	9.3	14.4	ne	8.6	ne

ne indicates non-estimatable

genotype × environment interaction for anthesis biomass reduced genetic variance and heritability for WSC content in all three populations (Table 1).

QTL mapping was undertaken on all three populations in both years. Large phenotypic variation and moderate to high narrow-sense heritabilities allowed good resolution of QTL associated with WSC concentration. Resulting genetic models accounted for between 42 and 76% of the phenotypic variance consistent with the narrow-sense heritability estimates reported above. There was little evidence for epistatic interactions but some evidence for QTL × environment interaction particularly in the S/T population. For the three populations, suggestive ($P < 0.10$) and significant ($P < 0.05$) QTL were identified on chromosomes 1A, 2B, 2D, 3D, 4B, 7A, 7B and 7D (C/H); 1A, 1B, 2B, 4A, 5B, 6B and 7B (S/T); and, 1B, 2D, 3B, 4B, 5B, 7A and 7B (C/K). The group 1, 2, 4 and particularly 7 chromosomes were consistent in their contribution to genotypic variation in WSC concentration. The large number of QTL observed for WSC in wheat was consistent with high numbers of QTL previously reported for a rice population grown in a single environment (Takai et al. 2005). Despite strong genotypic correlations for WSC concentration and content (Table 2), few QTL were identified for WSC content (data not shown). This probably reflects the lower heritability and reduced phenotype-genotype correlation for WSC content in the three populations (Table 1).

Table 2. Genetic correlations between WSC concentration and other agronomic traits for three wheat mapping populations. Cranbrook/Halberd (C/H), Sunco/Tasman (S/T) and CD87/Katepwa (C/K). * and ** indicate genetic correlation is significantly different from zero at P = 0.05 and 0.01, respectively

Population	WSC content	Grain yield	Grain weight	Grain Diameter	Screenings	N concentration
C/H	0.80^{**}	0.20ns	0.66^{**}	0.52^{**}	-0.58^{**}	0.07ns
S/T	0.87^{**}	0.33^{*}	0.51^{**}	0.48^{**}	-0.53^{**}	-0.56^{**}
C/K	0.88^{**}	0.64^{**}	0.63^{**}	0.49^{**}	-0.35^{**}	-0.78^{**}

Table 3. Correlated change in a range of agronomic characteristics with retrospective, divergent selection for low and high water soluble carbohydrate (WSC) concentration in three populations evaluated in rainfed environments with supplemental irrigation. Cranbrook/Halberd (C/H), Sunco/Tasman (S/T) and CD87/Katepwa (C/K). ¶, * and ** indicate low and high-selected WSC groups are significantly different at P = 0.10, 0.05 and 0.01, respectively. DC score is the decimal code score for plant development (smaller is later-flowering)

Population	Selected group	WSC conc.	WSC content	N	Grain yield	Harvest index	DC score	Plant height	Screenings	Grain weight	Grain diameter
		($g\ kg^{-1}$)	(gm^{-2})	($g\ kg^{-1}$)	(tha^{-1})			(cm)	(%)	(mg)	(mm)
C/H	High	274	320	1.11	5.70	0.42	60	91	3.16	42.7	2.90
	Low	199	238	1.16	5.51	0.35	48	107	4.17	36.6	2.74
	T	**	**	*	¶	**	**	**	**	**	**
S/T	High	247	285	1.34	6.20	0.44	65	74	0.81	40.7	2.83
	Low	175	195	1.58	6.04	0.41	64	74	5.39	37.4	2.71
	t	**	**	**	¶	**	ns	ns	**	**	**
C/K	High	265	309	1.20	5.86	0.42	68	75	1.20	40.5	2.86
	Low	164	197	1.41	5.57	0.36	43	67	3.06	35.7	2.71
	t	**	**	**	*	**	**	ns	*	**	**

Genotypic differences in WSC concentration were associated with changes in a number of agronomic traits. Higher WSC concentration was genetically correlated with higher WSC content and grain yield, larger grain size and fewer screenings (Table 2). Retrospective selection into high and low WSC concentration groups resulted in group mean changes of similar direction to that indicated through genetic correlation (cf. Table 2 and Table 3). For example, high-WSC selected groups commonly produced larger grain and fewer screenings. Similarly, selection for higher WSC was commonly associated with earlier flowering and reduced stature (Table 3). The negative genotypic association between WSC concentration and both plant height and anthesis date has been observed in other wheat populations (C. Jenkins and R. Shorter pers. comm. 2004). It suggests that breeders screening for high WSC should be careful to monitor lines for plant height and development. Monitoring should be simple given both plant height and anthesis date are highly heritable. N concentration and content at anthesis were lower for the high WSC-selected lines. However, this lower anthesis N was not necessarily associated with lower grain protein concentration at maturity (L. Tabe pers. comm. 2005) indicating the potential for development of high WSC, high grain protein varieties. Indeed, high WSC, high grain protein, milling wheats (e.g. H45 and Westonia) are in commercial use in Australia.

CONCLUSIONS

This study has identified QTL associated with genotypic variation for WSC concentration in wheat. These QTL were generally of small genetic effect but were often repeatable across both populations and years. The QTL of largest genetic effect have potential in marker-aided selection. However, the polygenic control of WSC concentration will reduce the effectiveness of QTL-based marker-assisted selection for high WSC in wheat. The data indicate that selection for reduced stature and/or earlier flowering will also result in correlated increases in higher WSC in some breeding populations. These parameters would need to be monitored or controlled in order to evaluate the effects of other QTL. The high narrow-sense heritabilities reported for WSC concentration in all three random wheat populations suggest the potential for modest genetic gain using phenotypic selection. Selection for high WSC was associated with marginally greater grain yields, significantly larger grain size and fewer screenings. The extent to which these effects are likely to improve wheat performance under drought needs further evaluation.

REFERENCES

Asseng S, van Herwaarden AF (2003) Analysis of the benefits to wheat yield from assimilates stored prior to grain filling in a range of environments. Plant Soil 256:217–229

Kammholz SJ, Campbell AW, Sutherland MW, Hollamby G, Martin P, Eastwood R, Barclay I, Wilson R, Brennan P, Sheppard J (2001) Establishment and characterisation of wheat genetic mapping populations. Aust J Agr Res 52:1079–1088

Kao CH, Zeng ZB, Teasdale RD (1999) Multiple interval mapping for quantitative trait loci. Genetics 152:1203–1216

Lehmensiek A, Eckermann PJ, Verbyla AP, Appels R, Sutherland MW, Daggard GE (2005) Curation of wheat maps to improve map accuracy and QTL detection. Aust J Agr Res 56:1347–1354

Ruuska S, van Herwaarden A, Jenkins C, Tabe L, Lewis D, Gillespie V, Richards R, Rebetzke G (2004) In: Black C, Panozzo J, Rebetzke G (eds) Cereals 2004 Proc 54th Australian Cereal Chemistry Conference and 11th Wheat Breeders Assembly. (Cereal Chemistry Division RACI)

Schnyder H (1993) The role of carbohydrate storage and redistribution in the source-sink relations of wheat and barley during grain filling – a review. New Phytologist 123:233–245

Takai T, Fukata Y, Shiraiwa T, Horie T (2005) Time-related mapping of quantitative trait loci controlling grain-filling of rice (*Orzya sativa* L.). J Exp Bot 56:2107–2118

van Herwaarden AF, Angus JF, Richards RA, Farquhar GD (1998) Haying-off, the negative grain yield response of dryland wheat to nitrogen fertiliser II. Carbohydrate and protein dynamics. Aust J Agr Res 49:1083–1093

VARIATION FOR STAYGREEN TRAIT AND ITS ASSOCIATION WITH CANOPY TEMPERATURE DEPRESSION AND YIELD TRAITS UNDER TERMINAL HEAT STRESS IN WHEAT

M. KUMARI[1], V.P. SINGH[1], R. TRIPATHI[1] AND A.K. JOSHI[1,2,*]

[1] *Department of Genetics and Plant breeding, Institute of Agricultural Sciences, BHU, Varanasi-221005, UP, India*

[2] *International Maize and Wheat Improvement Center (CIMMYT) Apdo. Postal 6-641, C.P. 06600, D.F. Mexico*

[*] *E-mail: joshi_vns@yahoo.co.in*

Abstract: Nine hundred and sixty three wheat advanced lines from various sources, including Indian and CIMMYT germplasm, were screened for the presence or absence of staygreen (SG) trait during two cycles, 2003–05. Staygreen was evaluated based on visual scoring (0–9 scale) and a new parameter, Leaf Area under Decline (LAUD). Approximately 5.5% of the lines were found to show staygreen character, 10.6% were moderately staygreen, and the remaining showed some or no expression of this trait. From this germplasm, one hundred lines were selected with the objective to find the effect of staygreen on yield and yield traits. These lines were sown under three different sowing dates (timely, late and very late) for three consecutive years to find the association between staygreen trait and heat tolerance. Canopy Temperature Depression (CTD), used as selection criteria for heat tolerance, was recorded at 12h, 14h and 16h at 7days interval, on bright sunny days. Correlation study showed that LAUD and CTD were strongly correlated ($r = 0.90$). LAUD was also found to be significantly associated with yield traits like grain filling duration (GFD) ($r = 0.83$), grain yield ($r = 0.89$), biomass ($r = 0.84$), but its association with test weight was non-significant. CTD also displayed significant correlation with yield traits like GFD ($r = 0.78$), grain yield ($r = 0.84$) and biomass ($r = 0.81$). There was significant association between grain yield, biomass, and GFD under all the sowing dates but the association between CTD, LAUD and test weight was significant only under very late sown conditions. Genotype x Year interaction was found to be non-significant for LAUD and CTD. Genotype x Year x Sowing date was non-significant for LAUD but significant for CTD. T-test done to compare SG and non-SG genotypes was found to be significant for all the traits for both the years and under all sowing dates, except for test weight, under timely sown condition. This investigation revealed that substantial variation exists for SG trait in wheat and there is significant difference between SG and non-SG

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 357–363.

genotypes for CTD. Hence, SG trait along with CTD can be used as effective selection criteria for tolerance to heat stress

Keywords: staygreen, heat stress

INTRODUCTION

Terminal heat is a major abiotic stress affecting yield in wheat. Under heat stress, the photosynthetic process is affected especially during grain filling stage when demand for assimilates is the greatest. Stay-green character is an important trait that allows plants to retain their leaves in active photosynthetic state when subjected to stress conditions (Rosenow et al. 1983). Hence, this trait, which is believed to affect radiation use efficiency, could be important under heat stress (Reynolds et al. 2001). Presence of stay-green trait in different crops (Thomas and Howarth 2000) has been widely used in breeding for heat tolerance, and as an indicator of drought tolerance (Walulu et al. 1994, Rosenow 1987).

CTD has been used as an efficient selection criterion to assess heat tolerance since one single reading integrates scores of leaves (Reynolds et al. 1994, 1998, Fischer et al. 1998, Amani et al. 1996). Though association between stay-green trait and yield has been reported, there is no study investigating association of stay-green trait with CTD in any crop. Hence, the present investigation was carried out to explore the variation for stay-green trait in wheat, and also to determine its association with CTD and yield traits, under different sowing conditions.

MATERIALS AND METHODS

Germplam Used

The 963 wheat lines included in the first experiment were previously studied (Joshi and Chand 2002, Joshi et al. 2004) with regards to spot blotch disease of wheat. Each of these lines was hand sown using a randomized complete block design with three replications in a four row plot of 3 m length with 25 cm spacing between the rows and 5 cm between seeds. The material was tested at the research station of Banaras Hindu University, Varanasi, India (North Eastern Plains Zone, 25.2 °N and 83.0 °E) for four consecutive seasons, 2001–02, 2002–03, 2003–04 and 2004–05. Recommended fertilization (120 kg N: 60 kg P_2O_5: 40 kg K_2O ha^{-1}) was followed. From the lines screened, 100 genotypes showing varying expressions of stay-green were evaluated for three years (2002–03, 2003–04 and 2004–05) under three different sowing dates.

Recording of Observation for Stay-Green

Stay-green trait was measured following two approaches viz., 0–9 scale scoring for greenness of leaves and spike at late dough stage, and leaf area under decline

(LAUD) (Joshi 2003). Based on the difference between leaf and spike scores (0-9), the genotypes were grouped as non-stay-green (0-1), moderately non-stay-green ($>1-<2$), moderately stay-green ($>2-<3$) and stay-green (>3). For estimating LAUD, greenness of flag leaf and spike was recorded using 0–9 scale at 5 days interval, starting from late milk stage (GS 77, Zadoks scale) (Zadoks et al. 1974), when both spikes and leaves were green, till physiological maturity was attained. The calculation was done using the modified formula (Joshi 2003) for Area Under Disease Progress Curve (van der Plank 1963) as given below:

$$LAUD = \sum_{i=1}^{a} [\{(\frac{Y_i + Y_{(i+1)}}{2})\} x (t_{(i+1)} - t_i)]$$

Where, Y_i = Ratio of % green area under spike and flag leaf at time t_i
$t_{(i+1)} - t_i$ = Time (days) between two readings
Y was calculated as under:
Y = % green area of leaf - % green area of spike

Based on LAUD values, the tested lines were grouped into four categories: <20 as non-stay-green, $>20-<40$ as moderately non-stay-green, $>40-<60$ as moderately stay-green and >60 as stay-green. For selected genotypes, data on CTD, LAUD, yield, biomass, test weight, and grain filling duration (GFD) were recorded.

Recording of Data for CTD and Yield Traits

Canopy temperature was recorded on per plot basis (4 row plot) using hand held Infrared Thermometer on bright sunny days at 12h, 14h, and 16h at 7 days interval, starting from anthesis till late milk stage. CTD was calculated using the formula: CTD = Air temperature – Canopy temperature. Yield and yield traits (days to anthesis, days to maturity, 1000 grain weight, biomass and grain yield) were recorded for all the genotypes, at the three sowing dates. Grain filling duration was calculated as difference between days to anthesis and days to maturity.

Data analysis: For germplasm, least significant difference (LSD) between stay-green and non-stay-green genotypes was estimated. Statistical analysis using LAUD values, CTD, yield and yield traits was done for 100 genotypes, in multiple factor split plot design, in which year was taken as main plot factor, sowing dates as sub-plot factor and genotypes as sub-sub plot factor. Simple linear correlation coefficient was calculated to determine the association among traits. T-test was done to compare difference between stay-green and non-stay-green genotypes.

RESULTS

A significant variation for stay-green trait, as measured by 0–9 scale and LAUD, was observed in the tested lines. Based on LAUD score, approximately 5.5% of the germplasm was found stay-green, 10.5% moderately stay-green, 20.4% as

Table 1. Performance of 963 wheat germplasm lines for stay-green trait tested during 2001–02 to 2004–05

Stay green class	Leaf area under decline (LAUD)			Visual score (Difference of 0–9 scores of flag leaf and spike)		
	Mean score	No. of genotypes	% of total	Mean score	No. of genotypes	% of total
Non-SG	9.46	611	63.45	0.67	619	64.28
Mod non-SG	25.88	197	20.46	2.00	194	20.15
Mod SG	49.84	102	10.59	3.00	99	10.28
SG	66.00	53	5.50	4.67	51	5.30
LSD (1%)	0.327*			0.145*		
(5%)	0.798**			0.354**		

*,** Indicates significant t values at $P < 0.05$ and $P < 0.01$ respectively.

moderately non-stay-green and 83.4% as non-stay-green (Table 1). Analysis of variance of 100 genotypes showed that there was significant variation among the genotypes, for all the traits studied (Table 2). Year x genotype interaction was non significant for CTD, LAUD and GFD but significant for other traits. Sowing date x genotype interaction was also significant for all the traits. However, genotype x year x sowing date was non significant for LAUD but not for CTD (Table 2).

Stay-green genotypes displayed higher CTD values in all the sowing dates. The correlation between LAUD and CTD was substantially high and positive ($r = +0.9$). Compared to timely sown condition, there was significant decline in yield, biomass, GFD and test weight in late and very late sown conditions, for all the genotypes but the decline was comparatively lesser for genotypes showing stay-green trait.

Table 2. Analysis of variance for CTD, LAUD and yield traits tested on 100 wheat lines under three sowing dates during three years (2002–03 to 2004–05)

Source of variation	Df	Mean sum of squares					
		CTD	LAUD	1000 gr.wt.	GFD	Biomass	Yield
Replication	2	0.050	10.34	53.763	53.076	1.196	0.076
Year (A)	2	1.918*	58.32*	133.117*	859.742*	2.762*	0.526^{ns}
Error (a)	4	0.045	0.01	2.597	25.076	0.0002	0.064
Sowing time (B)	2	1572.65**	42602.07**	48162.73**	33835.9**	95.184**	19.635**
AxB	4	8.451**	184.32**	827.867**	687.742**	0.043**	0.031^{ns}
Error (b)	12	0.038	1.53	27.305	14.076	0.0034	0.023
Genotype (C)	99	26.616**	9334.79**	42.458**	286.951**	1.431**	0.318**
AxC	198	0.002^{ns}	0.03^{ns}	1.559**	0.208^{ns}	0.0013**	0.001**
BxC	198	2.393**	74.29**	30.448**	25.732**	0.003**	0.002**
AxBxC	396	0.012**	0.04^{ns}	1.559**	0.208^{ns}	0.0001*	0.001**
Error©	1766	0.003	2.70	1.064	0.758	0.0001	0.00005
Total	2699	3.490	571.28	61.370	58.167	0.188	0.0403

Table 3. Comparison of CTD & LAUD of SG and non-SG wheat genotypes in 3 years at 3 sowing dates

Environment		CTD				LAUD			
Year	Sowing time	SG	non-SG	t-test	S.E	SG	non-SG	t-test	S.E
2003	Timely	4.24	2.67	12.22**	0.12	60.11	20.48	30.28**	1.29
	Late	5.92	4.22	18.12**	0.10	52.46	13.33	30.16**	1.27
	Very Late	7.64	5.28	19.44**	0.13	42.02	6.12	25.28**	1.36
2004	Timely	4.31	2.87	12.34**	0.12	61.14	20.78	31.30**	1.29
	Late	6.06	4.29	18.25**	0.10	52.98	13.49	31.19**	1.27
	Very Late	7.88	5.34	19.92**	0.13	42.06	6.16	26.30**	1.36
2005	Timely	4.00	2.71	11.03**	0.12	59.49	20.12	28.38**	1.39
	late	5.83	4.18	16.97**	0.10	52.48	13.12	31.85**	1.24
	Very Late	8.16	5.47	21.17**	0.13	42.38	6.44	23.78	1.51

** Indicates significant t values at $P < 0.01$ respectively.

Association between LAUD and yield traits like grain yield (+0.88), biomass (+0.84), and GFD (+0.83) was found significant and positive, however its association with test weight was not significant. CTD was also found to be positively associated with grain yield (+0.84), biomass (+0.81), GFD (+0.78) but not with test weight. T-test done to compare the performance of stay-green and non stay-green genotypes (Table 3) also displayed a significant difference for all the traits for both the years and under all three sowing dates, except for 1000 grain weight under timely sown conditions.

DISCUSSION

Stay-green is the ability of plants to remain green for longer time than non-stay-green lines, thereby contributing photosynthates for a longer time towards grain development. Stay-green trait has also been reported to confer heat tolerance (Reynolds et al. 2002). Germplasm screened in this study showed significant variation for stay-green trait. Visual rating of stay-green is quick to perform in the field on plot basis using 0-9 scale, designating it an important trait for plant breeders to screen large number of progenies (Xu et al. 2000). But a single reading tends to overestimate the result. However, LAUD appears to be more reliable as it is based on several readings integrated over time and is also easy to calculate. CTD is also very simple to record and a single reading integrates the temperature of scores of leaves.

In the studied germplasm, CTD value was high for stay-green genotypes. This could be due to transpirational cooling effect of stay-green genotypes resulting in lowering of canopy temperature with respect to air temperature. Low CTD value can also be ascribed to delayed senescence in wheat. A strong correlation between

LAUD and CTD under all sowing conditions indicated that LAUD can be used as effective screening criteria for determining tolerance to terminal heat stress in wheat.

Genotype x sowing date interaction was present and was significant for all the traits. Fischer et al. (1998) also found significant cultivar x date interaction for CTD but not for cultivar x year interaction. There was significant decline in yield, biomass, GFD and test weight due to heat stress in late and very late conditions, but the decline was less in genotypes displaying this trait. The t- test also showed significant differences (Table 3) between stay-green and non-stay-green genotypes for all the traits viz; CTD, grain yield, biomass, and GFD except for test weight, under timely sown condition. Research on other crops has also identified this relationship. Thomas and Howarth (2000) found that stay-green was associated with yield increase in sorghum. Positive correlation of stay-green trait with high grain yield has been found in sorghum (Rosenow et al. 1983; Vietor et al. 1989, Evangelista and Tangonan 1990), soybean (Phillips et al. 1984) and maize (Duvick 1984, Russel 1986, Ceppi et al. 1987). CTD also showed high genetic correlation with yield traits (biomass, grain yield and GFD), indicating that the trait is heritable and therefore amenable to early generation selection. Amani et al. (1996) and Fischer et al. (1998) have reported significant correlation of CTD with grain yield and biomass.

The results of this investigation suggest that there is significant difference between stay-green and non-stay green genotypes and, stay-green trait along with CTD, can be used as effective selection criteria for promoting tolerance to heat stress in wheat.

ACKNOWLEDGEMENTS

The financial support rendered by Council of Scientific and Industrial Research (CSIR), New Delhi, India for carrying out this research work is highly acknowledged.

REFERENCES

Amani I, Fischer RA, Reynolds MP (1996) Canopy temperature depression association with yield of irrigated spring wheat cultivars in hot climate. J Agron Crop Sci 176:119–129

Ceppi D, Sala M, Gentinetta E, Verderio A, Motto M (1987) Genotypic dependent leaf senescence in maize. Plant Physiol 85:720–725

Duvick DN (1984) Genetic contribution to yield gains of US hybrid maize 1930–1980. In: Fehr WR (ed) Genetic contributions to yield gains of five major crop plants, CSSA special publication 7. Crop Science Society of America, Madison, pp15–45

Evangelista CC, Tangonan NG (1990) Reaction of 31 non-senescent sorghum genotypes to stalk rot complex in Southern Philippines. Trop Pest Manag 36:214–215

Fischer RA, Rees D, Sayre KD, Lu ZM, Condon AG, Saavedra AL (1998) Wheat yield progress associated with higher stomatal conductance and photosynthetic rate, and cooler canopies. Crop Sci 38:1467–1475

Joshi AK (2003) Development of physiological approaches for breeding wheat varieties suited to different heat stress environment. Progress Report, NATP (ICAR) Project P-2793, BHU, Varanasi, India

Joshi AK, Chand R (2002) Variation and inheritance of leaf angle and its relationship with resistance to spot blotch in wheat (*Triticum aestivum*). Euphytica 123:221–228

Joshi AK, Chand R, Kumar S, Singh RP (2004) Leaf tip necrosis: A phenotypic marker associated with resistance to spot blotch disease in wheat. Crop Sci 44:792–796

Phillips DA, Pierce RO, Edie SA, Foster KW, Knowles PF (1984) Delayed leaf senescence in soybean. Crop Sci 24:518–522

Reynolds MP (2002) Physiological approaches to wheat breeding. In: Curtis BC, Rajaram S, Gomez Macpherson H (eds) Bread wheat: Improvement and production, Food and Agriculture Organization, Rome, Italy, pp118–140

Reynolds MP, Bolota M, Delgado MIB, Amani I, Fischer RA (1994) Physiological and morphological traits associated with spring wheat yield under hot, irrigated conditions. Aust J Plant Physiol 21: 717–730

Reynolds MP, Nagarajan S, Razzaque MA, Ageeb OAA (2001) Breeding for adaptation to environmental factors: Heat tolerance. In: Reynolds MP, Ortiz Monasterio JI, McNab A (eds) Application of physiology in wheat breeding, pp124–135

Reynolds MP, Singh RP, Ibrahim OA, Ageeb A, Quick JS (1998) Evaluating physiological traits to complement empirical selection for wheat in warm environments. Euphytica 100:84–95

Rosenow DT (1987) Breeding sorghum for drought resistance. In: Menyonga JM, Bezuneh T, Youdeowei A (eds) Proc. Int. Drought Symp. Food and grain production in semi arid Africa, Nairobi, 83–89

Rosenow DT, Quisenberry JE, Wendt CW, Clark LE (1983) Drought tolerant sorghum and cotton germplasm. Agr Water Manag 7:207–222

Russel WA. (1986) Contribution of breeding maize improvement in the United States, 1920s–1980s. Iowa State J Res 61:5–34

Thomas H, Howarth CJ (2000) Five ways to stay-green. J Exp Bot 51:329–337

van der Plank JE (1963) Plant diseases: Epidemics and control. Academic Press, New York

Vietor DM, Cralle HT, Miller FR (1989) Partitioning of ^{14}C-photosynthate and biomass in relation to senescence characteristics of sorghum. Crop Sci 29:1049–1053

Walulu RS, Rosenow DT, Wester DR, Nguyen HT (1994) Inheritance of the stay-green trait in sorghum. Crop Sci 34:970–972

Xu W, Rosenow DT, Nguyen HT (2000) Stay-green trait in grain sorghum: Relationship between visual rating and leaf chlorophyll concentration. Plant Breed 119:365–367

Zadoks JC, Chang TT, Konzak CR (1974) A decimal code for the growth stages of cereals. Weed Res 14:415–421

INFLUENCE OF HEAT STRESS ON WHEAT GRAIN CHARACTERISTICS AND PROTEIN MOLECULAR WEIGHT DISTRIBUTION

M. CASTRO[1,2,*], C.J. PETERSON[1], M. DALLA RIZZA[3], P. DÍAZ DELLAVALLE[3], D. VÁZQUEZ[2], V. IBÁÑEZ[2] AND A. ROSS[1]

[1] *Oregon State University, Dept. of Crop Science, Corvallis, OR 97331, USA*
[2] *National Agriculture Research Institute (INIA), La Estanzuela, CC 39173, Colonia, Uruguay*
[3] *National Agriculture Research Institute (INIA), Las Brujas, CC 33085, Canelones, Uruguay*
[*] *E-mail: mcastro@inia.org.uy*

Abstract: Wheat (*Triticum aestivum* L.) plants exposed to high temperatures (>35 °C) during ripening show altered agronomic and grain quality characteristics. Seasonal variation in quality creates difficulties in the marketing and processing of grain. Improving the genetic adaptation of wheat cultivars to heat stress is an important objective in breeding programs. Some genotypes have been reported to have a thermo tolerant response and could be used as genetic sources for heat tolerance. Fourteen spring wheat genotypes (Debeira, Estanzuela Cardenal, Estanzuela Pelón 90, INIA Boyero, INIA Caburé, INIA Churrinche, INIA Cóndor, INIA Mirlo, Pavón 76, Trigo 3, Ventnor, LE 2262, LE 2265, LE 2290) were evaluated in INIA La Estanzuela, Uruguay, to characterize their response to high temperatures. Variation in the duration and timing of heat stress was assessed in two greenhouse experiments. Grain protein concentration increased with heat stress, having the greatest effect when stress was imposed early in grain fill. High temperatures reduced the length of the grain fill period. A longer duration of heat stress resulted in the shortest grain fill period, but there was no difference in grain fill duration with respect to timing of the stress. Heat stress reduced thousand kernel weights without any effect attributable to the duration or timing of stress. Heat stress did not noticeably influence the protein quality of a selected subset of six wheat genotypes, measured using high-performance liquid chromatography (HPLC). However, significant genotype x treatment interaction was detected. INIA Churrinche showed a decrease in the ratio of polymeric to monomeric gluten proteins in response to heat stress. For Estanzuela Pelón 90, INIA Cóndor, and Trigo 3, the ratio was unchanged, and for Debeira and Pavón 76, the ratio increased. Cultivars with relatively stable grain characteristics and protein molecular weight distribution were identified and could be used as genetic sources for improving resistance to heat stress

Keywords: heat stress, protein molecular weight, grain characteristic

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 365–371

INTRODUCTION

High growing temperatures reduce the duration of all developmental stages in wheat (Shpiler and Blum 1986). Kernel weight also is affected by high temperatures, which further contributes to a decrease in grain yield (Wardlaw et al. 2002). Heat stress, even for a few days during grain filling, can have a major impact on protein composition. Graybosch et al. (1995) showed that protein quality, as measured by SDS sedimentation volumes and size-exclusion high-performance liquid chromatography is highly influenced by high temperatures and relative humidity during grain filling. Timing and duration of heat stress during grain filling have been shown to be important sources of variation in dough properties (Blumenthal et al. 1993, Stone and Nicolas 1995, Wardlaw et al. 2002). Heat stress during grain filling may affect the synthesis, accumulation and/or assembly of gluten proteins (Panozzo 1997). Very high temperature (e.g. short episodes of a few days at $> 35\,^{\circ}C$) produces grain with weaker-than-expected dough properties (Randall and Moss 1990, Blumenthal et al. 1993, Stone et al. 1997). This reduction in strength could be due to a lower ratio of glutenin:gliadin. According to Blumenthal et al. (1993), and Stone et al. (1997), there is an altered glutenin:gliadin ratio after heat stress, either because of a higher rate of monomeric protein synthesis, or reduction in the synthesis of glutenins. Given that seasonal variation in quality creates difficulties in the marketing and processing of grain (Peterson et al. 1992, 1998), the stability of quality is a very important goal. There are reports (Stone and Nicolas 1995) that genetic variation in response to heat stress among varieties has been observed. A few genotypes show either a small change or an increase in the glutenin:gliadin ratio. Such lines would be expected to have some consistent dough properties and may be a genetic source for further improving heat tolerance. The purpose of this research was to assess changes in grain characteristics and protein molecular weight distribution induced by timing and duration of heat stress during the grain filling period of selected wheat genotypes. The results will contribute to better understanding of the impact of heat stress on processing quality, leading to identification of heat tolerant wheat genotypes with more stable and consistent industrial quality.

MATERIALS AND METHODS

Fourteen spring wheat genotypes (Debeira, Estanzuela Cardenal, Estanzuela Pelón 90, INIA Boyero, INIA Caburé, INIA Churrinche, INIA Cóndor, INIA Mirlo, Pavón 76, Trigo 3, Ventnor, LE 2262, LE 2265, LE 2290) representing a broad range of genetic diversity in grain yield and bread-making quality were identified for this study. Plants were grown in the greenhouse, with a daytime maximum temperature of $24\,^{\circ}C$ and a nighttime minimum temperature of $16\,^{\circ}C$. Heads were tagged to record the date of anthesis. Two replicated experiments were conducted for the growth regimes described in Table 1. Experiment 1 was planted in 2001 and designed to examine impact of the duration of heat stress. Experiment 2 was planted

Table 1. Growth regimes for plants of different wheat genotypes during grain development

Experiment	Treatment	Regimen	Temperature (°C)[a]
1	Control	Moderate daytime temperature	24/16
	25 DAA (1w)	One week high daytime temperature starting 25 days after anthesis (DAA)	35/25
	25 DAA (2w)	Two weeks high daytime temperature starting 25 days after anthesis (DAA)	35/25
2	Control	Moderate daytime temperature	24/16
	15 DAA (1w)	One week high daytime temperature starting 15 days after anthesis (DAA)	35/25
	25 DAA (1w)	One week high daytime temperature starting 25 days after anthesis (DAA)	35/25

[a] Day/night maximum; 24/16 regimen maintained for 6 hr (day) and 12 hr (night), separated by intermediate 3-hr periods of gradual increase or decrease of temperature in the range of 16 to 24 °C. 35/25 regimen maintained for 5 hr (day) and 13 hr (night), separated by intermediate 3-hr periods of gradual increase or decrease of temperature (range of 25 to 35 °C) and intensity of artificial light.

in 2002 and intended to examine the influence of heat stress at different stages of kernel development. Following exposure to the high temperature regime, plants were placed again in the greenhouse along with the control plants until maturity. Date of physiological maturity was recorded when the peduncle of the spike started to change color from green to light yellow. Grain filling period (GF) was calculated as number of days between anthesis and physiological maturity. Thousand kernel weight (TKW) was reported in grams. Grain protein concentration was measured by near infrared spectrophotometer (NIRSystem 6500, Foss Inc., Silver Spring, MD, USA), and reported on a 13.5% moisture basis.

A subset of six genotypes (Debeira, Estanzuela Pelón 90, INIA Churrinche, INIA Cóndor, Pavón 76, and Trigo 3) were used to analyze ground whole wheat protein distribution. They were chosen based on previous information regarding stability of their quality attributes over different growing environments. Grain was milled into ground whole wheat using an UDY-Cyclone Sample Mill (model MS, UD Corporation, Boulder, Colorado, USA) equipped with a 1 mm opening size screen, and sieved (150 μm). Ground whole wheat protein molecular weight distribution (MWD) was measured by SE-HPLC, using an HPLC Äkta Purifier System (Amersham Pharmacia Biotech, Uppsala, Sweden), and a Superdex 200 HR 10/30 size exclusion analytical column (10 x 300 nm) (Dalla Rizza et al. 2005). Protein fractions were calculated as described by MacRitchie and Gupta (1993). The percentages of soluble polymeric protein (SPP) (mostly glutenin), monomeric protein (MP) (gliadin), and low molecular weight albumins and globulins (LMWAG) were determined from the total protein profile.

RESULTS AND DISCUSSION

Grain fill duration was reduced by an average of six days when heat stress duration was one week, and thirteen days when it lasted two weeks (Table 2). This is almost one day reduction of GF for each day of heat stress, when the stress was applied from 25 DAA.

Heat stress imposed at different stages (15 DAA or 25 DAA) had a similar effect on GF, averaging four days less than the Control (Table 3).

Heat stress caused a reduction in TKW in both experiments. Experiment 1 showed a 9.8% reduction in TKW (Table 2), and Experiment 2, a 7.3% reduction (Table 3). This decrease in GF and TKW could be associated with a shortening of the cell enlargement and dry matter accumulation phase in the grain, which occurs from 16 to 37 DAA (Wang and Gifford 1995). Heat stress increased grain protein concentration in both experiments (Tables 2 and 3). While the duration of heat stress did not show a differential increase in GP in Experiment 1, timing of

Table 2. Experiment 1 (duration of heat stress). Observed least square means for grain filling period (GF), thousand kernel weight (TKW), grain protein concentration (GP), for fourteen wheat genotypes; and soluble polymeric protein (SPP), monomeric protein (MP), low molecular weight albumins and globulins (LMWAG), and ratio SPP/MP, for a subset of six genotypes. Treatment = see Table 1

Treatment	GF (days)	TKW (g)	GP (%)	SPP (%)	MP (%)	LMWAG (%)	SPP MP^{-1}
Control	47	41	15.2	32.3	57.9	9.8	0.564
25 DAA^{++}	38**	37*	16.2*	32.4	58.2	9.5	0.563
25 DAA (1w)	41	37	16.2	32.6	58.2	9.2	0.565
25 DAA (2w)	34**	37	16.2	32.1	58.1	9.8	0.561

25 DAA^{++} = Average of heat treatments: 25 DAA (1w) and 25 DAA (2w)
Level of significance: *, $P < 0.05$; **, $P < 0.01$, for contrasts of Control vs. 25 DAA^{++}, and 25 DAA (1w) vs 25 DAA (2w).

Table 3. Experiment 2 (timing of heat stress). Observed least square means for grain filling period (GF), thousand kernel weight (TKW), grain protein concentration (GP), for fourteen wheat genotypes; and soluble polymeric protein (SPP), monomeric protein (MP), low molecular weight albumins and globulins (LMWAG), and ratio SPP MP^{-1}, for a subset of six genotypes. Treatment = see Table 1

Treatment	GF (days)	TKW (g)	GP (%)	SPP (%)	MP (%)	LMWAG (%)	SPP MP^{-1}
Control	37	41	17.7	24.7	66.6	8.7	0.377
15, 25 DAA^{++}	33**	38**	18.0	26.3	64.2	9.6	0.418
25 DAA (1w)	33	38	17.6	27.1	63.5	9.4	0.437
15 DAA (1w)	32	38	18.4*	25.5	64.8	9.7	0.398

15, 25 DAA^{++} = Average of heat treatments: 15 DAA (1w) and 25 DAA (1w)
Level of significance: *, $P < 0.05$; **, $P < 0.01$, for contrasts of Control vs. 15, 25 DAA^{++}, and 15 DAA (1w) vs 25 DAA (1w).

the stress showed a significant effect in Experiment 2. Stress during early stages of grain filling (15 DAA) resulted in the highest GP concentration (increase of 0.7%) compared with the control and the late stress treatment. Increase in grain nitrogen may reflect a reduction in grain starch content rather than a change in the quantity of nitrogen. Lower activity of the enzyme soluble starch synthase, possibly due to thermal denaturation, may be responsible for the reduced starch deposition in temperatures above 30 °C (Jenner 1994). Timing and duration of heat stress are expected to alter protein synthesis, which is evidenced by changes in the glutenin/gliadin ratio (Stone and Nicolas 1995). In this study, changes in grain

Table 4. Experiment 1. Observed least square means for soluble polymeric protein (SP) (%), monomeric protein (MP) (%), low molecular weight albumins and globulins, and SPP MP^{-1} ratio according the response to the duration of heat stress (one or two weeks) starting 25 DAA, in a subset of selected wheat genotypes

Genotypes	Treatment[A]	SPP (%)	MP (%)	LMWAG (%)	SPP MP^{-1}
Debeira	Control	37.0	52.4	10.3	0.704
	25 DAA^{++}	36.7	52.8	10.6	0.696
	25 DAA (1w)	35.3	54.7	10.0	0.645
	25 DAA (2w)	38.0	50.9†	11.1	0.747*
Estanzuela Pelón 90	Control	28.3	63.0	8.7	0.449
	25 DAA^{++}	27.7	63.2	9.1	0.439
	25 DAA (1w)	27.3	63.9	8.7	0.427
	25 DAA (2w)	28.1	62.4	9.4	0.451
INIA Churrinche	Control	36.5	55.0	8.5	0.665
	25 DAA^{++}	34.3	59.0*	6.8*	0.587†
	25 DAA (1w)	36.6	56.2	7.2	0.654
	25 DAA (2w)	31.9*	61.8**	6.3	0.519*
LE 2294	Control	30.9	58.8	10.3	0.525
	25 DAA^{++}	28.4	60.9	10.7	0.468
	25 DAA (1w)	30.0	59.6	10.4	0.504
	25 DAA (2w)	26.8	62.2	11.0	0.431
Pavón 76	Control	30.0	59.0	11.0	0.512
	25 DAA^{++}	35.1**	55.0*	10.0	0.640*
	25 DAA (1w)	34.9	55.5	9.6	0.630
	25 DAA (2w)	35.3	54.4	10.3	0.649
Trigo 3	Control	31.1	58.8	10.0	0.528
	25 DAA^{++}	31.9	58.2	10.0	0.548
	25 DAA (1w)	31.3	59.3	9.4	0.528
	25 DAA (2w)	32.4	57.1	10.5	0.568
General mean	Control	32.3	57.9	9.8	0.564
	25 DAA^{++}	32.4	58.2	9.5	0.563
	25 DAA (1w)	32.6	58.2	9.2	0.565
	25 DAA (2w)	32.1	58.1	9.8	0.561

[A] See Table 1

25 DAA^{++} = Average of heat treatments: 25 DAA (1w) and 25 DAA (2w)

Level of significance: †, $P < 0.10$; *, $P < 0.05$; **, $P < 0.01$, for contrasts of Control vs. 25 DAA^{++}, and 25 DAA (1w) vs 25 DAA (2w).

protein molecular weight distribution due to heat stress were relatively small and statistically non-significant. In Experiment 1, a significant treatment x genotype interaction was detected when duration of heat stress was assessed ($P < 0.05$). This interaction was due to changes in the direction of the response of genotypes to heat stress (Table 4).

In Experiment 2, a small but non-significant increase in soluble polymeric protein and decrease in monomeric protein was noted with heat stress (Table 3). There were no differences between stress treatments and no evidence of treatment x genotype interaction. In this experiment, GF was sixteen days shorter than Experiment 1 for the subset of six genotypes, which indicates that heat stress may have been applied too late in grain development, thereby minimizing the effect on protein synthesis. Thousand kernel weight, an important yield component variable, showed a significant treatment x genotype interaction in Experiment 2 ($P < 0.05$). In experiments where each variable had a significant treatment x genotype interaction genotypes were identified that varied in heat tolerance or sensitivity of TKW (data not shown for individual genotypes), and SPP/MP ratio (Table 4), INIA Churrinche appears to produce weaker dough than expected if subjected to heat stress during grain filling due to decreased SPP/MP ratio, even if there is no associated decrease in TKW. Estanzuela Pelón 90 and Trigo 3 may show decreased TKW when exposed to heat stress, but quality attributes, as indicated by SPP/MP ratio, seem to be more stable. Finally, Debeira, INIA Cóndor, and Pavón 76, show more stable kernel weight and stable SPP/MPP ratio. These are likely to be the best choices for production under heat stress or as promising parental lines for further genetic improvement in heat tolerance.

ACKNOWLEDGEMENTS

The project was funded by INIA (Uruguay); Technology Development Program-Dinacyt Fund, N° S/C/OP/07/24, Ministry of Culture and Education, Uruguay; and Oregon State University (USA). Genotypes were provided by Ing. Rubén Verges at INIA, Uruguay; Dr. Allan Fritz at KSU, USA; and Dr. M. Mohan Kohli, at CIMMYT. The authors are thankful to Drs. Hamid Naeem, Finlay MacRitchie, and Susan Altenbach for sharing their knowledge in conducting similar experiments. Contribution to 7th IWC was possible thanks to funds provided by CIMMYT, ICARDA, and FONTAGRO (IICA-BID-FTG/01-04-RG agreement).

REFERENCES

Blumenthal CS, Barlow EWR, Wrigley CW (1993) Growth environment and wheat quality: The effect of heat stress on dough properties and gluten proteins. J Cereal Sci 18:3–21

Dalla Rizza M, Díaz Dellavalle P, Vázquez D, Castro M (2005) Improved resolution of non silica-based size-exclusion HPLC column for wheat flour protein analyses. Cereal Chem 82:287–289

Graybosch RA, Peterson CJ, Baenziger PS, Shelton DR (1995) Environmental modification of hard red winter wheat flour protein composition. J Cereal Sci 22:45–51

Jenner CF (1994) Starch synthesis in the kernel of wheat under high temperature conditions. Aust J Plant Physiol 21:791–806

MacRitchie F, Gupta RB (1993) Functionality-composition relationships of flours as a result of variation in sulphur availability. Aust J Agr Res 44:1767–1774

Panozzo JF (1997) Grain filling and protein synthesis in wheat grown in environments with different temperature and soil conditions. PhD Thesis, University of New South Wales

Peterson CJ, Graybosch RA, Baenziger PS, Grombacher AW (1992) Genotype and environment effects on quality characteristics of hard red winter wheat. Crop Sci 32:98–103

Peterson CJ, Graybosch RA, Shelton DR, Baenziger PS (1998) Baking quality of hard winter wheat: Response of cultivars to environment in the Great Plains. Euphytica 100:157–162

Randall PJ, Moss HJ (1990) Some effects of temperature regime during grain filling on wheat quality. Aust J Agr Res 41:603–617

Shpiler L, Blum A (1986) Differential reaction of wheat cultivars to hot environments. Euphytica 35:483–492

Stone PJ, Nicolas ME (1995) A survey of the effects of high temperature during grain filling on yield and quality of 75 wheat cultivars. Aust J Agr Res 46:475–492

Stone PJ, Gras PW, Nicolas ME (1997) The influence of recovery temperature on the effects of a brief heat shock on wheat. III. Grain protein composition and dough properties. J Cereal Sci 25:129–141

Wang YP, Gifford RM (1995) A model of wheat grain growth and its applications to different temperatures and carbon dioxide levels. Aust J Plant Physiol 22:843–855

Wardlaw IF, Blumenthal C, Larroque O, Wrigley CW (2002). Contrasting effects of chronic heat stress and heat shock on kernel weight and flour quality in wheat. Funct Plant Biol 29:25–34

EXPRESSION QUANTITATIVE TRAIT LOCI MAPPING HEAT TOLERANCE DURING REPRODUCTIVE DEVELOPMENT IN WHEAT (*TRITICUM AESTIVUM*)

D. HAYS[1,*], E. MASON[1], J. HWA DO[1], M. MENZ[1] AND M. REYNOLDS[2]

[1] *Soil & Crop Sciences, Texas A&M University, College Station, Texas*

[2] *CIMMYT, Apdo. Postal 6-64, 06600 Mexico, D.F., Mexico*

[*] *E-mail: dbhays@neo.tamu.edu, m.reynolds@cgiar.org*

Abstract: High temperature during reproductive development is a major limitation to wheat production and end-use quality in the Southern Great Plains (USA) and to wheat production in many environments worldwide. We have initiated a project to integrate genotypic (QTL), phenotypic and transcript level data to identify genes controlling reproductive stage heat tolerance in heat tolerant genotypes of wheat as it relates to yield and end-use quality maintenance. Efforts have initially focused on building recombinant inbred lines (RILs) and cDNA libraries enriched, through suppressive subtractive hybridization, for genes induced by heat stress. The selected tissues for library construction included wheat heads and flag leaves isolated from plants subjected to heat stress 10 days after pollination. A heat tolerant spring wheat cultivar 'Halberd', and a susceptible winter wheat cultivar Cutter were used as models to define the two adaptive responses to heat stress (heat avoidance (susceptible) and heat tolerance). Over 1,920 unique ESTs have been sequenced. These genes include some potential regulatory proteins, heat shock proteins and lipid-transfer proteins, as well as many novel genes that may belong to uncharacterized pathways involved in response to heat stress. For example, a lipid transfer protein and an alpha amylase inhibitor remained stable during heat shock in the heat-tolerant cultivar Halberd. These genes were also highly expressed in the most heat tolerant RILs but not in the most susceptible RILs. Expression-QTL mapping results will be presented which link QTLs controlling heat tolerance to their regulation of discrete sets of the plant transcriptome

Keywords: heat tolerance, QTL, loci mapping

INTRODUCTION

Heat stress during reproductive development is a primary constraint to wheat production and profitability in many wheat-growing regions of the world. Additional yield reductions from the predicted rise in global temperatures and rising farming

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 373–382

input costs will add further strain to food supplies, the economic viability of farming and related farm-based businesses. In a recent international consultancy, leaders of national wheat programs identified heat tolerance as one of their major priorities (CIMMYT 1995). In heat-susceptible wheat genotypes, heat stress during reproductive development reduces photosynthesis and promotes premature senescence. As a consequence induced pollen sterility reduces yield, increases seed abortion and reduces test weight, flour yield, and dough quality. Susceptible wheat genotypes exhibit a 3% reduction in yield for every temperature degree rise above 15 °C (Wardlaw et al. 1989). Using this factor (3% yield loss/every 1 °C above 15 °C) we can calculate that most commercially-sown wheat cultivars in Texas would lose approximately 50% of their yield potential from exposure to 32 °C to 35 °C for one to two days during reproductive development. These temperatures are common both in the Southern Great Plains where roughly 30–40% of the USA's wheat crop is grown, and in the northern Negev region where more than 70% of Israel's wheat production occurs. Wheat genotypes with high levels of tolerance to high temperatures have been identified and are defined by maintenance of photosynthesis, chlorophyll content, and stomatal conductance (Yang et al. 2002). Yield of these genotypes is maintained through higher seed set, grain weight, and extended grain filling duration even at elevated temperatures (Yang et al. 2002). While these phenotypic traits have been associated with the quantitative inheritance of heat tolerance, identification of the key loci and understanding of the molecular and physiological basis of improved adaptation to high temperatures are lacking.

The primary goal of this project was to identify sources of reproductive stage heat tolerance in wheat, define the molecular basis of the heat tolerance, and introgress heat tolerance into new cultivars for Texas. Present efforts are focused on introgressing the heat tolerance conferred by 24 cultivars and germplasm lines from Australia, Israel, and CIMMYT (Mexico). This effort now represents 4000 F3-F7 recombinant inbred lines at various stages of screening. Heat tolerance screening has been conducted during pollination and early grain filling to identify sources of heat tolerance that do not exhibit sterility, and associated reduction in grain number per spike, grain weight, and end-use quality. Screening of the progeny derived from crosses between the heat tolerant parents and elite disease and insect resistant cultivars and germplasm has also been conducted. Heat tolerant progeny were identified by subjecting plants to heat stress during pollination and early grain development. Lines not exhibiting pollen sterility, seed abortion or shriveled grain due to heat stress are selected in the early generations; those lines able to maintain end-use quality in response to heat stress are selected in later generations. While these tests are the most critical they are inefficient and time consuming to apply on large numbers of recombinant inbred lines (RILs). The development of reliable molecular markers closely linked to the inheritance of heat tolerance would be an enormous benefit. The immediate goal is to identify molecular markers linked to loci conferring heat tolerance during pollination, early grain development and grain filling as it relates to end-use quality. The long-range goal is to identify

and understand the molecular and genetic events in wheat that controle the heat tolerant phenotype. The objectives of this study are to identify quantitative trait loci (QTLs) that control heat tolerance in recombinant inbred lines (RILs) and to determine the mechanism by which these loci confer improved adaptation to elevated temperatures. The central hypothesis of this application is that multiple genes mapping to distinct loci in the wheat genome control the stress response to heat in part through differential activation and expression of selected transcription factors and downstream networks. Determining if heat tolerance during pollination and at different stages of grain development is under separate genetic control is also of critical importance. Our preliminary evidence suggests that heat tolerance during these different stages is under separate genetic control. As part of this presentation, preliminary data will be presented aimed at mapping and defining the molecular basis of heat tolerance from the cultivars '7Cerros', 'SeriM82' (CIMMYT), and 'Halberd' (Australia). Two sets of recombinant inbred lines (RILs) from a cross between two well characterized heat tolerant and heat susceptible lines are being used to pursue the following objectives: 1. Identify molecular markers linked to the inheritance of QTLs regulating the development of the heat tolerant phenotype in wheat; 2. Link QTLs to the regulation of changes in gene expression (eQTL mapping) that contribute to improved plant performance during heat stress.

MATERIALS AND METHODS

Plant Material

The recombinant inbred population discussed in this report consisted of 62 recombinant inbred lines (RILs) derived from a cross between 'SeriM82' and '7Cerros' (obtained from Dr. Mathew Reynolds, CIMMYT, Mexico) and 65 RILs derived from a cross between heat tolerant 'Halberd' and heat susceptible 'Cutter'. Other larger RIL populations developed from the same heat tolerant sources crossed to multiple heat susceptible sources ('Karl 92', 'Len' and others) are also being used. Ten 36 cm pots for each RIL and both parents were seeded with two plants per pot. Pots consisted of 3/4 Lufkin sand-soil and 1/4 Metro Mix potting soil to maintain soil moisture. Each pot was supplied with 5 g OsmocoteTM and supplemented with Peters 20:20:20 (100mg each N, P, K supplied) every two weeks.

Heat Stress Treatment and Phenotyping Heat Tolerance

Plants were grown in growth rooms with 14 h day length with a 20 °C/16 °C day/night temperature cycle. The 1st, 2nd, and 3rd spikes on each plant were tagged for day of pollination (anther dehiscence). Ten days after pollination (for the main 1st spike), 5 pots (10 plants) of each RIL and the two parents were transferred to a heat treatment plant growth chambers with 14 h day length and a 38 °C/25 °C day/night heat stress for two days. At the same time, 5 pots (10 plants) of each

RIL and the two parents were transferred to a control treatment growth chambers set at 14 h day length with a 20 °C/16 °C day night cycle for two days. Following the two-day temperature treatment, plants were transferred back to growth rooms set at the original temperature and day length discussed above. Plants were watered on a regular cycle until all spikes reached maturity. The grain filling duration was recorded for each tagged spike. All tagged spikes for each plant were harvested individually; untagged spikes were bulked. Spikes were mechanically threshed and yield components were recorded. These included total grain weight and number per plant, single kernel weight per main spike, kernel number per main spike, total grain weight per main spike, and total grain number per spike.

Mapping

Molecular markers linked to the inheritance of QTLs heat tolerance in wheat are being identified. The independent contribution of each region, their gene action (additive/dominance) and interaction among genomic regions is being determined. A large set of well-defined simple sequence repeats (SSRs) are being analyzed in parents and RILs. Mapmaker v3 is used to construct the corresponding genetic maps. Phenotypic data for each RIL averaged across replications was used for the QTL and single-marker analysis, interval mapping and composite interval mapping using the software QTL Cartographer v2.0 (Basten et al. 2000). Epistatic interactions among QTLs were evaluated using Epistacy in SAS (Holland 1998).

Isolation of Unique Genes with Important Adaptive Functions During Heat Stress Using cDNA Subtraction

Genes induced in response to heat stress in flag leaves and seeds have been isolated and sequenced from each parent using cDNA Subtraction (PCR-Select cDNA Subtraction, Clontech, CA) (or suppression PCR). Two identical sets of 50 'Halberd', '7Cerros' and 'SeriM82' plants were grown separately in two identical $4' \times 8'$ environmental growth chambers (14h photoperiod set at 20 °C day/15 °C night). The primary spike for each plant was tagged for day of pollination (scored by appearance of anthers). At 10 DAP, plants in one growth chamber were heat stressed using a one day 38 °C day/25 °C night temperature regime. Plants in the control chamber were maintained at 20 °C day/16 °C night. The photoperiod was not changed. Seed and flag leaf tissue were collected from heat-treated and control plants at identical time intervals following the initiation of the heat stress. Total RNA used in the cDNA subtraction scheme was determined using the PCR-SelectTM cDNA Subtraction Kit (Clontech, Palo Alto, CA) as described in Hays et al. (2001). cDNA derived from heat stressed '7Cerros', 'SeriM82' and 'Halberd' seed and flag leaf tissue were used as the tester, and cDNA derived from control-treated seed and flag leaf tissue of the same cultivars, collected at identical time intervals, were used to eliminate constitutive house keeping genes or genes unaffected by heat stress. Over 2000 ESTs have been sequenced from this effort.

Plant Growth Conditions for RILs Used to Link QTLs to Regulation of Discrete Sets of the Plants Transcriptome

Five individual plants of each F8-F7 RIL (generated from crosses between 'Seri-M82' and '7Cerros' and 'Halberd' and 'Karl92'), each with discrete sets of QTLs controlling heat tolerance, were grown in large controlled environment growth rooms (14h photoperiod set at 20 °C day/15 °C night). The primary spike of each plant was tagged on the day of pollination (scored by appearance of anthers). At 10 DAP, multiple plants for each RIL were transferred to $4' \times 8'$ growth chambers set at the same photoperiod as the growth room from which they were moved. Heat stress was applied to plants as a three-day 38 °C day/25 °C night temperature regime. Seed/spike and flag leaf tissue were collected at limited time intervals following heat stress similar to the time course used for the PCR-SelectTM cDNA Subtraction experiment described above. Total RNA was extracted from a pool of tissue in the limited time course for each RIL. Extracted total RNA was used as a template for reverse Northerns and microarray/macroarray studies to link discrete QTLs with the expression of discrete sets of plant transcriptome.

Statistical Analysis Linking Changes in Gene Expression to QTLs Regulating Improved Adaptation to Heat Stress

All RILs used in this study will be included in the QTL analysis described previously. Using single-marker analysis in SAS or in QTL Cartographer and means of transcript levels, it is possible to identify associations between markers linked to QTLs and changes in gene expression of certain genes. This association can be indicative of the genes that underlie the identified QTLs. Depending on the number of QTLs controlling heat tolerance in '7Cerros', 'Seri-M82' and 'Halberd', it may not be possible to identify RILs with each QTL in isolation due to the small size of the RIL population. However, some will be identified and association between major QTLs and regulation of discrete sets of genes can be made.

RESULTS AND DISCUSSION

Characterization of heat tolerance sources. Using plant growth chambers, heat tolerance of several popular hard winter wheat varieties grown in the Southern Great Plains has been measured. When exposed to a one-day heat treatment of 40 °C 18 °C^{-1} (day/night), most varieties exhibited a 10 to 30% reduction in kernel number per spike and kernel weight, largely the result of increased seed abortion and an early transition to the dry seed stage, respectively (Fig. 1). Two hard wheat varieties, 'Halberd' (Australia-spring habit) and '7Cerros' (CIMMYT-spring habit), did not show these reductions. When temperatures rise above 30 °C, there is a progressive decrease in grain size. Smaller grain size results in a decrease in milling quality due to a reduction in the proportion of endosperm that can be extracted as flour. If the decrease in grain weight is severe, test weight will be reduced and

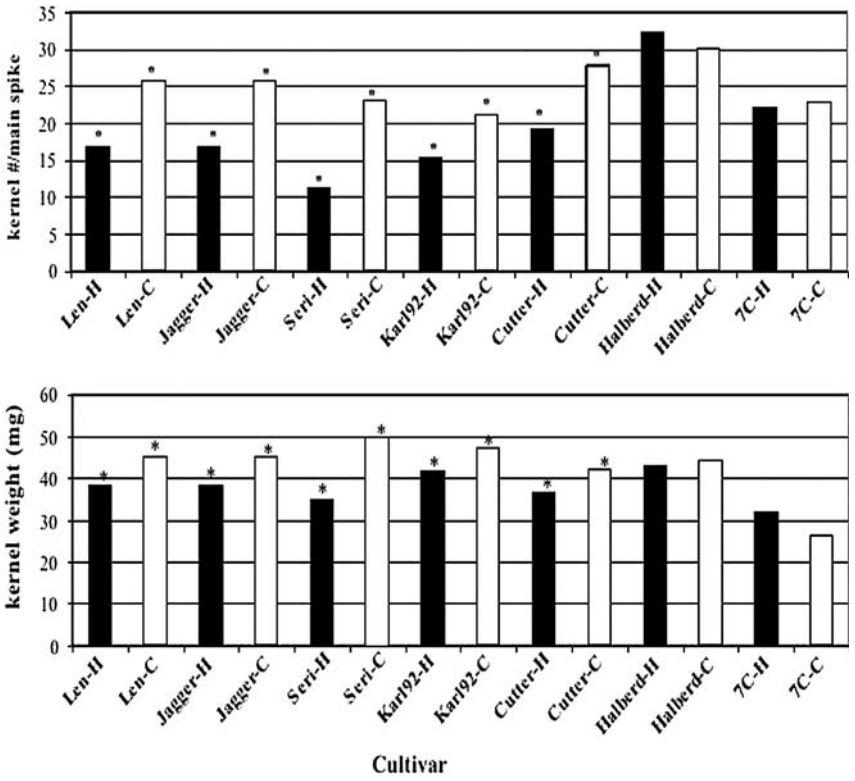


Figure 1. Grains per spike and 1000 kernel weights (g) of control and heat stressed wheat varieties popular in South Texas. Plants were treated when tagged seeds were 10 DAP with a one day 38 °C/25 °C day/night regime (dark bars). Controls were grown at 20 °C/15 °C day/night regime (white bars). Symbols above bars indicate a significant difference between the mean of the control versus heat treated plants for that variety

grain will fail to qualify for milling grades, thus reducing the farmers' economic return. 'Halberd' was previously identified as having a high degree of stability in end-use quality in response to heat stress (Wrigley et al. 1994). Temperatures of 30 to 40 °C are common in the Southern U.S. Great Plains during the wheat seed development period. Both the Texas PIs and CIMMYT have developed RILs from crosses between heat tolerant 'Halberd', and '7Cerros', which have previously been identified as heat tolerant (Reynolds et al. 2000) and the heat-susceptible varieties 'SeriM82' (susceptible under heat shock), 'Len', 'Cutter', and 'Karl 92', identified from our own studies and others (Yang et al. 2002).

Phenotypic Analysis of Heat Tolerance in Rils

Yield components were determined and correlated with total yield and total yield reduction percentage. Of the two parents, '7Cerros' was more resistant to heat stress when exposed to high temperatures. Of the 62 RILs, one in four lines exhibited

either no loss in kernel number or an increase in kernel number per main spike in response to heat stress during early kernel development (Fig. 2). While one in five RILs exhibited either no loss or an increase in the total number of kernels per plant (Fig. 3), one in 13 no loss in kernel weight of the main spike (Fig. 4), and one in 11 no change in total grain weight per plant (not shown).

These results indicate that inhibition of heat stress induced seed abortion in '7Cerros' is more simply inherited than other yield components. The 1:4 and 1:3 ratios indicate that heat stress induced seed abortion is regulated by a single dominant gene. On the other hand, the maintenance of kernel weight per main spike and kernel weight per plant (yield) in response to heat stress is controlled by multiple quantitative trait loci (QTLs). Similar results have been obtained for the heat tolerant line 'Halberd'.

A distinct set of RILs exhibited better resistance to heat stress induced seed abortion, while the other RILs are more resistant to heat stress induced reduction in kernel weight (not shown). There was little overlap between the two RIL sets in response to heat stress when the 1st spike was analyzed. However, the resistant RILs for these two heat stress responses during reproductive development (i.e. seed abortion, reduction in kernel weight) do overlap as they are both resistant to reduction in total yield or kernel weight per plant (not shown). Collectively, these

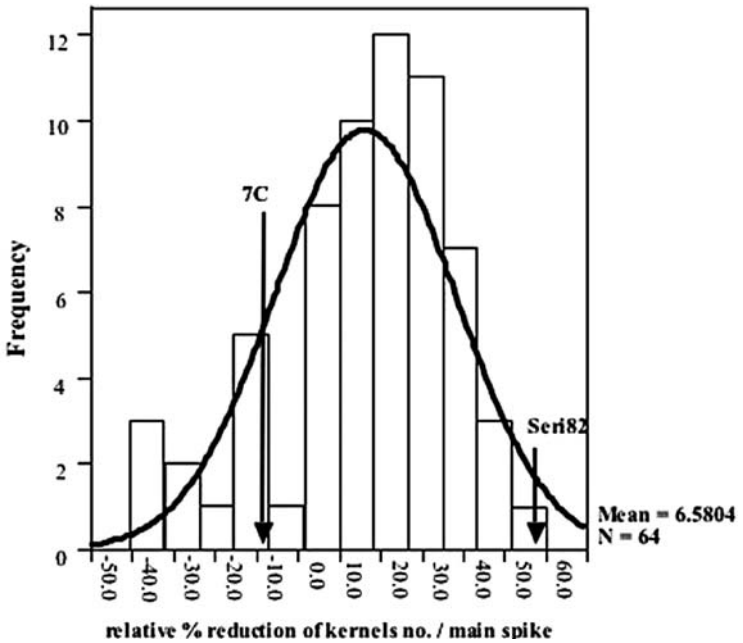


Figure 2. Frequency of RILs with various levels of seed abortion per 1st or main spike in response to heat stress 10 days after anthesis, expressed as percent reduction versus control plants. A negative number on the x-axis indicates no seed abortion, conversely a positive number indicates various levels of seed abortion relative to control treated RILs

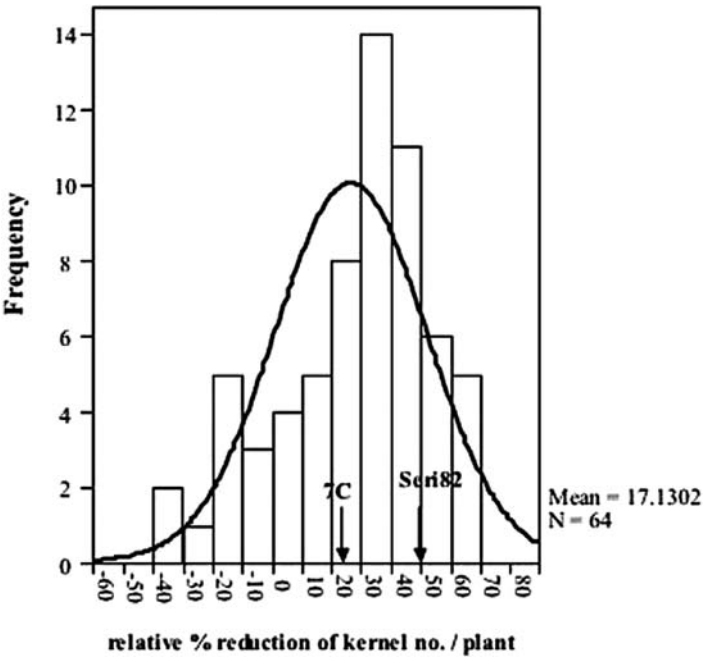


Figure 3. Frequency of RILs and percent reduction in average single kernel weight of the 1st or main spike in response to heat stress compared to the average single kernel weight from the control treated main spike. A negative number on the x-axis indicates no loss in grain weight, conversely a positive number indicates various levels of reduction in grain weight relative to control treated RILs

data indicate that both mechanisms contribute to yield, yet are possibly under separate genetic control. Table 1 illustrates the correlations between the various main spike yield components and total kernel weight per plant. Both kernel number per main spike and average kernel weight during heat stress were significantly correlated with heat tolerance as it relates to total plant yield under heat stress (kernel weight (wt)/plant). However the total kernel weight of the main spike was more strongly correlated with total yield per plant during heat stress (Table 1). Since both kernel number and kernel weight per main spike are components of total kernel weight per plant, the results indicate these traits may be under separate genetic control; therefore it is important to develop molecular markers for both components for use in marker-assisted breeding.

Gene Expression in Response to Heat Stress

So far over 1,920 unique genes that are induced in response to heat stress in the developing kernels of the heat tolerant parent 'Halberd' have been isolated and sequenced. While the largest portion of these genes are homologous to endosperm specific storage proteins, large numbers of the isolated genes show homology to

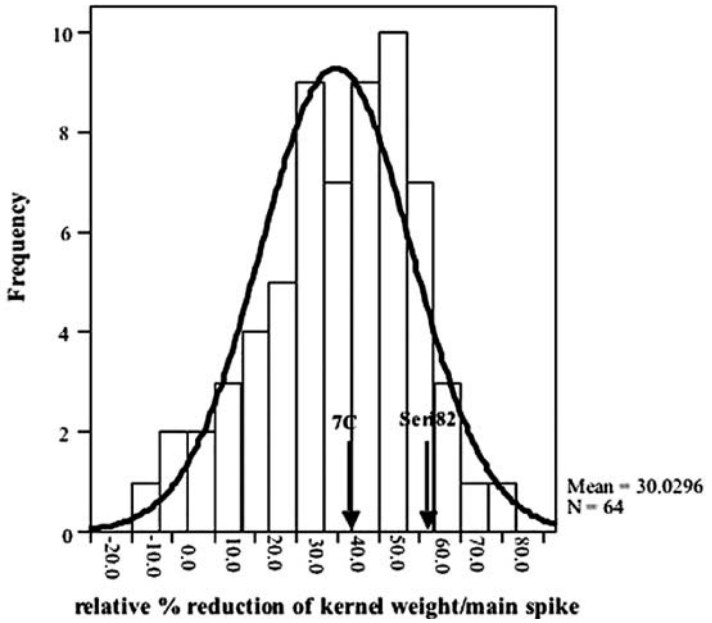


Figure 4. Frequency of RILs and percent reduction in kernel weight per plant (yield) in response to heat stress; values expressed as percent reduction versus control treated plants. A negative number on the x-axis indicates no seed abortion, conversely a positive number indicates various levels of seed abortion relative to control treated RILs

genes involved in inhibition of starch metabolism and membrane trafficking proteins involved in cuticular wax production. Expression studies indicate these genes are expressed at very high levels in response to heat stress. Genes such as ABC-type Zn2+ transport, lipid transfer proteins, nitrilase-associated proteins, HSP70 and others were induced from 400 to 1600 percent above control expression levels (Fig. 5). Linking the expression of these genes to specific heat tolerance QTLs

Table 1. Pearson Correlation Coefficients, N = 228 in 62 RILs from heat treatment. Highlighted numbers indicate yield components that have good correlations with total yield

	kernel # main spike^{-1}	kernel wt main spike^{-1}	kernel wt
kernel wt plant^{-1}	**0.5481**	**0.9069**	**0.7020**
	<0.0001	<0.0001	<0.0001
kernel # plant^{-1}	**0.7253**	0.6689	0.3308
	<0.0001	<0.0001	<0.0001
grain fill duration	0.0820	0.25246	0.26312
	<0.2174	<0.0001	<0.0001

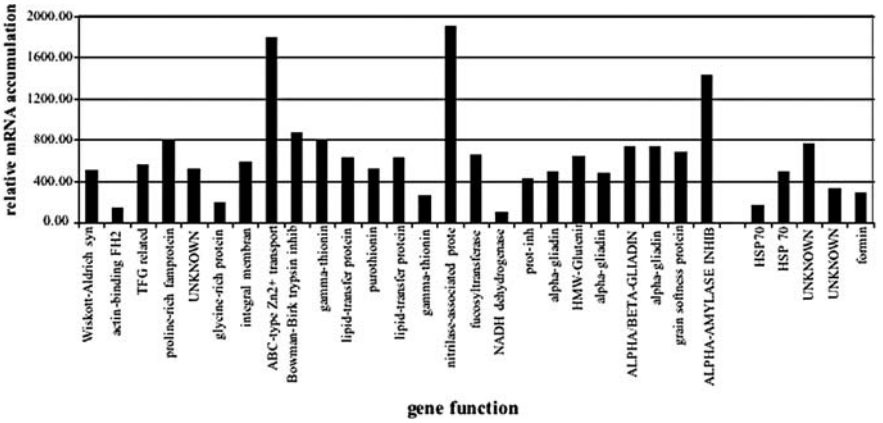


Figure 5. Relative mRNA accumulation of ESTs from 'Halberd' versus expression in control treated plants. Expression was normalized according to the expression of a constitutively expressed tubulin gene

found in discrete sets of RILs, as discussed earlier, will facilitate the separation of discrete sets of genes correlated with heat tolerance and reproductive stage yield stability from general response genes. This information will, in turn, be used to direct future functional genomic studies.

This research represents a small section of the total research effort being conducted and aimed at improving heat tolerance in wheat as it relates to improved yield and end-use quality. Similar studies on other sources of heat tolerance from Australia, CIMMYT and the Middle East are underway. While much of this effort has centered on transferring these sources of heat tolerance into cultivars adapted to Texas, a larger effort is also focused on developing tools to improve the efficiency of this breeding effort and understanding the molecular basis of heat tolerance.

REFERENCES

Hays DB, Skinner DZ (2001) Development of an expressed sequence tag (EST) library for Medicago sativa. Plant Sci 161:517–526

Basten CJ, Weir BS, Zeng ZB (2000) QTL Cartographer, version 1.14. Department of Statistics, NCSU, Raleigh, N. C.

Holland JB (1998) EPISTACY: A SAS program for detecting two-locus epistatic interactions using genetic marker. J Hered 89:374–375

Wardlaw IF, Dawson IA, Manibi P, Fewster R (1989) The tolerance of wheat to high temperature during reproductive growth. I. Survey procedures and general response patterns. Aust J Agr Res 40:1–13

Wrigley CW, Blumenthal C, Gras PW, Barlow EWR (1994) Temperature variation during grain filling and changes in wheat-grain quality. Aust J Plant Physiol 21:875–885

Yang J, Sears RG, Gill BS, Paulsen GM (2002) Genotypic differences in utilization of assimilate sources during maturation of wheat under chronic heat and heat shock. Euphytica 125:179–188

MOLECULAR BREEDING FOR SALT TOLERANCE, PRE-HARVEST SPROUTING RESISTANCE AND DISEASE RESISTANCE USING SYNTHETIC HEXAPLOID WHEATS, GENETIC TRANSFORMATION, AND ASSOCIATED MOLECULAR MARKERS

M. VAN GINKEL[1,2,*], F. OGBONNAYA[1,2], M. IMTIAZ[1,2], C. RAMAGE[1,2], M.G. BORGOGNONE[1], F. DRECCER[1,4], J. EDER[1], M. EMMERLING[1,2], P. HEARNDEN[2,3], E. LAGUDAH[6], A. PELLEGRINESCHI[2,5], R. TRETHOWAN[5], J. WILSON[1,2] AND G. SPANGENBERG[1,2]

[1] *Primary Industries Research Victoria, Department of Primary Industries, Victoria, Australia*
[2] *Molecular Plant Breeding Cooperative Research Centre, Victoria, Australia*
[3] *School of Agriculture and Wine, South Australia, Australia*
[4] *Present address: CSIRO Plant Industry, Queensland, Australia*
[5] *International Maize and Wheat Improvement Centre (CIMMYT), Mexico DF, Mexico*
[6] *CSIRO Plant Industry, Australian Capital Territory, Australia*
[*] *E-mail: maarten.vanginkel@dpi.vic.gov.au*

PRE-HARVEST SPROUTING RESISTANCE AND SALT TOLERANCE

INTRODUCTION

Pre-harvest sprouting (PHS) is a serious problem in major wheat producing areas in Australia. Reported average losses across the wheat crop are about AU$30–40 million annually. Less than 2% of current Australian wheat cultivars possess resistance. We focus on the potential of *Ae. tauschii*.

In Australia the potential area for dryland salinity" is estimated at 5.7 million hectares, and forecast to reach 17 million ha by 2050. At present, efforts are underway to transfer salinity tolerance from synthetic hexaploid (SH) to develop superior salt tolerant wheat germplasm.

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 383–385.

MATERIALS AND METHODS

For the PHS studies two accessions of *Ae. tauschii* were chosen, based on their levels of dormancy, and crossed with durum wheat to produce synthetic hexaploids Syn36 and Syn37 following colchicine treatment. Elite, Australian PHS susceptible bread wheat cultivars, Janz, Yitpi, Mitre, Lang were crossed with the resistant Syn 36 and moderately resistant Syn37. The RIL populations were grown under field conditions in Victoria, Australia and tested for sprouting by exposure to artificial rain in a rain-chamber, in which sprinklers provided 20 minutes of artificial precipitation every two hours for 20 minutes at a time. We measured three indices: germination index (GI) at days 2, 7, 14 and 21; whole head assay (sprouting index; SI) and visually sprouted seeds (VI). The two BC_1F_5RIL populations were also genotyped with SSR markers to identify genomic regions associated with these indices of resistance to PHS.

The salt tolerance studies were conducted using 54 primary synthetics, the wheat varieties Janz, Kharchia, and Westonia, two durum cultivars – Langdon and Wollaroi and the Langdon4D-4B substitution line. Materials were grown in a greenhouse flood and drain system and treated with 100 mM NaCl to give a final Na^+: Ca^{2+} of 15:1. Na^+ and K^+ concentrations of leaves were determined using a flame photometer on a fresh and dry weight basis.

RESULTS AND DISCUSSION

The PHS experiments identified RILs with phenotypic values outside the range of the parental genotypes, which can be differentiated statistically and repeatedly when exposed to more than seven days of high humidity and rain in the artificial rain-chamber. In the Syn36 x Janz BC_1F_5RIL an association between SI and VI of $r = 0.51$ was observed. The association between GI14 and VI was stronger ($r = 0.52$) than between GI14 and SI ($r = 0.35$) indicating that different mechanisms may contribute to PHS resistance. These mechanisms may need to be pyramided to produce germplasm with high levels of resistance to PHS. Our molecular characterization indicated that genomic regions on chromosomes 1D, 3D, 5D and 6D, mostly derived from *Ae. tauschii*, influenced PHS. The source of resistance on chromosome 6D was the susceptible cultivar Janz, which helps explain the presence of transgressive segregation in these populations.

The salt tolerance studies indentfied seven SH's that expressed significantly lower Na^+ values in the third leaf compared to Kharchia and Westonia. One SH expressed 57% less Na^+ than the salt tolerant cultivar Kharchia. The salt-sensitive *T. turgidum* cvs. Wollaroi and Langdon accumulated 1153% and 878% more sodium respectively, than Kharchia. Similar ranking was observed for the K^+/Na^+ ratio; the SHs with the highest sodium exclusion, also ranked lowest in for K^+/Na^+ ratio, but were amongst the highest K^+ accumulators. This shows that salt tolerance in Triticeae is associated with enhanced ability to discriminate between Na^+ and K^+ in soil solution, and to preferentially accumulate K^+ and exclude Na^+. This suite

of SH lines with elevated levels of Na^+ exclusion have been crossed with locally adapted Australian bread wheat cultivars, and linked molecular markers are being identified.

Transgenesis

Gene technology offers an attractive adjunct to the conventional wheat breeding toolbox. We have in place a routine wheat transgenesis pipeline, and novel genes and gene systems coding for enhanced performance under drought and disease resistance of an expected long-lasting nature are being transformed and tested.

Wheat transformation and regeneration take place using particle bombardment of zygotic wheat embryos (Bobwhite 26). The biolistic-mediated transformation process takes approximately 4–5 months to complete and consists of the following steps: 1) Year round wheat plant production: 12–16 weeks/cycle; 2) Zygotic embryo isolation/bombardment: 24 hours; 3) Regeneration and selection, approximately 8–10 weeks; 4) Transfer to greenhouse and molecular analysis, approximately 9–10 weeks. Target genes were identified based on performance in moisture stressed or disease challenged *in-planta* systems. Constitutive (*act1D*), tissue specific (*rbcs*) and abiotic stress inducible promoters (*rd29a*) were used in the development of Gateway™ enabled transformation vectors. High-throughput QPCR assays were developed for each candidate gene and used for molecular analysis of T_0 transgenic wheat plants. Transgenic wheat plants are categorized into high, medium and low expression and gene copy number classes for phenotypic analysis.

A total of 26 transformation vectors for expression of candidate genes targeted to extended tolerance for drought were created and 529 independent transgenic wheat plants generated. The first drought screens of T_2 wheat plants are scheduled for early 2006. They consist of a high-throughput screen to identify aberrant plant phenotypes, a greenhouse screen where physiological traits are determined, and field trials under drought.

In an analogous approach transformation vectors for expression of 11 candidate genes for elevated disease resistance have been created, and 538 independent transgenic wheat plants generated so far. The first fungal screens of T_2 wheat plants are scheduled for mid 2006.

ACKNOWLEDGEMENTS

This work was supported by the Victorian Department of Primary Industries, the Australian Molecular Plant Breeding Cooperative Research Centre, and the Grains Research and Development Corporation.

WHEAT BREEDING FOR SOIL ACIDITY AND ALUMINUM TOXICITY

E. VON BAER

Campex – Semillas Baer and AVELUP, Fundo El Hualle s/n, Cajón-Temuco, Chile
E-mail: infoseedsbaer@semillasbaer.cl

Abstract: The soils of southern and central-southern Chile where the national wheat production is concentrated are of volcanic origin, with low pH and with high exchangeable aluminum. Soils contain large amounts of phosphorous in organic form, however available phosphorous is low. The high content of free Al negatively affects the root growth of wheat varieties depending on their Al-sensitivity or Al-tolerance. The mechanism of tolerance is exclusion of toxic Al as a consequence of root excretion of queelant organic acids; roots of tolerant varieties are also protected by higher root mycorrhizal colonization. Crosses between sensitive and tolerant material and subsequent selection under low pH and high Al have produced high yielding tolerant cultivars. This paper outlines the development of tolerant wheat varieties, describes the germplasm used and discusses the selection process including including the use of growth chambers, greenhouse assays and field experiments

Keywords: Al-tolerant varieties, citric acid, rhizosphere, mycorrhizal colonization, segregant material

INTRODUCTION

The soils of southern and central-southern Chile (35 °S to 42 °S), are of volcanic origin and characterized by high acidity (pH range from 4.5 to 6) and high aluminum saturation approaching 30% without lime and 0–18% with applied lime. Nutrient availability including P is low. These constrictive characteristics are known as the soil acidity complex which often creates a chemical barrier reducing root growth and development; these roots become inefficient in absorbing nutrients and water (Reynolds et al. 2001).

Despite this, volcanic soils contain high levels of total P (2,000 to 4,000 mg kg^{-1}) but the availability of this nutrient is low (5–20 ppm) The unavailability of P to

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 387–393.

crop plants reduces wheat productivity in these areas. Low P availability and high exchangeable Al inhibits root growth with concomitant losses on grain yield and quality. This problem has been exacerbated by the massive use of urea as a source of cheap nitrogen; urea increases soil acidification. To overcome these limitations farmers need to apply large amounts of P fertilizers, apply lime and/or use more tolerant crop species. According to some authors and cited by Reynolds et al. (2001), the effect of the applications the lime is superficial.

Selection and development of genotypes with enhanced tolerance to acid soils and toxic levels of Al is the only reasonable solution to this problem (Reynolds et al. 2001)

The main mechanism of AL- tolerance is the excretion of queelant compounds in the rhizosphere which reduces the absorption phytotoxic Aluminum by roots. Among these compounds, citric and malic acids have been reported as the major short chain organic acids responsible of sequestering Al from the rhizospheric soil (Kochian et al. 2004 submitted). Another mechanism which has been recently suggested, is mycorrhizal protection against Al phytotoxicity (Kowlowsky and Berner 1989, Medeiros et al. 1994, Cumming and Ning 2003). Higher root mycorrhizal colonization was observed in Al-tolerant plants in comparison with sensitive ones (Borie and Rubio 1999). The mycorrhizal effect could be direct through a pH increase in the mycorrhizosphere and a concomitant decrease in Al^{+3} activity or indirect through the formation of a glomalin-Al complex. Glomalin is a recently discovered compound produced copiously by arbuscular mycorrhizal hyphae. Therefore arbuscular mycorrhiza could be an interesting Al detoxifing agent for plants growing in acidic soils. A major gene conferring tolerance to acid soils has also been identified in the D-genome (Reynolds et al. 2001).

MATERIAL AND METHODS

Wheat varieties of European origin were introduced (Germany, France) in the Gorbea location (S 39°, W 72°) in 1956 in soils with pH around 4.7–5.2 and a saturation of Aluminum of 13 to 30% and compared to local materials.

The national germplasm was characterized as having extensivable dough, high tillering and low yield (Castaño Colorado, Castaño Alemán (von Baer 1995)

The European germplasm with better adaptation to acid soil conditions can be divided according to their origin and cycle:

Spring: Heines Koga, Heines Peko and others.

Winter early maturing: Vilmorin 23, Vilmorin 29, Capelle Desprez, Ministré, etc.

Winter late maturing: Heine IV, Heine VII

The yields obtained were higher when the selected genotypes were tested on non acidic soils. Those lines that did well on both acidic and non-acidic soils were considered critical because of their potential for maintaining food security.

Under the soil conditions of Gorbea, a large part of the introduced material showed a yellowish colour at emergence, reduced vigor and poor tillering. However,

Table 1. Flexible basic outline according to the progenitors

1	Mother of quality without tolerance x father with tolerance.
2	F1, half tolerance.
3	F2, segregant material of distanced sowing, elimination of susceptible plants, harvests in bulk of the tolerant plants. Selection of the grains.
4	F3 Sowing of the bulk, harvests of spikes of half height to low, it thrashes of each spike in separate form (approx .200).
5	F4, separated sowing from the seed of each spike, observation for diseases, of the best (approx. 10%), are harvested spikes again.
6	F5, separated sowing from the seed of each spike, observation for type etc.
7	Depending on the adaptation obtained with the material, crosses with other lines or varieties that presented the wished characteristics.
8	F6 bulk harvests of the best line, sowing field trials in plots of 1 x 5 meters in a soil without restrictions (Cajón locality, soil with pH5.6).
9	F7 parallel trial of yield in Cajón and Gorbea soils. Field plots of 1x 5 meters with four replications. Evaluation of all plant and yield parameters.
10	F8 – F10. Regional trials according to the cycle of the obtained growings.
11	Multiplication and pilote industrial processing.

some lines were identified with green colour and vigorous emergence and they were selected for continued evaluation.

These selected materials were more vigorous and it was later found that this vigour was in fact better tolerance to acidity and aluminum toxicity and a higher association capacity with arbuscular micorrhiza fungi. Most of the lines were deficient in baking quality as some were derived from crosses with triticale and rye. Nevertheless, they formed the basis of wheat improvement efforts from the 1960's onwards. The progenitors used are outlined in Table 1.

RESULTS AND DISCUSSION

The results obtained in this study agree with those authors cited by Reynolds that Al tolerance in wheat is additive, with high heritability (see Table 2).

The varietal differences were clearly confirmed by the comparison of seedling relative root length (RRL) grown in nutrient solution culture (Gallardo et al. 1995) at pH 4.5 and 6.0 with increasing Al concentration at 21 days. In addition, soil bioassays were conducted in growth chambers to confirm wheat variety performance observed in nutrient solution culture.

In a field experiment carried out in Gorbea soil (1994/95), the following results were obtained, which correlate well with the results obtained in nutrient solution (see Table 3). The field test was conducted by the author at 4 locations in 1994/95.

In addition, the summary the experiments conducted last season (2004–2005), expressed as percentage of the yield of the variety OTTO, is shown in Table 4 and Fig. 1.

Table 2. Pedigree of the most important varieties

Name	Pedigree	Year
Intermedio	Ministré x Heines Peko	1968
Éxito	Weihnst x Olsson	1978
Export	Arin x Z 78755	1980
As	(H 46 x My 64)(Magdalena x Mirinoskaya808 (RxPonchxP14)	1983
Peneca	(H 46 x My 64)(Poncheau x Mag x Lee) x Klein Toledo	1983
Paleta	(Ack (DonxInt)(Ministré x Champlein)	1987
Otto	(Heines Koga x Éxito) Export	1990
Taita	(PalxFD)(Bt27xPal)	1994
Fama	Pavon (FD x Z 78755)	1995
Bingo	Taita x Amigo	2000
Puelche	Capo (Taita x Amigo)	2004

Table 3. Effect of lime over the yield of different varieties in kgs has^{-1}. (Gorbea 1994–95)

	0 Lime ton	1 Lime ton	% of increase
UFRO T-8	4.349	6.105	40
DALCAHUE	3.725	4.596	23
PITUFO	3.838	4.725	23
TAITA	5.950	6.823	15
OTTO	5.334	5.850	10
FAMA	6.110	6.357	4
AVERAGE	4.884	5.743	18

(Gallardo F, Borie F, Riquelme C, Santander I 1995)

Table 4. Percentage of the yield of OTTO variety (2004–05)

Place >>	% of Aluminum Saturation				Average %
	0.18 Cajon %	27.70 Gorbea %	0.41 Mafil %	0.70 Osorno %	
VARIETY					
OTTO	100.0	100.0	100.0	100.0	100.0
BARBARO	116.1	43.6	93.6	65.0	79.6
BT 151	123.8	95.3	108	139.3	116.6
BT 152	75.5	67.8	77	87.6	76.9
BT 158	130.0	39.3	100	92.5	90.4

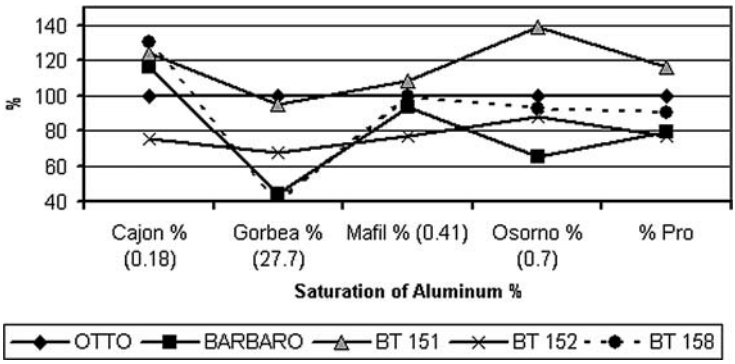


Figure 1. Cultivar in nutrition solution of short duration. Effects of aluminum toxicity in wheat yield 2004–2005

ADDITIONAL CONFIRMATION

Experimental studies in the growth chamber and with nutrient solutions carried out by Gallardo et al. (1995 and 1999) have demonstrated a great difference in growing behaviour between sensitive and tolerant wheat. Such differences were mainly expressed as increased root length of the tolerant varieties compared to sensitive lines. Plants were grown in nutrient solutions at 0, 50, 100 and 200 uM Al. (see Fig. 2 and 3).

Field experiments were grown in Gorbea and at three other locations both with and without additional lime (1 Ton ha^{-1}). Varieties selected in this way showed a high tolerance to acidity and free Aluminum.

Subsequent evaluation trials are carried out annually in Gorbea, Cajón and at seven other locations. Those varieties performing well across three years of trials

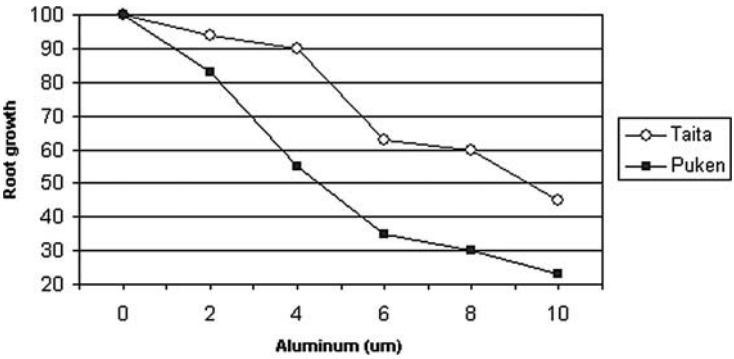


Figure 2. Effect of aluminum in the root growth of two varieties of wheat cultivated in nutritious solution during 4 days

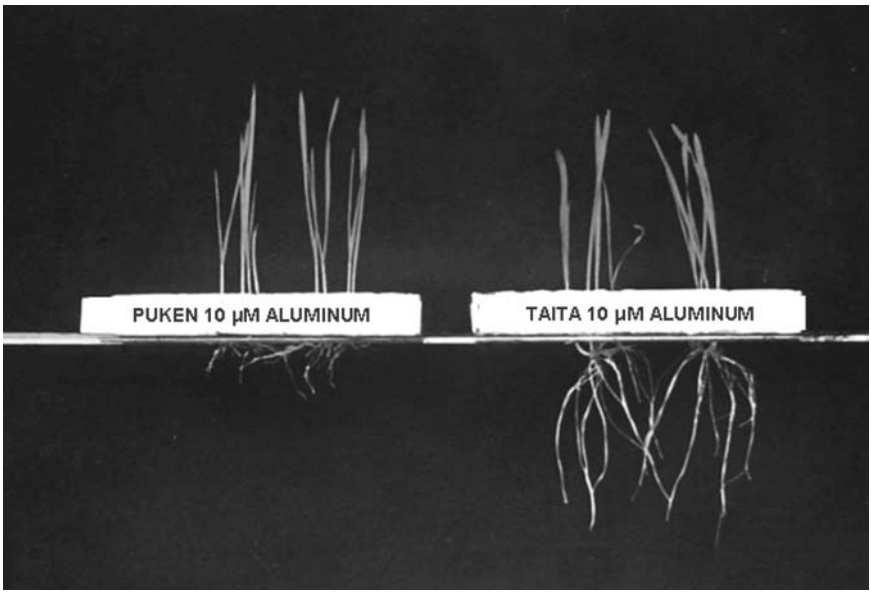


Figure 3. Cultivar in nutritious solution of short duration. Effects of Aluminum in two wheat varieties

are well adapted and offer farmers increased yield stability and Chilean consumers improved food security.

CONCLUSION

The results of our study show that it is possible to obtain wheat varieties with high yield potential and tolerance to low pH and aluminum toxicity. The mechanism of tolerance is heritable and indirectly improves yield stability and food security.

REFERENCES

Borie F, Rosa Rubio (1999) Effects of arbuscular mycorrhizae and living on growth and mineral acquisition of Al-tolerant and Al-sensitive barley cultivars. J Plant Nutr 22:121–137

Cumming JR, Ning N (2003) Arbuscular mycorrhizal fungi enhance aluminum resistance of broomsedge. J Exp Bot 54:1447–1459

Gallardo F, Borie F, Alvear M, von Baer E (1999) Evaluation of Aluminum tolerant of three barley cultivars by two short-term screening methods and field experiments. Soil Sci Plant Nutr 45:111–117

Gallardo F, Borie F, Riquelme C, Santander J (1995) Selección de especies y cultivares Tolerantes a Aluminio Revista Frontera Agrícola Año 3 N° 1, pp1–9

Kochian L, Hoekenga VOA, Piñeros M (2004) How do crop plants tolerate acid soils? Mechanism of Aluminum tolerance and Phosphorus efficiency. Annu Rev Plant Biol 55:459–493

Koslosky SD, Berner RR (1989) Interactive effects of aluminum, phosphorus and mycorrhiza on growth and nutrient uptake of panicum-virgatum (poaceae). Env Poll 61:107–125

Medeiros CA, Clark RB, Ellis JR (1994) Growth and nutrient uptake of sorghum cultivated with vesicular arbuscular mycorrhiza isolates at varying pH. Mycorrhiza 4:185–191.

Reynolds MP, Ortiz Monasterio JI, McNab A (2001) In: Reynolds MP, Ortiz Monasterio JI, McNab A (eds) Application of Physiology in Wheat Breeding, CIMMYT, Mexico, D.F.

von Baer E (1995) Variedades de trigo para la Zona Sur Temuco-Chile, Revista Frontera Agrícola Año 3 Nr.1 pp10–18

von Baer E (2003) Perspectivas de los cereales de la zona SUR, en la alimentación de salmones. Seminario Internacional Agricultura y Salmonicultura Fundación Chile Puerto Varas, Chile, 2 Octubre.

GENETIC VARIATION FOR SUBSOIL TOXICITIES IN HIGH pH SOILS

A.L. MILLAR*, A.J. RATHJEN AND D.S. COOPER

Discipline of Plant and Pest Science, School of Agriculture and Wine, University of Adelaide, PMB1, Glen Osmond, SA 5064, Australia

**E-mail: lison.millar@adelaide.edu.au*

Abstract: Alkaline soils (pH > 7) are common throughout the world in semi-arid to arid climates (<500mm rainfall/year) with soil types predominantly calcarosols (calcareous soils) and sodosols (sodic soils). The pH of calcareous soils is buffered in the range of pH 7.5 to 8.5 by the presence of $CaCO_3$, while sodic soils have a high exchangeable sodium percentage (ESP $\geq$ 6) and generally contain Na_2CO_3. The higher solubility of Na_2CO_3 compared to $CaCO_3$ increases the disassociation of CO_3^{2-} and HCO_3^- in sodic soils, leading to pH values > 8.5

Solution screening methods have been used for the identification of HCO_3^-/CO_3^{2-} tolerance in plants, with significant variation found in root length within both commercial and landrace lines. A moderate level of tolerance was found in much of the southern Australian bread wheat (*Triticum aestivum*) germplasm. Durum wheats (*Triticum turgidum*. ssp. durum) showed much lower tolerance to HCO_3^-/CO_3^{2-} ($\approx$ 50%), consistent with the shorter breeding history of durum wheat in southern Australia and their poor adaptation to alkaline soils; this is reflected in their confinement to higher rainfall regions. In field trials the durum variety Tamaroi ($\approx$ 89% of S.A. commercial production) suffered a > 1 t/ha yield penalty from increasingly higher subsoil pH (Cooper 2004). Several durum lines have been identified with significantly greater tolerance to HCO_3^-/CO_3^{2-} than Tamaroi

Significant correlations between HCO_3^-/CO_3^{2-} tolerance and yield have been identified in a double haploid bread wheat population RAC875/Cascades grown at a number of high pH field sites across S.A. A second population Frame/Yarralinka//Pugsley has high variation for HCO_3^-/CO_3^{2-} tolerance and is currently in field trials

Keywords: soils pH, toxicity

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 395–401

INTRODUCTION

In southern Australia cereal production is confined to dryland areas where soil and climate limitations restrict productivity. Soil mapping studies of South Australia identified dense clay subsoils with high sodicity, salinity and pH > 8.5 in 80% of the cropping region (Maschmedt 2002). Subsoils (> 30cm) typically ranged from pH 8.0 to 10.5, while topsoils (0 to 30cm) vary from acid to alkaline. Salinity (EC > 4dS/m) is commonly associated with sodic soils (ESP $\geq$ 6) and in southern Australia boron also accumulates naturally at depth. The prevalence of carbonates, boron and sodium toxicity, combined with various deficiencies and poor soil physical characteristics across much of the cropping region has been shown to restrict plant growth and yield.

Unlike soil deficiencies, nutrient toxicities are far more difficult to ameliorate and the identification of tolerant genotypes is often the most practical way to increase yield. Research on boron toxicity in the 1980s identified a gene on chromosome 7BL in the bread wheat *cv*. Halberd (Paull 1990). The incorporation of this gene for tolerance into elite breeding lines led to a substantial increase in wheat yield in boron affected areas, and remains the main source of tolerance in South Australia's breeding material. Similarly, boron tolerance was identified in a durum landrace Lingzhi Baimong Badamai in the mid 1990s. A boron tolerance gene located on chromosome 7BL and another gene of unknown location were incorporated into elite breeding material (Sansanee 1996).

Following the success of the boron research, attention was focused on the previously undefined problem of transient salinity in southern Australia and its potential affect on yield. While dryland salinity has received considerable attention due to the often dramatic visual effects of surface scalding, less than 5% of the cropping area in South Australia is likely to be affected by water-table induced salinity, whereas more than 60% is subject to transient salinity (Rengasamy pers. comm).

Unlike dryland salinity (seepage salinity), transient salinity is not associated with rising saline groundwater, but with the cyclic movement of naturally occurring soil salt and low levels of soil water. Transient salinity intensity fluctuates with season and rainfall, with movement of salt into the root zone as the soil dries having a two-fold affect. Crops intolerant to sodium suffer physiological problems, which are exacerbated by reduced water availability induced by increased osmotic potential. In South Australia's winter-spring growing season periods of extended drought are common, even in above average rainfall years.

Australian bread wheats have generally been shown to be low Na^+ accumulators (Paull et al. 1994), but the extent of the problem of transient salinity in South Australia was not fully recognised until soils were mapped (McCord 1995, Maschmedt 2002). In the root zone an ECe (electrical conductivity of the saturation extract) of 4 to 16 dS m^{-1} was common across most of the cereal belt, supporting the notion that a number of older varieties persisted in some areas due to their Na^+ tolerance and lower Na^+ accumulation (Rathjen et al. 1999). Further support for the adverse affect of transient salinity was observed in durum wheat trials, where significant yield losses were associated with high tissue Na^+ accumulation. Later

research found durum wheat accumulated, on average, 10 times more Na^+ than most Australian bread wheats (Cooper 2004), and to some extent explains the poor adaptation of durum wheat to lower rainfall regions of South Australia.

A low sodium accumulating durum landrace (Na149) was identified by Munns et al. (2000) and the exclusion gene on chromosome 2A was subsequently backcrossed into elite durum varieties (Cooper 2004, Munns et al. 2003). The exclusion gene reduced the accumulation of Na^+ to a level similar to bread wheats, and trials on transient salinity sites have sometimes provided a significant yield increase over the recurrent parent (Cooper 2004).

The incorporation of boron tolerant and sodium exclusion genes have not always resulted in a yield advantage in the field as other stresses are often dominant and mask these effects. Few attempts have been made to identify genetic variation for tolerance to HCO_3^-/CO_3^{2-} in gramineous crops. However, it has long been recognised in South Australia that some varieties, notably the bread wheat *cv*. Krichauff, continuously out-performed lines with similar breeding histories in these highly alkaline soils. Initial solution screening studies by Lui and Rathjen (1998) found that decades of selection for superior yielding bread wheats (*Triticum aestivum*) on these highly alkaline soils has unconsciously led to a moderate level of HCO_3^-/CO_3^{2-} tolerance in many of the locally bred varieties, with Krichauff appearing to be the most tolerant. More recently, attention has been focused on durum wheats (*Triticum turgidum spp* durum) due to their poor adaptation to South Australian conditions and much lower tolerance to HCO_3^-/CO_3^{2-}. The breeding goal is to combine boron, sodium and HCO_3^-/CO_3^{2-} tolerance with other biotic resistances in an attempt to improve the adaptation of the durum crop.

MATERIALS AND METHODS

Germplasm

Bread wheat and durum wheat seeds were sourced from both the Durum Breeding Program at the University of Adelaide, and the Australian Winter Cereal Collection (AWCC) in Tamworth. The double haploid population RAC875/Cascades, comprised of 93 lines, was provided by Hugh Wallwork (MPBCRC). The Frame/Yarralinka//Pugsley population was developed by single-seed descent by Anthony Rathjen (University of Adelaide) and consists of 150 lines. Plants were glasshouse grown (18–30 °C) at UC (University of California) in potting mix in 250mm poly pots and harvested approximately three months after planting.

Bicarbonate Solution Screening

Plants were screened for HCO_3^-/CO_3^{2-} tolerance in the laboratory using solution culture. Experiments were conducted in 25L plastic tanks, using suspended plastic trays (330mm × 120mm) with 120 rectangular holes. A 250mm × 15mm airstone was attached to the bottom of each tank and connected through a 4mm tube

to an aquarium pump to aerate the solution. Air was initially passed through a concentrated (10M) KOH solution to remove CO_2, to assist in maintaining constant pH. Seeds were sterilised in 2% NaHOCl for 10 minutes and rinsed thoroughly in reverse-osmosis deionised (RO) water. Three seeds per hole were placed directly onto the plastic trays, allowing 120 lines to be tested per tray. Trays containing seeds were cool-treated in the refrigerator for three days at 4 °C and germinated for two days at 20 °C. The sprouted seed (root length approx. 15mm) were then transferred to the treatment tanks. The tanks were placed on laboratory benches at room temperature. The treatment solution contained 5mM $NaHCO_3$, 1mM Na_2CO_3 and 5mM $CaCO_3$, in 22L RO water, maintained at pH 9.1–9.2 by daily adjustment with the addition of Na_2CO_3. Root length was measured after 10 days growth. All experiments were conducted with three replications, including controls of RO water, and results presented as relative root lengths (RRL).

Field Trails

Sites were chosen across the South Australian cereal zone representing a range of subsoil pH values. Sites included Buckleboo (pH 8.8–9.2), Pt. Pirie (pH 8.2–8.6), Winulta (pH 8.0–8.7), Roseworthy (pH 8.6–9.0), Angas Valley (pH 8.9–9.5), Claypans (pH 8.6–8.9) and Coonalpyn (pH 8.6–9.1). Bread wheat lines were sown at a rate of 30g $plot^{-1}$ (60kg ha^{-1}) and durum wheat at 35g $plot^{-1}$ (70kg ha^{-1}). Each plot was 4 rows wide and 6 metres long with 15cm inter-row spacing. Plots were arranged in 15 bays and 15 or 30 columns. Plots are separated by 30cm between columns and pathways of two metres sprayed with a knockdown herbicide in the period between head emergence and anthesis, reducing plot length to four metres. Fertiliser (di-ammonia phosphate) was applied at seeding at a rate of 80kg ha^{-1} and broadleaf herbicides applied when necessary. Trials were sown throughout June 2004 and harvested in December 2004.

Soil Sampling

Soil samples were extracted from every second plot, at a depth of 0–10cm and 30–40cm using a soil coring rig from the University of Adelaide. All samples were measured for EC (electrical conductivity) and pH in a 1:5 soil paste extract. The measurement of soil pH has been used as an estimate of soil bicarbonate/carbonate.

RESULTS AND DISCUSSION

The screening of both bread and durum wheat germplasm in HCO_3^-/CO_3^{2-} solution, maintained at pH 9.2, supported the original findings of Lui and Rathjen (1998). Significant variation exists for HCO_3^-/CO_3^{2-} toxicity in Australian bread wheat varieties with *cv*. Krichauff identified as the most tolerant. Krichauff had a RRL approximately 2.5 times greater than the least tolerant variety Excalibur from the 50 varieties tested (Table 1). Similarly, significant variation was identified in 20

Table 1. Relative root length (RRL) of a selection of durum and bread wheat lines assessed for HCO_3^-/CO_3^{2-} tolerance

Bread Wheat	RRL	Durum	RRL
Krichauff	47.1	Kalka	36.4
Dollarbird	42.3	Kalti 4	34.4
Westonia	37.2	Arrivato	31.4
Kukri	30.9	Helidur	30.1
Yitpi	30.7	Bellaroi	29.5
Halberd	28.8	Renville	27.6
Wyalkatchem	28.4	Gunderoi	22.6
Frame	28.3	Aconchi	22.5
Janz	27.0	Tamaroi	21.1
Molineux	24.6	Kyle	19.3
Pugsley	24.2	Altar 84	17.5
H45	23.6	Kronos	15.6
Excalibur	20.0	Yallaroi	15.3

international durum wheat varieties, with the South Australian bred variety Kalka the most tolerant. The tolerance of Kalka was approximately 2.4 times greater then the least tolerant variety Yallaroi among the lines tested. Durum wheats on average were far more intolerant to HCO_3^-/CO_3^{2-} toxicity than the bread wheats, largely reflecting the long breeding history of bread wheats in South Australia in highly alkaline environments.

The development of the South Australian durum industry in the early 1990s and a more concerted breeding approach has resulted in improved adaptation to southern Australian soil and climatic conditions. Progress has been rapid due to use of many of the technologies developed for the assessment of bread wheat lines and considerable knowledge of the soil constraints in the South Australian farming districts. In part, the poor adaptation of durum wheats has been a consequence of their inability to perform on highly alkaline soils, and results of screening for HCO_3^-/CO_3^{2-} tolerance support the hypothesis that intolerance to HCO_3^-/CO_3^{2-} may be partly responsible.

In 2004 the durum wheat variety Tamaroi accounted for approximately 89% of grain produced in South Australia (Australian Wheat Board). Screening identified Tamaroi as highly intolerant to HCO_3^-/CO_3^{2-} toxicity with a RRL of 21 percent. Furthermore, field trials conducted by Cooper (2004) concluded that Tamaroi suffered a $> 1\,t\,ha^{-1}$ grain yield reduction from a subsoil pH increase of 0.5 units (Fig. 1).

Six hundred durum landraces and pre-breeding lines were tested for tolerance to HCO_3^-/CO_3^{2-} in an effort to find new sources of variability; only 8 lines were identified as having a significantly greater RRL than Kalka (Table 2). Six of the identified lines have been used for the development of durum populations for use in genetic studies. Material is currently at the F_3 stage.

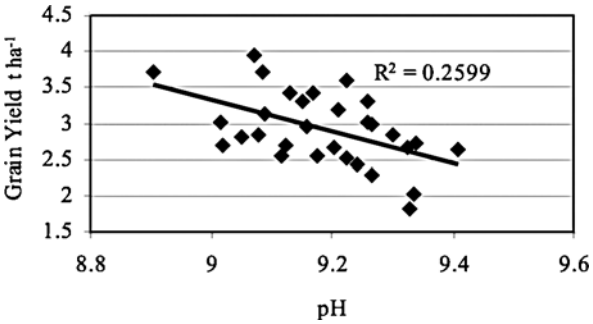


Figure 1. Subsoil pH (30–60cm deep) and grain yield (t/ha) of Tamaroi durum, Redhill 2001 (Cooper, 2004)

Two pre-existing bread wheat populations were used to measure the influence of HCO_3^-/CO_3^{2-} tolerance under field conditions. The double haploid mapping population RAC875/Cascades was found to have significant variation for HCO_3^-/CO_3^{2-} tolerance in solution, with a RRL ranging from 27 to 48 percent. A significant positive correlation (0.05% level) was identified between HCO_3^-/CO_3^{2-} tolerance and grain yield at three field sites. The correlation between soil pH and grain yield was weak, with significance at the 0.10% level. However, measurements in 2004 were strongly influenced by extended periods of water stress.

The bread wheat population Frame/Yarralinka//Pugsley was developed through single-seed descent from parents with a moderate and low level of HCO_3^-/CO_3^{2-} tolerance. The resulting population had a RRL ranging from 44 to 87 percent in HCO_3^-/CO_3^{2-} solution. In 2005/06 the population was grown in field trials at 8 sites ranging in subsoil pH from 8.0 to 9.5. The population is currently awaiting harvest in December, but is showing promise, being without the maturity or height issues which confound the RAC875/Cascades population data.

Table 2. Durum germplasm identified with tolerance to HCO_3^-/CO_3^{2-}

Line	RRL
(R622Sh*Tm)*WLYY9Tm2)/4/5	53.7
(C8MMDYk*BTWLYY9)*Tm)/4/5	51.7
(Bezbazak*LY#Tm)/2	49.5
Iraq 15/8	46.7
Borlaug/3	44.8
Grandumi Saman/3	38.6
Kalka	36.6
Tamaroi	21.1
Krichauff (Bread wheat)	47.1

The release of the durum wheat variety Kalka in 2003, developed by the Durum Breeding Program (Adelaide) has been a substantial step forward in the adaptation of durum to South Australian conditions. Kalka combines the boron genes identified in Lingzhi Baimong Badamai, with a moderate level of HCO_3^-/CO_3^{2-} tolerance to provide a marginally higher grain yield than Tamaroi. Similar experiences with BT-Schomburgk (boron tolerant), which had only a marginal yield advantage over the recurrent parent Schomburgk, have shown that after another cycle of crossing and selection a substantial increase in grain yield, through better adaptation, can be achieved.

REFERENCES

Cooper DS (2004) Genetics and agronomy of transient salinity in *Triticum durum* and *T. aestivum*. PhD thesis. University of Adelaide, Adelaide, SA

Lui CY, Rathjen AJ (1998) Tolerance of wheat genotypes to bicarbonate ions. In: Proceeding 9th Int. Wheat Genetics Symp. Saskatoon, Canada. pp 51–53

Maschmedt D (2002) Atlas of key soil and landscape attributes. Compact Disc. Dept. Water, Land, Biodiversity Conservation, South Australia

McCord AK (1995) A description of land in the southern Mallee of South Australia. Primary Industries of South Australia

Munns R, Hare RA, James RA, Rebetzke GJ (2000) Genetic variation for improving the salt tolerance of durum wheat. Aust J Agr Res 51: 69–74

Munns R, Rebetzke GJ, Husain S, James RA, Hare RA (2003) Genetic control of sodium exclusion in durum wheat. Aust J Agr Res 54: 627–635

Paull JG (1990) Genetic studies on the tolerance of wheat to high concentrations of boron. PhD thesis, University of Adelaide.

Paull JG, Liu CY, Rathjen AJ (1994) In: Paull JG et al.(eds), Proceeding 7th Assembly Wheat Breeding Society of Australia, Adelaide pp 229–232

Rathjen AJ, Brand JD, Lui CY, Paull JG, Cooper D (1999) Breeding for tolerance to soil toxicities. In: Proceeding 11th Aust Plant Breeding Conf, vol 1. Adelaide

Sansanee J (1996) Genetics of boron tolerance in durum wheat. PhD thesis. University of Adelaide.

DETERMINING THE SALT TOLERANCE OF TRITICALE DISOMIC ADDITION (*THINOPYRUM* ADDITIONS) LINES

W.C. BOTES* AND G.F. MARAIS

Department of Genetics, Faculty of Agriculture and Forestry Sciences, Stellenbosch University
**E-mail: wcb@sun.ac.za*

Abstract: Biotic and abiotic stresses constitute an increasing problem, and in arid and semi-arid regions salinity and drought stress are especially severe. Saline soils can be treated, but it is expensive and temporary. An obvious solution is to modify the plant to cope with salinity. Unfortunately the genetic and physiological bases of salt tolerance are not well understood as the trait is under polygenic control in most crop species including cereals. Accessible sources of salt tolerance genes are hard to come by. *Thinopyrum distichum* ($2n = 28$) is an indigenous grass native to the coast of the Western Cape province of South Africa. A project was initiated to transfer the salt tolerance of this grass to commercially cultivated triticale and wheat. Disomic additions were made of specific *Th. distichum* chromosomes believed to contribute to salt tolerance. This study developed and optimized screening techniques to differentiate among these addition lines with the aim of determining their potential contribution to salinity tolerance. Methods included growth and/or yield assessment, detection of damage or tolerance symptoms, and quantification of physiological changes. The results indicated that methods to detect growth or yield reduction gave the best overall correlation with salinity tolerance

Keywords: salt tolerance, triticale, stress

INTRODUCTION

Salinity constitutes an ever increasing threat to global food production and security, and limits production in many farming systems including arid and semi-arid regions. Estimates indicate that a 2.7% increase in world grain production is needed annually to sustain the growth in world population that will exceed 8.5 billion people by 2020 (Mujeeb Kazi and Rajaram 2002). Currently 19.5% of the world's arable land and approximately 9% of South African cultivated land are affected by salinity (Munns 2002). Although affected soils can be treated, it is generally very expensive and

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 403–409.

usually only temporary (Ashraf 1994). A possible solution is to genetically adapt plants to cope with salinity. However, the genetic, physiological and biochemical bases for salt tolerance are not well understood (Omielan et al. 1991). This is largely attributed to the polygenic control of salt tolerance in plants such as wheat (Asharaf 1994) and rice (Gregorio and Senadhira 1993). The *Thinopyrum* species are a rich source of novel genes for resistance to biotic stresses (rust diseases in wheat, etc.) and tolerance to abiotic stresses (salinity tolerance, etc.) (Wang et al. 2003; Mujeeb-Kazi and Rajaram 2002).

Thinopyrum distichum ($2n = 28$) is a hardy, slow growing, maritime grass, native to the coast of the Western Cape province of South Africa (Fig. 1) and is well adapted to the highly saline conditions of the coastal sand dunes (Pienaar and de 1990). Littlejohn (1988) reported hybridization of *Thinopyrum* species with *Triticum* parents; the hybrids were no more salt tolerant than the *Triticum* parents and it appeared that the genes controlling salt tolerance were suppressed by the wheat genomes. Subsequently Marais (1998) decided to hybridize *Thinopyrum* species with a cultivated cereal that allowed expression of the salt tolerance genes. Disomic additions of specific *Th. distichum* chromosomes believed to contribute to salt tolerance were subsequently made with triticale (cultivar Rex). In a programme aimed at transferring salt tolerance genes from *Th. distichum* to triticale, Marais et al. (2003) determined that chromosomes $2J_1^d$, $3J_1^d$, $4J_1^d$, $5J_1^d$ and $7J_1^d$ carried genes conferring possible salinity tolerance (Fig. 2). The purpose of this study is to: 1) Find and optimize effective screening techniques that can differentiate among specific disomic additions lines and 2) Quantify the potential contribution of these disomic addition lines to salinity tolerance. Screening methods considered included measuring growth and/or yield depression, determination of damage or tolerance symptoms, and monitoring of physiological changes.

Figure 1. Map depicting the geographic distribution of *Thinopyrum distichum* along the coast of the Western Cape province of South Africa

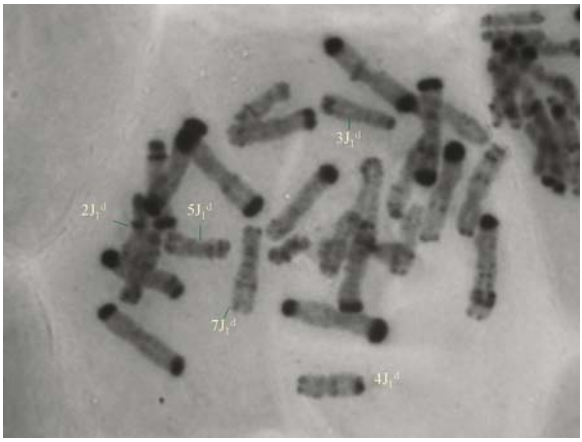


Figure 2. Identification of *Thinopyrum distichum* chromosomes that determine its salt tolerance

MATERIALS AND METHODS

Plant Material

Genotypes used during experiments are indicated in Table 1. Each experiment comprised the *Th. distichum* x triticale disomic addition lines and triticale, rye and bread wheat cultivars which were used as controls. The identification of more tolerant addition lines allowed numbers to be reduced in subsequent experiments providing a more intensive evaluation. In total 7 experiments were performed (Table 2).

Table 1. Genotypes used during experiments

Genotypes	Experiments				
	Exp 1,2	Exp 3	Exp 4	Exp 5	Exp 6,7
Thinopyrum distichum x triticale disomic additions:	1, 3, 5–9, 11, 13, 15–31, 56, 70	1, 3, 5–9, 11, 13, 15–31, 56, 70	1, 3, 5–9, 11, 13, 15–31, 56, 70	1, 3, 5–9, 11, 13, 15–31, 56, 70	9, 14, 28, 56
Triticale cultivars:	Rex Usgen 19	Rex Usgen 19	Rex	Rex Tobie Ibis	Rex
Rye cultivars:	Henoch	Henoch	Henoch	Henoch	Henoch
Bread wheat cultivars:	SST 57	SST 57	SST 57	SST 57	SST 57

A total of 7 experiments were performed. After identifying more tolerant genotypes the number of addition lines was decreased making it possible to use labour and resource intensive methods (as indicated in Table 2).

Table 2. Methods using during experiments vantages are also listed (adapted from Munns et al., 2003)

Methods	Experiment number	Controls	Osmotic or salt-specific effect	Length of treatment (weeks)	Advantages	Disadvantages
Based on damage and/or tolerance to very high salinity levels						
Survival	1, 2	No	Both	2–8	Tolerant genotypes stand out	Destructive
Germination	3	Yes	Osmotic	1	Large numbers easily handled	Do not always relate to field
Chlorophyll content	2	No	Both	2–4	Not destructive	Labour intensive
Based on growth or yield						
Leaf elongation	4, 5	Yes	Osmotic	2–4	Not destructive	Labour intensive
Biomass	5, 6, 7	Yes	Both	4–8	More likely to relate to field	Labour/ resource intensive
Yield	4, 5	Yes	Both	6–12	More likely to relate to field	Labour/ resource intensive
Based on physiological mechanism						
Proline	1, 2, 4	yes	Osmotic	4	Single easy analysis	Do not always relate to field

Indicated in the Table is the experiment number, whether a control treatment is needed, if the response measured is due to osmotic or salt specific effect, and how long the treatment needed to run. Advantages and disad

Growth and Planting Conditions

All seeds were germinated before planting (for 3–6 days). Experiments were conducted under glasshouse conditions with the temperature ranging from 12–26 °C with 8–10h natural sunlight. Initially experiments were conducted in individual pots. However, these were replaced by an automated hydroponic subsoil irrigation system for subsequent experiments. NaCl was added in increments depending on the experiment and treatment using a $Na^+ : Ca^+ :: 10 : 1$ ratio.

Experiments Assessing Growth and/or Yield Depression

Methods employed during experiments 1–3 were based on growth and/or yield depression induced by very high salinity levels. Both experiments 1 and 2 consisted of 4 replicates in a randomized complete block (RCBD) design. Experiment 1 and 2 exposed plants to salt levels of up to 300mM. Seedlings were watered with a nutrient solution (containing 50mM NaCl) commencing 10 days after emergence (DAE), the NaCl concentration was then raised, in 50mM increments, to 300mM and continued with, twice a day, until the rye cultivar (Henoch) was dead. Plants were then classified on a scale of 1 (Henoch – dead) – 7 (high survival). In experiment 2, chlorophyll content of the leaves was also assessed to see its correlation (if any) with salinity tolerance. In experiment 3, plant material was germinated in Petri dishes and germination assessed. This experiment consisted of 2 treatments (0mM NaCl – control and 300mM NaCl – salt) with 4 replicates. Germination was assessed as a percentage of the control vs salt treatments.

Experiments Assessing Proline Content

Leaf samples were taken during experiments 1, 2 and 4 on specific dates and proline content measured according to a protocol adapted from Bates et al. (1973).

Experiments Assessing Growth and/or Yield

During this series of experiments (4, 5, 6 and 7) plants were sown in the automated hydroponic system. The experiments consisted of 3 treatments (0mM NaCl – control; 150mM NaCl – medium; 300mM NaCl – high) with 4 replicates in an RCBD design. Leaf elongation rate was assessed with experiments 4 and 5. The elongation rate of the third leaf was measured for a 4 week period (exp. 4) and 2 week period (exp. 2) respectively. Leaf elongation rates were calculated as a percentage of the controls. Grain yield was assessed during experiment 4 and 5, and whole plant biomass was calculated in experiments 5, 6 and 7. Correlations were established between different measurements.

Data Analysis

All experiments were analysed using Agrobase GII (version 12.5.1.) Analysis of variance (ANOVA) was performed and LSD calculated, enabling the comparison of means between genotypes. A P value of 0.05 or less was considered to be statistically significant.

RESULTS AND DISCUSSION

Experiments Assessing Growth and/or Yield Depression

Experiments based on survival under high NaCl levels (Exp. 1 and 2) provided a very efficient manner to assess large numbers of addition lines and differences among lines were found to be statistically significant. However, plants with intermediate salinity tolerance were not recoverable and the destructive nature of this type of testing has limited application in plant breeding. The question must also be asked whether exposure to such high levels of salinity is realistic. In contrast, the germination test was very easily performed, took only 1 week to conclude and large numbers of samples could potentially be handled. However, this test showed very poor correlation with salinity tolerance as assessed by later experiments focussing on biomass and/or yield. Chlorophyll assessment was also problematic and did not show consistent correlation with biomass production as measured in later experiments. Chlorophyll assessment was also very labour intensive and therefore expensive.

Experiments Assessing Proline Content

Proline content was easily measured and large numbers of samples were easily handled by the adapted Bates protocol. However results were variable and not repeatable over experiments or replications. Measuring proline content under drought conditions to ascertain drought resistance is common practise as proline content is primarily a measure of osmotic stress (Munns and Richard 2003). Since water is limited in these experiments, it is difficult to differentiate between drought stress and salinity stress in the early stages of development; therefore proline measurement was not as effective as initially envisaged.

Experiments Assessing Growth and/or Yield

Leaf elongation was measured in Experiments 4 and 5. Leaf elongation was not very reliable and did not correlate with biomass production under saline conditions. On-the-other-hand, biomass production gave very consistent, repeatable data, but under certain conditions (very high average temperature x salinity level) the addition lines became sterile, thus making it extremely difficult to use either total biomass or grain yield to assess salinity tolerance.

Current Status and Future Efforts

Measuring salinity tolerance under glasshouse conditions is not very reliable and does not always correlate with field conditions. Nevertheless, accurately quantifying the tolerance levels of different genotypes under glasshouse conditions is convenient (and possibly cheaper and quicker) way to assess plant material. This study has shown that several of the disomic addition lines possess significantly high level of salinity tolerance. Results indicated that $3J_1^d$ (Addition line 56 and 14) gave the biggest contribution to salinity tolerance, followed by $5J_1^d$ (Addition line 28) and $7J_1^d$ (Addition line 9). Future work concerning screening techniques will focus on biomass assessment, as it gave the most consistent results. Screening for Na^+ exclusion is also envisaged. The identified salt tolerant disomic addition lines and associated chromosomes are currently being used to introduce salinity tolerance into commercial cultivars of triticale and wheat.

ACKNOWLEDGEMENTS

We would like to thank the Winter Cereal Trust and National Research Foundation (South Africa) for financial assistance.

REFERENCES

Ashraf M (1994) Genetic variation for salinity tolerance in spring wheat. Hereditas 120: 99–104

Gregorio GB, Senadhira D (1993) Genetic analysis of salinity tolerance in rice. Theor Appl Genet 86: 333–338

Littlejohn GM (1988) Salt tolerance of amphiploids and derivatives of crosses between wheat and wild *Thinopyrum* species. In: Proc 7th Wheat Genet Congress, Cambridge, UK, 845–849

Marais GF, Marais AS (2003) Identification of *Thinopyrum distichum* chromosomes responsible for salt tolerance. South African Journal of Plant and Soil 20(3): 103–110

Marais GF, Marais AS, Badenhorst PC (1998) Hybridization of *Thinopyrum distichum* and *Secale cereale*. Plant Breed 117: 107–111

Mujeeb-Kazi A, Rajaram S (2002) Transferring alien genes from related species and genera for wheat improvement. http://www.fao.org/documents/show_cdr.asp?url_file=/DOCREP/006/Y4011E/y4011e0e.htm.

Munns R (2002) The impact of salinity stress. http://www.plantstress.com/Articles/salinity_i/salinity_i.htm.

Munns R, Richard AJ (2003) Screening methods for salinity tolerance: a case study with tetraploid wheat. Plant Soil 253: 201–218

Omielan JA, Epstein E, Dvorak J (1991) Salt tolerance and ionic relationships of wheat as affected by individual chromosomes of salt-tolerant *Lophopyrum elongatum*. Genome 34: 961–974

Pienaar R de V (1990) Wheat x *Thinopyrum* hybrids. In: Bajaj YPS (ed) Biotechnology in agriculture and forestry. Springer Verlag, Berlin, vol 13, pp 167–217

Wang RRC; Li XM, Hu ZM; Zhang JY; Larson SR; Zhang XY; Grieve CM, Shannon MC (2003) Development of salinity-tolerant wheat recombinant lines from a wheat addition line carrying a *Thinopyrum junceum* chromosome. Int J Plant Sci 164: 25–33

CURRENT AND FUTURE TRENDS OF WHEAT QUALITY NEEDS

R.J. PEÑA
CIMMYT. Apartado Postal 6-641, 06600 Mexico, D. F., Mexico
E-mail: j.pena@cgiar.org

Abstract: Wheat is one of the main sources of nutrients for humans and animals. Its wide adaptation to diverse agro-ecological conditions, its storability, and its complex chemical composition, are main attributes that have made wheat the most widely used crop in food processing. Wheat trading is vast, occurs world-wide, and is now more dynamic than ever. Present and predicted shifts in the composition and trading figures of the wheat export market, are strongly associated to economical and social (agricultural reforms, rural-urban population balance, labor force composition, food habits) changes occurring in mainly developing and emerging countries; the urban population shows and steady increase, so is the demand for convenience and novel food products. The wheat processing industry is becoming more efficient and versatile, and therefore requires more specific quality characteristics to produce distinct flour and food types. Wheat grades and classes have to be developed or further refined, to market wheat with specific and, above all, more uniform quality attributes. Therefore, and in order to maintain wheat production in agreement with growing population and consumers' demands, wheat-producing countries need to become more efficient in developing wheat cultivars possessing high productivity as well as specific quality attributes. On the other hand, there are nutritional and health issues associated with the food consumption of wheat. In the first case, the still large use of wheat as the main source of nutrients, particularly in the rural populations of WANA, CAC, and SA regions, makes it necessary to enhance (naturally or artificially) the nutritional value of wheat-based foods consumed by the more vulnerable (rural- and urban-poor) part of the population. Regarding health issues, change in food habits of mainly the urban population, particularly the increasing consumption of calorie- and fat-rich foods in conjunction with a decrease in the consumption of fiber-rich vegetables, is causing increasing health problems associated with obesity (hypertension, coronary disease, diabetes, etc.); promoting increases in consumption of whole wheat flour-based foods is one way to reduce associated health risks. However, a balanced diet combined with exercise is the best approach to reduce nutrient intake-related risks

Keywords: wheat trading, wheat sanitation, end use quality nutrition

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 411–424.

INTRODUCTION

Wheat is a major crop contributing importantly to the nutrient supply of the global population. From their total wheat supply, an average of 53% is consumed as food in the developed world and close to 85% in the developing countries (Table 1). People living in West Asia-North Africa (WANA) and Central Asia (CA) get more than 40% (in some CA countries close to 60%) of their calories and protein (Table 2). Ekboir and Morris (2004) estimated that wheat will continue playing a major role in food supply in the next decades (Table 1).

Wheat is a very versatile crop; it shows wide adaptation to diverse agro-ecological conditions and cropping technologies. The wheat grain, which can be stored relatively easy during long periods of time, posses unique and complex chemical composition that allows the manufacture of diverse products, achieved either from viscoelastic dough (leavened and unleavened bread and noodles, alimentary pasta)

Table 1. Trends in wheat supply and food use in the main regions of the world

Regions	Wheat supply (MT)		Food (%/year)	
	1997	2020[a]	1997	2020[a]
East Asia	124,4	159,8	86,6	84,9
South Asia	93,5	147,1	88,6	88,3
WANA[b]	75,1	111	75,4	74,9
Central Asia	14	18,2	69,5	69,1
Latin America	29,6	41,3	82,4	80,6
Sub-Saharan Africa	9,4	19,1	93,1	93,1
Developing countries	340,1	491,7	84,7	83,9
Developed countries	244,8	268,2	52,9	52,7

[a] Source: Ekboir and Morris (2004); [b] WANA: West Asia/North Africa.

Table 2. Wheat Per capita consumption an contribution to calorie and protein intake in main global regions/countries (2002)[a]

Region/Country	Per capita Kg/year	Calorie, % total intake	Protein, % total intake
Kyrgyzstan	225,3	58,5	52,4
WANA	154,2	41,2	46,3
Tunisia	196,2	48,3	54,4
EE & CIS-Europe	135,3	19,8	19,6
Latin America	73,8	19,1	18,7
S-, E-, & SE Asia	45	13,6	15,3
China	65,2	16,2	16,7
India	58,4	20,3	25,5
Developed countries	113,7	28,6	25,9
Developing countries	86,4	23,9	25,9

[a] Source: FAOSTAT (2003)

or from highly viscous pastes (Biscuits and pastry). These are main attributes that have made wheat a world-wide cultivated crop with a very important export market and vast use in the food processing industry.

Given the complexity of the theme, in this seminar only an overview of current and future needs in wheat quality will be presented in the contexts of wheat trading, processing requirements to prepare main traditional and industrialized wheat-based food types, and also considering nutritional and health issues.

GENERAL TRENDS IN WHEAT PRODUCTION, TRADE, DEMAND, AND CONSUMPTION IN THE WORLD

Global wheat production in the crop season 2003/04 was roughly 625 million ton; 347 and 279 million tons were produced in the developed and developing regions, respectively (Table 3). The group of developing countries continues to have production levels lower than those realized by the group of developed countries (FAO, 2005). The aggregate of countries known as emergent (Eastern Europe,

Table 3. Wheat production and trade in selected regions/countries of the world[a]

Region/Country	Production 2004[b] (million t)	Imports 2004/05[b] (million t)	Exports 2004/05[b] (million t)
Asia	253,6	49,9	10,8
China	92	8,4	0,3
India	72,1	0,1	1,5
Iran	14	0,2	–
CA	20	0.12[c]	5.2[c]
Turkey	20,7	–	2
North Africa	17,2	16,7	0,5
Egypt	7,2	7,7	–
Morocco	5,5	2,1	–
Sub-Saharan Africa	4,6	12,1	0,1
Latin America	27,5	17,7	13,2
Argentina	16	–	12,4
Brazil	5,7	5	–
Mexico	2,4	3,7	0,5
Europe	217,8	10,47	26,5
EU 25	137,3	7,1	13
Russian Federation	45,3	1,1	7,9
Ukraine	16,5	0,1	4,4
World	625,9	108,9	109,7
Developing C.	279	85,8	19,5
Developed C.	346,9	23,1	90,1

[a] Source: FAOSTAT (2005). Includes flour in wheat grain equivalent (excludes semolina).
[b] Estimate
[c] Source: FAOSTAT (2004). Figures for 2003/04

CIS-Europe and Central Asia), showed in the 2003/04 crop season a production close to 110 million tons of wheat (FAO, 2005).

Wheat production, and consequently wheat trading, is variable over the years. During the 2000–2004 period wheat production in Canada, China, India, and the USA showed a decreasing trend, while in Eastern Europe, the CIS, and WANA region, the good climate conditions and/or local policies favoring investments in agriculture and wheat farming, have resulted in increasing wheat production trends (FAO, 2005; FAOSTAT, 2005).

Traditional wheat importing countries in the developing world (except Argentina, Iran, and likely India) are expected to continue importing variable amounts of wheat in the near future. China reduced importantly its wheat imports, however, in spite of its production increase in 2003/04 (FAOSTAT, 2005), and due to increased liberation of wheat market and actual decrease in the profitability of the predominantly low-scale wheat growers, this country is expected to increase its wheat imports significantly (to 7–8 million tones). In contrast Brazil, which is at present the largest wheat importer in the world, is expected to experience an important reduction in wheat imports (FAOSTAT, 2005). This is forecasted as result of the announced construction of infrastructure to redistribute wheat production area to increase production while reducing transportation costs.

The share in the export market has been experiencing some significant changes during the past few years. While the major exporting countries: Australia, Canada, and USA, as a group lost around 15–18% of their share in the export market, the Eastern Europe-CIS-Europe regional aggregate increased its participation significantly (roughly 15% of the total). The Asia wheat-exporting countries as a group also gained up to 5% more of the export market share (Table 4).

Future trends and regional shifts in wheat trading (regional exports) patterns are difficult to predict over the coming 5–10 year period in part due to uncertainties in production and trading policies (particularly those associated with future subsidies to wheat farmers and with the establishments in emerging and developing countries of efficient marketing systems), but most importantly to regional climatic conditions affecting wheat production as well as the quality of the crop.

Table 4. Distribution of wheat export market during 2000–2003a

	Distribution (% of total export market)			
Region	2000	2001	2002	2003
Aus., Can., USA	55,7	52,7	43,2	43,3
EU-15	24,9	23,7	21,3	26,2
E. Europe-CIS-Europe	2,7	6,5	19,4	11,3
Latin America	10	10,1	8	6,3
S., E., & SE Asia	0,7	3,1	4,2	6,9
Central Asia	4,3	2,7	3,3	4,8
WANA	1,5	1,2	0,6	1,1

Source: FAOSTAT, 2005; FAO, 2005)

Wheat demands in developing countries are large now and are expected to continue increasing steadily in the coming years (Table 1). Wheat consumption increased by about 5.6 million tons/year in the last decade (Carter, 2002). It is estimated that in 2020 the populations of Asia and the WANA regions will remain the main wheat consumers in the world (Table 1). This trend in wheat consumption is very important for the wheat export market, since it cannot be satisfied by local wheat production (Ekboir and Morris, 2004).

THE IMPORTANCE OF GRAIN QUALITY IN WHEAT TRADING

Present and predicted shifts in the composition and trading figures of the wheat export market are strongly associated with economical and social (agricultural reforms, rural-urban population balance, labor force composition, food habits) changes occurring in mainly developing and emerging countries. Recent increases in wheat demand have been driven mainly by population growth and redistribution of the rural-urban population; increased migration to urban areas with the resulting decrease in agricultural labor force will remain as a steady increasing trend in developing countries (Table 5). The net result of the combination of factors such as increasing population, increased urbanization and its associated changes in dietary patterns is an increasing demand of wheat with specific quality attributes to satisfy the processing requirements of diverse wheat-based foods.

As a result of deregulation of wheat trading (no government intervention) that has been occurring in emerging and developing countries during the last decade; the wheat processing industry in countries obtaining large part of their supply from the export market is becoming more efficient and versatile, and therefore requires more specific quality characteristics to produce distinct flour and food types. Increased

Table 5. Current and predicted trends in population composition in the main regions of the developing world

Regions	Farm Labor[a] (% of total labor)		Urban Population (% of total population)		
	2000	2010	2000[b]	2010[a]	2020[a]
Sub-Saharan Africa	63	58	34	40	45
East Asia & Pacific	62	55	38	46	54
South Asia	59	53	27	31	36
W. Asia & N. Africa	30	25	56	60	65
Latin America	20	16	75	79	82
Developing countries	55	49	40	46	51
Industrialized countries	3	2	79	81	83

[a] Source: FAOSTAT (2003).
[b] Source: UNICEF (2003).

competition in the milling and baking industry has forced millers and bakers to become more conscious of grain sanitation and quality, as well as to become more knowledgeable of the wheat market.

Wheat Sanitation

Wheat buyers in many cases request phytosanitary certificates to ensure that the wheat is sound and represent no risk for the consumer. Among the most important risks are (Dexter and Worden, 2005):

- Pesticide residues. Maximum limits for shipments are outlined by FAO/WHO.
- Fungi associated toxins:
  - Mycotoxins (vomitoxin, DON, fumosins). Produced *Fusarium graminearum* (Fusarium head blight, scab). Highly toxic; the limits are 500 μg/kg in grain and products and 200 μg/kg for infant foods.
  - Alkaloids produced by *Claviceps purpurea* (ergot). Variable toxicity, rate of contamination is determined by ergot body content.
  - Ochratoxin A. Produced by *Penicillium* and *Aspergillus* during storage of high moisture grain. EU-regulation limit is 5 μg/kg for grain and 3 μg/kg for wheat-based food products.
- Cadmium concentration in durum wheat. High levels of Cadmium may occur in durum wheat (not in common wheat). Cadmium concentration is controlled by a single gene and therefore breeding for low cadmium durum wheat is possible (Dexter and Worden, 2005). Maximum levels in grain have been set (Codex Alimentarius) at 200 mg/kg.

Wheat export countries implementing reliable sanitation systems that allow fulfilling grain safety regulations will be better positioned in the export market. Sanitary and phytosanitary standards should encourage developing and emerging countries to modernize their exporting infrastructure and regulatory systems to maintain or improve access to the export market.

Wheat Grading and Quality Classification

Australia, Canada, USA. Wheat grading and quality classification allows traditional wheat exporters in Canada, Australia, and to a lesser extent in the USA, to offer wheat shipments with desirable quality traits. Australia and Canada in particular, posses unique highly regulated market systems allowing these countries to satisfy very specific quality demands of buyers in the export market. These countries are able to offer wheat to correct gluten strength as well as wheat with specific characteristics fitting leavened- and flat bread production, as well as Chinese- or Japanese-type noodles

European Union. The EU-25 produces wheat with medium hard, medium strong gluten quality (mainly due to high yield potential) suitable for the manufacture of heart breads, such as the typical baguette and soft wheat for biscuits (Bonjean, 2004). Some of the EU countries (UK, France) need to import hard wheat from

Canada and the USA to improve wheat for the local mechanized baking industry (Dobraszczyk, 2004).The EU-25 wheat surplus is exported mainly to countries of the North Africa- West Asia region.

Argentina. Argentina, a long time net wheat exporter of the Southern Cone, has not yet being able to offer its wheat based on quality attributes; Argentinean wheat supply consists of blends of different quality types and therefore it gets low price in the export market (Cuniberti and Otamendi, 2005). Argentina recently established a wheat classification system based on end-use quality; the aim is to preserve or improve market share as well as to obtain better market prices. There are three quality groups: TDA1, TDA2, and TDA3, with strong, intermediate, and weak gluten types, respectively, directed to the mechanized (pan bread and buns in long fermentation process) and no mechanized (French, variety breads and crackers in short fermentation process) bread making industries of countries in the Southern Cone region (Cuniberti and Otamendi, 2005).

Kazakhstan. The main wheat exporter in CA is Kazakhstan. This country has a wheat classification system based on hardness, protein and wet gluten content. Medium hard to hard wheat with strong to weak gluten is exported mainly to countries in Central Asia and the Caucasus. The main quality problem in the Central Asia region is the poor gluten extensibility characterizing main commercial cultivars. Wheat exports from Kazakhstan are limited now but could increase by improving wheat quality, particularly gluten quality (Alexander, 2003), which is partly influenced by poor crop fertilization.

CIS-Europe. Russia and Ukraine, main exporting countries of the CIS-Europe aggregate, follow the same wheat grading system. Wheat classification is based primarily on gluten content. Class 1 and 2 are top quality classes (higher gluten content), class 3 is considered suitable for standard bread, and class 4 includes feed wheat (Alexander, 2003a; Sequeira, 2005). The gluten viscoelastic properties of wheat from the CIS countries are generally inferior for modern mechanized bread making.

Non traditional emerging and developing countries still need to improve wheat quality as well as develop reliable grading and classification systems to segregate wheat shipments based on functional properties such as hardness, protein content and gluten quality. This is necessary to offer value-added wheat shipments and compete with fewer disadvantages with the main wheat exporters. However, Australia, Canada and USA, may give one step ahead by offering identity preservation of wheat varieties with specific and uniform quality attributes.

Some grain attributes which may add value to wheat exports shipments are:

- **Grain characteristics**. Vitrousness, soundness (no sprout damage), hardness, color, and protein content (additionally, yellow pigment content in the case of durum wheat).
- **Flour properties**. Gluten strength and extensibility, starch paste viscosity.

Breeders from emerging and developing wheat-exporting countries cannot longer ignore wheat quality improvement. Even more, achieving yield and quality stability across environments, and tolerance to abiotic stresses (heat, drought, winter-kill, and

sprouting-promoting conditions) and resistance to diseases (Fusarium, Sunny pest, among others) affecting directly grain quality, need to be their combined objective.

THE IMPORTANCE OF GRAIN QUALITY IN WHEAT-PRODUCING DEVELOPING COUNTRIES

For some wheat producers (particularly those living in developed countries) wheat farming represents a profitable business, while for many others living in developing countries it represents subsistence and some times the main source of nutrients. In the latter case, if wheat imports increase and local production decreases, then more rural to urban migration will occur. Therefore, and in order to maintain wheat production in agreement with rural to urban composition, wheat-producing countries need to become more efficient in developing wheat cultivars that combine high productivity with desirable quality attributes. In this way farmers could have the opportunity of using part of the crop to prepare traditional foods and the bulk of it as a cash crop satisfying the quality demands of the market. In countries were most of the workforce is dependent on agriculture, improvement in productivity of the crop and of the land, building rural infrastructure, and government support for accessing credit and extension services are necessary for ensuring food security and for improving the livelihoods of small-scale farmers.

In the developing world, wheat imports are usually directed to satisfy the needs of the processing industry and concomitantly the end-product quality demanded mostly by the urban population. In contrast, local wheat production is aimed to satisfy the needs of both, rural and urban populations. The wheat quality requirements to prepare (household or village levels) acceptable traditional wheat-based foods, (leavened and flat breads; flour noodles regional dishes) in the rural areas are different to those required to prepare the same products at the industrial level. Better dough properties and end-product quality uniformity are usually required in the latter case.

Consumption of traditional foods is still very important in the world, but especially in rural areas of countries in Asia, WANA and LA. Some examples:
Wheat Quality in China. Across East and Southeast Asia, rapid economic growth and urbanization are creating significant changes in dietary patterns. As incomes rise, households tend to increase their consumption of wheat, especially in the form of convenience foods (fast, ready-to-eat foods and frozen, ready-to-heat/boil foods). As a result of these trends and population growth, wheat demand in Asia is projected to rise significantly (Table 1). In recent years, China has experienced a gradual change from a centrally planned to a more market-oriented economy. At present most wheat is consumed by the rural population, which accounts for 65% of the total population (FAO, 2004). Rural households are able to adjust ingredients to satisfy the processing attributes of handmade breads and noodles. However, rapidly rising populations and intense rural-to-urban migration are exerting pressure on the wheat-based food supply in the cities, particularly of noodles and breads (steamed and oven-baked) supplied by the industry and supermarkets. One problem facing

farmers and the industry is that the shift to a more market-oriented production system has affected the acceptability of locally produced wheat, traditionally low and inconsistent in quality attributes. The processing industry requires the improved quality needed for mechanized dough making and baking or steaming. Currently high quality flour accounts for about 10% of demand, but is expected to increase to as much as 50%, as more high quality varieties become available (Alexander 2003b). As the wheat milling industry was able to purchase wheat on the export market, it determined grain quality characteristics to satisfy the baking processes and established quality standards for local wheat.

According to Wang et al (2004), the predicted flour use in 5–10 years should shift as follows: noodles and steamed bread, from 80 to 76%; fried and roasted products, from 12 to 8%; and baked products, from 8 to 16%. At present, 2 million tons of imported wheat is used to produce baked bread; in 10 years, the requirement could rise to 4.0–5.0 million tons. Wheat imports are used mainly in coastal areas with large milling capacities. Mills in those areas are increasing the production of flours with specific attributes (Wang et al, 2004). Other trends are decreased household use of flour; increased mechanized production and restaurant use of flour; frozen and non-frozen foods purchased in supermarkets (noodles and steamed bread); and widespread use of additives (Wang et al, 2004). If wheat production is to become sustainable, quality must improve; production costs must be reduced; and realistic, reliable quality standards need to be established (Wang et al, 2004). Breeding programs in China are responding by developing new varieties with improved quality traits (hardness, protein, sprouting, polyphenol oxidase, PPO, and starch quality).

Wheat Quality in India. Indian agricultural policy has centered on ensuring the supply of basic staples at a low price, mainly by providing minimum support prices for food grains and protecting the industry from foreign competition. India is now the second largest wheat producer in the world (Misra 2004). India exported wheat to 30 countries, thus becoming the seventh largest exporter in the world (Misra 2004). However, the big challenge is yet to come, since India is predicted to require approximately 109 million tons by 2020 to feed its rising population. Agriculture is vital to rural livelihoods in India because 72% of the population lives in villages (FAO, 2004). It is estimated that 85% of wheat in India is consumed in the form of *chapati*, an unleavened, flat, circular bread. *Chapati* is essentially homemade. Bread wheat *chapati* is made from *atta*, whole-meal flour (wheat is ground by stone mill), although more recently small mills in suburban and urban areas, and large industrial mills in the cities provide a large proportion of the *atta* used to make *chapatis*, mainly for convenience, particularly of household wives. Besides *chapatis*, other food types, which also have an increasing demand, are produced and consumed in large suburban and urban areas. Approximately 1.1 million tons of ready-to-eat products (hamburger, pizza, and flour noodles) and biscuits are produced per year (Misra 2004). Wheat varieties usually have large and plump grain; wheat crops with high (>13.5%) and with low (<10%) protein contents are available to satisfy the requirements for specific products.

Although varieties currently grown in India satisfy quality requirements for making traditional foods, the increasing consumption of non-traditional wheat-based foods in urban areas and the range of quality types required for the export market make it necessary to determine the specific quality requirements for different food types. In response to the increasing demand for specific wheat quality types locally and on the export market, breeding programs are directing efforts towards enhancing quality, especially protein quantity and gluten quality (gluten strength is the major quality criterion in India) (Misra 2004). To suit processing needs, Indian wheat varieties have been classified into different categories based on protein content and gluten strength. Systematic separation of each class into grades has shown encouraging results. Also, areas where a variety could reach the optimum expression of its quality traits for a given product are being identified. Farmers are being encouraged to grow product-specific varieties through contractual farming, and some millers have started making payments based on protein content and test weight. In India the following quality types have been defined: Indian Hard White/Amber wheat, with protein above 13% and strong, extensible gluten is recommended for making good quality leavened breads (pan-type, two-layered flat, hamburger buns), vermicelli, and porridge; Indian Medium Hard White/Amber wheat, with 10–12% protein, low PPO activity, and medium-strong, slightly extensible gluten is recommended for making *chapati* and other flat breads (tandoori, nan, and parantha), crackers, flour noodles, and sweet traditional dishes; Indian Soft White/Red wheat, with les than 10% protein is good for making high quality biscuits (cookies); Indian Durum wheat, protein content above 12%, strong gluten, high yellow pigment content, and a high percent of vitreous kernels, is recommended for making good quality pasta products as well as sweet and salted porridge. Durum wheat contributes around 3% to total wheat production in India.

West Asia/North Africa (WANA). Wheat production in the WANA region exceeds 52 million tons while imports are roughly 20 million tons (FAOSTAT 2005). By 2020, wheat production and demand (or supply) are expected to reach 47–48 and 84–85 million tons, respectively (Ekboir and Morris 2003). Consequently, satisfying wheat demands in the region will require relying heavily on imports. Over the past decade several countries in WANA have gradually initiated globalization by liberalizing some sectors, including the milling and baking industries (Alexander 2001, 2002a,b; 2004; Troxel 2000, Wylie 2000). As a result, flour trading within and outside the region has become very dynamic (Troxel 2000, Wylie 2000). Flour with specific quality attributes for non-traditional, western-type convenience foods and for home baking of pre-formulated mixes and frozen dough is starting to gain interest in the milling and baking industries of West Asia (Troxel 2000). Wheat is the main cereal crop in West Asia, where it is consumed in large quantities (170–230 kg per capita), mainly as yeast-leavened bread. Dense breads with high satiation capacity are actually the main component of a meal among the resource-poor rural population. Egypt produces soft white wheat, most (66%) of which is used for direct consumption on-farm (Alexander 2001). European-style bread is very popular in North Africa; it may account for 15–30% of total flour use. However, flat

breads are the preferred type of bread, with 40–70% of flour used to produce them (Alexander 2001, 2002a). Flat breads can be single-layered (Arabic tanoori, Iranian sangak) or double-layered (e.g., the Egyptian baladi and Arabic or pita bread). All flat breads are made from viscoelastic doughs prepared with 80–95% wheat flour or with composite flours that combine wheat and other cereal grains (e.g., maize in Egypt). Most flat breads remain ethnic foods made at home using traditional clay or brick ovens, or cooked on a hot clay or metal plate. Others fit the needs of the fast food and/or convenience food industry (e. g., baladi and pita breads). Durum wheat flour, alone or blended with other flours, is widely used in Mediterranean countries for making dense leavened breads (Quaglia 1988). In the WANA region, 50–60% of all durum wheat is consumed as single- or double-layered flat bread, and as dense, leavened bread (Williams 1985. Couscous (steam-cooked granules of agglomerated semolina) and burghoul (durum grain is soaked and then boiled in the excess water, then dried, rubbed to remove the bran and cracked to produce coarse pieces) are two of the most important traditional foods in the WANA region (Williams 1985, Qaroonі 1994). Large-scale automated production of couscous and burghoul is found in large urban areas in the WANA region and the USA (Qaroonі 1994). Durum burghoul has a characteristic yellow colour and hard texture not obtainable with common wheat. Regional breeders, aiming to develop good quality European-style leavened and flat breads, target white, hard-to-medium grained wheat with high milling extraction and intermediate flour protein (10–13%) (Wrigley 1991). Hard-to-medium-hard wheat with medium-strong and extensible gluten is suitable for flat-type breads such as the two-layered Arabic baladi, and the single-layered tanoori (Qaroonі 1996).

Wheat Quality in Latin America. Argentina, Brazil, and Mexico together produce 87% of all wheat produced in Latin America. Argentina is self-sufficient and the only net wheat exporter in the region; Brazil and Mexico export 1.2 and 0.6 million tons of bread wheat and durum wheat, respectively. The milling industry in LA imports wheat from Argentina (6 million tons), USA (7.7 million tons), and other countries (Canada, France, Russia, Ukraine, and Australia) (5.3 million tons). In Latin America wheat is consumed mainly as European-style leavened breads (both salty and sweet) prepared mostly in small bakeries, although supermarket bakeries are producing an increasingly bigger share. Pan-type bread and hamburger/hot dog buns follow in importance, and then alimentary pasta. Wheat consumption is associated mainly with industrialized food production serving both rural and urban populations. Because wheat is produced mostly as a cash crop, very little is consumed on-farm. The milling industry is grouped in the Latin American Milling Association. The association provides the means for sharing experiences and technology, and promotes business among milling enterprises to help make the sector more efficient, modern, and profitable. In Argentina, Brazil, and Mexico grain quality classification system is based on gluten strength types; strong, medium and weak gluten types are directed to mechanized, semimechanazied, and non mechanized bread making processing, respectively. In wheat producing countries of LA, grain quality is in general being improved through attention to genetic

factors associated with grain hardness, gluten strength and extensibility, and grain sprouting, particularly in southern Brazil and Uruguay, where getting hard, plump, non-sprouted grain is difficult.

WHEAT QUALITY IN HEALTH AND NUTRITION

Globally more than three billion people are affected with micronutrient deficiencies resulting in problems with human health, mental development and productivity (Kennedy et al, 2003). Vitamin and mineral deficiencies affect mainly children and pregnant women. Malnutrition is not limited to the poor; poor nutrition leads to health problems caused by nutrient deficiency (undernourishment), eating too much (overnourishment) or eating unbalanced meals. There are health and nutritional issues associated with wheat consumption. In this paper, the health issue is addressed in relation to overnourishment leading to obesity-related health problems, while nutrition is treated in the context of undernourishment associated with insufficient consumption of nutrients or with unbalanced diets.

Health

Change in food habits of mainly the urban population, particularly the increasing consumption of calorie- and fat-rich foods in conjunction with a decrease in the consumption of fiber-rich vegetables and fruits, is causing increasing health problems associated with obesity (hypertension, coronary disease, diabetes, etc.). Although the problem of obesity has been associated with the consumption of cereal products, particularly with refined wheat flours, it is true that it is not the consumption of wheat flour but the large amounts of food consumed, the unbalance of the diet, and the low activity of the individual what causes weight gain. Foods that control blood sugar levels (low absorption of simple sugars) are associated with delayed hunger. Feeling satiation after a meal may reduce daily calorie intake. Future wheat-based product development is expected to focus on satiation without sacrificing nutrition. In this context, the ability of resistant (to digestion) starches (high amylose starch) and fiber (Erickson, 2005) used as low-carbohydrate ingredients (Patil, 2004) to produce satiation and therefore reduce food intake, is expected to play an important role. Promoting increases in consumption of whole wheat flour-based foods, fortified and added with fiber is one way to reduce obesity-associated health risks.

Nutrition

More than 800 million people do not have enough food to meet their basic daily energy needs. Women and children in the less developed countries are particularly vulnerable to diseases, anemia, impaired cognitive abilities, and premature death associated with the consumption of diets poor in micronutrients such as Vitamin A, iodine, iron and zinc. Wheat flour fortification with Fe, Zn, folic acid, and vitamins

is a simple and effective way to make impact in the nutrition of the consumer; flour is fortified in 63 countries and some others are being encouraged to follow (Lyddon, 2004). An alternative initiative recently launched as the Harvest Plus Challenge Program is that enhancing the micronutrient concentration of, among other crops, wheat. The still large use of whole wheat flour or high extraction flours as the main source of nutrients, particularly in the rural populations of WANA, CAC, and SA regions, makes it feasible to enhance the nutritional value of wheat-based foods consumed by the more vulnerable (rural- and urban-poor) part of the population. The highest levels of zinc and iron in wheat come from landraces and wild relatives (*Triticum dicoccon* and *Aegilops tauschii*). These sources are used in conventional breeding to try to incorporate high levels of micronutrient in new wheat cultivars. In the first phase of the program wheat biofortification was targeted to Pakistan and India.

REFERENCES

Alexander M (2001) Focus on Egypt. World Grain 19(3):32–36
Alexander M (2002a) Focus on Iran. World Grain 20(9):16–21
Alexander M (2002b) Focus on Morocco. World Grain 20(10):18–22
Alexander M (2003) Focus on Kazakhstan. World Grain 21(6):18–23
Alexander M (2003a) Focus on Ukraine. World Grain 21(3):18–23
Alexander M (2003b) Focus on China. World Grain 21(1):16–21
Alexander M (2004) Focus on Saudi Arabia. World Grain 23(9):18–23
Bonjean A (2004) Update of Wheat Industry in European Union. In: Unpublished oral presentation in, International Wheat Quality Conference. From Molecular Improvement to Consumers Needs. May 29–31, 2004. Beijing, China (Abstarct book, p 10)
Carter AC (2002) Current and future trends in the global wheat market. In:. Ekboir J (ed) CIMMYT 2000–2001 World Wheat Overview and Outlook: Developing No-till Packages for Small-Scale Farmers, CIMMYT, Mexico, DF, 45–51
Cuniberti M, Otamendi M (2005) Classification of Argentinean wheat. In: Chung OK, Lookhart GL (eds) Third International Wheat Quality Conference. May 22–26, 2005, Manhattan KS, USA. Grain Industry Alliance, Manhattan, KS, USA, pp 333–340
Dexter JE, Worden GC (2005) Trends in demand for wheat. In: Chung OK, Lookhart GL, (eds) Third International Wheat Quality Conference. May 22–26, 2005, Manhattan KS, USA. Grain Industry Alliance, Manhattan, KS, USA, pp 309–321
Dobraszczyk B (2004) New methods for discrimination of wheat baking quality based on rheological and polymer molecular structure-function relationships in gluten. In: Unpublished oral presentation in, International Wheat Quality Conference. From Molecular Improvement to Consumers Needs. May 29–31, 2004. Beijing, China (Abstarct book p 28)
Ekboir J, Morris M (2004) Projected trends in supply and demand of wheat and maize. In: CIMMYT (International Maize and Wheat Improvement Center). 2004. Global Trends Influencing CIMMYT's Future. Prepared by the Global Trends Task Force in support of Strategic Planning at CIMMYT. CIMMYT, Mexico, DF, pp 27–34
Erikson W (2005) The baker's toolbox for developing health-promoting products. Cereal Foods World 50:6–8
FAOSTAT (2002–2005) www.fao.org/
FAO (2004) The State of Food and Agriculture. FAO Agricultural series No. 35, p 169
FAO (2005) Food Outlook, September 2005, www.fao.org/giews/
Kennedy G, Nantel G, Shetty P (2003) The scourage of "hidden hunger": global dimensions of micronutirent deficiencies. Food, Nutrition and Agriculture 32:8–16

Lyddon C (2004) Flour millers join to fight malnutrition. World Grain 23(9):42–45
Misra BK (2004) Wheat in India. Unpublished working paper, Indian Agricultural Research Institute, New Dehli-110012, India
Patil SK (2004) Resistant starches as low-carb ingredients—current application and issues. Creal Foods World 49:292–294
Qarooni J (1994) Historic and present production, milling and baking industries in the countries of the Middle East and North Africa. Special Publication of the Department of Grain Science and Industry, Kansas State University, Manhattan
Quaglia GB (1988) Other durum wheat products. In: Fabriani G, Lintas C (eds) Durum Wheat: Chemistry and Technology. Am. Assoc. Cereal Chem.: St. Paul, MN, pp 263–282
Sequeira M (2005) Focus on Russia. World Grain (January, 2005) 23(1):16–20
Troxel M (2000) Regional review: The Middle East. World Grain 18(8):38–44
Wang S, He ZH, Zhou Y (2004) Update of wheat industry in China. Unpublished oral presentation in International Wheat Quality Conference. From Molecular Improvement to Consumers Needs. May 29–31, 2004, Beijing, China (Abstract, p 7)
Williams PC (1985) Survey of wheat flours used in the Near East. Rachis 4:17–20
Wylie S (2000) An oasis. National flour mills in UAE leads the manufacturing revolution in the Arabian Gulf. World Grain 18(8):46–50

MITIGATING THE DAMAGING EFFECTS OF GROWTH AND STORAGE CONDITIONS ON GRAIN QUALITY

C.W. WRIGLEY

Wheat CRC and Food Science Australia, PO Box 52, North Ryde 1670 (Sydney), Australia
E-mail: Colin.Wrigley@csiro.au

Abstract: Today's grain market requires growers to maximize both yield and processing quality. Wheat breeding, the first focus in working towards these two objectives, promises the delivery of yield-related advantages, as well as providing varieties "tailor-made" for the various downstream processing requirements. Growth and storage conditions are next in determining grain quality at the flour mill. While we may have no control over many aspects of growth environment, the damaging effects may be mitigated, for example, by attempting to predict and forestall the risk factors, and by breeding as a means of "building in" genetic tolerance to environmental hazards. Significant loss of dough strength can be caused by heat stress, i.e., a few very hot days (> 35 °C) during grain filling. Nevertheless, some genotypes are less affected than others, opening the possibility of selecting for tolerance to the dough-weakening effects of heat shock. Growers have the added opportunity of using statistical weather data to sow early enough to reduce the risk that heat stress may reduce both yield and quality. Buyers may also use information about climate fluctuations to select regions with grain of suitable quality. During prolonged storage, on the other hand, elevated temperatures appear to cause an increase in dough-strength potential. In this case, grain-moisture content is an important factor, interactive with storage temperature. Adequate water in the intermediate growth stage of the plant may affect the outcome of grain-protein content. On the other hand, rain at harvest leads to the risk of sprout damage, and the storage of moist grain is an obvious source of spoilage. Plant nutrition is a basic aspect of growth environment that affects grain quality. The economics of fertilizer use may be based on grain-yield considerations, plus maximizing protein content and thus premium payments. As a result, nitrogen fertilizer may be used, but in excess, this action may cause sulfur to be limiting, leading to a loss of dough-forming quality (especially extensibility). A test of N:S ratio in harvested grain can serve as an indicator of sulfur deficiency, a ratio of over 17:1 being considered abnormal. Finally, the approach of precision agriculture may offer possibilities of overcoming quality losses due to growth conditions. It offers the opportunities of applying variable inputs of fertilizer and of selective harvesting to optimize grain quality

Keywords: environmental effects, heat tolerance, protein content, dough strength, storage conditions, milling quality, nitrogen, sulfur

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 425–439.

INTRODUCTION

The specification of variety is the basis of segregation for grain quality in many wheat-growing countries. Generally, a small group of varieties is specified because their quality range is expected to satisfy the market requirements for each specific wheat grade (Cracknell and Williams 2004). This approach thus defines the genetic basis of grain quality (the genotype, "G"), as built in by the breeder. However, growth conditions (the environment, "E") are likely to cause significant changes to this genetic potential, with the result that the precise quality of the harvested grain is difficult to predict, depending on regions and seasons of growth. Storage conditions also affect grain quality. In addition, interactions occur between genotype and environment ('G X E'), due to the different reactions of specific varieties to the environment, some varieties being more tolerant than others to extremes of growth or harvest conditions.

Critical to the final quality of wheat-based foods are the wheat grower's management of grain production and growth-environment factors, plus contributions relating to harvesting, transport and storage, even before the miller contributes by turning the grain into flour. Grain quality at the mill is thus the result of the interaction of genotype with environment (G X E), involving all the environmental factors from sowing to delivery to the mill. The G X E interaction is potentially different for each aspect of grain quality, as is shown in Figure 1 by the position of the various quality attributes across the G X E width of the diagram.

Obviously, the variety of wheat sown makes no contribution to the presence of weed seeds or stones in the harvested grain, nor to the possible presence in it of pesticides; these factors relate solely to growth conditions and management. Thus "Contaminants" and "Pesticide residues" appear at the extreme right of Figure 1. On the left-hand side, "Grain hardness" is shown to be primarily determined by variety. Genotype makes a significant contribution to "Milling quality" (it is thus

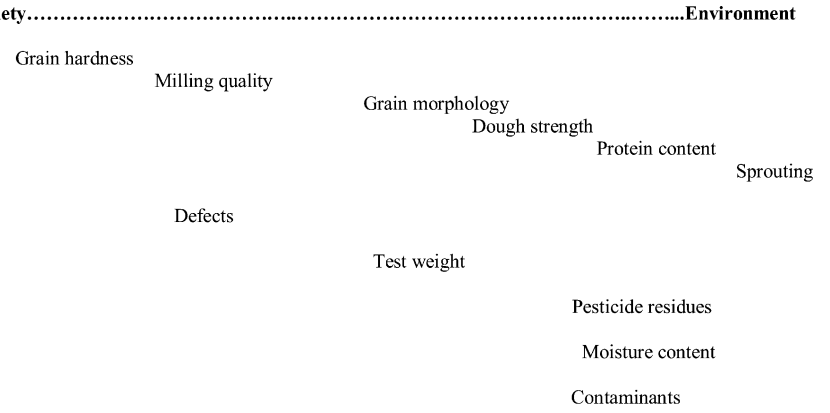


Figure 1. Grain-quality attributes, listed in position from left to right, based on the relative influences of genotype and growth environment on each attribute. Adapted from Wrigley and Batey (2003)

well to the left of the diagram), but if there are stress conditions, the environmental influence would be greater than is indicated by this position in the diagram. Grain size and shape ("Grain morphology") may be used for varietal identification, but significant environmental influences reduce the potential value of this approach.

"Dough strength" (mid-way in Figure 1) can be considered to have significant contributions from both variety and growth conditions, as those listed in Table 1. While analysis of variety is certainly important, the extent of the contribution of growth environment depends on how extreme are the growth conditions in a specific region and season. Additionally, there is the possibility that the variety involved may have a measure of tolerance to the particular growth stress. Thus, the breeder may be able to help in mitigating the detrimental effects of environmental factors. Even though breeding cannot have any influence over growth environment, it is possible for the breeder to select for tolerance to environmental stresses. An example is tolerance to pre-harvest sprouting, which for some varieties means that rain at harvest does not cause a rise in alpha-amylase activity as would be expected for many varieties. This presentation reviews some of the published literature on these topics, plus focusing on specifically Australian research findings and crop statistics.

Table 1. Environmental factors (abiotic) known to affect grain quality and dough strength

Environmental factors	Likely effects on quality	Possibilities for mitigation of damaging effects
1. Plant nutrition-Nitrogen	Variations in protein content and thus in processing quality and in market value	Tissue testing to plan N-fertilizer use Crop rotation with legumes Predict % protein by climate
2. Plant nutrition Sulfur	High N:S ratios relate poor extensibility and lower baking quality	Test N and S in grain to determine possible sulfur deficiency Use of S-containing fertilizers
3. Plant nutrition Micronutrients	Poorer quality possible for Cu deficiency. No agreed outcome for other micronutrients	Fertilizer use, after soil and grain testing
4. Modest temperature variations (15–30 °C)	Increases expected in dough strength with temperature rise in this range	Choice of growing region, based on expected growth temperatures
5. Heat stress (a few days of maxima > 35 °C)	Higher grain-protein content Significant dough weakening, depending on genotype	Select for genotypic tolerance Predict dough-weakening based on climate details and genotype
6. Rainfall during winter and at harvest	Lower grain-protein content Sprout damage	Predict changes in protein content Select for genotypic dormancy Test before harvest for amylase
7. Drought (several days of water stress during grain filling)	Higher grain-protein content, but probably little change in protein quality	Irrigation; select for genotypic tolerance

PHYSICAL AND MILLING QUALITY

Various types of stress factors are potentially detrimental to all aspects of grain quality. Stresses are most likely to involve combinations of heat and drought, but they also relate to biotic stress involving pathogens and disease, and competition from weeds. Frost at flowering is a likely cause of empty florets and shrunken kernels, plus the possibility of elevated alpha-amylase levels for these kernels. Stress conditions produce low test weights and extreme levels of screenings (e.g., tested as the percentage of grains that pass through the 2-mm-wide slots in a sieve), due to many of the florets being poorly filled and due to many grains being shrunken, narrow and light weight (Sharma and Anderson 2005). The presence of small grains results in less endosperm being available; consequentially, milling quality and flour yields are reduced (Posner and Hibbs 2005). The level of screenings is influenced by season, crop management and perhaps also, to genotype.

To a limited extent, management can help to minimize the effects of stress on physical aspects of grain quality (Anderson et al. 1995, Kettlewell 1996). This may involve late planting of a variety with longer maturity to avoid frost at flowering; irrigation, if available, would mitigate the stress of drought. Grain from extremely stressed plants is generally down-graded due to low test weight or high screenings; as a result, such deliveries are unlikely to be accepted into a milling grade. Thus, other quality attributes would not be relevant if the grain is relegated to feedbreak grade.

GRAIN-PROTEIN CONTENT

Soil nutrition has an obvious effect on grain-protein content, mainly relating to nitrogen fertilizer (Table 1). For example, the manipulation of nitrogen fertilizer was a significant factor in the success, a few years ago, in making it possible for high-protein grain of the Prime Hard grade to be grown in the southern NSW region of eastern Australia (Allen 1999), where grain of only modest protein content had traditionally been grown. The timing of fertilizer application (in relation to rainfall) was as important as the amounts used and other such cases (Wurst 1999). Another Australian example of managing nitrogen fertilizer is the need to target a protein "window" of 9% to 11% for the noodle grade in Western Australia (Sharma and Anderson 2005). Fertilizer application has been effectively complemented by nitrogen fixation using crop rotation with legumes, such as lupins and pea crops. Protein content is a major factor in determining wheat prices, being well known to contribute to dough properties. The ability to predict the protein content of the crop prior to harvest is potentially valuable for the forward marketing of grain, for planning the allocation of storage areas, and to facilitate the logistics of transport and sea-board loading.

The influence of climatic fluctuations on protein content were studied by Correll et al. (1994) in a statistical survey of the protein content of silo receivals of wheat and of barley in South Australia over several seasons. Whereas environmental factors

during grain filling might be expected to be the most critical, the modeling exercise showed that rainfall during winter (May to September in Australia) was a significant factor together with spring heat (as days > 30 °C in October and November). The model narrowed the prediction for the protein content of grain being received at a specific silo from ±2%, based on the site mean, to ±0.8%, based on the climate conditions of the specific season. Thus, as a season progressed, the prediction could be narrowed for a specific silo, as climate data could be incorporated into the model right up to the time of harvest.

PROTEIN QUALITY

"Protein quality" is important when the grain proceeds into most forms of processing, although the "single number" of protein content is critical to determining wheat classes and in marketing. Protein quality determines the dough properties needed in different forms for the many diverse foods made from wheat flour – bread (by various processes), noodles (many forms), steamed breads, Arabic flat breads, pastries, cookies and starch-gluten manufacture. This attribute is more difficult to quantify than the amount of protein in the grain, yet it is of greater practical value to the food processor. Table 1 lists major environmental factors likely to alter the genetic potential for grain quality.

PLANT NUTRITION

Sulfur deficiency has been shown to reduce baking quality, especially for crops grown in Europe. Surveys of grain grown in Britain during the 1980s and 1990s showed that there has been a gradual fall in the sulfur status of the harvested grain and thus of the soils (Zhao et al. 1995). As a result, sulfur deficiency has been blamed in some regions for poorer bread-making quality (Byers et al. 1987, Haneklaus et al. 1992). Sulfur deficiency has been attributed to excessive use of nitrogen fertiliser, to improved control on air pollution in industrial regions (thus reducing the adventitious provision of sulfur from the air, e.g., Europe), and to the use of fertilizers that lack sulfur, such as anhydrous ammonia and urea. In Australian field trials, sulfur deficiency has been demonstrated to impair dough properties extensibility (Moss et al. 1983, Randall and Wrigley 1986).

Indications of sulfur deficiency may be obtained by the analysis of sulfur and nitrogen levels in the harvested grain. Admittedly, this diagnostic test of harvested grain provides results "after the event", when it is already too late to remedy the deficiency for that crop, but in time to rectify the deficiency before the next planting. If the level of sulfur in the harvested grain is lower than a ratio of nitrogen-to-sulfur of 17:1, there is the indication that sulfur is limiting in the soil, and that the grain is likely to produce dough with poorer grain quality than would be expected for the particular combination of variety and protein content (Randall and Wrigley 1986). This situation also indicates that sulfur-containing fertilizers are needed before wheat is grown again at this site.

A range of micronutrients, especially copper, phosphorus and potassium, may also be needed to maximize grain yield, but there is no general agreement that these elements are critical to grain quality.

MODEST TEMPERATURES DURING GRAIN FILLING

Wheat is a temperate cereal, for which the ideal growth temperatures are often quoted as being 15–25 °C. Occasional literature reports of "increased temperatures" having strengthening effects on dough properties must be examined carefully, because in some regions, "high temperatures" mean up to 30 °C. In Australian glass-house studies, Randall and Moss (1990) reported that increases up to 30 °C in daily mean temperature during grain filling generally increased dough strength for cv. Olympic wheat. In support of this general conclusion, grain of the variety Condor, grown in New South Wales (central eastern Australia), was observed to give consistently greater dough strength, compared to samples grown in cooler regions further south (Victoria). This result was attributed to the effect of the warmer growth conditions in NSW (Archer and O'Brien 1987).

In Europe, progressive increases in dough strength have been associated with progressively rising temperatures in the range of 15–35 °C for daily maxima during grain filling (Schipper et al. 1986). Uhlen et al. (1998) also demonstrated the positive effects on dough strength of increasing temperatures in the range 9–21 °C during kernel development. Corbellini et al. (1998) demonstrated similar dough-strengthening results for both bread and durum wheats (four varieties of each at four sites in Italy) as a result of growth temperatures well below 30 °C, compared to warmer growth temperatures (maxima of 30–35 °C) (Figure 2). However, plants subjected to temperatures in the range 35–40 °C, even for short periods, revealed a lower P/L ratio (a weakening of dough properties).

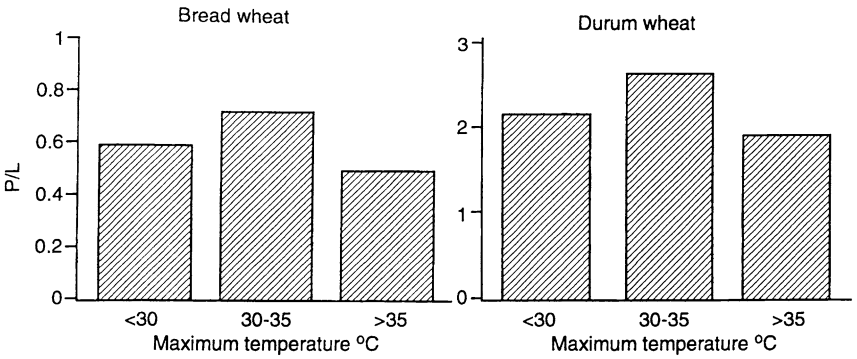


Figure 2. Mean dough strength results (Alveograph) for four varieties of bread wheat and four durum varieties grown at four sites in Italy, with sowing times and sites selected to provide the three growth-temperature ranges indicated. Adapted from Corbellini et al. (1998)

HIGH TEMPERATURES DURING GRAIN FILLING

Deleterious effects of high temperatures during grain filling on grain quality have been reported by many authors for wheats (both hexaploid and durum) grown in different parts of the world (e.g., Ciaffi et al. 1996, Corbellini et al. 1998, Graybosch et al. 1995, Stone and Nicholas 1996). A very early report of Finney and Fryer (1958) for growth conditions in the American mid-west stated: "Loaf volume and mixing time decreased with accumulated degrees Fahrenheit above 90 °F (> 32 °C) during the last 15 days of the fruiting period." Their comment that this association was "51 to 84%, depending on variety" alludes to the subsequent finding that there is naturally occurring tolerance to the effect of heat stress on dough strength. It is experimentally difficult to study the effects of growth temperatures, especially when it is clearly more significant to do so in the field; inevitably, expected heat conditions do not eventuate, or perhaps both control and test crops experience heat stress. Nevertheless, several sources of evidence have been reported.

Anecdotal Evidence

In Australia, there have been many 'anecdotal accounts' that high temperatures have been the basis of quality problems. Some of these have been described in the introductions to conference papers, e.g., by Blumenthal et al. (1990). The need for a solution of this type of problem is epitomized by statements from millers and bakers, such as: "We can cope with many quality problems, but we do not like "surprises" – unexpected fluctuations in quality; for example, when the combination of variety and protein content gives unexpected dough properties." In many of these cases, the root cause has later been attributed to heat stress during grain filling.

Long-term Statistics

The dough strength (as Rmax) of Prime Hard wheat in eastern Australia has been shown progressive decreases according to the degree of heat stress in those in seasons, based on analyses of crop reports over 29 years (Figure 3) (Blumenthal et al. 1991a). In this case, heat stress was determined as the cumulative hours over 35 °C during the grain filling period at three grain-receival sites.

Field Trials

Observations of heat have often been possible by planting varying the date of sowing. Thus, different crops of the same variety may be grown at the same site; the early sown crop provides control grain, whereas the later-sown crop has experienced heat stress. This was the approach taken by Corbellini et al. (1998) to obtain the result (Figure 2) that growth temperatures of > 35 °C caused dough weakening. Similar results were reported by Graybosch et al. (1995) for 30 hard red winter wheats grown in 17 Nebraska environments in 1990 and 1991. Protein

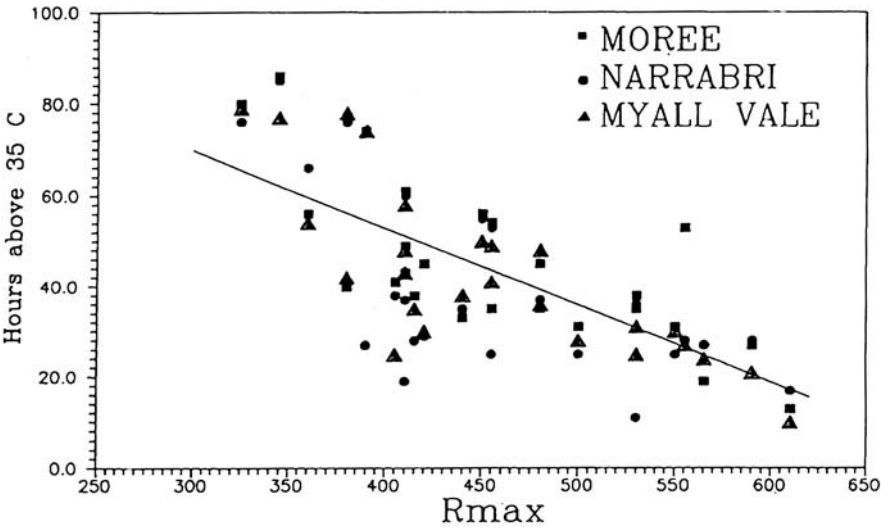


Figure 3. Variations in dough strength (as Rmax) with heat stress (as cumulative hours > 35 °C) for crops of Australian Prime Hard wheat over 29 successive seasons. The correlation coefficient is −0.79 ($P < 0.001$). Adapted from Wrigley et al. (1994a)

quality (SDS sedimentation and protein composition) was highly influenced by growth temperatures, with quality increasing with exposure to daily maxima over 32 °C for less than 60 hours. Beyond this level of heat exposure, protein quality fell dramatically. Heat-shock episodes in late November, at four sites near Narrabri (northern NSW, Australia), were observed to cause considerable losses in dough strength for crops of three Prime Hard varieties (Table 2) (Blumenthal et al. 1991b). The loss was greatest for the latest-sown trials, which were still immature (dough stage) at the time of the heat episode. Heat shock caused similar results for crops of four Australian varieties at several sites (Blumenthal et al. 1991a). Grain, early sown

Table 2. Loss of dough strength (as Rmax) with stage of grain filling due to heat-stress episodes, starting 28 November near Narrabri, NSW. Adapted from Blumenthal et al. (1991b)

Variety (*HMW alleles*)	Rmax for grain harvested before 28 Nov (Mean of 4 sites)	Rmax for grain from plants near harvest ripeness at 28 Nov (Mean of 2 sites)	Rmax for grain from plants at 15–35% grain moisture at 28 Nov (Means of 4 sites)
Stage of heat stress:-	No heat stress	At late maturity	At mid grain filling
Songlen (*a b,f a*)	391	300	227
Cook (*a b a*)	438	350	211
Kite (*b i a*)	419	343	216

and early harvested, escaped an episode of heat stress (28 October to 1 November) with daily maxima of 36°, 39°, 39°, 37° and 37 °C.

Another instance of heat stress in the field occurred for crops from field trials of the Plant Breeding Institute of the University of Sydney, Narrabri (Table 3). Only one of two crops had heat stress (four consecutive days of > 35 °C. Dough weakening due to heat shock was much more evident for the first three of the varieties listed in Table 3, than for the variety Sunstate, which showed a degree of tolerance to the dough-weakening effects of heat shock. Table 3 shows results only for Rmax, but a broader range of testing showed that the heat episode had greatly reduced dough properties for the three susceptible varieties based on several other measures of dough quality. The losses of dough strength were matched by the biochemical result of lower % 'unextractable' polymeric protein, indicating a reduction in the proportion of large polymeric glutenin.

Glass-House and Growth-Cabinet Experiments

Because of the difficulties in controlling field trials, researchers have conducted experiments in the glass-house and in growth-cabinets, where combinations of temperature and moisture availability can be closely controlled. The research of Randall and Moss (1990) centered on glass-house grown plants of three varieties; temperatures above 30 °C produced grain that showed weaker doughs than the control samples. In contrast to field-grown crops, the small amounts of grain produced in glass-house experiments make it difficult to perform reliable dough tests.

The development of a direct-drive two-gram Mixograph has facilitated the analysis of small flour samples from growth cabinet experiments, such as the mixing curves shown in Figure 4. For both varieties shown, the heat-stressed samples had slightly higher protein contents. Halberd is shown to be tolerant to the effects of heat stress on dough properties. In contrast, the susceptibility of cv. Ella is shown by its shorter time to peak resistance and the rapid breakdown after the peak for the heat-stress mixing curve. These two sets of Mixograms exemplify the wider range of genotypic reactions to the effects of heat shock on dough quality shown for similar experiments with 44 bread-wheat varieties (Blumenthal et al. 1995a).

Table 3. Loss of dough strength (as Rmax) for grain harvested with and without three days'heat stress, at two sites in northern NSW (Spring Ridge and North Star, respectively)

Variety (*HMW alleles*)	North Star *No heat stress*	Spring Ridge *Heat stressed*	Change in dough strength due to heat
	Rmax	Rmax	Rmax
Janz (*a b a*)	600	225	62% loss
Banks (*b b a*)	590	190	68% loss
Sunco (*a b a*)	550	205	63% loss
Sunstate (*aid*)	660	380	42% loss

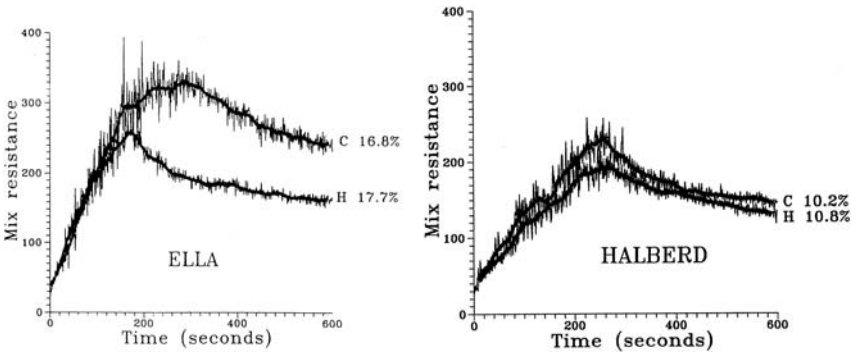


Figure 4. Mixograms for flour milled from grain of plants that had experienced heat shock in growth cabinets (three days at 40 °C during grain filling), designated H, compared to the non-shocked controls (C). The susceptible variety Ella has *Glu-1* alleles *abd*, and Halberd (tolerant) has *acd*

GENOTYPIC TOLERANCE TO HEAT STRESS

Comparison of the results for these 44 genotypes (plus one durum) showed a continuous range of reactions from tolerance to susceptibility. Most of the more tolerant genotypes had the 5 + 10 pair of high-molecular-weight (HMW) glutenin subunits, whereas the 2 + 12 pair of subunits was most common in the susceptible genotypes. The statistical analysis of this observation is shown in Table 4. Although the 2 + 12 lines tended to have higher protein contents than the 5 + 10 ones, the former were significantly more susceptible to heat stress than the 5 + 10 lines, as shown by the greater decrease in time-to-peak (mix time) and the more rapid breakdown for the heat-stressed 2 + 12 lines. This distinction is evident in the results from several other heat-stress experiments, as summarized in Table 5. Most of the entries in the susceptible column are for genotypes with the *a* allele (subunits 2 + 12) for the *Glu-1* locus (Ella, in Figure 4, in an exception), whereas all these tolerant varieties have the *Glu-D1d* allele (subunits 5 + 10).

The relationship between heat tolerance and the Glu-D1 alleles was tested further with the biotypes of Australian varieties Kewell, Avocet, Warigal and Lance, which

Table 4. Comparison of genotypes carrying either 2 + 12 or 5 + 10 HMW glutenin subunits in 44 bread-wheat genotypes tested in growth-cabinet experiments by Blumenthal et al. (1995a). The effects of heat shock on dough strength in the Mixograph are shown as means of differences between the values for heat-shocked and control values (HS – C). The significance of differences between the two groups of genotypes is shown as P values

Attribute	*Glu-D1a* (Subunits 2 + 12)	*Glu-D1d* (Subunits 5 + 10)	P
Grain protein content (%) as HS – C	2.4	1.7	0.05
Mix time to peak (sec.) as HS – C	−44	−4	0.001
Breakdown after peak as HS – C	4.5	−0.2	<0.001

Table 5. Comparison of varieties that are either *Glu-D1a* or *Glu-D1d* with respect to the effects of heat stress on dough properties. The HMW alleles of the varieties are shown (in the appropriate tolerant or susceptible columns) as the *Glu-1* alleles for the three genomes (A, B and D)

Variety	*Glu-1*alleles		Source of data
	Tolerant	Susceptible	
Songlen	–	*a b,f a*	Table 2, Blumenthal et al. (1991a)
Cook	–	*a b a*	–
Kite	–	*b i a*	–
Janz	–	*a b a*	Table 3
Banks	–	*b b a*	–
Sunco		*a b a*	–
Sunstate	*a i d*	–	–
Ella	–	*a b d*	Figure 4
Halberd	*a c d*	–	–
Egret	*c b d*	–	Stone and Nicholas (1996)
Oxley	–	*b b a*	–
Fang	*c i d*	–	Skylas et al. (2001)
Grebe	*c c d*	–	–
Wyuna	–	*b i a*	–
Batavia	–	*a b a*	–
Trigo 1	–	*c b a*	Wardlaw et al. (2002)
Lyallpur	*a i d*	–	–

each exist as a pair of naturally occurring biotypes, expressing either the *a* or *d* allele for the *Glu-D1* locus. These biotypes have been isolated, so that lines of each are available having either HMW subunits 2 + 12 (*Glu-D1a*) or subunits 5 + 10 (*Glu-D1d*). Plants of these pairs of genotypes were grown in the glasshouse, some left as controls and some heat stressed for a few days at 40 °C. For the doughs from un-stressed plants, the greater strength of the 5 + 10 biotypes is evident for all four varieties. For all four of the 2 + 12 biotypes, heat shock produced a considerable loss of dough strength. For the 5 + 10 biotypes of first three varieties in Table 6,

Table 6. Dough strength (as mix time in seconds to peak resistance) for *Glu-D1* biotypes of four varieties, comparing the effects of heat shock during grain filling. Statistical-significance figures compare the heat-shock reactions (stressed – control) of the *Glu-D1a* biotypes *versus* the reactions of the *Glu-D1d* biotypes

Variety	*Glu-D1a* Subunits 2 + 12		*Glu-D1d* Subunits 5 + 10		Significance of *a* versus *d*
	Control	Heat shocked	Control	Heat shocked	
Kewell	151	125	228	235	$P < 0.05$
Avocet	191	142	242	261	$P < 0.05$
Warigal	215	155	262	253	$P < 0.05$
Lance	267	202	388	301	Not significant

dough strength changed very little as a result of heat stress. For the 5 + 10 biotype of Lance, there was a significant loss of dough strength after heat stress.

Nevertheless, the hypothesis (Blumenthal et al. 1995b) that the *Glu-D1d* allele (subunits 5 + 10) is associated with heat tolerance (and subunits 2 + 12 with susceptibility) is upheld for three of the four pairs of biotypes, with high statistical significance. If these relationships are found to be general in further sets of genotypes, the implications are widespread for breeding (providing direction in screening for heat-stress tolerance) and in predicting the effects of heat stress on crops, depending on the *Glu-D1* alleles of the varieties involved.

PROSPECTS FOR FUTURE CLIMATES

The results for cumulative heat-load experiments have demonstrated that shock treatments above about 36 °C have more disastrous effects on grain yield and on dough strength, compared with longer periods of heat of similar overall heat load. As a result, research efforts need to be concentrated on this type of heat stress. The stimulus for this research is been accentuated by forecasts that the frequency of such heat-shock episodes is likely to increase with the progressive onset of global warming.

These increases in temperature stresses are linked to the increasing levels of atmospheric carbon dioxide. Considerable increases in grain yield (6–35%) have been obtained for wheat grown in an atmosphere enriched with CO_2 to double the present level. The increased yields were due to increases in grain number, rather than grain size. Of most concern was the reduction in grain-protein content, ranging down to levels (below 8%) at which normal processing would be difficult (Blumenthal et al. 1996). Dough testing of the resulting grain showed that dough properties were reduced (especially extensibility) but interpreting the results was difficult due to low protein content. A dramatic change in grain composition was the considerable increase in the proportion of large (A-type) starch granules, which is also evident as a result of heat stress with little evidence of genotypic tolerance to this consequence (Blumenthal et al. 1995a, Panozzo and Eagles 1998).

STORAGE CONDITIONS AND DOUGH QUALITY

Environmental conditions are not only an important consideration during plant growth but they may also affect dough quality following the harvest of the grain. Long-term storage of grain can cause significant changes in grain quality as determined by dough and baking tests, depending on the storage conditions, mainly the temperature and moisture levels (Gras et al. 2000).

A series of laboratory experiments in grain storage, conducted over a six-year period, demonstrated that dough from wheat stored at 35 °C for periods of over 70 days showed progressively longer development times, lower extensibility and

higher resistance, producing loaves with significantly lower volumes. The experiments were also conducted with wheat stored under different levels of oxygen, but no correlation was found between oxygen concentration and alterations in dough properties.

Storage at 23 °C (or below) prevented the changes in grain quality that would otherwise have been caused by some months' storage at temperatures above 30 °C. The laboratory experiments demonstrated that this reduction in temperature could be achieved effectively for hot-delivered grain by aeration of the stored grain to reduce grain temperature to about 23 °C, provided this is achieved during the first 70 days of storage. However, reduction of the grain temperature to only about 29 °C was shown to be insufficient to prevent the quality changes. Aeration is a well-proven and relatively inexpensive technique which could be readily applied on a commercial scale to meet these aims. The provision of consistent quality flour, resulting from the implementation of this strategy, can deliver the benefits of decreased product reformulation and increased water absorption with consequent improvements in bread yield.

PRECISION AGRICULTURE

Various strategies are offered above for mitigating the damaging effects of fluctuations in environmental conditions. Some additional advantages in this respect are offered by precision agriculture – a "scientific approach to grain production" involving the use of global-positioning systems (GPS) in the combine during grain harvesting, whilst continuously recording the yield of grain (Ehlert et al. 2004, Wrigley 2005). As a result, areas of poor productivity are identified, so that remedial action may be taken, such as adjusting the levels and types of fertilizers applied, also using GPS to identify the relevant parts of the field.

Experiments were undertaken in Western Australia (Skerritt et al. 2002) to use these principles to optimize protein content and quality for noodle-wheat production (requiring protein content in a "window" of between 9.5 and 11.5% for a variety having suitable starch quality. Despite the application of variable inputs of nitrogen fertilizer (30–90 kg/ha) to maximize yield evenly across the field, there were large variations in protein content, making some of the harvested grain unsuitable for the premium window. Protein quality (as SDS sedimentation volume) varied considerably, being greatest in the areas of high protein content. Starch quality for noodle production (as swelling volume) varied also greatly, being negatively correlated with protein content. Nevertheless, good prospects have been demonstrated for selective harvesting to target parts of the crop of suitable protein content, while avoiding parts known to be damaged, e.g., sprout damaged. This may involve using an NIR protein monitor mounted beside the grain-flow sensor on the bin intake of the combine. Alternatively, pre-harvest sampling and analysis of grain from the standing crop can be used to map trends in protein content and on-the-spot kits are available to test for sprout damage (Skerritt and Heywood 2000, Wrigley and Driver 2005).

REFERENCES

Allen H (1999) Australian Prime Hard Wheat in South-Eastern Australia. A Growers' Guide. Quality Wheat CRC, Sydney, Australia.

Anderson WK, Crosbie GB, Lemsom K (1995) Production practices for high protein, hard wheat in Western Australia. Aust J Exp Agr 35:589–595.

Archer MJ, O'Brien L (1987) A comparison study of the quality status of Condor wheat grown in northern Victoria and southern New South Wales. Aust J Agr Res 38:465–471.

Blumenthal C, Batey IL, Bekes F, Wrigley, CW, Barlow EWR (1990) The effects of heat stress on the quality and protein composition of wheat. In Westcott T, Williams Y, Ryker R (eds) Proceedings of the 39th Australian Cereal Chemistry Conference, 11–14 September 1989, Perth, WA. Royal Australian Chemical Institute, Melbourne, pp 110–112.

Blumenthal CS, Batey IL, Bekes F, Wrigley CW, Barlow EWR (1991a) Seasonal changes in wheat-grain quality due to temperature fluctuations (> 30°) during grain filling. Aust J Agr Res 42:21–30.

Blumenthal CS, Bekes F, Batey IL, Wrigley CW, Moss HJ, Mares DJ, Barlow EWR (1991b) Interpretation of grain quality results from variety trials with reference to high temperature stress. Aust J Agr Res 42:325–334.

Blumenthal, C, Bekes, F, Gras, P.W, Barlow, E.W.R. and Wrigley, C.W. 1995a. Identification of wheat genotypes tolerant to the effects of heat stress on grain quality. Cereal Chem.72:539–544.

Blumenthal C, Gras PW, Bekes F, Barlow EWR, Wrigley CW (1995b) Possible role for the *Glu-D1* locus with respect to tolerance to dough-quality change after heat stress. Cereal Chem 72:135–136.

Blumenthal C, Rawson HM, McKenzie E, Gras PW, Barlow EWR, Wrigley CW (1996) Changes in the grain quality of field-grown wheat due to doubling the level of atmospheric carbon dioxide. Cereal Chem 73:762–76.

Byers M, McGrath P, Webster R (1987) A survey of the sulphur content of wheat grown in Britain. J Sci Food Agric 38:151–160.

Ciaffi M, Tozzi L, Borghi B, Corbellini M, Lafiandra D (1996) Effect of heat shock during grain filling on the gluten protein composition of bread wheat. J Cereal Sci 24:91–100.

Corbellini M, Mazza L, Ciaffi M, Lafiandra D, Borghi B (1998) Effect of heat shock during grain filling on protein composition and technological quality of wheats. Euphytica 100:147–154.

Correll R, Butler J, Spouncer L, Wrigley CW (1994) The relationship between grain-protein content of wheat and barley and temperatures during grain filling. Aust J Plant Physiol 21:869–73.

Cracknell RL, Williams RM (2004) Wheat: Grading and segregation. In: Wrigley C, Corke H, Walker C (eds) Encyclopedia of Grain Science. Elsevier, Oxford, UK, pp 355–363.

Ehlert D, Schmerler J, Voelker U (2004) Variable rate nitrogen fertilisation of winter wheat based on a crop density sensor. Precis Agr 5:263.

Finney KF, Fryer HC (1958) Effect on loaf volume of high temperatures during the fruiting period of wheat. Agron J 50:28–34.

Gras PW, Bekes F, Kaur S, Lewis DA, O'Riordan B, Suter DAI, Thomson WKT (2000) How and why to keep grain quality constant. In: Wootton M, Batey IL, Wrigley CW (eds) Cereals 2000. Proceedings of the 50th Australian Cereal Chemistry Conference. Royal Australian Chemical Institute, Melbourne, pp 526–530

Graybosch RA, Peterson CJ, Baezinger PS, Shelton DR (1995) Environmental modification of hard red winter wheat flour protein composition. J Cereal Sci 22:45–51.

Haneklaus S, Evans E, Schug E (1992) Baking quality and sulphur of wheat. I. Influence of grain sulphur and protein concentrations on loaf volume. Sulphur in Agriculture 16:31–34.

Kettlewell PS (1996) Agronomy and cereal quality. In: Henry RJ, Kettlewell PS (eds) Cereal Grain Quality. Chapman and Hall, London, pp 407–437.

Moss HJ, Randall PJ, Wrigley CW (1983) Alteration to grain, flour and dough quality in three wheat types with variation in soil sulfur supply. J Cereal Sci 1:255–264.

Panozzo JF, Eagles HA (1998) Cultivar and environmental effects on quality characters in wheat. I. Starch. Aust J Agr Res 49:757–766.

Posner ES, Hibbs AN (2005) Wheat Flour Milling. American Association of Cereal Chemists, St Paul, MN.

Randall PJ, Moss HJ (1990) Some effects of temperature regime during grain filling in wheat quality. Aust J Agr Res 41:603–617.

Randall PJ, Wrigley CW (1986) Effects of sulfur deficiency on the yield, composition and quality of grain from cereals, oil seeds and legumes. Adv Cereal Sci Technol 8:171–206.

Schipper A, Jahn-Deesbach W, Weipert D (1986) Untersuchungen zum Klimateinfluss auf die Weizenqualitat. Getreide, Mehl und Brot 40:99–103.

Sharma DL, Anderson WK (2005) Small grain screenings in wheat: interactions of cultivars with seasons, site, and management practices. Aust J Agr Res 55:797–809.

Skerritt JH, Heywood RH (2000) A five-minute field test for on-farm detection of pre-harvest sprouting in wheat. Crop Sci 40:742–756.

Skerritt JH, Adams ML, Cook SE, Naglis G (2002) Within-field variation in wheat quality: implications for precision agricultural measurements. Aust J Agr Res 53:1229–1242.

Skylas DJ, Cordwell SJ, Butow B, Walsh BJ, Wrigley CW (2001) Identification of polypeptides associated with the heat-shock reaction of wheat endosperm, using the proteome approach. In: Wootton M, Batey IL, Wrigley CW (eds) Cereals 2000. Proceedings of the 11th International ICC Cereals and Bread Congress. Royal Australian Chemical Institute, Melbourne, pp 377–381.

Stone PJ, Nicholas ME (1996) Effect of timing during grain filling on two wheat varieties differing in heat tolerance. II. Fractional protein accumulation. Aust J Plant Physiol 23:739–749.

Uhlen AK, Hafskjold R, Kalhovd AH, Sahlstrom S, Longva A, Magnus EM (1998) Effects of cultivar and temperature during grain filling on wheat protein content, composition, and dough mixing properties. Cereal Chem 75:460–465.

Wardlaw IF, Blumenthal C, Larroque O, Wrigley CW (2002) Contrasting effects of heat stress and heat shock on kernel weight and on flour quality in wheat. Funct Plant Biol 29:25–34.

Wrigley CW (2005) Precision agriculture – a means of improving grain quality? Cereal Foods World 50:143–144.

Wrigley CW, Batey IL (2003) Assessing grain quality. In: Bread Making: Improving Quality. Cauvain SP (ed) Woodhead Publishing Ltd, Cambridge, UK, pp 71–96

Wrigley CW, Driver F (2005) On-the-spot determination of sprout damage in cereal grains. Poster presentation. Book of Abstracts, 7th International Wheat Conference, 27 Nov–2 Dec 2005, Mar del Plata, Argentina, p 281.

Wrigley CW, Blumenthal CS, Gras PW, Barlow EWR (1994) Temperature variation during grain filling and changes in wheat-grain quality and chemistry. Aust J Plant Physiol 21:875–85.

Wurst M (1999) Nitrogen Management for Wheat and Malting Barley. Quality Wheat CRC, Sydney.

Zhao FJ, McGrath SP, Crosland AR (1995) Changes in the sulphur status of British wheat grain in the last decade, and its geographical distribution. J Sci Food Agr 68:507–514.

MOLECULAR AND BIOCHEMICAL CHARACTERIZATION OF PUROINDOLINE A AND B ALLELES IN CHINESE IMPROVED CULTIVARS AND LANDRACES

Z.H. HE[1,2,*], F. CHEN[1], X.C. XIA[1], L.Q. XIA[1], X.Y. ZHANG[1], M. LILLEMO[3] AND C.F. MORRIS[4]

[1]*Institute of Crop Science/National Wheat Improvement Center, Chinese Academy of Agricultural Sciences (CAAS), Zhongguancun South Street 12, Beijing 100081, China*

[2]*International Maize and Wheat Improvement Center (CIMMYT) China Office, c/o CAAS, Zhongguancun South Street 12, Beijing 100081, China*

[3]*Department of Plant and Environmental Sciences, Norwegian University of Life Sciences, P.O. Box 5003, N-1432 Ås, Norway*

[4]*USDA-ARS Western Wheat Quality Laboratory, P.O. Box 646394, Washington State University, Pullman, WA 99164-6394, USA*

[*]*E-mail: zhhe@public3.bta.net.cn - xiaxianchun@caas.net.cn*

Abstract: Kernel hardness conditioned by puroindoline genes has a profound effect on milling, baking and end-use quality of bread wheat. In this study, 251 current cultivars and advanced lines, 166 historical cultivars, and 219 landraces from China were investigated for their kernel hardness and puroindoline alleles using molecular and biochemical markers. The frequencies of soft, mixed and hard genotypes were 31.5%, 21.1%, and 47.4%, respectively, in current cultivars, 45.2%, 13.9% and 40.9%, respectively, in historical cultivars, whereas 42.7%, 24.3% and 33.0%, respectively, in Chinese landraces. Among hard wheat genotypes, frequencies of PINA null, *Pinb-D1b* and new allele *Pinb-D1p* genotypes were 43.8%, 12.3% and 39.7%, respectively, in hard wheat of landraces, while 48.5%, 36.8% and 14.7%, respectively, in historical hard wheats, and 13.4%, 76.5%, and 8.4%, respectively, in current cultivars. New alleles *Pinb-D1q* and *Pinb-D1t*, was identified in winter wheat Jingdong 11 and two landraces Guangtouxianmai and Hongmai, respectively. A new *Pina-D1* allele, designated *Pina-D1m*, was detected in the landrace Hongheshang. Among the PINA null genotypes, *Pina-D1l* was detected in five landraces, while another novel *Pina-D1* allele, designed as *Pina-D1n*, was identified in six landraces

Keywords: puroindoline a, puroindoline b, grain hardness, wheat characterization, landraces

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 441–448.

INTRODUCTION

Kernel texture, mainly controlled by one major locus (*Ha)* on the short arm of chromosome 5D, has a large effect on the end-use quality of bread wheat (*Triticum aestivum* L.) and is used for marketing classification (Morris 2002). A profound understanding of the genetic basis for kernel hardness resulted from the discovery of friabilin, a M_r 15-kDa protein (Greenwell and Schofield 1986). Friabilin was shown to mainly contain three components, puroindoline a (PINA), puroindoine b (PINB) and GSP-1, and puroindolines represent the molecular-genetic basis of kernel hardness (Morris et al. 1994, Morris 2002, Hogg et al. 2004). Genes coding for PINA and PINB were located at the *Ha* locus and designated as *Pina-D1* and *Pinb-D1*, respectively (Giroux and Morris 1997). When both genes are in their functional wild type form, grain texture is soft. Hard grain texture is the result of mutations in either the *Pina-D1* or *Pinb-D1* locus. Several *Pinb-D1* alleles have been reported in bread wheat, which result in a kernel hardness change from soft to hard (Lillemo and Morris 2000, Morris et al. 2001, Tranquilli et al. 2002, Xia et al. 2005, Chen et al. 2005, Ram et al. 2005). However, the most drastic effect on grain hardness is caused by the PINA-null allele that in several studies is shown to confer harder endosperm than for example the *Pinb-D1b* allele (Giroux et al. 2000, Martin et al. 2001, Cane et al. 2004).

China is the world's largest wheat producer and consumer. Chinese wheat germplasm are unselected populations for grain hardness since intensive selection for quality improvement did not start until the late 1990s. The objectives of the present study were to characterize the distribution of puroindoline alleles in Chinese cultivars from landraces to current cultivars, and to uncover the evolution of puroindoline alleles in Chinese wheats since the 1930s.

MATERIALS AND METHODS

In total, 251 leading cultivars and advanced lines from late 1980s to present, 166 historical cultivars developed from the 1950s to the early 1980s, and 219 landraces from 16 provinces, were used for the investigation of SKCS hardness and puroindoline alleles. They represent very well the landmark landraces and historical cultivars in China from the 1930s to 1980s, and current cultivars used for production. In addition to the Chinese accessions, 12 exotic cultivars, were also included, since they were the most frequently used introductions in Chinese breeding programs before the 1980s (Zhuang 2003). The 251 current cultivars and advanced lines were sown at Anyang Experiment Station of CAAS in 2001–02 and 2003–04 seasons. The 219 landraces, 166 historical cultivars, and 12 introduced wheats were planted at Luoyang Agricultural Research Institute in the 2002–03 season according to local management practices. After harvest, all wheat samples were cleaned. Falling number tests indicated that they were free of sprouting damage.

Kernel hardness was measured with 300-kernel samples of each genotype using the Perten Single Kernel Characterization System (SKCS) 4100, following the manufacturer's operation procedure (Perten Instruments North America Inc., Springfield, IL). Mean, standard deviation, and distribution of SKCS hardness data were used to classify the genotypes tested into soft, mixed, and hard types. The SKCS produces a four-class frequency distribution of hardness data for each cultivar with class limits of <33, 34–46, 47–59, and >60.

Details of DNA isolation and PCR characterization of puroindoline alleles, isolation of Triton-soluble proteins and SDS-PAGE detection of PINA null genotypes, and DNA sequencing were reported previously (Lagudah et al. 1991, Gautier et al. 1994, Giroux and Morris 1997, 1998, Lillemo and Morris 2000, Morris and Massa 2003, Xia et al. 2005, Chen et al. 2005).

SAS 8.0 software and LSD multiple comparison were used to compute averages of SKCS hardness index.

RESULTS AND DISCUSSION

Distributions of SKCS Grain Hardness and Puroindoline Alleles in Chinese Wheats

As presented in Table 1, frequencies of soft, mixed and hard genotypes were 42.7%, 24.3% and 33.0%, respectively, in Chinese landraces, and 45.2%, 13.9% and 40.9%, respectively, in historical cultivars. In comparison with landraces and historical cultivars, the frequency of hard genotypes in current cultivars increases notably (62.5%), with a corresponding decrease in the frequencies of soft and mixed hard types (25.1% and 12.4%). As presented in Table 2, frequencies of PINA null, *Pinb-D1b*, and *Pinb-D1p* were 43.8%, 12.3% and 39.7%, respectively, in hard landraces, and 48.5%, 36.8% and 14.7%, respectively, in hard historical cultivars. Compared with landraces and historical cultivars, frequencies of PINA null and *Pinb-D1p* was significantly reduced, while the frequency of *Pinb-D1b* was largely increased in the current cultivars. Historical cultivars were mostly developed by crossing Chinese landraces with introduced cultivars, and current wheat cultivars were predominantly developed by intercrossing historical cultivars

Table 1. Frequencies of three hardness types in Chinese landraces, historical and current cultivars, and introduced wheats

Type	Sample No.	Soft (%)	Mixed (%)	Hard (%)
Landraces	219	42.7	24.3	33.0
Historical cultivars	166	45.2	13.9	40.9
Current cultivars	251	25.1	12.4	62.5
Introduced cultivars	12	33.4	8.3	58.3
Total	648	35.2	16.7	48.1

Table 2. Frequencies of different puroindoline alleles in Chinese landraces, historical, and current wheat cultivars

Genotype	Phenotype	No.	Landrace (%)	Historical cultivar (%)	Current cultivar (%)
PINA null[a]	Hard	81	43.8	48.5	13.4
Pina-D1m/Pinb-D1a	Hard	1	1.4	0	0
Pina-D1a/Pinb-D1b	Hard	159	12.3	36.8	76.5
Pina-D1a/Pinb-D1d	Hard	2	0	0	1.7
Pina-D1a/Pinb-D1p	Hard	52	39.7	14.7	8.4
Pina-D1a/Pinb-D1t	Hard	2	2.8	0	0

[a] PINA null is composed of Pina-D1b/Pinb-D1a, Pina-D1l/Pinb-D1a and Pina-D1n/Pinb-D1a

or improved elite advanced lines (He et al. 2001). Among the parents of popular wheat cultivars, 26 Chinese landraces and 12 introduced cultivars were used most frequently in breeding programs (Zhuang 2003), in which 54.1% of landrace cultivars and 58.3% of introduced cultivars belong to hard types. The 12 introduced parents can be divided into two groups. Four Italian cultivars including Abbondanza, Ardito, Funo, and St1472/506 are of the soft type and widely used in breeding programs in the southern part of the Yellow and Huai Valleys (Zone II) and Yangtze regions (Zones III and IV). The other eight introduced parents are hard types with the predominant *Pinb-D1b* allele, and they were widely used in northern China (Zones I and II). From Chinese landraces and historical cultivars to currently popular cultivars, the reduction of soft and mixed wheats and increase of hard wheats are largely due to the increasing use of hard type parents and a favorable selection of hard genotypes in breeding programs although soft types are also acceptable.

Pinb-D1p, characterized as a new *Pinb* frame-shift mutation resulting in PINB null (Xia et al. 2005), was identified in 39 landraces and historical cultivars with SKCS hardness index ranging from 50 to 79. Further analysis indicated that the *Pina* gene in these genotypes with *Pinb-D1p* was wild type (*Pina-D1a*), based on SDS-PAGE of Triton X-114 soluble proteins and sequencing of *Pina* fragment. Most of *Pinb-D1p* genotypes in historical cultivars could be traced back to landraces, based on pedigree information. Another new allele *Pinb-D1q* was found in winter wheat cultivar Jingdong 11 (Chen et al. 2005).

Detailed data on the influence of three puroindoline mutations (PINA null, *Pinb-D1b* and *Pinb-D1p*) on kernel hardness was shown in Fig. 1. Among these puroindoline mutations, SKCS hardness index of PINA null (70.3) was significantly higher than that of both *Pinb-D1b* (61.6) and *Pinb-D1p* (60.5). The SKCS hardness of PINA null is also much higher than that of *Pinb-D1b*. The presence of the very hard PINA null is high in Chinese landraces. More work is needed to understand this since most wheat was milled by manual methods in the major wheat growing areas before the 1970s.

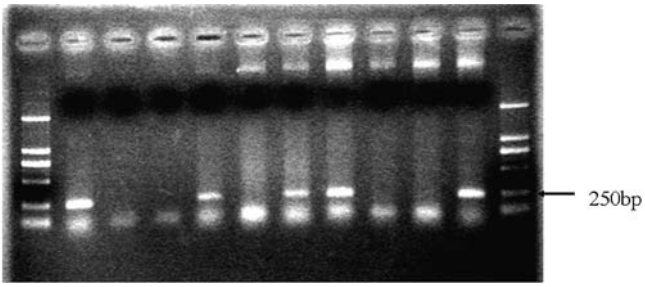


Figure 1. Detection of *Pinb-D1b* allele by PCR specific primers From left to right, DL2 000 marker (lane 1 and 12), Chinese Spring (lane 2 and 3), Jinnan 17 (lane 4 and 5), Yuandong 107(lane 6 and 7), CA9648 (lane 8 and 9), Jindong 8 (lane 10 and 11)

Novel Puroindoline A and B Alleles Involved in a Single Nucleotide Substitution

Two hard landraces, Guangtouxianmai and Hongmai, originating from Xiuwen County, Guizhou Province, did not belong to any of the genotypes *Pinb-D1b, Pina-D1b* and *Pinb-D1c* based on PCR amplification with *Pinb-D1b* specific primer sets, SDS-PAGE of Triton X-114 soluble proteins and site specific cleavage of PCR-amplified *Pinb* fragment. Subsequent DNA sequencing of the *Pinb* gene from 10 kernels of each of the two landraces revealed a point mutation, involving a base G to C substitution at the 226th nucleotide in the coding sequence of the *Pinb* gene, which results in a glycine (GGC) to arginine (CGC) substitution at position 47 in the deduced amino acid sequence of puroindoline b. Sequencing *Pina*-amplified fragments indicated that both landraces possessed the wild-type *Pina* allele. The *Pinb* allele in these two landraces was different from any one of previously reported *Pinb* mutations. The single nucleotide mutation with G to C substitution at the 226th nucleotide of *Pinb* locus could be designated as *Pinb-D1t* according to the 2005 Supplement of the Wheat Gene Catalogue (McIntosh et al 2005). The SKCS hardness index (means±SD) and frequency distribution of Guangtouxianmai and Hongmai were 68 ± 16, 64 ± 15, and 3–9–13–75, 3–6–23–68, respectively. Both of them were classified as class 1, a hard type.

A hard wheat landrace Hongheshang, originating from Guanyun County, Jiangsu Province, did not belong to any known mutation, either. Sequencing the *Pinb* gene in Hongheshang indicated that it had the wild type allele (*Pinb-D1a)*. However, sequencing of *Pina* revealed a new *Pina-D1* mutation, involving a base C to T substitution at the 187th nucleotide in the coding sequence of the *Pina* gene, resulting in a proline (CCG) to serine (TCG) substitution at position 35 in the deduced amino acid sequence of puroindoline a (Table 3). The single nucleotide mutation with C to T substitution at the 187th nucleotide of *Pina* gene could be designated as *Pina-D1m* according to the 2005 Supplement of the Wheat Gene Catalogue (McIntosh et al 2005). The SKCS hardness index (means±SD) and frequency distribution of Hongheshang were 73 ± 13 and 1–3–9–87. It was classified as class 1, a hard type.

Table 3. Nucleotide and deduced amino acid sequence changes of new puroindoline-a alleles detected in Chinese wheat landraces

Allele	Position
	34 35 36 37 38 39 40 41 42 43 ..... 51 52 53 54 55 56 57 58 59 60 61 62
Pina-D1a (Soft)	TTC CCG GTC ACC TGG CGT TGG TGG AAA TGG ..... CTC CTT
	GGG GAG TGT TGC AGT CGG CTC GGC CAA ATG
	F P V T W R W W K W ..... L L G E C C S R L G Q M
Pina-D1l (Hard)	TTC CCG GTC ACC TGG CGT TGG TGG AAA TGG ..... CTC CTT
	GGG GAG TGT TGC AGT CGG CTC GGC –AAA TG
	F P V T W R W W K W ..... L L G E C C S R L G **K**
Pina-D1m (Hard)	TTC **T**CG GTC ACC TGG CGT TGG TGG AAA TGG ..... CTC CTT
	GGG GAG TGT TGC AGT CGG CTC GGC CAA ATG
	F **S** V T W R W W K W ..... L L G E C C S R L G Q M
Pina-D1n (Hard)	TTC CCG GTC ACC TGG CGT TGG TGG AAA T**A**G ..... CTC CTT
	GGG GAG TGT TGC AGT CGG CTC GGC CAA ATG
	F P V T W R W W K * ..... L L G E C C S R L G Q M

Molecular Basis of the PINA-null Genotype

Of these 65 PINA null genotypes, PCR fragments of the *Pina* gene could be amplified in 11 landraces, but not in other 54 accessions with *Pina* specific primers for the coding region. Sequencing *Pinb* of the 11 landraces indicated that they had the wild-type allele (*Pinb-D1a*). Subsequently, sequencing their *Pina* gene revealed a cytosine deletion at position 265 in five PINA-null landraces including Sanyuehuang, Baikezaomai, and Xiaoyuhua from Jiangsu province, Chengduguangtou from Sichuan province and Guangtouxiaomai from Guangxi province. This mutation leads to a shift in the open reading frame (ORF) in the coding region of the *Pina* gene, and subsequently a stop codon at position 93 in the deduced amino acid sequence of PINA. This mutation is identical to the allele previously detected and described as *Pina-D1c* by Gazza et al. (2005), which we have renamed as *Pina-D1l* according to the 2005 Supplement of the Wheat Gene Catalogue (McIntosh et al. 2005) (Table 3). Moreover, a single nucleotide substitution was detected in the coding region of the *Pina* gene in six other PINA-null landraces, i.e., Xianmai, Zhuantoubaike, Baimangchun (two cultivars with a common name from Jianhu County and Guanyun County, respectively), Yazuizi and Yazuixiaomai, characterized by a G to A change at position 212, resulting in tryptophan-43 to a 'stop' codon (Table 3). This new *Pina-D1* allele was designated as *Pina-D1n* according to the 2005 Supplement of the Wheat Gene Catalogue (McIntosh et al. 2005).

Previously, the PINA null (*Pina-D1b*) was the only hardness mutation known at the *Pina-D1* locus, and simply described as a lack of PINA protein expression (Giroux and Morris 1997, 1998, Lillemo et al. 2000, Morris et al. 2001). We checked the sequence of *Pina* in common wheat and found that the 267th position is a base A instead of C, and a cytosine deletion at the 267th position at *Pina* locus reported by Gazza et al. (2005) should be at the 265th position, which is consistent with the

sequencing results of *Pina* in the present study. The name *Pina-D1c* designated by Gazza et al. (2005) was in conflict with a previous report for an allele in *Ae. tauschii* by Gedye et al. (2004). Therefore, the PINA-null type due to a cytosine deletion at position 265 in the coding region of the *Pina* gene was renamed as *Pina-D1l* (McIntosh et al. 2005).

ACKNOWLEDGEMENTS

This project was funded by the National Basic Research Program (2002CB11300) and National Natural Science Foundation of China (30260061).

REFERENCES

Cane K, Spackman M, Eagles HA (2004) Puroindoline genes and their effects on grains quality traits in southern Australian wheat cultivars. Aust J Agr Res 55:89–95

Chen F, He ZH, Xia XC, Lillemo M, Morris CF (2005) A new puroindoline b mutation present in Chinese winter wheat cultivar Jingdong 11. J Cereal Sci 42:267–269

Gautier MF, Aleman ME, Guirao A, Marion D, Joudrier P (1994) *Triticum aestivum* puroindolines, two basic cysteine rich seed proteins: cDNA sequence analysis and developmental gene expression. Plant Mol Biol 25:43–57

Gazza L, Nocente F, Ng PKW, Pogna NE (2005) Genetic and biochemical analysis of common wheat cultivars lacking puroindoline a. Theor Appl Genet 110:470–478

Gedye KR, Morris CF, Bettge AD (2004) Determination and evaluation of the sequence and testural effects of the puroindoline a and puroindoline b genes in a population of synthetic hexaploid wheat. Theor Appl Genet 109:1597–1063

Giroux MJ, Morris CF (1997) A glycine to serine change in puroindoline b is asssociated with wheat grain hardness and low levels of starch-surface friabilin. Theor Appl Genet 95:857–864

Giroux MJ, Morris CF (1998) Wheat grain hardness results from highly conserved mutations in friabilin components puroindoline a and b. Proc Natl Acad Sci USA 95:6262–6266

Giroux MJ, Talbert L, Habernicht DK, Lanning A, Hemphill A, Martin JM (2000) Association of puroindoline sequence type and grain hardness in hard red spring wheat. Crop Sci 40:370–374

Greenwell P, Schofield JD (1986) A starch granule protein associated with endosperm softness in wheat. Cereal Chem 63:379–380

He ZH, Rajaram S, Xin ZY, Huang GZ (2001) A history of wheat breeding in China. CIMMYT, Mexico, DF, pp 1–94

Hogg AC, Sripo T, Beecher B, Martin JM, Gorpux MJ (2004) Wheat puroindolines interact to form friabilin and control wheat grain hardness. Theor Appl Genet 108:1089–1097

Lagudah ES, Appels R, Mcneil D (1991) The *Nor-D3* locus of *Triticum tauschii*: natural variation and genetic linkage to markers in chromosome 5. Genome 34:387–395

Lillemo M, Morris CF (2000) A leucine to proline mutation in puroindoline b is frequently present in hard wheats from Northern Europe. Theor Appl Genet 100:1100–1107

Martin JM, Frohberg RC, Morris CF, Talbert LE, Giroux MJ (2001) Milling and bread baking traits associated with puroindoline sequence type in hard red spring wheat. Crop Sci 41:228–234

McIntosh RA, Devos KM, Dubcovsky J, Rogers WJ, Morris CF, Appels R, Anderson OD (2005) Catalogue of gene symbols for wheat: 2005 supplement, published online at: http://wheat.pw.usda.gov/ggpages/wgc/2005 upd.html

Morris CF (2002) Puroindolines: the molecular genetic basis of wheat grain hardness. Plant Mol Biol 48:633–647

Morris CF, Massa AN (2003) Puroindoline genotype of the U.S. national institute of standards & technology reference material 8441, wheat hardness. Cereal Chem 80:674–678

Morris CF, Greenblatt GA, Bettge AD, Malkawi HI (1994) Isolation and characterization of multiple forms of friabilin. J Cereal Sci 20:167–174

Morris CF, Lillemo M, Simeone MC, Giroux MJ, Babb SL, Kimberlee KK (2001) Prevalence of puroindoline grain hardness genotypes among historically significant North American spring and winter wheats. Crop Sci 41:218–228

Ram S, Jain N, Shoran J, Singh R (2005) New frame shift mutation in puroindoline b in Indian wheat cultivars Hyb65 and NI5439. J Plant Biochem Biotechnol 14:45–48

Tranquilli G, Heaton J, Chicaiza O, Dubcovsky J (2002) Substitutions and deletions of genes related to grain hardness in wheat and their effect on grain texture. Crop Sci. 42:1812–1817

Xia LQ, Chen F, He ZH, Chen XM, Morris CF (2005) Occurrence of puroindoline alleles in Chinese winter wheats. Cereal Chem 82:38–43

Zhuang QS (2003) Chinese wheat improvement and pedigree analysis. Chinese Agriculture Press. Beijing, pp 1–681

INTRODUCTION OF D-GENOME RELATED GLUTEN PROTEINS INTO DURUM WHEAT

D. LAFIANDRA[1,*], C. CEOLONI[1], R. CAROZZA[1], B. MARGIOTTA[2], M. URBANO[2], G. COLAPRICO[2] AND M.G. D'EGIDIO[3]

[1]*Department of Agrobiology & Agrochemistry, University of Tuscia, Viterbo (Italy)*
[2]*Institute of Plant Genetics, C.N.R., Bari (Italy)*
[3]*Experimental Institute for Cereal Research, Rome (Italy)*
[*]*E-mail: lafiandr@unitus.it*

Abstract: Genes encoding D-genome related gliadins and glutenin subunits have been transferred to durum wheat through chromosome engineering. In particular, segments containing genes corresponding to the pairs of high-molecular-weight glutenin subunits (HMW-GS) 5 + 10 or 2 + 12, normally present at the *Glu-D1* locus in bread wheat, have been introgressed into chromosome 1A of different durum wheat cultivars, replacing the *null* allele present at the *Glu-A1* locus. Using the same approach or the bread wheat cultivar Perzivan, carrying a translocation involving the short arm of chromosomes 1A and 1D, the two alleles present at *Gli-D1/Glu-D3* loci in most bread wheat cultivars, encoding gliadin and low molecular weight glutenin subunits (LMW-GS), have also been introduced into durum wheat

Keywords: gluten proteins, HMW-glutenins, D-genome, durum wheat

INTRODUCTION

Gluten proteins, encoded by a series of loci on the group 1 and 6 chromosomes, are the major determinants of technological properties in bread and durum wheat (Shewry et al. 2003). They are mainly composed by two protein fractions, termed gliadins and glutenins. While glutenins comprise polypeptides assembled into high molecular mass polymers stabilized by inter-chain disulphide bonds, gliadins are monomeric proteins that interact with other gluten proteins by non-covalent forces. The glutenin polymers are responsible for the elastic properties of gluten and dough, with strong doughs containing greater amounts of high molecular mass polymers. Dough strength is under genetic control, with allelic variation for glutenin

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 449–454.

subunits, termed high- (HMW-GS) and low-molecular-weight glutenin subunits (LMW-GS), correlated with dough strength properties. HMW-GS are encoded by genes located on the long arms of group 1 chromosomes (*Glu-1* loci), whereas those corresponding to LMW-GS are located on the short arms of the same group of chromosomes, tightly linked to genes encoding gamma and omega gliadins (*Glu-3/Gli-1* loci). In durum wheat dough strength is particularly dependent on LMW–GS encoded by the *Glu-B3* locus, with two major allelic forms being associated with good (LMW-2) and poor (LMW-1) quality, respectively. The differential effect observed with the two types of LMW-GS is considered to be purely quantitative, with the LMW-2 allele being expressed more strongly than the LMW-1 allele (Masci et al. 1995).

Though durum wheat is mostly used for pasta production, and its breadmaking quality could be inferior to that of bread wheat, its use for the preparation of different kinds of bread is widespread in many Mediterranean countries.

Liu et al. (1995) using the D-genome disomic substitution lines of the durum wheat cultivar Langdon have demonstrated a large effect of chromosome 1D substitutions on glutenin amount, SDS sedimentation value, mixing time and peak resistance value. This evidence stimulated various attempts aimed at transferring chromosomal segments containing genes encoding D-genome related gliadins and glutenins into durum wheat. Lukaszewsky and Curtis (1992, 1994), through chromosome engineering, transferred bread wheat 1D chromosome segments carrying *Glu-1* genes for the HMW-GS 5 + 10 or 2 + 12 pairs, encoded by genes present at the bread wheat *Glu-D1* locus, to chromosomes 1R and 1A of triticale, and subsequently incorporated the latter transfer into durum wheat germplasm replacing the *null* allele present at the *Glu-A1* locus (Lukaszewsky 2003). Chromosome engineering was similarly used to transfer D-genome related gluten proteins directly to durum wheat (Ceoloni et al. 1993). As a result, two recombinant lines were isolated in which the 5 + 10 allele and the *Gli-D1/Glu-D3* genes were separately transferred into the 1AL and 1AS arm, respectively, of the recipient durum wheat (Ceoloni et al. 1996, Vitellozzi et al. 1997).

A different approach was used by Pogna et al. (1996). These authors, using the bread wheat cultivar Perzivan, which contains a translocated segment on the short arm of chromosome 1A carrying genes for gliadins and LMW-GS normally present at the *Gli-D1/Glu-D3* loci, introduced these proteins into durum wheat.

The production of a more complete set of recombinant lines, carrying D-genome related HMW-GS and LMW-GS in different durum wheat backgrounds with allelic differences at the *Glu-B1* and *Glu-B3* loci, is described in the present paper.

MATERIALS AND METHODS

The durum wheat lines with various D-genome introductions used in the present work were described in Ceoloni and Jauhar (2006). Electrophoretic separation of glutenin subunits was performed on 10% polyacrylamide gels.

Determination of unextractable polymeric proteins (%UPP) was carried out by size-exclusion high performance liquid chromatography (SE-HPLC) as reported by Batey et al. (1991), using a Biosep-SEC-S4000 column (Phenomenex).

Standard procedures were followed for evaluation of quality traits such as SDS, gluten index and alveographic measurements.

RESULTS AND DISCUSSION

Electrophoretic separation of gluten proteins extracted from different durum wheat cultivars and derived lines in which either the pair of HMW-GS 5 + 10 or 2 + 12 are present is reported in Fig. 1. The various durum wheat cultivars used possess different HMW-GS alleles at the *Glu-B1* locus, numbered according to Payne and Lawrence (1983). Additionally, the two biotypes present in the durum wheat cultivar Lira, differing in gliadin and LMW-GS encoded by linked genes at the *Gli-B1/Glu-B3* loci, were also used. These two allelic types, designated as 42/LMW-1 and 45/LMW-2, are associated to poor or good pasta making properties, respectively, as mentioned in the introduction.

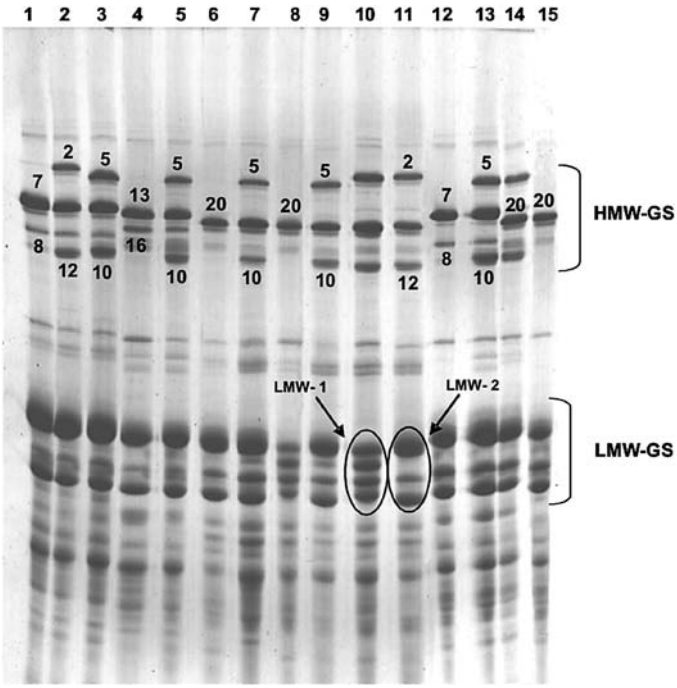


Figure 1. SDS-PAGE of durum wheat cultivars Svevo (1), Duramba (4), Lira (6, 8, 10, 12), Simeto (12), Cappelli (15) and recombinants carrying the 5 + 10 or 2 + 12 HMW-GS, combined with different *Glu-B1* and *Glu-B3* allelic variants

In the durum wheat cultivar Svevo the two major alleles present in bread wheat at the *Gli-D1/Glu-D3* loci, termed Chinese Spring (CS) and Cheyenne (CNN)–types (Masci et al. 1991, Lafiandra et al. 2000) were also introduced.

The CNN-type allele was introduced using 1AL.1AS/1DS translocation present in the bread wheat cultivar Perzivan, whereas the CS-type allele was introduced in durum wheat through *ph1* mediated chromosome engineering by Ceoloni et al. (1993, 1996). Subsequent crosses and backcrosses allowed the transfer of this allele into the Svevo background.

In bread wheat the relative amount of unextractable polymeric proteins (which correspond to the larger sized polymers) present in total polymeric protein, determined by SE-HPLC, has a strong positive effect on dough strength parameters (Gupta et al. 1993). Flours with a greater percentage of larger sized polymers (%UPP) show a greater dough strength and elasticity compared to those with a smaller %UPP. Determination of the amount of %UPP by SE-HPLC on the recipient cv. Svevo and on the four recombinant lines demonstrated that the introduction of the D-genome related HMW-GS and LMW-GS results in a significant increase of this parameter, with the larger effect exerted by the line possessing the HMW-GS 5 + 10 pair.

Determination of the relative amount of HMW-GS and LMW-GS in the unextractable polymeric proteins showed that replacement of the *Gli-A1/Glu-A3* locus with the *Gli-D1/Glu-D3* loci (CNN- or CS-type allele), produced a decrease of the HMW-GS/LMW-GS ratio, indicating that the D-genome alleles contribute with a greater amount of subunits to the formation of the glutenin polymers.

Recombinant lines carrying the 1AL.1AS/1DS (PS) or the 1AL.1AS/1DL (PL) translocations and possessing either HMW-GS 7 + 8 or 20 at the *Glu-B1* locus were also produced. These two alleles have been shown to differentially affect breadmaking properties, with the first being superior to the latter (Margiotta et al. 2000).

Quality data on these lines and on their sibs lacking any 1D segment indicate that, irrespective of the relative contribution of the different *Glu-B1* HMW-GS, gluten quality is significantly affected by the presence of the D-genome related proteins. SDS-sedimentation values result higher in the PL (30–50%) than in the PS lines (10–30%), while Gluten Index appears to increase to a similar degree in both recombinants compared to the controls.

Alveographic measurements are also considerably influenced by the two types of D-genome encoded proteins. As a whole, P values (a measure of tenacity of the dough) increase (more in PL than in PS), whereas L values (dough extensibility) show a considerable decrease only in PL lines, and W (dough strength) a more significant increase in PS lines. More equilibrated ratios between tenacity and extensibility seem thus to result from the presence of the LMW-GS encoded by *Glu-D3* genes rather than from that of the *Glu-D1*-encoded HMW-GS 5 + 10 pair.

CONCLUSIONS

The present work demonstrates that chromosome engineering, enabling production of lines expressing an array of additional glutenin subunits, represents an efficient tool to finely manipulate gluten quality in durum wheat.

The addition of HMW-GS 5 + 10 leads to an increase in the amount of high molecular mass glutenin polymers, with similar effects also produced by the 2 + 12 pair or by LMW-GS.

Substitution of *Gli-A1/Glu-A3* with the *Gli-D1/Glu-D3* loci, either CNN- or CS-types, results in an increase of the amount of LMW-GS, which might lead to an improvement of dough properties.

Recently, Klindworth et al. (2005) reported quality data of BC2F5 derived 1AS.1AL-1DL translocation lines selected from the breeding materials obtained by Joppa et al. (1998). These lines were characterised by the presence of the 5 + 10 alleles and segregated for the LMW-GS associated at the *Gli-B1/Glu-B3* loci. Such lines exhibited significant differences in agronomic and quality characteristics, with translocation lines having the LMW-1 type, normally associated to poor technological properties, exhibiting better mixing stability and loaf volume than lines possessing the LMW-2 type allele.

Complete biochemical and technological analyses of the material described in the present paper will allow to further clarify the individual contribution of D-genome glutenin genes and alleles to durum wheat gluten quality parameters, and also to better explore the possibility to improve by these means its breadmaking properties.

REFERENCES

Batey IL, Gupta RB, MacRitchie F (1991) Use of size-exclusion high-performance liquid chromatography in the study of wheat flour proteins: an improved chromatographic procedure. Cereal Chem 68:207–209

Ceoloni C, Jauhar PP (2006) Chromosome engineering of the durum wheat genome: Strategies and applications of potential breeding value. In: Singh RJ, Jauhar PP (eds) vol 2 Genetic Resources, Chromosome Engineering, and Crop Improvement: Cereals. CRC Press, Taylor & Francis group, pp 27-59

Ceoloni C, Ciaffi M, Lafiandra D, Giorgi B (1995) Chromosome engineering as a means of transferring 1D storage protein genes from common to durum wheat. In: Li ZS, Xin ZY (eds) Proceedings of the 8th International Wheat Genetics Symposium, 20–25 July 1993, Beijing, China. China Agricultural Scientech Press, Beijing, pp 159–163

Ceoloni C, Biagetti M, Ciaffi M, Forte P, Pasquini M (1996) Wheat chromosome engineering at the 4x level: the potential of different alien gene transfers into *durum* wheat. Euphytica 89:87–97

Gupta RB, Khan K, MacRitchie F (1993) Biochemical basis of flour properties in bread wheats. I. Effects of variation in the quantity and size distribution of polymeric protein. J Cereal Sci 18:23–41

Joppa LR, Klindworth DL, Hareland GA (1998) Transfer of high molecular weight glutenins from spring wheat to durum wheat. In: Slinkard AE (ed) Proc. 9th International Wheat Genetics Symposium, 2–7 August 1998, Saskatoon, Sask., Canada. University Extension Press, University of Saskatchewan, pp 257–260

Klindworth DL, Hareland GA, Elias ME, Xu SS (2005) Agronomic and quality characteristics of 1AS.1AL-1DL translocation lines of durum wheat carrying glutenin allele *Glu-D1d*. Crop Sci 45:77–84

Lafiandra D, Margiotta B, Colaprico G, Masci S, Roth MR, MacRitchie F (2000) Introduction of the D-genome related high- and low-Mr glutenin subunits into durum wheat and their effect on technological properties. In: Shewry PR, Tatham AS (eds) Wheat Gluten: Proceedings of the 7th International Workshop Gluten, 2–6 April 2000, Bristol, UK. The Royal Society of Chemistry, Cambridge, pp 51–54

Liu CY, Rathijen AJ, Shepherd KW, Gras PW, Giles LC (1995) Grain quality and yield characteristics of D-genome disomic substitution lines in Langdon (*Triticum turgidum* var. *durum*) Plant Breed 114:34–39

Lukaszewski AJ (2003) Registration of six germplasms of durum wheat with introgressions of the *Glu-D1* locus. Crop Sci 43:1138–1139

Lukaszewski AJ, Curtis CA (1992) Transfer of the *Glu-D1* gene from chromosome 1D of bread wheat to chromosome 1R in hexaploid triticale. Plant Breed 109:203–210

Lukaszewski AJ, Curtis CA (1994) Transfer of the *Glu-D1* gene from chromosome 1D of bread wheat to chromosome 1A in hexaploid triticale. Plant Breed 112:177–182

Margiotta B, Pfluger L, Roth MR, MacRitchie F, Lafiandra D (2000) Isogenic bread wheat lines differing in number and type of high Mr glutenin subunits. In: Shewry PR, Tatham AS (eds) Wheat Gluten: Proceedings of the 7th International Workshop Gluten, 2–6 April 2000, Bristol, UK. The Royal Society of Chemistry, Cambridge, pp 29–33

Masci SM, Lew EJ-L, Lafiandra D, Porceddu E, Kasarda DD (1995) Characterization of low-molecular-weight glutenin subunits in durum wheat by RP-HPLC and N-terminal sequencing. Cereal Chem 72:100–104

Masci SM, Porceddu E, Colaprico G, Lafiandra D (1991) Comparison between the B and D subunits coded by the *Glu-D3* locus in two common wheat biotypes (cv. Newton) differing for technological characteristics. J Cereal Sci 14:35–46

Payne PI, Lawrence GJ (1983) Catalogue of alleles for the complex gene loci, *Glu-A1, Glu-B1* and *Glu-D1* which code for high-molecular-weight subunits of glutenin in hexaploid wheat. Cereal Res Commun 11:29–35

Pogna NE, Mazza M, Redaelli R, Ng PKW (1996) Gluten quality and storage protein composition of durum wheat lines containing the *Gli-D1/Glu-D3* loci. In: Wrigley CW (ed) Gluten 96, Proceedings of the 6th International Gluten Workshop, 1–6 September 1996, Sydney, Australia. Royal Australian Chemical Institute, Melbourne, pp 18–22

Shewry PR, Halford NG, Lafiandra D (2003) The genetics of wheat gluten proteins. Adv Genet 43:111–184

Vitellozzi F, Ciaffi M, Dominici L, Ceoloni C (1997) Isolation of a chromosomally engineered durum wheat line carrying the common wheat *Glu-D1d* allele. Agronomie 17:413–417

NATURAL VARIATION AND IDENTIFICATION OF MICROELEMENTS CONTENT IN SEEDS OF EINKORN WHEAT (*TRITICUM MONOCOCCUM*)

H. OZKAN[1,*], A. BRANDOLINI[2], A. TORUN[3], S. ALTINTAS[1], S. EKER[3], B. KILIAN[4], H.J. BRAUN[5], F. SALAMINI[6] AND I. CAKMAK[7]

[1]*University of Cukurova, Faculty of Agriculture. Department of Filed Crops, Adana, Turkey*
[2]*Istituto Sperimentale per la Cerealicoltura – CRA, Via R. Forlani 3, 26866 S. Angelo L. (LO), Italy*
[3]*University of Cukurova, Faculty of Agriculture. Department of Soil Science, Adana, Turkey*
[4]*Max-Planck-Institut für Züchtungsforschung Carl-von-Linné-Weg 1050829 Köln*
[5]*CIMMYT, POB 39 Emek, 06511 Ankara, Turkey*
[6]*Parco Tecnologico Padano, Polo Universitario, via Einstein 2 Llocalità Cascina Codazza, 26900 Lodi, Italy*
[7]*Sabanci University, Faculty of Engineering and Natural Sciences, Istanbul, Turkey*
[*]*E-mail: hozkan@cu.edu.tr*

Abstract: Micronutrient deficiencies in human beings are common problems, especially in developing world. Among the micronutrient deficiencies, zinc (Zn) and iron (Fe) deficiencies are particularly important affecting severely health of humans. Major reason for the widespread occurrence of micronutrient deficiencies in human beings is the high and monotonous consumption of cereal-based foods with very low content of micronutrients. An increase in concentration of Zn and Fe in grain is, therefore, a high-priority research area. Exploitation of large genetic variation for Zn and Fe existing in cereals germplasm is an important approach to minimize the extent of Zn and Fe deficiencies in developing world. In the present study, the variation for seed content of micronutrients (Zn, Fe, Mn and Cu) in 54 accessions of einkorn wheat (*Triticum monococcum*) was tested. The accessions have been first grown under same field conditions in 2 locations in Turkey, and the seeds obtained from the field trials were analyzed for micronutrients. In addition, a mapping population with 168 recombinant inbred lines which were grown in 4 locations in Germany, Turkey and Italy has also been tested for the variation of micronutrients in seeds and analyzed for identification of QTLs associated with micronutrient content in seeds

The results obtained showed existence of large genotypic variation in content of micronutrients. The contents of Zn and Fe among the 54 einkorn wheat accessions varied from 0.21 to 2.16 μg seed^{-1} for Zn with an average of 1.19 μg seed^{-1} and from 0.54 to 3.09 μg seed^{-1} for Fe with an average of 1.15 μg seed^{-1}. There was a close positive relationship between seed contents of Fe and Zn. The genetic basis of this variation was elucidated by QTL analysis, using a mapping population comprising 168 recombinant inbred lines that was developed from a cross between 2 cultivated

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 455–462

Einkorn genotypes (e.g., ID-362 bread-making quality poor and ID-331 bread-making quality good). From the parents ID-362 had always more Zn than the other parent in all four locations. The four locations presented different mean values, varying from 1.09 to 2.16 μg seed^{-1} for Zn content, from 0.83 to 1.97 μg seed^{-1} for Fe content, from 1.43 to 1.97 μg/seed^{-1} for Mn content and from 0.14 and to 0.24 μg seed^{-1} for Cu content. Pooling the results of the four trials, a major QTL, common to all four microelements and explaining from 10 to 30% of the variation (depending on the mineral assayed), was observed only on the chromosome 5, and not on the other chromosomes. The Einkorn germplasm tested had a significant variation for micronutrients, especially Zn and this variation could be exploited inbreeding programs. Chromosome 5 likely carries the genes affecting micronutrient accumulation in Einkorn seeds

Keywords: *Triticum monococcum*, micronutrients, zinc deficiency, iron deficiency

INTRODUCTION

Currently, half of the world population suffers from micronutrient deficiencies, especially Fe and Zn. Zinc and Fe deficiencies cause very serious health problems such as impairments in the immune system, physical growth, mental and cognitive development and increases in anemia, morbidity and mortality (Welch and Graham 1999, Hotz and Brown 2004). As a main source of calorie intake, cereal-based foods are extensively consumed in the developing world. However, cereals are inherently very poor both in concentration and bioavailability of Zn and Fe in seeds. Welch and Graham (1999) reported that cereal grains are the primary source of Fe and Zn for people in developing countries; however, intakes do not satisfy their mineral requirements. Increasing the total amount and bioavailability of Zn and Fe in food crops is, therefore, a big challenge.

One major approach to minimize micronutrient deficiencies in human beings in developing countries is the selection and development of new plant genotypes with high grain density of micro nutrients in edible parts. Existence of large genetic variation for micronutrients in seeds is essential for a successful breeding program aiming at development of micronutrient-rich new plant genotypes. Several authors have reported a large genotypic variation for Zn and Fe in different cereal species (Peterson et al. 1986, Rengel et al. 1999, Graham et al. 1999, Cakmak et al. 2000). A number of recent studies deal with genetic variation for micro-element content in seeds, such as in bean (Beebe et al. 2000), rice (Gregorio et al. 2000), wheat (Ortiz-Monasterio and Graham 2000, Cakmak et al. 2004) and maize (Banziger and Long 2000). In cultivated wheats, variation in seed Zn and Fe concentration is relatively small and seems to be not promising for a genetic improvement of wheat (Rengel et al. 1999, Cakmak et al. 2004). Compared to cultivated wheat cultivars, wild and primitive wheats, such as *Triticum monococcum, T. dicoccon* and *T. dicoccoides*, were found to be much more promising genetic donors for micronutrients (Cakmak et al. 2000, 2004, Ortiz-Monasterio and Graham 2000). Among wild

wheat germplasm, the emmer wheat, *T. dicoccoides*, showed the largest variation and the highest concentration of micronutrients, especially for Zn, and is considered a promising genetic source to improve Zn and Fe concentrations of wheat seeds (Cakmak et al. 2004). However, little is known for the diploid wheat *T. mocococcum*. Einkorn wheat produces protein equal to durum when grown under adverse condition (Vallega 1979). In addition, the seed amino acid composition in Einkorn is similar to those of other wheats, irrespective of a very large variation in total proteins (Acquistucci et al. 1995). *T. monococcum* was also found to contain high levels of both protein and carotenoids (Borghi et al. 1996). According to Borghi et al. (1996). *T. monococcum* genotypes contain nearly 7 times more carotenoids than cultivated wheat. It is important to study the genetic potential of *T. monococcum* and the mapping populations derived from *T. monococcum* for micronutrients and to characterize the localization of genes and QTLs involved in micronutrient accumulation in seeds.

The main objectives of this study were a) to determine the degree of genetic variability for micro-elements in Einkorn wheat accessions; and b) to identify QTL associated with microelements content (particularly Zn and Fe) in Einkorn wheat, using 168 recombinant inbred lines derived from a cross between ID 362 (poor breadmaking quality) and ID 1331 (good breadmaking quality).

MATERIALS AND METHODS

In the present study, seeds of 54 accession of Einkorn *(T. monococcum)* were used for analysis of Zn, Fe, Mn and Cu. These accessions were kindly obtained from USDA. All lines were grown in 2003–2004 at Adana, Turkey, in two contrasting environments (upland and lowland conditions). The seeds obtained from the field trials were dehulled and analyzed for Zn, Fe, Mn and Cu by using inductively coupled plasma-atomic emission spectrometry (ICP-AES). Seed samples were digested by using a microwave digesting system and then subjected to ICP tests. The measurements were checked using the certified mineral nutrients values in durum wheat flour samples obtained from the National Institute of Standards and Technology. The reference materials used was the durum wheat flour (8436).

The Map

A mapping population has been used to study genetic variation for micronutrients and to identify QTLs which are associated with high micronutrient concentration in seeds. The original consensus map (Fig. 1, Taenzler et al. 2002) was built from two populations of 117 and 168 F_2 plants, respectively, from which F_3 families were derived. Population 1 (117 progenies) was derived from a cross between ID 49, a wild Einkorn line (T. m. ssp. boeoticum), and ID 69, a free-threshing, cultivated Einkorn (T. m. ssp. monococcum var. sinskajae). Population

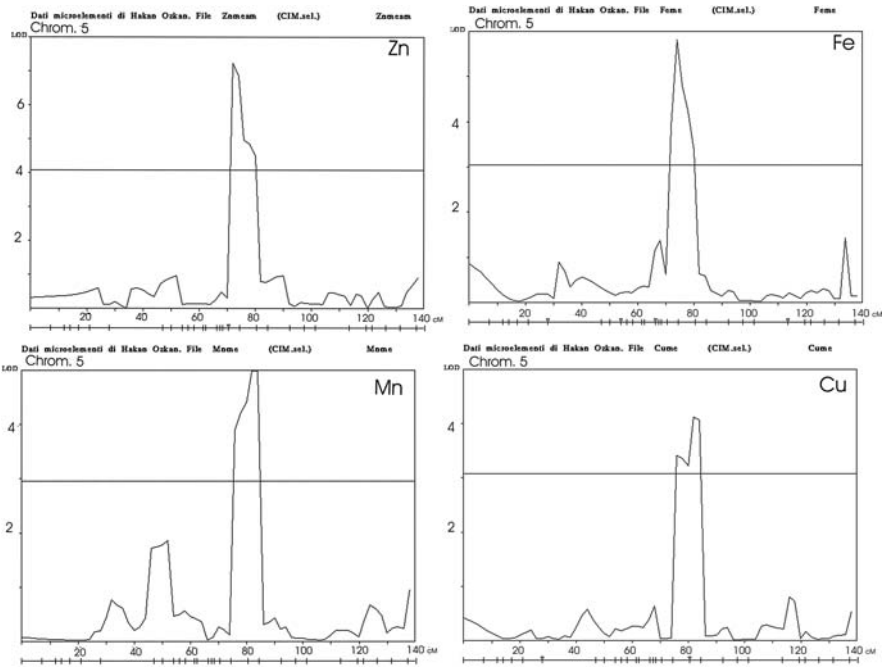


Figure 1. Localisation on einkorn chromosome 5 of QTLs controlling Zn, Fe, Mn and Cu content. The analyses are based on the average contents of four locations. The LOD thresholds corresponding to $P \leq 0.05$ (solid lines) are 4.09, 3.04, 2.96 and 3.08, respectively, while the LOD thresholds corresponding to $P \leq 0.01$ (dotted lines) are 5.22, 4.10, 3.76 and 4.44, respectively

2 (168 progenies) was derived from a cross between two cultivated Einkorn lines with different breadmaking quality, ID 362 (poor quality) and ID 1331 (good quality). The integrated map, based on the data sets for both populations and including 477 markers (32 RFLPs, 438 AFLPs, one morphological and six storage-proteins), was assembled using JoinMap version 2.0 (Stam and Van Ooijen 1995). Its total length is of 856 cM, with an average distance of 1.8 cM between markers.

Microelements Content

To assess microelements content, F3-derived families of Population 2 were grown in Sant'Angelo Lodigiano (Italy) in 1998 (S98) and 2004 (S04), in Cologne (Germany) in 2003 (K04) and in Adana (Turkey) in 2004 (A04), in 10 m^2 plots, following the agronomic practices described by Castagna et al. (1995). After harvest, the seeds were manually dehulled and microelements content assessed as above described. Data obtained for F_3–derived families were taken as indicators of values for individual F_2 plants.

QTL Analysis

To localize the QTLs responsible for microelements content, the chromosome marker order determined by JoinMap was transfered to the computer program PLABQTL (Utz and Melchinger 1996), together with laboratory data (Fe, Zn, Mn and Cu content) of Population 2. The numbers of markers mapped in Population 2 and considered in our QTL analysis were 36, 28, 33, 18, 37, 26 and 13, respectively, for linkage groups 1 to 7 (data not shown). The allelic average substitution value of the chromosome fragment hosting the microelement-related gene was determined by halving the differences between the genotypic values of the two homozygotic classes.

RESULTS AND DISCUSSION

Genetic Variation for Microelements between Einkorn Accessions

The analysis of microelements concentration and content in seeds of einkorn accessions grown in Adana, in two environments, are presented in Table 1. Clear variation was observed in seed micronutrient concentrations among the accessions. Zinc content of the seeds varied from 0.21 to 2.16 μg seed^{-1}, with an average of 1.21 μg seed^{-1} and Fe content varied from 0.54 to 3.09 μg seed^{-1}, with an average of 1.27 μg seed^{-1}. A similar variation was also found with Mn, but in the case of Cu the genetic variation was lesser.

There was a significant ($p \leq 0.05$) positive correlation between microelements in two locations, most noticeably for Zn, which correlated with Fe, Mn and Cu (data not shown). Similar results were reported by Cakmak et al. (2004) for Zn and Fe. The correlation between Fe and Zn in grain was also reported by Peterson et al. (1986) and Graham et al. (1999). This may point to common genetic mechanisms controlling Zn and Fe uptake and seed deposition. Correlations among microelements indicate that the improvement of one micronutrient (e.g. Zn) may

Table 1. Concentration and content of Zn, Fe, Mn and Cu in seeds of 54 *Triticum monococcum* accessions grown in two different place

Locations	Concentration (mg kg^{-1} dry wt.)							
	Zn		Fe		Mn		Cu	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Upland	51	36–76	43	32–61	42	26–60	6.6	4.1–10
Lowland	59	44–84	51	35–85	56	31–92	6.2	3.9–9.1
	Content (μg seed^{-1})							
	Zn		Fe		Mn		Cu	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Upland	1.21	0.37–2.07	1.02	0.54–2.07	1.01	0.53–2.08	0.16	0.10–0.23
Lowland	1.17	0.21–2.16	1.27	0.62–3.09	1.32	0.54–2.23	0.41	0.10–2.56

simultaneously improve the content of other micronutrients (e.g. Fe). The variation for Zn, Fe, Mn and Cu content in seed was much greater when compared to the variation found for the concentration (Table 1). These results suggest that 1) ample genetic variation is detected in the progeny of crosses if the parents have different microelements contents; and 2) it is possible to develop new wheat cultivars with higher Zn and Fe content.

Mapping Population

In the ID 362 x ID 1331 mapping population, the content of four microelements was determined in four different locations: S98, K03, S04 and A04 (see Material and Methods). The parent ID 362 always showed higher content of microelements than the ID 1331 (Table 2); the average lowest and highest values over the locations were 2.06 vs 3.12 for Zn, 1.63 vs 1.80 for Fe, 1.76 vs 2.06 for Mn and 0.12 vs .018 for Cu.

The four locations differed in mean values of micronutrients, varying from 1.09 (A04) to 2.16 (S04) for Zn content, from 0.83 (A04) to 1.97 (K03) for Fe content, from 1.43 (K03) to 1.97 (S98) for Mn content and from 0.14 (S98 and A 04) to 0.24 (S04) for Cu content. Among progenies within locations, the values between samples with low and high microelements content varied 2– to 5–fold; averaged

Table 2. Average values (± s.e.) recorded for Population 2 (parents and F3-derived progenies) for microelements content. For F3 progenies, the field of variation covered by single progenies is also reported.2164839550

Micro element	Location					
	Genotype	S98	K03	S04	A04	Average
Zn	ID 362	2.91 ± 0.04	3.50 ± 0.00	2.95 ± 0.18	–*	3.12 ± 0.19
	ID 1331	2.40 ± 0.11	2.04 ± 0.34	1.74 ± 0.07	–	2.06 ± 0.19
	progenies	1.98 ± 0.03	1.50 ± 0.03	2.16 ± 0.03	1.09 ± 0.02	1.70 ± 0.02
	range	1.29–3.40	0.96–2.85	1.26–3.68	0.49–1.72	1.15–2.33
Fe	ID 362	1.89 ± 0.03	2.34 ± 0.44	1.18 ± 0.07	–	1.80 ± 0.34
	ID 1331	1.78 ± 0.17	2.03 ± 0.69	1.08 ± 0.03	–	1.63 ± 0.28
	progenies	1.46 ± 0.02	1.97 ± 0.04	1.15 ± 0.02	0.83 ± 0.02	1.36 ± 0.02
	range	0.92–4.63	0.99–4.59	0.71–1.99	0.33–1.31	0.96–1.96
Mn	ID 362	2.65 ± 0.07	1.71 ± 0.36	1.81 ± 0.11	–	2.06 ± 0.30
	ID 1331	2.30 ± 0.07	1.60 ± 0.15	1.39 ± 0.02	–	1.76 ± 0.28
	progenies	1.98 ± 0.03	1.43 ± 0.03	1.47 ± 0.02	1.70 ± 0.05	1.64 ± 0.02
	range	1.28–2.91	0.81–2.50	0.78–2.22	0.62–3.52	1.16–2.40
Cu	ID 362	0.06 ± 0.00	0.18 ± 0.02	0.31 ± 0.02	–	0.18 ± 0.07
	ID 1331	0.05 ± 0.01	0.12 ± 0.04	0.20 ± 0.01	–	0.12 ± 0.04
	progenies	0.14 ± 0.03	0.15 ± 0.00	0.24 ± 0.00	0.14 ± 0.00	0.17 ± 0.00
	range	0.04–0.21	0.01–0.27	0.16–0.38	0.06–0.26	0.11–0.24

* No parents analysed

over location, the progenies with high microelements content showed values double than those with low content. The frequency distribution of Zn, Fe, Mn and Cu level showed considerable transgression in both directions for all microelements (data not shown). This suggests that both accessions carry genes with alleles contributing to an increased content for all microelements tested.

QTL Analysis

For all the traits considered, two LOD score thresholds were computed, the first corresponding to a significance level of $P \leq 0.05$ and varying between 2.96 and 4.08, and the second corresponding to a significance level of $P \leq 0.01$ and varying between 3.76 and 5.22, depending on the microelement considered. The analyses revealed a major QTL on chromosome 5, insisting in the same interval and present in two environments (S98 and K03) for Zn and Mn, and only in K03 for Fe. For Zn and Mn content, second QTL was detected on chromosome 1 in one location (K03). No QTLs were evident for Cu content, even though smaller peaks were present in the same chromosome 5 position of the other microelements. The analyses carried out by pooling the data of the four locations confirmed the existence of a strong QTL ($P \leq 0.05$) between 71 cM and 86cM from the tip of the short arm of chromosome 5 that is associated with Zn, Fe, Mn and Cu content. Three out of four QTLs (for Zn, Fe and Mn) were significant also at LOD scores corresponding to $P \leq 0.01$ (Fig. 1).

The substitution value of the allele originating from ID 362 varied between +0.30 (for Cu) and +0.42 (for Zn), indicating that the allelic segment encompassing the region of the QTL in ID 362 induces an increase of 0.30 to 0.42 units (depending on the microelement) compared with the same allelic fragment of ID 1331. Minor dominance effects (+0.15 and +0.12) of complementary sign to the additive effect were observed in the case of Zn and Fe, respectively.

The results obtained suggest that the *T. monococcum* is a promising genetic resource for working genetic variation and identification of genes for micronutrients, especially Zn. Studies are on-going to collect more information on the localization of genes affecting content of micronutrients (especially Zn) in different genetic stocks derived from *T. monococcum*.

ACKNOWLEDGEMENTS

The authors are grateful to Biofortification Challenge Program (www.harvestplus.org) and the State Planning Organization of the Turkish Republic (www.dpt.gov.tr) for the financial support.

REFERENCES

Acquistucci R, D'Egidio MG, Vallega V (1995) Amino acid composition of selected strains of diploid wheat, *Triticum monococcum* L. Cereal Chem 72:213–216

Bangizer M, Long J (2000) The potential for increasing the iron and zinc density of maize through plant breeding. Food Nutr Bull 21:397–400

Beebe S, Gonzalez AV, Rengifo J (2000) Research on trace minerals in the common bean. Food Nutr Bull 21:387–391

Borghi B, Castagna R, Corbellini M, Heun M, Salamini F (1996) Breadmaking quality of Einkorn wheat. Cereal Chem 73:208–214

Cakmak I, Ozkan H, Braun HJ, Welch RM, Romheld V (2000) Zinc and iron concentrations in seeds of wild, primitive and modern wheats. Food Nutr Bull 21(4):401–403

Cakmak I, Torun A, Millet E, Feldman M, Fahima T, Korol A, Nevo E, Braun HJ, Ozkan H (2004) Triticum dicoccoides: An important genetic resource for increasing zinc and iron concentration in modern cultivated wheat. Soil Sci Plant Nutr 50(7):1047–1054

Castagna R, Borghi B, Di Fonzo N, Heun M, Salamini F (1995) Yield and related of Einkorn (*Triticum monococcum* ssp. monococcum) in different environments. Eur J Agr 4:371–378

Graham RD, Senadhira D, Beede SE, Iglesias C, Ortiz-Monasterio I (1999) Breeding for micronutrient density in edible portions of staple food crops: conventional approaches. Field Crops Res 60:57–80

Gregorio GB, Senadhira H, Htut H, Graham RD (2000) Breeding for trace mineral density in rice. Food Nutr Bull 21:382–386

Hotz C, Brown KH (2004) Assessment of the risk of zinc deficiency in populations and options for its control. Food Nutr Bull 25:94

Ortiz-Monasterio I, Graham RD (2000) Breeding for trace minerals in wheat. Food Nutr Bull 21:392–396

Peterson CJ, Johnson VA, Mattern PJ (1986) Influence of cultivar and environment on mineral and protein concentrations of wheat flour, bran and grain. Cereal Chem 63:118–186

Rengel Z, Batten GD, Crowley DE (1999) Agronomic approaches for improving the micronutrient density in edible portions of staple field crops. Field Crops Res 60:27–40

Stam P, Van Ooijen JW (1995) Join Map Version 2.0. Software fort he calculation of genetic linkage maps. Wageningen, The Netherlands, CPRO-DLO

Taenzler B, Esposti RF, Vaccino P, Brandolini A, Effgen S, Heun M, Schafer-Pregl R, Borghi B, Salamini F (2002) Molecular linkage map of Einkorn wheat: mapping of storage-protein and soft-glume genes and bread-making quality QTLs. Genet Res 80:131–143

Utz HF, Melchinger AE (1996) PLABQTL: a program for composite interval mapping of QTL. Journal of Quantitative Trait Loci 2:1

Welch RM, Graham RD (1999) A new paradigm for world agriculture: meeting human needs. Productive, sustainable, nutritious. Field Crops Res 60:1–10

Vallega V (1979) Field performance of varieties of *Triticum monococcum, T. durum*, and *Hordeum vulgare* grown at two locations. Genet Agr 33:363–370

MOLECULAR WEIGHT DISTRIBUTION OF GLUTEN PROTEINS

O.R. LARROQUE[1,2,*], P. SHARP[2] AND F. BEKES[1]

[1] *CSIRO Plant Industry, GPO Box 1600, Canberra ACT 2601, Australia*
[2] *University of Sydney, Plant Breeding Institute, PMB11, Camden NSW 2570, Australia*
[*] *E-mail: oscar.larroque@csiro.au*

Abstract: Gluten confers on flour the ability to produce viscoelastic doughs. The molecular weight distribution (MWD) of gluten proteins is one of the main determinants of physical dough properties. Size-exclusion high performance liquid chromatography (SE-HPLC) is routinely used to evaluate this parameter. A proper, highly efficient solubilization protocol plays a vital role in the determination of MWD. Coupling SE-HPLC with multi-angle laser light scattering (MALLS) has brought the possibility of unravelling new information about these polymers. Therefore, finding a solubilization method to study the polymeric structures of gluten by MALLS was our main objective. We used short term extraction with 50% aqueous acetonitrile added with 0.05% triflouracetic acid. This gave better reproducibility than extractants containing sodium dodecyl sulfate (SDS), with similar efficiency. Using flour extracts from the Australian wheat cvs. Chara and Banks we collected 15 sub-fractions from the first peak of the SE-HPLC chromatograms to determine the molecular weight average (Mw) of the fractions, with and without sonication. Similar Mw was found between sonicated and non-sonicated samples for all fractions except the first one (void volume) where the sonicated sample showed higher values. We also found that the majority of high molecular weight glutenin subunits (HMW-GS) eluted in a much narrower sector of the polymeric peak when compared with previous findings. Based on these findings, a solubilization method combining SE-HPLC/MALLS and RP-HPLC was developed

Keywords: molecular weight, protein, biochemical markers

INTRODUCTION

Gluten is a unique compound made up from glutenins and gliadins, the major storage proteins in wheat endosperm. It confers on flour the ability to produce viscoelastic doughs. Both glutenin and gliadin fractions are important contributors to the rheological properties of dough. Whilst glutenin provides elasticity due to

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 463–469.

its polymeric structure, the monomeric component of gluten (gliadin fraction) is responsible for the viscous behaviour. In a way, gliadins act as "plasticizers" or "solvents" for glutenins. A proper balance between them is essential for good quality doughs and final products (Gianibelli et al. 2001). This balance is usually evaluated by SE-HPLC analysis of total protein extracts that provide the relative amounts of monomeric and polymeric protein (Bietz 1984). Nevertheless, the above measurement is not enough to accurately predict quality differences between wheat flours. The other very important aspect to be considered is the molecular weight distribution (MWD) of polymeric protein (Gupta et al. 1993).

A proper, highly efficient solubilization protocol plays a vital role in the determination of MWD. Nevertheless, thorough solubilization is achieved by sonication, a process that hinders the study of the real sizes involved. Furthermore, due to the exclusion limit of the SEC columns, all material of molecular weight higher than about 1×10^7 Da is co-eluted in the exclusion volume limiting the determination of the MWD. The upper molecular weight limit of glutenin as a result cannot be accurately determined (Jumel et al. 1996).

Detection by the use of concentration detectors (UV at 214 nm) has proven quite effective in determining the approximate molecular weight of individual peaks using molecular weight standards. In more recent years, coupling SE-HPLC with multi-angle laser light scattering (MALLS), an absolute method to determine the molecular weight, shape, and conformation of polymers (Wyatt 1993), has brought the possibility of unravelling new information about these polymers. More importantly, for SE-HPLC measurements MALLS can also provide some information on the void volume peak of the chromatographic separation, namely molecular weight and radii of gyration averages.

Therefore, finding a solubilization method to study the polymeric structures of gluten by MALLS was our first objective. Because the usual mobile phase in our laboratory and elsewhere for SE-HPLC analysis is 50% ACN/0.05%-0.07% TFA, we tested the possibility of using it as protein extractant, which would provide a few advantages: a much simpler component system for MALLS detection, no SDS residues for the analysis of SEC-collected fractions through RP-HPLC and longer column life.

To further analyse and interpret the MWD of these proteins, the collection of sub-fractions within the polymeric area of the SEC chromatogram was performed (Batey et al. 1991, Larroque et al. 1997). In order to test new fraction collection protocols we aim at taking another look into this topic.

Finally, by achieving the previous aims we would be able to develop a new methodology to provide as many quantitative and qualitative biochemical markers as possible from one single extract analysis in order to better, quicker understand the MWD of gluten.

MATERIALS AND METHODS

Plant material: Australian wheat cultivars Chara ($2^*, 7^{OE} + 8^*, 2 + 12$) a strong hard wheat and Banks ($2^*, 7 + 8, 2 + 12$) a medium-strength hard wheat were used in this study.

Size Exclusion HPLC: Samples were extracted using the procedure of Batey et al. (1991) for total protein and Gupta et al. (1993), for SDS-soluble (without sonication) polymeric protein and SDS-soluble (with sonication) polymeric protein with modifications by Larroque and Bekes (2000). Sonication was performed at 25% amplitude (9W) using a Branson digital sonifier (model 450, Branson Ultrasonics Corp., Danbury, CT) equipped with a tapered micro tip (end diameter of 3.2 mm). The end of the micro tip was placed at just over the pellet. A Shodex Protein KW-804 column was used throughout the study.

MALLS: A MiniDawn (Wyatt Technology, Santa Barbara, CA, USA) system with 3 detection angles at 45, 90 and 135 degrees in combination with a UV detector was used throughout the study.

SDS-PAGE: Total proteins were fractionated in vertical SDS-PAGE slab gels in a discontinuous buffer system using a 4% acrylamide stacking gel and a 10% acrylamide separating gel ($160 \times 200 \times 0.75$mm) contained 10% (w v^{-1}) acrylamide, 0.13% (w v^{-1}) bisacrylamide, 0.1% SDS and 375 mM Tris made to pH 8.5 with HCl.

RESULTS AND DISCUSSION

The use of 50% acetonitrile with 0.05% trifluoracetic acid gave acceptable overall extraction levels in our study. For both cultivars, extraction levels closer to 90% were achieved with the aid of sonication. In comparison, results for the SDS-based solvent were over 95% (Table 1), in agreement with Singh et al. (1990).

More recently, Bean and Lookhart (2001) established that 50% ACN/0.1% TFA showed extraction rates of 57.4% compared with 66.3% of 1% SDS/NaPhosphate, pH 7.0 for short term extraction without the use of sonication. In terms of long term extraction (24 h shaking, no sonication), the same authors indicated that the ability of ACN to extract more protein was very limited (up 2.7%), particularly compared to SDS (up 13.7%). In our analysis, using cvs. Banks and Chara for short and long extraction, we found similar results (Table 2).

The Percentage of Unextractable Polymeric Protein (%UPP) technique is a sequential two-step solubilisation process in which larger polymeric structures

Table 1. Comparison between extraction rates for wheat cv. Chara and Banks. a) 50% aqueous acetonitrile + 0.05 TFA and b) 0.5% SDS 0.05M phosphate buffer.*Protein content determined on pellet after sonication. Average of two replicates

	a	b
Banks flour	100	100
Banks*	89.57	97.58
Chara flour	100	100
Chara*	89.79	95.75

Table 2. Changes in %UPP: comparison between short (1′, 15′) and long (12h, 24h) first step (SnS) solubilization of protein extracts from cultivars Chara and Banks. Average of two replicates. SDS: 0.5% SDS/NaPhos pH 7 buffer; ACN: 50% aqueous acetonitrile + 0.05% TFA. SS: area under the curve of soluble with sonication extract (2nd step); SnS: area under the curve of soluble without sonication extract (1st step)

first step solubilization	%UPP (SS/(SnS+SS))*100			
	Chara/SDS	Chara/ACN	Banks/SDS	Banks/ACN
1′	61.75	57.90	54.91	51.08
15′	54.69	59.26	49.53	50.23
12 h	50.66	57.49	39.20	50.81
24 h	46.84	55.47	34.16	45.20
% change				
15′–24h	−14.36	–6.40	−31.04	−10.01

remain in the pellet after a mild first extraction and are subsequently extracted with the aid of sonication (Gupta et al. 1993). A decrease in %UPP is associated, in this case, with an improved solubilization of soluble without sonication (SnS) polymers in the first step of the extraction protocol. The better performance of SDS-based solvents could be based on a more thorough disruption of non-covalent interactions. %UPP is a good indicator of polymer size distribution and therefore a good marker for dough strength. Either of both solvents is able to differentiate between the stronger flour (cv. Chara) and the medium-strength one (cv. Banks). Since both cultivars only differ at the expression level of subunit Glu1Bx7 in terms of HMW-GS, it is possible that the presence of the over-expressed subunit in cv. Chara is responsible for glutenin that is more difficult to solubilize in the longer term.

The next step was to compare the Mw average of soluble with sonication (SS) and soluble without sonication (SnS) samples in the void volume fraction. By doing that, we would be able to evaluate the effect of sonication on the final size of the polymers in the excluded peak. The comparison clearly indicated that even with the use of sonication, the size of the extracted SS polymer is larger than SnS. This is independent of the cultivar being assessed (Table 3). In the case of cv. Banks the SS Mw average for the excluded peak was higher than in cv. Chara. On the other hand, Chara showed larger SnS Mw average values. Whilst the rate for SS/SnS was 5.04 in cv Banks, in cv Chara the value was much lower at 3.09 (Table 3).

When a large wheat collection set was analysed, different ratios were also found (data not shown). Sub-fractions from the eluted SS extracts were also analysed in order to determine to a much closer level the average Mw of the larger polymeric structures. The first sub-fraction, corresponding to the earlier eluting material within the void peak, had MW averages of 1.950 e + 07 and 2.106e + 07 for Chara and Banks respectively.

Table 3. MALLS measurements from SS and SnS void volume fractions of extracts from cvs. Banks and Chara. SS-sub1, sub2 and sub3: sub-fractions within the void volume fraction of SS. %E: Percentage error determined by Astra software. M: molecular weight R: radii of gyration; n: number average; w: weight average; z: Z average

Banks	SS total	%E	SS sub-1	%E	SS sub-2	%E	SS sub-3	%E	SnS	%E	SS/SnS
(M)n $\times 10^6$	4.923	0.6	13.66	1	4.291	0.4	3.538	0.5	1.479	0.8	
(M)w $\times 10^6$	13.07	2	21.06	3	8.205	1.2	6.876	1.8	2.59	1.7	5.05
(M)z $\times 10^6$	36.66	7	35.62	15	21.64	4	19.18	7	6.691	5	
Rn	31	1.8	53.6	1.4	28.7	1.6	25.9	2.4	30	2.7	
Rw	44.9	1.7	63.5	2.5	38.5	1.4	35.2	2.1	39	2.3	
Rz	73.7	2.6	79.9	4	61.2	1.6	58.7	2	60	2	
Chara	SS	%E	SS-sub-1	%E	SS-sub-2	%E	SS-sub-3	%E	SnS	%E	SS/SnS
(M)n $\times 10^6$	4.454	0.5	13.1	0.8	3.878	0.6	3.006	0.7	1.719	1	
(M)w $\times 10^6$	11.2	1.2	19.5	1.5	7.516	1.2	6.18	1.3	3.622	1.6	3.09
(M)z $\times 10^6$	32.81	4	31.69	4	19.89	4	18.69	5	1.15	5	
Rn	30.3	1.7	51.2	1.1	26.7	2.8	23.2	4	23.8	5	
Rw	41.8	1.3	60.6	1.3	35.6	1.9	32.5	2.2	34.2	2.9	
Rz	68.2	1.6	76.5	1.6	58.1	1.7	55.8	1.4	60.3	1.9	

SDS-PAGE analysis of reduced fractions indicated that majority of high molecular weight glutenin subunits (HMW-GS) eluted in a much narrower sector of the polymeric peak when compared with previous findings (Larroque et al. 1997). Under our running conditions, the presence of HMW-GS was very scarce from around 8.15 mL of eluted volume. This represented about 3/4 of the total elution volume for the polymeric peak and coincided with the presence of two smaller peaks adjacent to the large gliadin peak. Similar results were found in cv. Banks (data not shown). This may be due to the improvements in the collection protocol, the column used and the extraction conditions. This finding would be particularly important in selecting appropriate, more meaningful, areas under the curve of the chromatograms for the determination of the %UPP value.

In order to improve the evaluation of technological properties of wheat flours a new, faster methodology was developed taking into account some of the results obtained in this study. Briefly, a single SE-HPLC determination of SnS-SE with MALLS detection was followed by a RP-HPLC analysis of the pellet (SS-RP) under reduced conditions. Since it was possible to relate areas under the curve in the SE and RP separations (using BSA as standard), the %UPP and Glutenin/Gliadin ratio could also be established. For the determination of the SS/SnS ratio, a second SE-HPLC+MALLS determination of SS extract was performed.

ACKNOWLEDGEMENTS

The University of Sydney PRSS scheme and CSIRO Plant Industry supported ORL to attend the Conference.

REFERENCES

Batey IL, Gupta RB, MacRitchie F (1991) Use of size-exclusion high-performance liquid chromatography in the study of wheat flour proteins: An improved chromatographic procedure. Cereal Chem 68:207–209

Bean SR, Lookhart GL (2001) Factors influencing the characterization of gluten proteins by size-exclusion chromatography and multiangle laser light scattering (SEC-MALLS). Cereal Chem 78:608–618

Bietz J (1984) Analysis of wheat gluten proteins by high-performance liquid chromatography. Bakers Digest 58:15–17, 20–21, 32

Gianibelli MC, Larroque OR, MacRitchie F, Wrigley CW (2001) Biochemical, genetic, and molecular characterization of wheat glutenin and its component subunits. Cereal Chem 78:635–646

Gupta RB, Khan K, MacRitchie F (1993) Biochemical basis of flour properties in bread wheats. I. Effects of variation in the quantity and size distribution of polymeric protein. J Cereal Sci 18:23–41

Jumel K, Fiebrig I, Harding SE (1996) Rapid size distribution and purity analysis of gastric mucus glycoproteins by size exclusion chromatography/multi angle light scattering. Int J Bio Macromol 18:133–139

Larroque OR, Gianibelli MC, Batey IL, MacRitchie F (1997) Electrophoretic characterization of fraction collected from gluten protein extracts subjected to size-exclusion high-performance liquid chromatography. Electrophoresis 18:1064–1067

Larroque OR, Bekes F (2000) Rapid size-exclusion chromatography analysis of molecular size distribution for wheat endosperm protein. Cereal Chem 77:451–453

Singh NK, Donovan GR, Batey IL, MacRitchie F (1990) Use of sonication and size-exclusion HPLC in the study of wheat flour proteins. I. Dissolution of total proteins in unreduced form. Cereal Chem 67:150–161

Wyatt PJ (1993) Light scattering and the absolute characterization of macromolecules. Anal Chim Acta 272:1–40

GLUTENIN AND GLIADIN ALLELIC VARIATION AND THEIR RELATIONSHIP TO BREAD-MAKING QUALITY IN WHEAT CULTIVARS GROWN IN GERMANY

F.J. ZELLER*, G. WENZEL AND L.K. SAI HSAM

Technical University of Munich, Center for Life and Food Sciences, Institute of Plant Breeding, Am Hochanger 2, 85350 Freising-Weihenstephan, Germany

**E-mail: Zeller@wzw.tum.de*

Abstract: Glutenins and gliadins are the most important protein fractions for bread-making quality. Commercial German wheat cultivars were classified for their high molecular weight glutenin (HMW), low molecular weight (LMW) and gliadin allelic constituents employing the standard sodium dodecyl sulphate (SDS-PAGE) and acid (A-PAGE) polyacrylamide gel electrophoresis methods, respectively and their relationships to bread loaf volumes were conducted. Six glutenin (*Glu-1*) alleles, *Glu-A1a, Glu-A1c, Glu-B1c, Glu-B1d, Glu-D1a* and *Glu-D1c* are important in determining the loaf volume variabilty. The effects of low molecular weight (*Glu-3)* and gliadin (*Gli-1*) alleles were also observed, among which the rare gliadin alleles *Gli-B1c* and *Gli-D1g* appear to be found exclusively in high grade quality wheat groups

Keywords: HMW glutenins, LMW glutenins, Gliadins, Baking quality, Loaf volume

INTRODUCTION

Classification of wheat cultivars into quality groups has a long tradition in Europe. In Germany, commercial varieties are grouped into four quality categories: Elite (E), quality (A), bread (B) and special (C) wheats based on rheological and loaf volume determined by the standard Rapid Mix Test (RMT). Differences in bread-making quality have been attributed to the qualitative and quantitative composition of the storage gluten proteins in the endosperm of the wheat kernels. The most important protein fractions, the gliadins and glutenins are the primary determinants of dough extensibility and dough elasticity, respectively (Payne 1987). Glutenins are

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 471–477.

multimeric aggregates of high molecular weight (HMW) and low molecular weight (LMW) subunits. HMW glutenin subunits (HMW-GSs) are encoded by *Glu-A1*, *Glu-B1* and *Glu-D1* on the long arm of homoeologous group-1 chromosomes 1A, 1B and 1D, respectively, while LMW-GSs are encoded by *Glu-A3*, *Glu-B3* and *Glu-D3* on the short arm of these chromosomes. The main gliadin-coding loci, *Gli-1* and *Gli-2*, are located on the short arms of the homoeologous chromosomes of the first and sixth group, respectively (Payne 1987), and the *Gli-1* loci are tightly linked to the corresponding *Glu-3* loci. HMW-GSs that confer good bread-making quality are well documented (Payne et al. 1987, Hsam et al. 2001, Tohidfar et al. 2004), however, the roles of LMW-GSs and gliadins on bread-making quality has been studied less intensively (Maruyama-Funatsuki 2004).

In Germany, winter wheats predominate over spring wheats with a seed production area of 65,800 ha of winter wheat in 2005, as compared to 2,070 ha for spring wheat. The production of winter wheat is currently 23.2 Mt. Moreover, the past decades have witnessed an increased emphasis of breeding for bread-making qualities in addition to agronomic and biotic stress resistance characteristics. The number of the best baking quality group, the elite (E) wheats has increased from six among the 68 registered cultivars in 1993 to 20 from the 117 winter wheat cultivars that are registered in the current National List of commercial cultivars (BSA 2005). A new cultivar must meet a prescribed minimum level of quality (Table1) before being registered for release. Previous selection methods for quality using rheological properties are now being supplemented by standard SDS-PAGE electrophoretic method. The current study reports on the allelic variation of the prolamines occurring in the commercially grown German wheat gene pool, with the objective to establish an evaluation scheme for earlier selection of bread-making quality.

MATERIALS AND METHODS

A set of 155 winter wheat varieties grown in the years 1997 to 2005 was selected and analysed. Gliadin analysis was performed using 70% ethanol extraction and A-PAGE, whereas HMW-GS and LMW-GS were visualized by SDS-PAGE employing the methods described by Yan et al. (2003). HMW-GS were numbered according to Payne and Lawrence (1983), while allele designation of LMW-GS and gliadin followed the nomenclature of Jackson et al. (1996). Evaluation of quality parameters involving falling numbers, protein content, SDS-sedimentation values and baking volumes (RMT) followed the methods of ICC-Standards (1994). SPSS programmes were used for statistical analyses.

RESULTS AND DISCUSSION

New cultivars with superior nutritional quality and resistance to diseases, as required by the European Union (EU) regulations are now widely grown in Germany. Among the important cultivars grown in 2005 (Table 1), Tommi has the greatest

Table 1. Quality characteristics of important cultivars grown in Germany in 2005

Cultivar	Year release	Quality group	Falling No. (s)	Seed Protein (%)	Sedi-value	RMT BV[1]	Yield	*Glu-A1*	*Glu-B1*	*Glu-D1*
Akteur	2003	E	+++[2]	+++	+++	8	−	1	7+9	5+10
Bussard	1990	E	(+)	++	+++	9	−−−	1	7+9	5+10
Enorm	2002	E	++	+	+++	8	−	1	7	5+10
Monopol	1975	E	+	++	+++	9	−−−	1	7+9	5+10
Astron	1989	A	+	+	+++	6	−	1	7+9	5+10
Cubus	2002	A	++	(−)	++	6	+++	N	7	5+10
Ellvis	2002	A	+++	o	(+)	6	+	N	7+9	2+12
Magnus	2000	A	+	(−)	(+)	6	+	N	22	5+10
Tommi	2002	A	+	(+)	++	6	++	1	6+8	2+12
Transit	1990	A	+++	+	(+)	7	(+)	1	6+8	2+12
Campari	2003	B	(+)	(−)	o	5	++	N	6+8	5+10
Dekan	1999	B	+	(−)	(+)	4	++	N	6+8	5+10
Hybred	2003	B	+	(−)	(+)	4	+++	1	6+8	5+10
Limes	2003	B	+	(−)	(−)	4	++	1	7	5+10
Certo	1999	C	+	(−)	(−)	3	+++	N	6+8	2+12
Hermann	2004	CK	(+)	+	+	2	+++	N	7	2+12

[1] bread loaf volume using a scale of 1–9, scale 5 = 622–651ml;
[2] +++ very high, + high, (+) medium to high, o medium, (−) medium to low, − low, −−− very low

seed production area followed by Dekan and Cubus (BSA 2005). Although the current trend is to utilize high yielding A-cultivars, earlier registered E-cultivars such as Monopol and Bussard are still grown.

Two different approaches are being used to correlate bread-making potential with HMW-GS composition. At each *Glu-1* locus, Payne et al. (1987) assigned superior quality scores for HMW-GS based on SDS-sedimentation value to *Glu-A1a* (band1), *Glu-B1b* (7 + 8) as well as *Glu-B1i* (17 + 18) and *Glu-D1c* (5 + 10), respectively. Ng and Bushuk (1988) used the HMW-GS composition to develop an equation for predicting the unit loaf volume based on protein contents.

In the present study, the frequencies of the HMW-GS *Glu-1* alleles varied among the cultivars analysed. Among them *Glu-A1c* (N) and *Glu-D1c* (5 + 10) occur at 74% and 59%, respectively (Table 2). Moreover, the frequency of HMW-GS allele *Glu-B1i* (17 + 18) is increasing in German wheat cultivars. Until 1997, Alidos was the only registered E-cultivar to possess this HMW-GS composition. Recently registered cultivars, including E-cultivars Altos, Creativ and Qualibo, as well as A-cultivar Boomer all carry the allele *Glu-B1i*. Among the allele combination, that of *Glu-A1a* (band1), *Glu-B1c* (7 + 9) and *Glu-D1d* (5 + 10) has the highest RMT bread volume, whereas the allelic combination *Glu-A1c* (N), *Glu-B1d* (6 + 8) and *Glu-D1a* (2 + 12) is associated with the lowest RMT bread volume, respectively. The HMW-GS combination 1, 7 + 9, 5 + 10 are found mostly in the E and A-cultivars. It appears that selection for quality wheat based on SDS-sedimentation method, as being practiced by German breeders unconciously selected for corresponding superior glutenin alleles.

Similar analyses were made for alleles determining LMW-GS and gliadin compositions. Four alleles, namely *Glu-A3a*, *Glu-A3d*, *Glu-A3e*, and *Glu-A3f* were detected at *Glu-A3* locus, whereas *Glu-D3a* and *Glu-D3c* were observed at the *Glu-D3* locus.

Table 2. Influence of *Glu-1* alleles on rapid mix test bread volume of 155 winter wheat cultivars

No. of cultivars	HMW-GS	*Glu-A1*	*Glu-B1*	*Glu-D1*	RMT- BV[1] (mean values)
36	1	a	–	–	6.4
5	2	b	–	–	6.2
114	N	c	–	–	5.3
10	7	–	A	–	5.3
11	7 + 8	–	B	–	5.5
62	7 + 9	–	C	–	6.5
64	6 + 8	–	D	–	4.5
1	20	–	E	–	6.0
7	17 + 18	–	I	–	6.5
61	2 + 12	–	–	A	4.8
3	3 + 12	–	–	B	4.7
91	5 + 10	–	–	C	6.3

[1] bread volume using a scale of 1–9, scale 5 = 622–651 ml

Six alleles involving *Glu-B3c*, *Glu-B3d*, *Glu-B3f*, *Glu-B3g*, *Glu-B3h* and *Glu-B3j* were found at the *Glu-B3* locus. Among them *Glu-A3f* was detected in 10% of the cultivars and *Glu-B3g* occurred in 70% of the cultivars. Both were found to be associated with higher bread volume. A total of 22 alleles was scored at the *Gli 1* loci, five at *Gli-A1* (*a,b,f,l,m,o*), nine at *Gli-B1* (*b,c,d,e,f,g,h,l,s*), and seven at the *Gli-D1* (*a,b,d,g,i,k,l*) loci, respectively.

Alleles *Gli-B1c* and *Gli-D1g* are presumed to be associated with superior bread quality as they are found mostly in the E and A-cultivars possessing a high bread loaf volume. The allele *Gli-B1l*, which is the secalin locus on the T1BL.1RS wheat-rye translocation occurred in 20% of the commercial cultivars and produced a low loaf volume.

The relationship of the 46 prolamine alleles detected in the set of analysed wheat cultivars was associated to the respective bread loaf volumes by using hierarchical cluster analysis. Two clusters were evidenced, with one involving the loaf volume and eleven prolamine alleles (Fig. 1). Among the *Glu-1* alleles are *Glu-A1a* (band 1), *Glu-B1c* (7 + 9) and *Glu-D1d* (5 + 10). Furthermore, these three alleles showed a significant correlation value of $r = 0.28^{**}$, $r = 0.39^{**}$ and $r = 0.51^{**}$ ($P = 0.001$) with loaf volume. It may be inferred that the HMW-GS combination of 1, 7 + 9, 5 + 10 showed a positive influence on the expansion of bread loaf volume. In addition, LMW-GS alleles *Glu-A3a*, *Glu-B3g, Glu-D3c* and *Gli-1* alleles *Gli-A1a*, *Gli-A1f*, *Gli-B1f* and *Gli-D1b* also belong to this cluster.

The second larger cluster involving alleles *Glu-B1d* (6 + 8), *Glu-D1a* (2 + 12), *Gli-B1l* (secalin) and other *Gli-1* alleles may probably exert a negative influence on the bread-making quality. The correlation between RMT bread loaf volume and the above mentioned three alleles are $r = -0.63^{**}$, $r = -0.47^{**}$, and $r = -0.38^{**}$, respectively. The remaining seven *Gli-1* alleles are *Gli-A1b*, *Gli-A1o, Gli-B1e*, *Gli-B1h*, *Gli-D1a*, *Gli-D1d* and *Gli-D1g*. It was unexpected to find the allele *Gli-D1g* in this cluster as it was present only in E-and A-cultivars. Similarly, the LMW-GS allele *Glu-A3d*, which was previously reported to have a positive effect on dough extensibility (Morel et al. 1994) is included in this group. However, it may be pointed out that the positive or negative influence of an allele on baking quality, either by its presence or absence may not be detected as much as when it is in combination with other alleles. Individual combination of the alleles showed that interactions between *Glu-1* and *Glu-3* as well as *Gli-1* alleles may be present. This interaction may be in the form of an epistatic effect or partial dominance and that favourable alleles may inhibit or compensate the effects of others. In this aspect the quality scoring system, including the 'rye-correction' method of Payne et al. (1987), which assumed an additive effect of the alleles may not be applicable to German wheat cultivars that were analysed in this study. Nevertheless, the superior effect of certain *Glu-1* alleles such as *Glu-B1c* (7 + 9) and *Glu-D1d* (5 + 10) on bread-making is convincing and should be further investigated in different genetic backgrounds. In addition, as wild species, particularly *Aegilops*

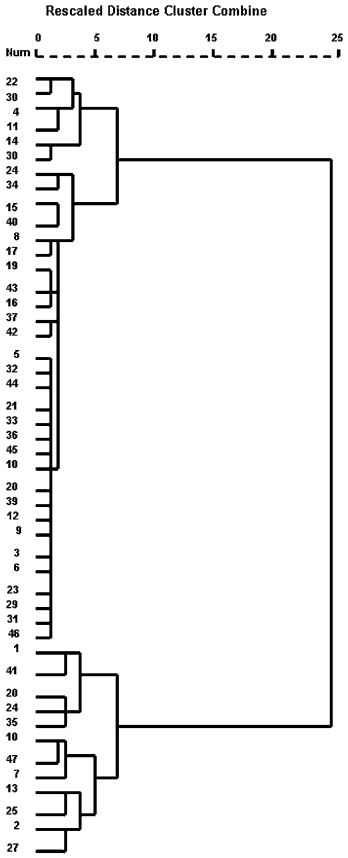


Figure 1. Cluster analysis of the 46 prolamine alleles (*Glu-1, Glu-3, Gli-1*) and RMT bread volume of commercial wheat cultivars grown in Germany. Description of variables: 1–3 *Glu-A1*(*c*, *a*, *b*); 4–9 *Glu-B1*(*d,a,b,c,h,i*); 10–12 *Glu-D1* (*d*, *a*, *b*); 13–16 *Glu-A3* (*a*, *d*, *e*, *f*); 17–22 *Glu-B3* (*c,d,e,g,h,j*); 23–24 *Glu-D3* (*a*, *c*);25–30 *GliA1* (*a*, *b*, *f*, *l*, *m*, *o*); 31–39 *Gli-B1*(*a,c,d,e,f,g,h,l,s*); 40–46 *Gli-D1*(*a,b,d,g,i,k,l*); 47 bread loaf volume

tauschii, the D-genome donor of common wheat possessed a significant influence on baking quality (Hsam et al. 2001) the search for novel allelic variation (Yan et al. 2003) for the improvement of bread-making quality should be further considered.

ACKNOWLEDGEMENTS

Financial support by the Ministry of Nutrition, Agriculture and Forestry (BML) and Community for the Enhancement of Private German Plant Breeding (GFP), and technical assistence of Sigrid Hiesch are gratefully acknowledged.

REFERENCES

BSA (2005) Beschreibende Sortenliste. Bundessortenamt Hannover, Deutscher Landwirts-chaftsverlag GmbH

Hsam SLK, Kieffer R, Zeller FJ (2001) Significance of *Aegilops tauschii* glutenin genes on breadmaking properties of wheat. Cereal Chem 78:521–525

ICC (1994) Standard methods. International association for cereal science and technology Moritz Schäfer, Detmold, Germany

Jackson EA, Morel M-H, Sontag-Strohm T, Branlard G, Metakovsky EV, Redaelli R (1996) Proposal for combining the classification systems of alleles of *Gli-1* and *Glu-3* loci in bread wheat (*Triticum aestivum* L.) J Genet Breed 50:321–336

Maruyama-Funatsuki W, Takata K, Nishio Z, Tabiki E, Yahata E, Kato A, Saito K, Funatsuki H, Yamauchi H (2004) Identification of low molecular weight glutenin subunits of wheat associated with bread-making quality. Plant Breed 123:355–360

Morel MH, Bonicel J, Melas V, Autran JC (1994) Multiple approach (IEF, SDS-PAGE and A-PAGE) on the composition of LMW subunits and its effect on dough properties. In: Gluten Proteins 1993, Association of Cereal Research, Detmold, pp 244–254

Payne PI (1987) Genetics of wheat storage proteins and the effect of allelic variation on bread-making quality. Annu Rev Plant Physiol 38:141–153

Payne PI, Lawrence GJ (1983) Catalogue of alleles for the complex gene loci, *Glu-A1*, *Glu-B1, Glu-D1* which code for high-molecular-weight subunits of glutenin in hexaploid wheat. Cereal Res Commun 11:29–35

Payne PI, Nightingale MA, Krattiger AF, Holt LM (1987) The relationship between the HMW glutenin subunit composition and the bread-making quality of British-grown wheat varieties. J Sci Food Agr 40:51–65

Ng PKW, Bushuk W (1988) Statistical relationships between HMW subunits of glutenin and bread-making quality of Canadian-grown wheats. Cereal Chem 65:408–413

Tohidfar G, Mohammadi M, Ghareyazie B, Mohammadi SA (2004) Relationships between HMW-GS and breadmaking quality in advanced wheat lines. Cereal Foods World 49:28–34

Yan Y, Hsam SLK, Yu J, Jiang Y, Zeller FJ (2003) Allelic variation of the HMW glutenin subunits in *Aegilops tauschii* accessions detected by SDS-PAGE, A-PAGE and capillary electrophoresis. Euphytica 130:377–385

BREEDING FOR BREADMAKING QUALITY USING OVEREXPRESSED HMW GLUTENIN SUBUNITS IN WHEAT (*TRITICUM AESTIVUM* L.)

Z. BEDŐ[1,*], M. RAKSZEGI[1], L. LANG[1], E. KERESZTÉNYI[1], I. BARACSKAI[1] AND F. BÉKÉS[2]

[1]*Agricultural Research Institute of the Hungarian*[Academy] *of Sciences, 2462 Martonvásár, 2 Brunszvik St., Hungary*

[2]*CSIRO, Plant Science Industry, GPO Box 1600, Canberra, ACT, 2601, Australia*

[*]*E-mail: bedoz@mail.mgki.hu*

Abstract: In the course of breeding, a number of genetic resources have been used to investigate the effect of the overexpressed allelic form of the Bx7 high molecular weight glutenin encoded by Glu-B1 on dough strength, stability and extensibility. Biochemical marker selection was carried out using RP-HPLC on breeding lines in the F_3–F_4 and F_5–F_7 generations, developed using parental lines overexpressing storage proteins, in order to detect the overexpression of the Bx7 HMW glutenin subunit. In early generations lines were selected that had mean values of dough strength (R_{max}) and area under the curve (A) substantially exceeding those recorded for the original set of breeding material using Kieffer's Texture Analyser. The values of R_{max} rose from 16.0 to 23.3 and those of A from 984 to 1403 on average in the selected lines. Correlation analysis indicated that a medium strong, significant correlation was found for the resistance and stability of the dough and the area under the curve. The results of rheological analysis on selected lines overexpressing Bx7 show that the ratio of genotypes with good breadmaking quality increased during both periods of selection, but breeding for HMW glutenin overexpression alone is not sufficient for an improvement in breadmaking quality. It can be concluded that overexpressed allelic forms could be useful means of breeding for improvements in traits influencing technological quality, especially dough strength and stability. Compared to genetic resources with high protein content, these overexpressed forms do not make a significant contribution to increases in protein content and dough extensibility

Keywords: overexpressed alleles, HMW glutenins, dough strenght

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 479–485.

INTRODUCTION

Common wheat (*Triticum aestivum* L.) exhibits considerable variability in storage protein composition and in the quantity of each component. Research has provided valuable information on the composition of high and low molecular weight (HMW and LMW) glutenins and gliadins, on the quantities of the various components and on their interactions (Payne et al. 1987). It has also contributed to the improvement of breadmaking quality and to the better understanding of its biochemical background. Analysis has revealed that, among the dough properties, HMW glutenins have the greatest influence on dough strength, which is affected not only by the composition, but also by the quantities of individual HMW glutenin subunits. This finding drew attention to overexpressed forms. Among the naturally occurring overexpressed HMW glutenins, outstanding importance is attached to the Glu-B1-encoded subunit Bx7 ($Bx7^{oe}$) which, according to D'Ovidio et al. (1997), leads to greater protein expression in the variety Red River 68 as the result of doubled DNA band intensity. Overexpressed Bx7 subunits have also been detected in many other wheat genotypes, both in old landraces and in modern varieties (Marchylo et al. 1992, Butow et al. 2004).

Genetic transformation is a new tool that can be used to increase the amounts of HMW subunit protein. The 1Ax1 transgene resulted in improved breadmaking quality, while the 1Dx5 transgene resulted in a decrease (Darlington et al. 2003). The line B73-6-1, produced in the UK (Barro et al. 1997), contains 10–15 additional copies of the HMW subunit 1Dx5 transgene, resulting in a four-fold increase in the amount of encoded protein (Rooke et al. 1999, Barro et al. 1997). When this line was studied in a continental climate, significant changes occurred in the Dx/Dy, HMW/LMW and glutenin/gliadin ratios due to the unbalanced x to y ratio of HMW glutenins (Rakszegi et al. 2005).

We studied the population and sublines of an old Hungarian variety, Bánkúti 1201, and considerable variation was detected within the population for the HMW glutenin composition, the overexpression of the Bx7 HMW glutenin subunit and the unextractable polymeric protein % (UPP%) (Juhász et al. 2003). Marchylo et al. (1992) reported that at least two types of Bx7 protein existed in different varieties, and designated the Cheyenne type, leading to normal protein production, as 7^* and the overexpressed Glenlea type as 7.

The present experiments were designed to analyse the effect of various genetic sources overexpressing Bx7 on breadmaking quality during breeding. Biochemical markers were used to analyse genotypes in early generations and developed lines produced from sources overexpressing the Bx7 HMW glutenin storage protein in order to breed genotypes with good technological quality.

MATERIALS AND METHODS

Genotypes overexpressing the Bx7 storage protein were crossed with wheat genotypes whose HMW glutenin subunit compositions are known. In the first part of the experiment 409 genotypes in early (F_3, F_4) generations were selected

from crosses with the parents Glenlea and N93-3026. This breeding stock was analysed using biochemical marker selection (RP-HPLC), after which the technological quality parameters of selected lines were recorded using Kieffer's Texture Analyser. This instrument provides information on the rheological properties of the dough, particularly the resistance to extension and the extensibility.

In the second part of the experiment, 45 F_5–F_7 lines from combinations produced using Bánkúti 1201, Glenlea and N93-3026 were analysed, after complex agronomic selection, with RP-HPLC and with the farinograph method (ICC 115 standard).

The composition of the HMW glutenin subunits was examined by means of SDS polyacrylamide gel electrophoresis (Jackson et al. 1996), the quantity of subunits using RP-HPLC (Marchylo et al. 1989) and the protein content with a Percon Inframatic 8611 instrument. The control varieties used for the identification of genotypes overexpressing the Bx7 subunit were Glenlea, already proved to overexpress this subunit, and, in the second experiment, Glenlea and Red River 68.

RESULTS AND DISCUSSION

Characterisation of Parental Lines

The Bx7 HMW glutenin production of the parental lines used to produce the combinations is presented in Table 1. The results show that in the Canadian spring wheat variety Glenlea the Bx7 subunit storage protein made up 37.2% of the HMW glutenin and 10.6% of the total glutenin. For the other parental lines these ratios were 22.0–25.0% and 5.5–7.5%, respectively. Interesting results were obtained for the HMW/LMW glutenin ratios of the parental lines. Data similar to those of Glenlea were recorded for Alföld, Fertödi 293 and Mv Emma. A lower ratio was observed for Obriy, a Ukrainian variety with excellent breadmaking quality and Red River 68 in its parentage, and Mv Palotás. Mv Verbunkos, a variety bred in Martonvásár, showed a considerably increased HMW/LMW glutenin ratio.

The Kieffer Texture Analyser used for the technological analysis of the parental lines works on a principle similar to that of the extensograph. The characteristic points of the curve, which resembles an extensogram, are the maximum resistance (R_{max}), the extensibility (E) and the deformation work, or area under the curve (A). The highest values of R_{max} and A were obtained for Mv Emma and Glenlea.

The data confirmed that the HMW glutenin composition of Glenlea differed substantially from that of the other parental lines. It should be noted, however, that all the lines crossed with Glenlea were winter types, and exhibited considerable variation for other technological quality parameters.

The Bánkúti 1201 population also used as a crossing partner was divided into sublines in our previous experiments on the basis of HMW glutenin subunit composition, Bx7 overexpression and UPP% (Juhász et al. 2003). It can be seen from the data in Table 2 that this old variety population exhibited a normal level of Bx7 production, which was in good agreement with the values obtained using the Kieffer Texture

Table 1. Glutenin characteristics and rheological properties of the parental lines

Parents	Bx7/ HMW (%)	Bx7/ glutenin (%)	HMW/ LMW ratio	Max. resistance (Rmax)	Extensibility (E)	Area under the curve (A)	HMW glutenin composition
Glenlea	37.2	10.6	0.43	32.5	80.6	1976	2* 7+8 5+10
Obriy	22.0	5.5	0.31	19.1	67.5	1026	2*/N 7+8 5+10
Alföld	24.9	6.8	0.40	18.9	78.3	1116	2* 7+9 5+10
Fertödi 293	22.0	6.2	0.42	5.7	61.4	376	1 7+9 2+12
Mv 2	22.9	6.3	0.40	13.8	69.0	814	2* 7+8 5+10
Mv Emma	24.7	7.4	0.46	47.3	56.8	2335	1/*N* 7+9 5+10
Ukrainka Od.	22.7	7.2	0.48	26.7	61.5	1374	2*/1 7+9 5+10
Mv Verbunkos	25.0	7.5	0.60	10.8	68.9	720	2* 7+9 2+12
Mv Palotás	24.6	6.0	0.35	19.7	80.6	1290	2* 7+9 5+10

Table 2. HMW glutenin composition, level of Bx7 overexpression, rheological properties of Bánkuti 1201 and its subline

Genotypes	HMW glutenin composition	Bx7/HMW glutenin (%)	Rmax (g)	Extensibility (mm)	Area (A)
Glenlea (st.)	2* 7+8 5+10	37.8	27.2	69.8	1498
Red River 68 (st.)	1 7+8 5+10	33.0	35.4	74.1	2092
Bánkúti1201 population*	2*/1 7+8/9 2/3+12	22.3	13.0	60.1	850
Bánkúti 1201-9154-95	2* 7+8 2+12	36.9	21.7	80.3	1593

* Note: HMW glutenin compositon is heterogeneous

Analyser. However, the Bx7 overproduction, R_{max} value and area under the curve of a line selected from this population proved that the variability existing within the population could be exploited to select overexpressing genotypes. The dough strength of the overexpressing line Bánkúti 1201-9154-95 was similar to that of Glenlea and Red River 68, which also overexpress Bx7.

Selection in Early Generations

After examining lines in the early F_3 generation, a second cycle of selection revealed 18 genotypes overexpressing Bx7, where the ratio of Bx7 protein within the total HMW glutenin exceeded that of Glenlea. The rheological properties of genotypes previously analysed using RP-HPLC were then examined using the Texture Analyser, to determine their dough strength and extensibility. A large proportion of the genotypes that overexpressed Bx7 were found to have excellent rheological properties, confirming that biochemical marker-assisted selection for Bx7 overexpression can be used to select wheat genotypes with excellent dough strength in early generations. While the average R_{max} value of the lines selected in the first cycle was 16.0, this value increased to 23.3 in the second cycle of selection, representing a substantial breeding gain. The improvement in extensibility was considerably less, being 71.7 in the first cycle and 76.5 in the second. However, there was an increase in the area under the curve, from 984 in the first cycle to 1403 for lines selected in the second cycle.

The results of correlation analysis between rheological traits and overexpression indicate that the Bx7 storage protein content is correlated ($r^2 = 0.63$) to the resistance of the dough to extension (R_{max}), while a similar, moderately strong correlation ($r^2 = 0.65$) can also be detected between the area under the curve and Bx7 overexpression

The protein content of the selected genotypes was checked using a Percon Inframatic 8611 instrument. Several of the genotypes tested were found to have outstandingly high values. Correlation analysis was carried out on the protein contents of all the F_3 lines. As expected, there was no demonstrable correlation between the total protein content and the Bx7 storage protein ratio ($r^2 = 0.21$), but a close correlation

was found between the HMW/LMW ratio and the Bx7 overexpression in terms of total glutenin ($r^2 = 0.83$).

Selection of Developed Lines

Glenlea, N93-3026 and Bánkúti 1201 were each used as parents in crossing combinations with adapted parental genotypes. Their F_5–F_7 lines were subjected to agronomic selection in order to produce adaptable genotypes with good breadmaking quality. The ratio of Bx7 protein averaged 24.2% in the tested lines, which is lower than that of the Bx7 overexpressing genotypes used in these crosses, but similar to that of samples taken from the Bánkúti 1201 population. Despite this mean value, genotypes were selected from 7 of the 45 developed lines that exhibited Bx7 overexpression similar to that of Glenlea, Red River 68 and the overexpressing Bánkúti 1201-9154-95 genotype. The rheological parameters were analysed using the farinograph method, where the stability value of the curve demonstrates gluten strength. Table 3 shows the farinograph results of the two best lines: one was developed from a Bánkúti 1201 combination (MvC116/05) and the other from a Glenlea cross (MvB3539/05). It can be seen from the farinograph analysis that the curve stability of MvC116/05 was greater than that of either the Bánkúti 1201 population or the B9154-95 overexpressing genotype, and approached that of Red River 68 and Glenlea.

It can be concluded from the results that overexpressed allelic forms could be important tools in breeding for improvements in traits influencing technological quality, especially dough strength and stability. Selection in early generations allows genotypes overexpressing Bx7 to be selected on a wider basis, but it is still possible to carry out successful selection on adaptive developed lines after agronomic selection. The results of rheological analysis on genotypes overexpressing Bx7 demonstrates that the ratio of genotypes with good breadmaking quality increased substantially during both periods of selection, but breeding for this property alone is not sufficient for an improvement in breadmaking quality.

Table 3. Farinographic results of the Bx7 overexpressed developed lines and genotypes

Developed lines and genotypes	Bx7/HMW	Far. Curve stability (sec)	ICC quality number	Water absorption (%)	Development time (sec)
Bánkúti1201 population	22,3	6,2	99	63.7	6.0
Bánkúti 1201-9154-95	36,9	12,2	200	64.2	19.5
Mv C 116/05	33,1	16,1	200	58.0	20.0
Mv B 3539/05	34,7	12,4	172	65.6	10.7
Glenlea (st.)	34,1	17,6	200	61.2	20.0
Red River 68 (st.)	33,0	16,6	200	62.3	19.9

ACKNOWLEDGEMENTS

The Martonvásár institute receives grant-aided support from the Ministry of Agriculture and Rural Development FVM-46028/2004 and has carried out a successful project in the framework of Collaborative Agreement with CSIRO, Plant Science Industry, Canberra, Australia.

REFERENCES

Barro F, Rooke L, Békés F, Gras P, Tatham AS, Fido R, Lazzeri PA, Shewry PR, Barcelo P (1997) Transformation of wheat with high molecular weight subunit genes results in improved functional properties. Nat Biotechnol 15:1295–1299

Butow BJ, Gale KR, Ikea J, Juhász A, Bedö Z, Tamás L, Gianibelli MC (2004) Dissemination of the highly expressed Bx7 glutenin subunit (Glu–B1al allele) in wheat as revealed by novel PCR markers and RP-HPLC. Theor Appl Genet 109:1525–1535

Darlington H, Fido R, Tatham AS, Jones H, Salmon SE, Shewry PR (2003) Milling and baking properties of field grown wheat expressing HMW subunit transgenes. J Cereal Sci 38:301–306

D'Ovidio R, Masci S, Porceddu E, Kasarda DD (1997) Duplication of the Bx7 high –molecular-weight glutenin subunit gene in bread wheat (*Triticum aestivum* L.) cultivar 'Red River 68'. Plant Breed 116:525–531

Jackson EA, Morel MH, Strohm T, Branlard G, Metakovsky EV, Redaelli R (1996) Proposal for combining the classification systems of alleles of Gli-1 and Glu-3 loci in bread wheat (*Triticum aestivum* L.). J Genet Breed 50:321–336

Juhász A, Larroque OR, Tamás L, Hsam SLK, Zeller FJ, Békés F, Bedö Z (2003) Bánkúti 1201 – an old Hungarian wheat variety with special storage protein composition. Theor Appl Genet 107:697–704

Marchylo BA, Kruger J, Hatcher DW (1989) Quantitative reversed-phase high-performance liquid chromatographic analysis of wheat storage proteins as a potential quality prediction tool. J Cereal Sci 9:113–130

Marchylo BA, Lukow OM, Kruger JE (1992) Quantitative variation in high molecular weight glutenin subunit 7 in some Canadian wheat. J Cereal Sci 15:29–37

Payne PI, Lawrence GJ, Nightingale MA, Krattiger AF, Holt LM (1987) The relationship between HMW subunit composition and bread-making quality of British-grown varieties. J Sci Food Agr 40:51–60

Rakszegi M, Békés F, Láng L, Tamás L, Shewry PR, Bedö Z (2005) Technological quality of transgenic wheat expressing an increased amount of a HMW glutenin subunit. J Cereal Sci 42:15–23

Rooke L, Békés F, Fido R, Barro F, Gras P, Tatham AS, Barcelo P, Lazzeri P, Shewry PR (1999) Overexpression of a gluten protein in transgenic wheat results in greatly increased dough strength. J Cereal Sci 30:115–120

NUTRITIONAL AND BAKING QUALITY OF LOW PHYTIC ACID WHEAT

M.J. GUTTIERI*, K.M. PETERSON AND E.J. SOUZA

University of Idaho, Aberdeen Research and Extension Center, P.O. Box 870, Aberdeen, ID, USA, 83210

**E-mail: mgttri@uidaho.edu*

Abstract: Phytic acid is the major storage form of phosphorus in wheat grain. Non-ruminant animals cannot utilize phytic acid phosphorus, and phytic acid reduces the nutritional availability of important minerals. We have identified a wheat mutant (*Lpa1-1*) with reduced phytic acid P and increased inorganic P (P_i). Ideally, LPA wheats will have improved micronutrient availability without detrimental effects on baking quality. To test this hypothesis, the mutant phenotype was transferred via backcrossing into the hard red spring wheat cultivar 'Grandin.' Wild-type (WT) and low phytic acid (LPA) sib selections from two backcross families were grown in replicated, irrigated yield trials at Aberdeen, ID in 2003 and 2004. Total P, P_i, and phytic acid P (PAP) were measured in grain and in fractions obtained after milling on a Quadrumat Sr. experimental mill. Elemental concentrations (Ca, Cu, Fe, Mg, Mn, P, S, and Zn) were measured in flour and bran fractions by ICP mass spectrometry. Total P concentration in grain of WT and LPA sib lines was similar. However, the distribution of P between phytic acid and P_i was altered: P_i in LPA grain was up to 340% of WT grain, and PAP in LPA grain was reduced to as low as 65% of the concentration in WT grain. This difference in P composition of grain was reflected in flour: P_i in break and reduction flours of LPA wheat was 3- to 4-times the concentration in break and reduction flours from WT wheat. Total P concentration in LPA flours was 20% greater than in WT flours. Mineral concentrations in bran and shorts of LPA and WT wheats were similar. However, magnesium concentrations in LPA break and reduction flours were significantly greater than in WT flours. The LPA genotype had little effect on concentrations of other minerals. Increases in P and Mg concentration in LPA flours were manifested in greater flour ash concentration. Flour ash of WT flours averaged 3.86 g kg^{-1}; flour ash of LPA flours averaged 4.38 g kg^{-1}. Protein concentration of LPA and WT flours was similar. However, LPA flours had a longer time to mixograph peak and greater mixograph peak height than WT flours. Bread loaf volume of LPA and WT flours was similar. The results of this study indicate that the LPA trait can produce flours with greater P_i and Mg concentration and little effect on bread flour functionality

Keywords: phytic acid, P concentration, micronutrients

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 487–493.

INTRODUCTION

Phytic acid is the major storage form of phosphorus in wheat grain. Non-ruminant animals cannot utilize phytic acid phosphorus, and phytic acid reduces the nutritional availability of important minerals. We have identified a wheat mutant (*lpa1-1*) with reduced phytic acid P and increased inorganic P (P_i). Ideally, low phytic acid (LPA) wheats will have improved micronutrient availability without detrimental effects on baking quality.

MATERIALS AND METHODS

To test this hypothesis, the mutant phenotype was transferred via backcrossing into the hard red spring wheat cultivar 'Grandin'. F_2 plants of two BC_3 sib families with the pedigree Grandin*4/Js-12 LPA were grown in the greenhouse. A preliminary evaluation of high inorganic phosphorus (HIP) phenotype to eliminate heterozygous genotypes was conducted on F_3 seed from each F_2 plant. HIP phenotype of individual kernels was evaluated as described previously (Guttieri et al. 2004). $F_{2:3}$ selections identified as homozygous HIP phenotype and selections identified as homozygous wild-type phenotype were hand-planted in the field in 2002. Field-grown seed was evaluated for uniformity of HIP phenotype. Fourteen $F_{2:4}$ selections were advanced into replicated yield trials in 2003, including three strong HIP phenotype selections and three wild-type (WT) HIP selections derived from one of the two initial BC_3 families (C family), and five moderate HIP phenotype selections and three WT HIP selections from the other of the two initial BC_3 sib families (B family). Wild-type (WT) and LPA sib selections from two backcross families were grown in replicated, irrigated yield trials at Aberdeen, ID in 2003 and 2004.

Grain from each plot was tempered by the standard American Association of Cereal Chemistry (AACC, 2000) method 26–10. Tempered grain was milled using a Brabender Quadrumat Senior Mill (AACC method 26–21A). Total P, P_i, and phytic acid P (PAP) were measured in grain and in fractions obtained after milling. Total P was determined by digestion of dried samples (0.15 g to 0.3 g) in 2 ml concentrated sulfuric acid and 30% hydrogen peroxide at 120 °C until solutions were clear and colorless and all traces of peroxide were gone. Digested samples were cooled to room temperature and diluted to a standard volume of 25 ml. P content was assayed on microtiter plates with a modification of the Chen's reagent (Chen et al. 1956). Due to the sulfuric acid content of the digests, Chen's reagent was prepared with substitution of water for sulfuric acid. Sulfuric acid was added to standards and to samples requiring further dilution to provide the appropriate concentration of 0.6 N sulfuric acid in all wells. P_i was determined by a variation of the Chen method, modified for use on microtiter plates. Briefly, dried samples (0.5 g) were extracted in 10 ml of 12.5% (w/V) trichloroacetic acid containing 25 mM $MgCl_2$ at 4 °C overnight with continuous shaking, then centrifuged at 4 °C and 5000 g for 15 minutes. The supernatant was removed and brought to a standard

volume of 25 ml with distilled water. Extracts were assayed with equal volumes of Chen's reagent along with prepared potassium phosphate standards and reagent blank on microtiter plates. After 1 hour incubation at room temperature, plates were read at 820 nm on a Dynatech Laboratories MRX microplate reader. Phytic acid content was determined by the method of Haug and Lantzsch (1983) adapted for use on microtiter plates. Dried samples were extracted at 4 °C overnight with continuous shaking in 0.2 N HCl. Samples were centrifuged at 5000 g at 4 °C for 15 minutes, and the extract was brought to a standard volume with 0.2 N HCl. Sample extracts and prepared standards were then treated as follows: 1.0 ml of extract (or known standard sodium phytate solution) was incubated in a boiling water bath for 15 minutes with 1.0 ml of 415 μM ferric ammonium chloride prepared in 0.2 N HCl, cooled in an ice bath for 15 minutes and mixed well. Samples were assayed directly on microtiter plates in a ratio of 120 μl sample (or prepared sodium phytate standard) to 180 μl 2, 2′-bipyridine:thioglycolic acid solution, and read without delay at 530 nm on a Dynatech Laboratories MRX microplate reader. All samples were plated in quadruplicate, and each analysis performed in duplicate.

Mineral element composition of milling fractions was determined by the University of Idaho Analytical Services Laboratory using a Perkin-Elmer Optima 3200 ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometer) to quantify aqueous constituents following nitric acid digestion of the milling fractions. Mineral concentrations tested included Al, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, Pb, S, V, Zn, and Y.

Break and reduction flour fractions were combined to produce a patent flour for bread quality evaluations. The University of Idaho Wheat Quality Laboratory conducted flour protein, flour ash, mixograph, and bread loaf evaluations as described by Souza et al. (1993) and in accordance with AACC standard protocols (AACC 2000).

RESULTS AND DISCUSSION

Total P concentration in grain of WT and LPA sib lines was similar. However, the distribution of P between phytic acid and P_i was altered: P_i in LPA grain was up to 340% of WT grain, and PAP in LPA grain was reduced to as low as 65% of the concentration in WT grain (Fig. 1). This difference in P composition of grain was reflected in flour: P_i in break and reduction flours of LPA wheat was 3- to 4-times the concentration in break and reduction flours from WT wheat (Table 1). Total P concentration in LPA flours was 20% greater than in WT flours.

Concentrations of individual minerals in bran and shorts of LPA and WT wheats were similar. The LPA genotype had little effect on concentrations of minerals other than Mg, Mn, and P. Break flour Mg concentration was 22% greater in C family LPA flours than in WT flours (Table 2). Break flour concentrations of Mn in WT and lpa genotypes of the C family were 5.59 and 6.48 mg kg^{-1}, respectively, an increase of 16% in LPA flours. Total mineral concentrations (sum of all measured

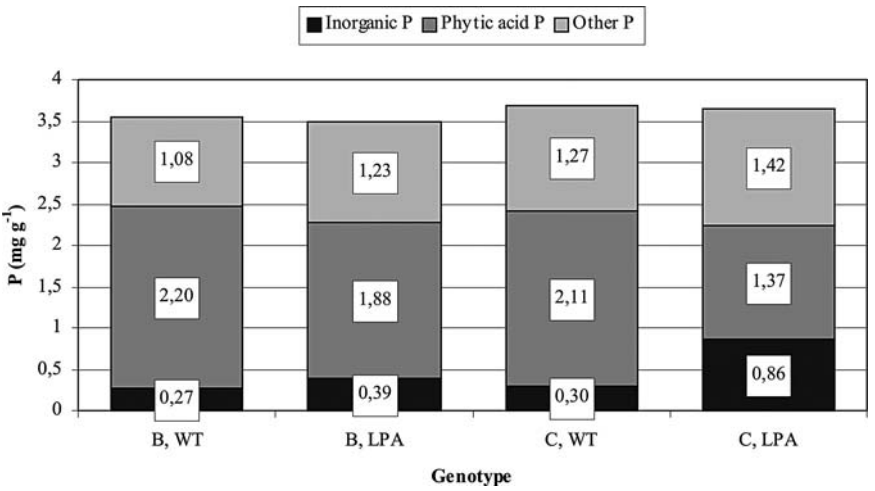


Figure 1. Phosphorus distribution in whole wheat meal of wild-type (WT) and low phytic acid (LPA) hard red spring wheats from two BC_4F_1-derived families (Family B, Family C) grown in Aberdeen, ID in 2003 and 2004

minerals) were 311 and 354 mg kg^{-1} greater in break and reduction flour fractions, respectively, of LPA genotypes relative to WT genotypes of the C family.

Flour ash concentration was significantly greater in LPA flours than in WT flours of the C family (Table 3). This increase in flour ash may reduce acceptance of LPA wheats by some flour millers. Importantly, approximately 65% of this increase in flour ash is directly accounted for by the increased total mineral concentration in break and reduction fractions of the flour.

Protein concentration of LPA and WT flours was similar (Table 4). Flour extraction was slightly lower from LPA grain than from WT grain. However, flour extractions obtained for LPA wheats on the Quadrumat Sr. mill would nonetheless be considered excellent within the University of Idaho wheat breeding program. LPA flours had a longer time to mixograph peak and greater mixograph peak height than WT flours. Both of these differences in mixing characteristics are considered desirable in the baking industry in the United States. Mixing tolerance and mixograph water absorption were unaffected by genotype. Bread loaf volume of LPA and WT flours was similar.

Therefore, the LPA trait increased the mineral concentration of flour, primarily through increases in P and Mg concentration. The elevated mineral concentration in LPA flours contributed substantially to increased flour ash concentration, which may limit adoption by some millers. Other milling and baking parameters were affected to a limited extent, and generally in a favorable direction. The phytic acid concentration in all flour fractions was reduced and the inorganic P, which is nutritionally more available to non-ruminant animals, was increased. The decrease

Table 1. Phosphorus composition of milling fractions from low phytic acid (LPA) and wild-type (WT) hard red spring wheat grown in Aberdeen, ID in 2003 and 2004

	Total P				Phytic acid P				Inorganic P			
	Bran	Shorts	Break flour	Reduct. flour	Bran	Shorts	Break flour	Reduct. flour	Bran	Shorts	Break flour	Reduct. flour
					mg g^{-1}							
Family B												
WT	11.30	6.04	1.07	0.96	8.22	4.04	0.28	0.22	0.72	0.42	0.06	0.06
LPA	9.97	5.93	1.07	0.97	7.25	3.82	0.26	0.20	0.96	0.53	0.10	0.09
Contrast WT vs lpa	**	ns	ns	ns	*	ns	*	ns	ns	ns	ns	ns
Family C												
WT	10.79	6.04	1.07	0.97	8.28	4.00	0.28	0.22	0.78	0.46	0.08	0.07
LPA	10.71	5.82	1.29	1.15	5.90	3.15	0.23	0.16	1.85	0.93	0.25	0.27
Contrast WT vs lpa	ns	ns	**	**	**	**	**	**	**	**	**	**
Mixed-effects analysis of variance F-value												
Family (F)	ns	Ns	18.3**	12.7**	ns	12.8**	5.0*	6.5*	15.2**	8.1*	19.2**	15.2**
Genotype (G)	5.1*	Ns	20.9**	14.0**	33.1**	28.2**	35.2**	25.7**	29.3**	14.9**	27.1**	24.4**
F x G	ns	Ns	20.8**	11.8**	5.9*	9.8**	5.4*	5.1*	11.7**	5.5*	12.7**	12.3**

*, ** indicate significance at $p < 0.05$ and 0.01, respectively

Table 2. Magnesium concentration in milling fractions from low phytic acid (LPA) and wild-type (WT) hard red spring wheat

	Magnesium concentration			
	Bran	Shorts	Break flour	Reduct. flour
	mg g^{-1}			
Family B				
WT	4970	2740	362	308
LPA	4830	2690	365	329
Contrast WT vs lpa	ns	ns	ns	ns
Family C				
WT	4970	2630	331	270
LPA	4660	2500	402	247
Contrast WT vs lpa	ns	ns	**	**
	Mixed-effects analysis of variance F-value			
Family (F)	ns	5.6*	ns	ns
Genotype (G)	ns	ns	12.7**	23.5**
F x G	ns	ns	10.4**	7.6*

*, ** indicate significance at $p < 0.05$ and 0.01, respectively

Table 3. Flour ash concentration of low phytic acid (LPA) and wild-type (WT) hard red spring wheat grown at Aberdeen, ID in 2003 and 2004

	Flour ash concentration
	mg kg^{-1}
Family B	
WT	3.91
LPA	4.01
Contrast WT vs lpa	Ns
Family C	
WT	3.86
LPA	4.38
Contrast WT vs lpa	**
	Mixed-effects analysis of variance F-value
Family (F)	2.9
Genotype (G)	11.0**
F x G	5.0*

Table 4. Milling and baking quality of wild-type (WT) and low phytic acid (LPA) hard red spring wheats grown in 2003 and 2004 at Aberdeen, ID

	Flour protein	Flour yield	Mixograph				Bread loaf volume
			Peak time	Peak height	Tolerance	Absorption	
	g kg^{-1}		min	cm	degrees	ml kg^{-1}	cc
Family B							
WT (N = 3)	136	705	2.2	6.7	67.5	632	1242
LPA (N = 5)	131	702	2.5	6.8	65.7	624	1216
(standard error)	(2)	(3)	(0.2)	(0.1)	(2)	(3)	(30)
Family C							
WT (N = 3)	135	708	2.2	6.7	69.8	629	1217
LPA (N = 3)	135	697	2.8	6.9	67.0	631	1247
(standard error)	(2)	(4)	(0.2)	(0.1)	(2)	(3)	(32)
Mixed effects analysis of variance F-value							
Family	0.4	0.1	0.7	1.0	1.2	0.3	0.0
Genotype	1.1	5.1*	6.0*	10.1**	2.0	0.8	0.0
Family x Genotype	1.8	1.6	0.4	0.3	0.1	2.4	1.6

in phytic acid concentration in the bran fraction may be important in reducing waste P from animals fed bran from flour mills.

ACKNOWLEDGEMENTS

Katherine O'Brien, Manager, University of Idaho Wheat Quality Laboratory. USDA NRI Grant No. 2002-35503-12546, Project No. IDA00204-CG.

REFERENCES

American Association of Cereal Chemists (2000) Approved Methods of the AACC, 10th edn. American Association of Cereal Chemists, St. Paul, MN, USA

Chen PS, Toribara TY, Warner H (1956) Microdetermination of phosphorus. Anal Chem 28:1756–1758

Guttieri MJ, Bowen D, Dorsch JA, Raboy V, Souza E (2004) Identification and characterization of a low phytic acid wheat. Crop Sci 44 (erratum 1505):418–424

Haug W, Lantzsch HJ (1983) Sensitive method for the rapid determination of phytate in cereals and cereal products. J Sci Food Agr 34:1423–1426

Souza EJ, Tyler JM, Kephart KD, Kruk M (1993) Genetic improvement in milling and baking quality of hard red spring wheat cultivars. Cereal Chem 70:280–285

LONG-TERM BREEDING FOR BREAD MAKING QUALITY IN WHEAT

S. DENCIC*, B. KOBILJSKI, N. MLADENOV, N. HRISTOV AND M. PAVLOVIC

Institute of Field and Vegetable Crops, 21000 Novi Sad, Serbia and Montenegro
**E-mail: dencic@ifvcns.ns.ac.yu*

Abstract: Six quality traits related to bread making have been analyzed in 37 winter wheat genotypes released in the past 50 years in the former Yugoslavia and the present S&M. Regarding cultivars in production, 4 periods were defined, first from 1955 to 1973, second from 1974 to 1982, third from 1983 to 1992 and fourth from 1992 to 2005. The objective was to determine direction and magnitude of changes in milling and baking quality traits of winter wheat since domestic breeding efforts were initiated. Improvements were made in test weight, flour yield, protein content and bread yield while no significant change was detected for 1000 kernel weight and wet gluten. In general, the last period was superior with regard to the above traits compared with the first and the second one. A total of 11 alleles have been identified at the three loci, *Glu-A1*, *Glu-B1*, and *Glu-D1*. In the first period, the most frequent glutenin subunits composition was 1, 7 + 8, 2 + 12 while in the last period it was 2*, 7 + 9, 5 + 10. The achieved quality improvement was greatly due to the cultivar Bezostaya 1 which served as a parent in many cultivars, especially the ones that dominated in the third and fourth periods

Keywords: winter wheat, Milling quality, Baking quality, HMW glutenins, Germplasm

INTRODUCTION

Hard red wheat is used primarily to produce yeast-leavened bread products. "Quality" of wheat cannot be expressed in terms of one or two traits. Traits used as indicators of milling quality include: test weight, 1000-kernel weight and flour yield. Indicators of baking quality include many traits such as grain and flour protein content, wet gluten, water absorption, mixing time, mixing tolerance, extensibility, loaf volume and crumb grain and color (Finney et al. 1987, Czuchajowska and Paszczynska 1996). Some of these traits have moderate to high heritability (Loffler and Busch 1982, Snape et al. 1993).

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 495–501

Two major classes of glutenin polypeptides have been identified in wheat endosperm, designated as HMW-GS and LMW-GS. The genes coding for HMW-GS are located on the long arms of chromosomes 1A, 1B and 1D at the *Glu-A1*, *Glu-B1* and *Glu-D1* loci, respectively (Payne 1987). The work of Payne et al. (1980) provides evidence of a strong association between the presence of certain alleles coding for HMW-GS and bread-making quality.

Both milling and baking quality traits can vary tremendously in any wheat genotype depending on the production environment (Mladenov et al. 2001). However, end-use quality traits are important selection criteria in all wheat breeding programs. The generally negative correlations between protein content and grain yield may not be valid for varieties selected for yield and quality traits and released over time.

We analyzed milling and baking traits of 37 winter wheat genotypes introduced or developed from 1955 to 2005. Our objective was to determine the changes in quality that occurred due to breeding over past 50 years.

MATERIALS AND METHODS

Milling and baking quality traits were analyzed in 37 winter wheat genotypes representing past 50 years. Regarding cultivars in production, 4 periods were defined. Milling and baking quality traits were analyzed in ten dominant varieties per period. The following cultivars were analyzed:

1st period (1955–1973): Abondanca, Autonomija, Produtore, Fortunato, Leonardo, Etoil de Choisy, San Pastore, Libellula, Bezostaya 1 and Bankuty 1205;

2nd period (1974–1982): Sava, Zlatna Dolina, Biserka, Sanja, Miljenka, Partizanka, KG 56, Novosadska rana 2, Bosanka and Novosadska rana 1;

3rd period (1983–1992): Lasta, Baranjka, Jugoslavija, Super zlatna, Balkan, Skopljanka, Novosadska rana 2, Sana, KG 56, Zagrepcanka 2;

4th period (1993–2005): Evropa, Francuska, Italija, Lasta, Evropa 90, Pobeda, Renesansa, Novosadska rana 5, Pesma and Rusija.

Data analyzed were collected from official documents issued by Ministry of Agriculture or other relevant agencies, institutes and faculties.

The following quality traits were analyzed: test weight (TW), 1000-kernel weight (TKW), flour yield (FY), grain protein content (GP), wet gluten (WG) and bread yield per g $(100\,g)^{-1}$ of flour (BY).

The analysis of variance (ANOVA) and linear regression were used for statistical analyses.

RESULTS AND DISCUSSION

Short history of wheat breeding in the former Yugoslavia. Before World War I, wheat grown in the former Yugoslavia consisted mostly of native populations. These populations were different ecotypes of *Triticum aestivum*, which were either native to these regions or were imported during migrations and by trade.

After World War I, wheat breeding transitioned from mass to individual selection from native populations as well as from foreign variety populations. Using the old local population Banatka, D. Sarf selected the cultivars Rumska Crvenka and Krusevacka 22. The cultivar Maksimirski prolifik 39 and many advanced lines developed in Zagreb and Krizevci come from the population Sirban Prolific. The cultivar Krizevacka 9 was developed from the Italian cultivar Cologna (Tavcar 1929).

Later on, again, a transition was made to crossing native populations with newly developed cultivars and with foreign cultivars. In Novi Sad, the Canadian cultivar Marquis was crossed with the native cultivar Banatka to produce the varieties Novosadska 1439/3 and Novosadska 1446. Crossing Dakota with a native bearded wheat from Cuprija resulted in the cultivar Krusevacka 2217 (Mirzinski 1953). The cultivar U-1 was developed by crossing the Italian cultivar Carlotta Strampelli with Marquis (Borojevic and Potocanac 1966).

After World War II, wheat breeding continued with much greater intensity. The main trend was to cross cultivars developed between the wars with foreign cultivars. The objective was to obtain genotypes with productivity 5–10% higher than the widespread cultivar U-1, the standard in the western regions, and the cultivar Bankuty 1205, the standard in the eastern regions of the country.

As Yugoslavian wheat production could not wait for new cultivars, it was decided that cultivars should be sought in foreign countries. Therefore, a considerable number of Italian cultivars and several Austrian and Greek cultivars were tested. The experimental results in the first two years showed that some Italian cultivars possessed high yielding capacity. The contribution of Italian cultivars to wheat production in Yugoslavia was enormous. The cultivar San Pastore remained in production for 12 years (1957–1970), being a leading cultivar in the period 1961–1964. The cultivar Libellula was also in the group of leading cultivars, dominating Yugoslav wheat production from the mid-60s till the arrival of the first domestic high-yielding cultivars Sava and Zlatna Dolina (Dencic 1995). The genetic yield potentials of these cultivars were significantly above those in the other domestic and introduced foreign cultivars.

The cultivar Bezostaya 1 had a significant impact on the Yugoslavian wheat breeding as well as on commercial wheat production. It was grown for a long period in Yugoslavia (1960–1973) mostly because of its excellent bread-making quality and very good winterhardiness.

The cultivar Novosadska rana 2 was released in 1975. Superior in grain yield, quality and earliness, this cultivar became popular overnight, spreading to all parts of Yugoslavia as well as to considerable acreages in Hungary and Romania. The variety was grown from 1976 to 1992 (Misic 1989).

After the collapse of the former Yugoslavia, four institutions continued to work on wheat breeding in the new state (SR Yugoslavia, Serbia and Montenegro after 2003). Between 1992 and 2005, 123 winter and 23 spring wheat cultivars were developed.

Bread-making quality traits. The group of cultivars from last period (1992–2005) was superior in all traits except wet gluten in relation to the previous groups. The cultivars from the earliest period, mostly Italian genotypes, had the poorest performance (Table 1). There were no significant differences between the cultivars in the first two periods in either trait but one (Table 1). This fact is logical since the Italian cultivars (from the first period) served as ideotype for development of first Yugoslav high-yielding cultivars, Sava, Zlatna Dolina, Sanja, etc., which dominated the second period.

In the mid-1970s, emphasis was placed on breeding for bread-making quality resulting in many wheat cultivars with very good to excellent quality such as Balkan, Jugoslavija, Pobeda, Pesma, etc. These cultivars dominated in last two periods.

Considering individual traits in the last 50 years, we can see that no progress was made in the 1000-kernel weight and wet gluten. Furthermore, a negative regression coefficient was registered for the latter trait (Table 1 and Fig. 1). This happened because 1000-kernel weight was disregarded as breeding criterion; instead, breeders opted for increased number of grains when breeding for increased yield potential. In fact, breeders treated 1000-kernel weight as yield component rather than as a bread-making quality trait. Likewise, wet glutena was not treated as a direct breeding criterion in the first three periods because breeders placed focus on parameters from extensigraphs and farinographs. The parameters produced by these instruments pertained more to gluten quality than gluten quantity.

Unlike the above traits, increasing trends were registered for test weight, flour yield, protein content and bread yield for last 50 years. However, not all differences between the periods were significant. As these traits served as criteria in wheat trade, it was logical that breeders placed them in center of attention.

It must also be taken into account that the characteristics of the analyzed periods were masked by certain cultivars whose quality traits were diametrically opposed to the traits of other cultivars which formed a dominant group in a given period. For example, in the first period (1955–1973), Italian cultivars with very poor quality were grown at more than 90% of the total wheat acreage while the two high-quality cultivars, Bankuty 1205 and Bezostaya 1, covered very small areas.

Table 1. Means for six milling and baking quality traits for 37 wheat cultivars grown in the 4 periods from 1955 to 2005

Period	Test weight	1000-kernel weight (gr)	Yield Flour %	Protein Content %	Wet Gluten %	Bread Yield gr (100gr) $^{-1}$ flour
I (1955–1973)	76.66	36.60	70.43	11.22	30.21	135.7
II (1974–1982)	78.02	34.66	69.05	12.52	30.33	135.8
III (1983–1992)	80.08	36.04	71.11	12.63	27.67	135.7
IV (1993–2005)	81.93	37.11	75.98	12.97	29.68	138.4
LSD 0.05	3.24	2.63	2.47	0.64	3.18	1.73
0.01	4.38	3.56	3.34	0.86	4.32	2.34

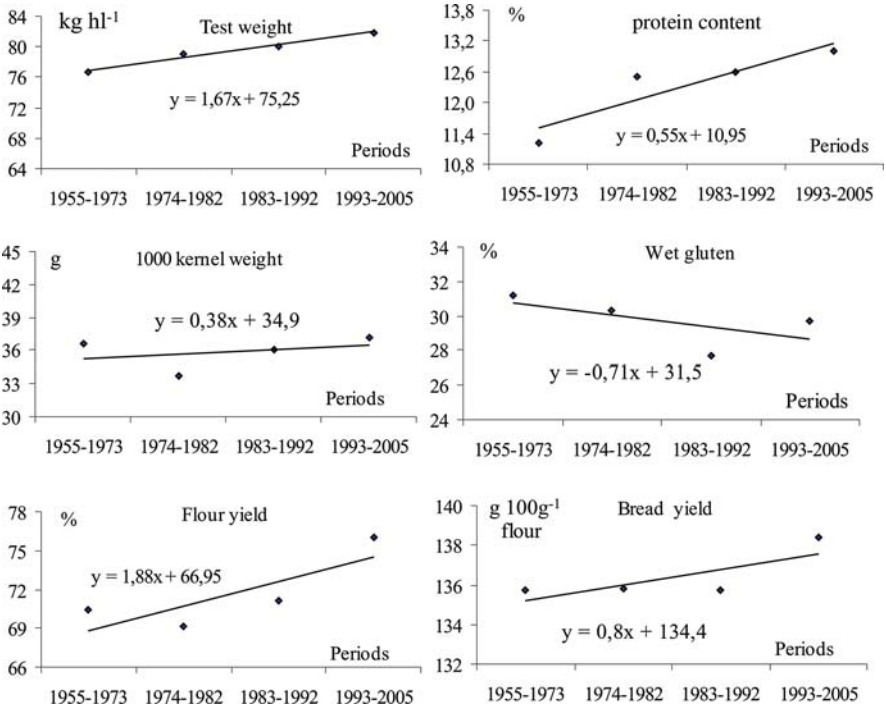


Figure 1. Regression lines for six bread-making quality traits

High-molecular Weight Glutenin Subunits

HMW-GS were detected in all 37 wheat cultivars that were prevalent in wheat production in last 50 years. A total of 11 alleles were identified at the three loci, *Glu-A1*, *Glu-B1* and *Glu-D1* (Table 2). Frequencies of HMW-GS varied through the analyzed periods. In the first period, the most frequent glutenin subunits composition was 1, 7 + 8, 2 + 12 while in the last period it was 2*, 7 + 9, 5 + 10 (Table 2). The former composition is associated with poor bread-making quality while the latter implied good quality (Pogna et al. 1992; Dencic et al. 1993). In the last period (1993–2005), all analyzed cultivars had a single allele (c) at the *Glu-A1* locus coding for glutenin subunit 7 + 9. This is the result of the influence of the cultivar Bezostaya 1, which was a parent to many cultivars that dominated the last two periods, and which contained that allele. Changes also occurred at the *Glu-D1* locus. In the first period, the subunit 2 + 12, a carrier of inferior quality, was largely predominant. This predominance dwindled over time to 30%, in favor of the subunit 5 + 10, a well-known carrier of good quality (Payne et al. 1980; Pogna et al. 1992; Dencic et al. 1993), which took 70% in the last period (Table 2).

Table 2. Percentages of HMW-GS in the wheat genotypes dominating in wheat production in last 50 years in the former Yugoslavia

Period	High-molecular weight glutenin subunits										
	Glu-A1			*Glu-B1*						*Glu-D1*	
	N	1	2*	7	7+8	7+9	6+8	20	14+15	2+12	5+10
I (1955/73)	40	50	10	0	60	20	0	20	0	90	10
II (1974/82)	20	60	20	20	30	10	20	10	10	70	30
III (1983/92)	30	30	40	10	10	30	40	0	10	40	60
IV (1993/05)	40	10	50	0	0	100	0	0	0	30	70

Germplasm

The germplasm used for the development of the 37 wheat cultivars that prevailed in the production over last 50 years was variable and included genotypes from many countries. The Italian cultivars from the first period had been developed mostly by crossing old Italian genotypes, such as Damiano, Mentana, Ardito, etc. In the second period, two types of parents were included in the development of the dominant cultivars. The first group of parents included Italian cultivars such as Fortunato, Leonardo, Villa Glori; the second group included two Russian cultivars, Bezostaya 1 and Mironovska 808. The cultivars from the third and fourth periods were developed by crossing many genetically divergent parents originating from Italy, Yugoslavia, Russia, USA, France, Canada, Hungary, etc. Nevertheless, Bezostaya 1 was one of the parents to more than 50% of cultivars in the third and forth periods, what explains the improved bread making quality in these periods.

REFERENCES

Borojevic S, Potocanac J (1966) The Development of the yugoslav program for creating high-yielding wheat varieties. 5th Yugoslav symposium on research on wheat, Novi Sad, pp 1–48

Czuchajowska Z, Paszczynska B (1996) Is wet gluten good for baking? Cereal Chem 7(4):483–489

Dencic S (1995) Rezultati oplemenjivanja psenice u Novom Sadu. Periodical of scientific research on field and vegetable crops, Novi Sad, 23:103–114

Dencic S, Vapa L, Kovacev-Djolai M (1993) Relationship between Glu-genes, bread-making quality, and grain yield in wheat. In: Li ZS, Xin ZY (eds) Proceedings of the 8th International wheat genetics symposium, 20–25 July 1993, Beijing, China, vol 2. China Agricultural Scientech Press, Beijing,1125–1129

Finney KF, Yamazaki WT, Youngs VI, Rubenthaler GI (1987) Quality of hard, soft, and durum wheats. In: Heyne EG (ed) Wheat and wheat improvement, 2nd edn. ASA, Madison, WI, pp 677–748

Loffler CM, Busch RH (1982) Selection for grain protein, grain yield, and nitrogen partitioning efficiency in hard red spring wheat. Crop Sci 22:591–595

Misic T (1989) Psenice Jugoslavije, proizvodnja, prinosi, selekcije sorte. U: Zita Jugoslavije, izdavac Jugoslovenski fond za zita. 45–158. (in Serbo-Croatian)

Mirzinski J (1953) Prikaz prvih selekcija ozime psenice Zavoda za polj. istrazivanja u Krusevcu. Arhiv za polj. nuke 11:45–74. (in Serbo-Croatian)

Mladenov N, Przulj N, Hristov N, Djuric V, Milovanovic M (2001) Cultivar-by-environment interaction for wheat quality traits in semiarid conditions. Cereal Chem 78(3):363–367

Payne PI (1987) Genetics of wheat storage proteins and the effect of allelic variation on bread-making quality. Annu Rev Plant Physiol 38:141–153

Payne PI, Harris PA, Low CN, Holt LM, Blackman JA (1980) The high-molecular-weight subunits of glutenin: structure, genetics and relationship to bread-making quality. Ann Technol Agr 29:309–320

Pogna NE, Readelli F, Dackevitch T, Curioni A, Dal Belin Perufo A (1992) Benefits from genetics and molecular biology to improve the end use properties of cereals. In: Feillet P (ed) Cereal chemistry and technology: A long past and bright future. INRA, Montpellier, France, pp 83–93

Snape JW, Hyne V, Aitken K (1993) Targeting genes in wheat using marker mediated approaches. In: Li ZS, Xin ZY (eds) Proceedings of the 8th International wheat genetics symposium, 20–25 July 1993, Beijing, China. China Agricultural Scientech Press, Beijing, pp 749–759

Tavcar A (1929) Problem uzgoja psenicnih sorata otpornih protiv *P. gram. tritici*. Glas. polj. i voda, V. 20. (in Serbo-Croatian)

THE GENETICS OF SOFT WHEAT QUALITY: IMPROVING BREEDING EFFICIENCY

E. SOUZA* AND M. GUTTIERI

University of Idaho, Aberdeen Research and Extension Center, P.O. Box 870, Aberdeen, ID 83210, USA

* *E-mail: esouza@uidaho.edu*

Abstract: Soft wheat quality in breeding programs can be selected either directly through baking tests or indirectly through rheological tests or biochemical markers. We have evaluated the efficiency of selection using rheological tests: solvent retention capacity test (SRC), whole grain SDS-sedimentation test, mixographs, and alveograph. Based on testing in the USA with inbred lines derived from different crosses of spring wheats, we have concluded that the SRC test (AACC test 64-11) can improve selection efficiency in breeding programs because: 1) heritabilities are often above 70%, 2) the SRC tests directly relate to industrial baking parameters, and 3) SRC values change in predictably based on the segregation of genes with known effects on end-use quality

Keywords: Soft wheat, quality improvement

INTRODUCTION

Soft endosperm wheats traditionally are used for processed foods that require low water absorption flour, typically 48% to 55%. Gluten requirements for soft wheat flour are typically less demanding than that required for hard wheats used in bread baking. Yet, soft wheats in many applications require gluten strength to retain dough extensibility during the forming and baking of pastry products, particularly leavened products such as soda crackers (Slade and Levine, 1994). Low water absorption flours are produced by milling grain of soft endosperm wheat. Soft endosperm is conditioned by a single locus, *Ha*, which encodes tandem genes for puroindolines (Giroux and Morris, 1998). Environment and minor gene effects modify the hardness of the endosperm and subsequently flour water absorption. To select improved soft wheats, plant breeders use a series of tests to select for softer endosperm wheats with lower water absorption, including alkaline water absorption

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 503–508.

test (AACC 56–10), alveograph (AACC 54–30A), and sugar snap cookie (AACC 10–52). The problem underlying these selection strategies is that, in addition to selecting for softer wheats, breeders also select for weaker gluten wheats (Souza et al. 1994, Guttieri et al. 2001). Often, the strongest response to selection is loss of gluten strength, leaving other components of water absorption, such as damaged starch and pentosans relatively unaltered (Souza et al. 1994). Milling yield is another component of the soft wheat quality that is difficult to address in breeding. By definition, soft wheats should have low energy requirements for flour milling with a large fraction of high quality flour released from the first mill rolls (called break rolls). The standard experimental measure to assess break flour requires the use of an experimental milling of tempered grain such as the AACC 26–10. For a breeding program, tempering and experimental milling represents a significant bottleneck for improving soft wheat quality due to the small number of samples that can be processed. In the University of Idaho Quality laboratory, we can temper and mill only 32 samples per day, 4 days per week. In contrast, hard wheat breeding programs have rapid, accurate, and heritable quality tests that have produced systematic improvements in the milling and baking quality of bread wheats (Cox et al. 1989, Souza et al. 1993). This paper outlines some of our attempts to develop fast standard protocols for soft wheat quality improvement.

Analysis of Flour

The AACC protocol 56–11, solvent retention capacity (SRC), was developed as an analytical tool for analyzing flour entering a bakery. At its simplest, the SRC indirectly measures damaged starch, pentosan content, and gluten strength based on the absorption of specific solvents by flour. Sodium carbonate (5%) solvent measures damaged starch in flour; 50% sucrose measures water soluble pentosan in flour, and absorption of 5% lactic acid measures gluten strength. We have published several modifications to the method to increase its speed and flexibility for breeding programs (Guttieri et al. 2001). A full descriptions of the modifications are posted at our program website (www.agls.uidaho.edu/cerealsci) and are demonstrated on a free training video (DVD format) described at the website. The most important modifications have been the use of repeating dispensers to replace weighing solvents and automated agitation using an orbital shaker (rocking shakers are not sufficiently chaotic for SRC analysis). In addition, electronic data capture from balances has reduced transcriptional errors common with processing large numbers of samples for SRC flour evaluations.

The attractive aspect of the SRC is the relatively low cost for equipment and reagents to conduct the evaluation. Another paper within this section reports on evaluations of EMBRAPA soft wheats using a standard benchtop microfuge (US $300). For breeding purposes, measures of quality that have relatively minor components of genotype x environment interaction are particularly helpful because, with use of appropriate check cultivars, selection of superior genotypes can be accomplished with testing in one to several trials. We have found that the SRC accurately

ranks genotypes for their solvent retention capacity with only minimal genotype x environment interaction (Guttieri et al. 2001, Guttieri et al. 2003, Guttieri and Souza 2003). For example, among 25 elite soft wheats, Guttieri et al. (2001) found less than 10% of total variation could be attributed to genotype x environment interaction for sodium carbonate, sucrose, and lactic acid SRC, with the major source of variation in all three solvents deriving from differences among genotypes. Guttieri and Souza (2003) reported similar findings for unselected breeding populations.

One additional important advantage of the SRC test is the ability to evaluate soft wheat quality despite pre-harvest sprouting. Pre-harvest sprouting produces α-amylase enzymes in the flour and typically obscures the inherent soft wheat characteristics of the flour. Theoretically, the sodium carbonate measure of damaged starch should be unaffected by the α-amylase activity caused by pre-harvest sprouting because the primary factor determining the absorption of sodium carbonate SRC is physically sheared starch rather than enzymatically degraded starch. Samples of genotypes harvested in fields without pre-harvest sprouting and with pre-harvest sprouting present were used to test this hypothesis. Figures 1 and 2 show the correlations found in two studies of soft white wheat comparing testing the correlations of sodium carbonate SRC in flours from fields with and without field pre-harvest sprouting. We are following up to use measures of pre-harvest sprouting (rapid viscoanalyzer data) to confirm the independence of SRC data from pre-harvest sprouting.

Milling

The University of Idaho has used a Quadrumat Senior mill system to evaluate milling yield of soft wheats. It has two experimental mill heads and produces both break and reduction flour. We still use it for our advanced breeding line evaluation

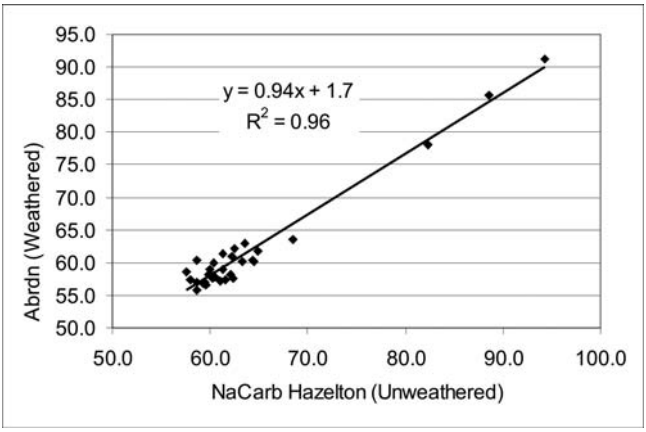


Figure 1. Comparison of sodium carbonate SRC in elite soft white spring wheats produced in Idaho in 2004 at two irrigated locations, Hazelton, which was harvested prior to fall rains, and Aberdeen, which was harvested following pre-harvest rains. (University of Idaho samples)

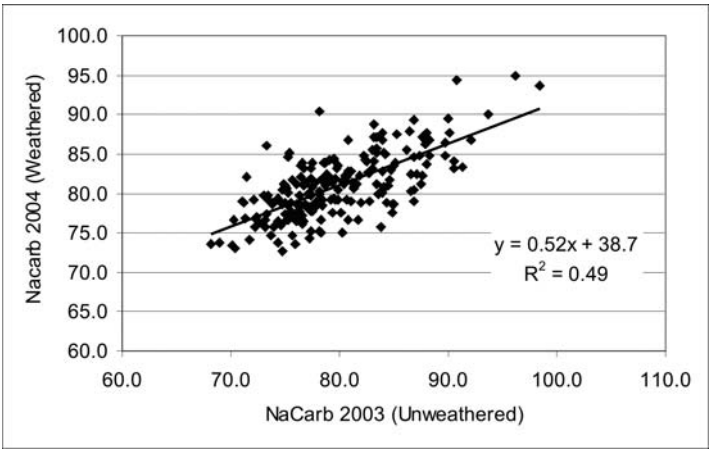


Figure 2. Comparison of Sodium carbonate SRC in a recombinant inbred population of Cayuga X Caledonia grown in 2003 with normal harvest (unweathered) and 2004 with rainfall prior to harvests weathered, Ithaca NY 2003 and 2004. (Samples courtesy of Mark Sorrells, Cornell University)

when finished patent flour is required for evaluation. But because of the limited throughput of the Quadrumat Senior mill, we sought a faster protocol that minimized starch damage. Our laboratory has available a Quadrumat Junior mill with a single mill head that can produce a low-grade flour with poor separation of bran. But due to the simpler flow and smaller sample size, the Junior mill is able to more than quadruple the per-day sample number. Tempering can improve bran separation, but tempering is time consuming: grain moisture must be measured and an aliquot of water added to each sample. Environmental tempering is a protocol used at the Western Wheat Quality Laboratory in the first half of the 20th Century. With Doug Engle of that laboratory, we have developed the following equipment and protocol to speed tempering. We are using a sealed cabinet with a home humidifier and a small portable heater with a thermostat. The humidifier keeps the cabinet at about 80% humidity and the heater maintains a temperature of 24°C. Samples (40 g) are tempered for 4 days in coin envelopes organized in open rack. Following environmental tempering, samples are milled in a Quadrumat Junior mill. Although the length of time to temper and mill a sample is doubled, the number of samples per day that can be milled is more than quadrupled and the amount of work per sample is reduced to a minimal effort.

For smaller grain samples, whole grain analysis offers the opportunity to apply SRC analysis to plant and family selections. Bettge et al. (2002) developed a whole grain version of the SRC analysis. A whole grain sample ground in a Udy grinder with a standard size screen is analyzed using the same protocols as a flour SRC. Guttieri et al. (2004) found that the sodium carbonate and sucrose SRC values of whole grain samples were correlated with the values of flour SRC measures for the same lines. However, whole grain lactic acid SRC measures were poorly correlated

with flour lactic acid SRC due to the interference of bran in the gluten absorption (Guttieri et al. 2004). However, the standard SDS sedimentation of whole grain samples is highly correlated with flour lactic acid SRC and can act as a substitute for whole grain lactic acid SRC. Interestingly, as more products worldwide are manufactured with whole grain flour, it is increasingly important to select low water absorption whole grain flours for biscuits and crackers. Guttieri et al. (2004) found as much genetic variation for whole grain water absorption as for flour absorption, suggesting that a whole grain SRC may provide a useful tool for improving whole grain products.

Breeding Program Flow

Within our breeding program, we use selected bulks through early generations followed by pure-line selection, headrows, a year of unreplicated testing, and then multi-location replicated testing. The follow outline for quality testing is based on the findings described above (Table 1)

After F_9 breeding lines are entered into regional testing and evaluations are conducted at both USDA laboratories and the University of Idaho.

Table 1. Selection and tests used to improve breeding populations for soft wheat quality at the University of Idaho wheat breeding program

Generation	No./generation	Milling	Quality test
F_1, BC_1, or Double Cross	200	None	Molecular markers for puroindolines in some crosses
F_2, F_3	200	Udy mill (whole grain grind)	Whole grain sodium carbonate SRC and SDS sedimentation
F_4	100,000 heads	None	Visual selection for soft kernels. Selection for low polyphenol oxidase activity
F_5	1,500	Udy mill (whole grain grind)	Whole grain sodium carbonate SRC and SDS sedimentation
F_6	400	Environmental temper, Quadrumat Junior mill	Sodium carbonate and lactic acid SRC
F_7	50	Quadrumat Senior mill	Four solvent SRC
F_8	25	Quadrumat Senior mill	Four solvent SRC, cookie bake
F_9	12	Quadrumat Senior mill	Four solvent SRC, cookie bake

ACKNOWLEDGEMENTS

The research described in this paper was supported by Kraft Foods, the Idaho Wheat Commission, and the Idaho Agricultural Experiment Station.

REFERENCES

American Association of Cereal Chemists (2000) Approved Methods of the AACC, 10th edn. American Association of Cereal Chemists, Saint Paul, MN, USA

Bettge AD, Morris CF, Demacon VL, Kidwell KK (2002) Adaptation of AACC Method 56-11, solvent retention capacity for use as an early generation selection tool for cultivar development. Cereal Chem 79(5):670–674

Cox TS, Shogren MD, Sears RG, Martin TJ, Bolte LC (1989) Genetic improvement in milling and baking quality of hard red winter wheat cultivars, 1919 to 1988. Crop Sci 29:626–631

Giroux MJ, Morris CF (1998) Wheat grain hardness results from highly conserved mutations in the friabilin components puroindoline a and b. Proc Natl Acad Sci USA 95:6262–6266

Guttieri MJ, Souza E (2003) Sources of variation in the solvent retention capacity test of wheat flour. Crop Sci 43:1628–1633

Guttieri MJ, Bowen D, Gannon D, O'Brien K, Souza E (2001) Solvent retention capacities of irrigated soft white spring wheat flours. Crop Sci 41:1054–1061

Guttieri MJ, McLean R, Lanning SP, Talbert LE, Souza E (2003) Assessing environmental influences on solvent retention capacities of two soft white spring wheat cultivars. Cereal Chem 79:880–884

Guttieri MJ, Becker C, Souza E (2004) Application of wheat meal solvent retention capacity tests within soft wheat populations. Cereal Chem 81:261–266

Slade L, Levine H (1994) Structure and function relationships of cookie and cracker ingredients. In: Faridi H (ed) The science of cookie and cracker production. Chapman & Hall/AVI, New York, pp 23:141

Souza E, Tyler JM, Kephart KD, Kruk M (1993) Genetic improvement in milling and baking quality of hard red spring wheat cultivars. Cereal Chem 70:280–285

Souza E, Kruk M, Sunderman DW (1994) Association of sugar snap cookie quality with high molecular weight glutenin alleles in soft white spring wheats. Cereal Chem 71:601–605

WHEAT MICROEVOLUTION UNDER INTENSIVE BREEDING PROCESS IN THE NORTHERN CAUCASIAN REGION

L.A. BESPALOVA*, Y.M. PUCHKOV AND F.A. KOLESNIKOV

Krasnodar Lukyanenko Research Institute of Agriculture
**E-mail: lbespalova_l_a@rambler.ru*

Abstract: In 2005 it was the 50th Anniversary of the outstanding variety Bezostaya 1, which was developed at our Institute by Academician Pavel P. Lukyanenko. This variety had been grown in many countries of the world, covering in some years more than 13 million hectares and more than 160 million hectares during the whole period of its utilization. Due to high yielding, good grain quality and ability to adapt to a wide range of environmental and technological conditions this variety won the first place in the International Variety Trial in 1971 and 1972. After this Dr. Johnson considered this variety to be the best genotype for many wheat areas of the world. It is remarkable that among Bezostaya 1's progenitors there was an Argentine variety Klein 33, donor of *Rht* gene and leaf rust field resistance. 124 winter bread wheat varieties have been developed after Bezostaya 1. The major trends of Krasnodar breeding are balanced improvement of potential yielding and grain quality and development of great number of genetically and biologically different varieties, which helps to extend the crop's adaptation potential. Potential yielding of our new varieties Tanya, Doka, Ktasnodarskaya 99 averages 12 tons per hectare. The varieties like Delta, Deya, Moskvitch possess FHB and other fungal disease resistance; Zimorodok, Polovchanka, Knyazhna, Starshina, Soratnitsa, Selyanka, Russa are tolerant to late sowing, deficient, over-compacted and saline soils. New varieties have very good baking quality, middle to high protein content; differ in growing period within 7–12 days. Their frost-resistance is increased by 1–2 °C. Winter wheat microevolution in the Northern Caucasia can be characterized by the following properties: a) increase of coenosis capacity due to higher ear productivity and plant density; b) prolongation of grain filling period; c) attraction rate acceleration; d) plant nitrogen metabolism intensification; e) plant "economics" improvement

All these changes have been achieved during 50 years due to intraspecific and distant crossing within the system of complex step-wise hybridization and use of genetic "bridges" – synthetic amphidiploids

Keywords: wheat breeding, baking quality, Bezostaya 1 variety, *Triticum sphaerococcum*, synthetic amphiploids, dwarfing genes

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 509–517.

INTRODUCTION

In the Northern Caucasia priority in winter bread wheat breeding is set on high stable yielding and grain quality. During the last 50 years of the previous century wheat yielding in Krasnodar region has increased from 1.5 to 4.4 t ha^{-1} (Bespalova, 2003). In the last five years of this century this figure averaged 4.59 t ha^{-1}. According to our estimates, annual growth of yielding due to breeding achievements is 2.5 percent. A great contribution to this progress is made by the varieties developed by academician Pavel Lukyanenko during 1930–1973. His breeding methods are internationally known (Lukyanenko, 1932, 1946, 1956, 1959, 1967). They are based on environmental-genetic concepts. Large-scale hybridization and early-stage selection of simple hybrids and back-cross hybrids developed according to the parameters of his model helped him to create varieties with a new level of adaptability. Lukyanenko's variety Bezostaya 1 combining high level of adaptability and extraordinary longevity with high grain quality (Kronstad, 1996) has been commercially grown for 50 years. The varieties Kavkaz and Avrora being the carriers of 1BL/1RS translocation from rye have been used for breeding many modern varieties (Rajaram, 2000). The principal attribute of bread-making wheat – grain quality – is gradually falling down even in commercial production. There are several reasons for this: oversaturation of crop rotation system with high-yielding grain- and technical crops, high level of NPK carry-over, progressive decrease of soil fertility, lower mineral and organic fertilizer input (Kalinenko, 1999). Under these conditions varieties of Bezostaya 1 type cannot guarantee high-quality grain production (with protein content of 14 percent and higher, crude gluten content – 28 percent and higher, flour strength > 280, farinograph index > 80).

To negotiate this negative tendency special emphasis is currently put on breeding for grain quality through application of various methods and sources.

MATERIALS AND METHODS

Breeding for grain quality is carried out on the basis of generation-specific evaluation of breeding material – a method developed at our institute (Tarasenko, Puchkov, 1977; Kazartseva et al., 2001). F_3 and F_4 plants are evaluated and selected according to their SDS-sedimentation, grain hardness and protein content; F_4, F_5 plants – according to their protein content, gluten content and quality, grain hardness and flour strength; F_5, F_7 plants – according to all the abovementioned parameters as well as to the results of their full technological analysis. In addition, F_4 and F_5 plants are selected by their gliadine markers. The principal breeding method applied in Krasnodar is complex step-by-step hybridization and, as a rule, two- or more-times mass or individual selection starting from F_2 generation. In order to raise plant nitrogen status, accelerate nitrogen metabolism and its direct translocation to grain, and to increase stability of this process we exercise interspecific and intergeneric crossings (with *T. durum, T. sphaerococcum, Triticale*), use related wild species (*Ae. tauschii, Ae. speltoides, T. militinae, T. timopheevii*) via

synthetic amphidiploids and their translocation lines developed at the Biotechnology Laboratory of our Institute (Davoyan, 2005).

RESULTS AND DISCUSSION

Among thirty six currently released varieties twenty are graded as high-quality. They are developed through application of various methods, approaches and source material.

Genes for Dwarfness and Hybrid Dwarfness in Breeding High-quality Varieties

There exists an opinion (Pavlov, 1982; Bebyakin & Kotlyar, 1986) (and it is mostly true), that short-stem varieties, possessing high harvest index, as a rule, show low protein content. Our experiments have proved that breeding material with the recessive allele of Rht11 dwarfing gene (from Krasnodarskij karlik 1) possesses higher plant N concentration at the flowering stage compared to medium-height varieties (Bespalova & Kerimov, 1995). The difference in N concentration varies between 0.11–0.15 percent. At the same time, protein content in semi-dwarf varieties is by 0.8–1.2 percent lower due to higher yielding level and, consequently, lower N supply to grain. Inclusion into hybridization of source material with high level of direct N translocation which causes conversion of the derived metabolites into protein synthesis has enabled us to develop varieties combining high yielding and good grain quality.

Pobeda 50 is a variety of the above mentioned type (Table 1). According to its protein and gluten content, flour strength, rheological properties of dough it is graded as a baking strength improver and at the same time it itself produces high quality bread. A very early variety Esaul, due to application of the above

Table 1. Description of varieties with high bread-making quality, Krasnodar, 1999–2004

Variety	Skifyanka	Pobeda 50	Esaul	KN 001
Genotype	Rht11	Rht11 + FDT	Rht11 + FDT	Rht11 + D
Yielding, t ha^{-1}	7,6	8,2	8,5	8,9
Harvest index, %	45	45	42	42
Test weight, g I^{-1}	844	824	819	842
TKW, g	40,5	41,6	43,7	38,6
Vitreousness, %	79	74	84	82
Grain protein content, % (DM)	12,7	14,2	14,8	14,9
Flour gluten content, %	26,7	31,9	32,8	32,1
Gluten Deformation Index	63	70	63	65
Flour strength (alveograph value)	452	410	386	380
Valorimeter value	76	86	92	84
Loaf volume, ml	648	615	686	650
General bread-making score	4,8	4,6	5,0	4,8

Table 2. Protein content and yielding of Esaul under various agrotechnological conditions, Krasnodar, 2004–2005

Previous crop	Grain protein content, %(DM)			Yielding, t ha^{-1}		
	Esaul	Bezostaya 1	$LSD_{0.05}$	Esaul	Bezostaya 1	$LSD_{0.05}$
Rape	15, 1	14, 0	0.28	8, 9	6, 9	0.39
Wheat	15, 3	14, 7	0.32	6, 2	5, 2	0.54
Sunflower	13, 3	12, 7	0.21	7, 0	6, 1	0.46

mentioned system of grain quality improvement and a slight decrease of harvest index, demonstrates progress in all grain quality aspects including bread making. Esaul shows the highest quality values when grown after such forecrops which leave enough available nitrate and other macro-elements in the soil (Table 2). It also shows high stability of grain quality even if grown under poor conditions (late sowing, low fertilizer input). Its advantage over Bezostaya 1 is self-evident and highly significant. In the last years we have made an attempt to transfer genes for hybrid dwarfness from a mutant form, Karlik Istoka, developed at our Institute, in order to improve plant "economics". Our hypothesis concerning D-gene indirect positive effect on grain quality was confirmed. New experimental lines KN 001 along with high yielding show high protein and gluten content.

Use of the variety Atlas 66 for breeding high-quality varieties was initiated by Pavel Lukyanenko in 1971 when he crossed this variety with Kavkaz and Avrora. As a result a new line Lutescens 611h157 was developed showing high protein content but not very good bread-making properties. Further breeding for high protein content was based on hybridization with varieties and forms possessing high protein content, good or excellent bread-making properties (NS11-35, Obrij, 622h1265) and more efficient partitioning of nitrogen to grain (Pavlovka). As a result of 3–4 step-wise crossings, varieties Gorlitsa and Veda (Fig. 1) were developed showing, along with relatively high yielding and stress resistance, increased protein and gluten content and excellent bread-making quality. Gorlitsa is characterized by high and stable nitrate uptake ability both under favorable weather conditions and under draught stress with high temperature at grain filling period. Being tested in 75 variety trials, complex and environmental trials during two years (2003–2004) Veda performed yielding – 6.8 t ha^{-1}, protein content – 14.95 percent, gluten content – 28.4 percent with gluten referred to high-quality group (Table 3). In Environmental Trial 2005 Veda exceeded Bezostaya 1 in yielding by 2.38 t ha^{-1} and showed significant advantage in protein and gluten content.

Improving Grain Quality via *T. sphaerococcum* Perc

Great prospects of including *T. sphaerococcum* into wheat breeding for high quality have been predicted by outstanding *Triticum* researchers such as N.I. Vavilov (1935), P.M. Zhukovskij and M.M. Yakubtsiner (1947). In 1980s by

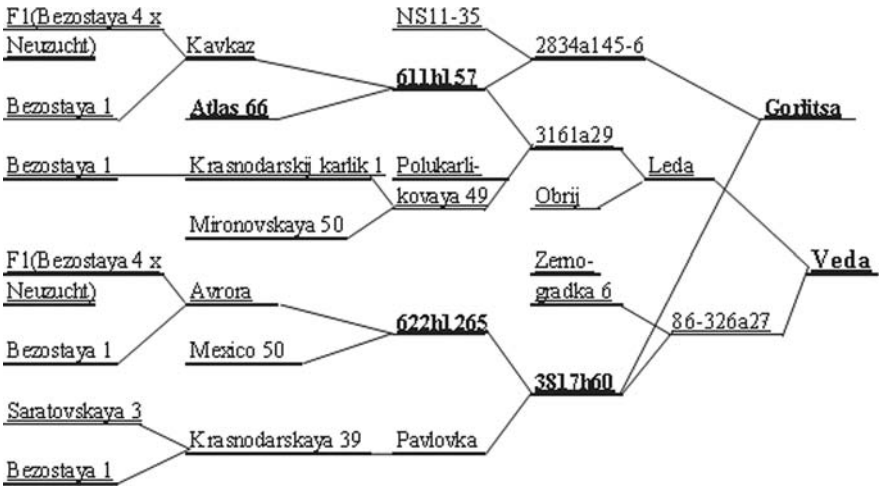


Figure 1. Pedigree of Gorlitsa and Veda

means of distant hybridization we have developed a series of *sphaerococcum* lines with very high values of all grain quality parameters. Further crossings with these lines and selection of alternative species within derived hybrid populations allowed us to single out high-quality lines of *sphaerococcum* type with improved adaptability characteristics and *T. aestivum* lines with improved grain quality parameters. One of the selected *T. sphaerococcum* lines became the variety Sharada. It has short (55–90 cm), relatively slim and very strong lodging-resistant stem. Leaf blades are short, wide, sharply narrowing to the tip, almost erect (leaf angle relative to the stem is 15–16°). The ear is compact, 4–7 cm long, shedding-resistant but at the same time easily thrashed. The grain is of spheric shape (which is almost perfect for

Table 3. Yielding and grain quality of Veda, Krasnodar, 2003–2005

Trait	(average for 75 trials)			
	Veda	Bezostaya 1	Difference	$LSD_{0.05}$
Yielding, t ha^{-1}	6,8	5,7	1, 1	0,26
Grain protein content, % (DM)	15,0	14,4	0, 6	0,18
Grain gluten content, %	28,4	28,1	0, 3	0,39
Gluten quality (GDI)	70	69	1, 0	1,8
	2005 (average for two trials)			
Yielding, t ha^{-1}	10,4	8,0	2, 4	0,41
Grain protein content, % (DM)	14,7	14,0	0, 7	0,34
Grain gluten content, %	27,1	26,0	1, 1	0,62
Gluten quality (GDI)	58	62	−4, 0	1,8

milling industry), relatively small, thousand kernel weight – 34 g. Protein content reaches 18.8 percent, crude gluten content – 38 percent with Gluten Deformation Index (GDI) corresponding to the 1st gluten quality group. However, sometimes gluten may be too strong (GDI – 45), though this positively effects mixing ability. Flour strength varies between 306 and 780 units as measured by Alveograph. Loaf volume may reach 650 ml (with no improver application). Bread-making score is very high (4.8–4.9).

This variety produces high-quality grain due to increased N uptake from vegetative organs and partitioning to grain, at lower harvest index (0.38–0.40), as well as due to its ability to accumulate high concentration of nitrate in leaves and stem at flowering stage (Kerimov, 2004). In grain yielding Sharada is inferior to the bread wheat check variety Skifyanka (*T. aestivum*) by 1.1 t ha^{-1} or 13.9 percent but significantly exceeds in grain quality (Table 4). A bread wheat variety Novokubanka developed through backcross *sphaerococcum* × *aestivum* exceeds in yielding the check variety Skifyanka by 0.9 t ha^{-1} and reliably exceeds the check in grain quality, though being inferior to Sharada in quality aspect.

When wheat grain is processed into flour or groats morphological properties of grain have a particular effect on technological process and quality of end-product. Sphere geometry is characterized by maximal volume at minimal surface; hence *sphaerococcum* wheat has high endosperm content and lower share of bran – the main low value offals. This permits to reduce losses at separation of bran and aleurone layer and without any additional energy input to increase flour extraction rate and groats production (Table 5). In groats quality parameters Sharada undoubtedly exceeds the hard bread wheat variety Pobeda 50, which has an oval-shape kernel.

Using genetic diversity of wheat congeners for increasing bread wheat pathogen resistance via synthetic amphidiploid *T. miguschovae* allowed us to simultaneously

Table 4. Description of varieties having *T. sphaerococcum* in their pedigree, Krasnodar

Trait	1995–1998			1999–2005		
	Novokubanka T. aestivum	Skifyanka (check) T. Aestivum	$LSD_{0.05}$	Sharada T. sphaerococcum	Skifyanka (check) T. aestivum	$LSD_{0.05}$
Yielding, t ha^{-1}	8,8	7,9	0,34	7,0	8,1	0,28
Grain protein content, % (DM)	14,0	13,5	0,31	16,8	12,7	0,36
Grain gluten content, %	29,2	27,6	0,78	33,8	23,3	0,55
Gluten quality (GDI)	75	68	2	62	65	2,3
Loaf volume, ml	632	607	31	686	635	28
General bread-making score	4,3	4,3		4,6	4,4	

Table 5. Technological properties of Sharada's grain, Krasnodar, 2003–2004

Milling					
	Extraction rate, %				
Variety	Coarse semolina	Medium semolina	Middlings	Dunst and flour	Total extraction
Sharada	**48,4**	16,8	4,2	14,1	**83,5**
Pobeda 50	40,2	16,7	5,1	16,6	78,6
$LSD_{0.05}$					2,0
Cracking					
	Bran removal product share, %				
Variety	Undamaged kernels	Cracked kernels	Meal	Total extraction	Whiteness
Sharada	**72,1**	2,2	17,8	**92,1**	34,8
Pobeda 50	66,3	2,2	19,8	88,3	35,4
$LSD_{0.05}$				1,8	

improve resistance and grain quality in new varieties (Table 6). Vrieties Zhirovka (1998) and Vostorg (2005) included into the State Register of breeding achievements exceed check varieties in yielding by 0.4 and 0.8 t ha^{-1} respectively. They produce grain with increased gluten and protein content, good dough rheology properties and high bread-making score. The advantage of new varieties is in their resistance to leaf and yellow rust (Vostorg is also resistant to stem rust), low susceptibility to powdery mildew and some viruses. They are also remarkable for prolonged green functioning of glumes and stem which causes yield and quality improvement.

Table 6. Description of varieties Zhirovka and Vostorg, developed with application of T. miguschovae synthetic amphidiploid, Krasnodar (* 1994–1998; ** 2001–2005)

Trait	*Zhirovka	*Skifyanka (check)	$LSD_{0.05}$	**Vostorg	**Pobeda 50 (check)	$LSD_{0.05}$
Yielding, t ha^{-1}	8,4	8,1	0,36	9,9	9,0	0,38
Harvest index	0.43	0.45	0.015	0.45	0.44	–
TKW, g	43,2	41,1	0,84	45,1	43,2	0,65
Grain gluten content, %	31,8	28,1	1,32	31,6	27,8	0,89
Grain protein content, % (DM)	14,6	13,5	0,62	14,8	13,8	0,43
Flour strength (alveograph value)	411	386	31	286	301	32
Valorimeter value	86	82	5	84	86	4
General bread-making score	4,4	4,3		4,3	4,4	

We also widely use triticale as a genetic "bridge" for transferring genetic information from rye and durum wheat to bread wheat. As evidenced by our study, 1BL/1RS translocation, as a rule, causes increase in grain protein and gluten content, though reducing flour strength and worsening dough quality (Bespalova et al, 1999). This is caused by increased N supply to grain due to higher concentration of nitrate in plant and improved root system functioning (direct N translocation at grain filling stage). Selection of lines without 1BL/1RS translocation among triticale × wheat hybrids helps to identify breeding material with increased and high protein and gluten content accompanied by good bread-making quality of grain. This may be supported by the example of variety Pamyat, developed through this method, and breeding lines KN 291.

CONCLUSION

Large-scale breeding for grain quality improvement (increasing protein and gluten content, improving dough and bread properties) through application of various methods and wide diversity of source material within the system of complex stepwise hybridization and strict evaluation and selection by quality parameters resulted in optimal final outcome.

REFERENCES

Bebyakin VM, Kotlyar LE (1986) Uslovia mineralnogo pitania i belkovistost zerna yarovoj myakgoj pshenitsy. 11. Reutilizatsia azota vegetativnykh organov zernom. Phiziologia i biochimia kulturnykh rastenij 1:30–35

Bespalova LA (2003) Adaptive breeding of winter bread wheat in Krasnodar. Vestnik regionalnoj seti po vnedreniu sortov pshenitsy i semenovodstvu: 114–119

Bespalova LA, Kerimov VR (1996) Breeding value of some winter common wheat cultivars with high protein content. Poster presentation. Book of Abstracts, 5th International Wheat Conference, 10–14 June 1996, Ankara, Turkey, p 231

Bespalova LA, Gritsaj TI, Zima VG, Kazartseva AT (1999) Vliyanie bloka gliadinov GLDiB3 na kachestvo zerna ozimoj myagkoj pshenitsy. Aktualnye problemy selektaii i semenovodstva zernovykh kultur Ugo-Vostochnogo regiona RF: 3536

Davoyan RO (1988) Sintezirovannyj gomolog myagkoj pshenitsy Triticum miguschovae kak istochnik ustojchivosti k listovoj rzhavchine. Selektsia i genetika zernovykh i zernobobovykh kultur: 3035

Jakubtsiner MM (1947) Selectsionnyj fond mirovigo sortimenta pshenits. Selektsia I semenovodstvo, 8

Kalinenko IG (1999) Selektsia ozimoj pshenitsy: 220

Kazartseva AY, Vorobjova RA, Domchenko MI (2001) Formirovanie vasokokachestvennogo zerna pshenitsy sortami selektsii KNIISH, peredannymi v GSI v 1991–2000 godah. Materialy nauchno-prakticheskoj konferentsii: 399–401

Kerimov VR (2004) Morphobiologicheskaya charakteristika istochnikov vysokogo kachestva zerna ozimoj pshenitsy. Avtoreferat: 24

Kronstad WE (1996) Agricultural development and wheat breeding in the 20th century. In: Braun HJ, Altay F, Kronstad WE, Beniwal SPS, McNab A (eds) Wheat: prospects for global improvement. Proceedings of the 5th international wheat conference, 10–14 June 1996, Ankara, Turkey. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 1–10

Lukyanenko PP (1932) Osnovnye itogi rabot po selektsii ozimoj pshenitsy I yachmenya (s 1920 g. po 1930 g.): 31

Lukyanenko PP (1946) Selektsia novykh sortov ozimoj pshenitsy dlya Kubani. Selektsia i semenovodstvo. Yubilijnyj nomer: 70–75

Lukyanenko PP (1956) Skreshchivanie geograficheski otdalennykh form v selektsii ozimoj pshenitay. Doklady VASHNIL 2:813

Lukyanenko PP (1959) Metody I resultaty selektsii ozimoj pshenitsy na Kubani. Sel. khoz-vo Sev Kavkaza. N°1:2732

Lukyanenko PP (1967) Itogi selektsii ozimoj pshenitsy na Kubani. Dostizhenia otechestvennoj selektsii: 71–95; Selektsia i semenovodstvo. N°5:14–20

Pavlov AN (1982) Phiziologicheskie prichiny, opredelyayushchie uroven nakoplenia belka v zerne razlichnykh genotipov pshenitsy. Phiziologia rastenij 4:767779

Rajaram S (2000) Prospects and promise of wheat breeding in the 21st century. In: Bedő Z, Láng L (eds) Wheat in a global environment. Proceedings of the 6th international wheat conference, 5–9 June, Budapest, Hungary. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 37–52

Tarasenko ND, Puchkov YM (1997) Otsenka kachestva zerna pshenitsy v protsesse selektsii. Sb. trudov. XII:58–62

Vavilov NI (1935) Nauchnye osnovy selectsii pshenitsy. M.-L.

Zhuchenko AA (2004) Resursnyj potentsial proizvodstva zerna v Rossii (teoria i praktika):1109

Zhukovskij PM (1971) Kulturnye rastenia i ikh sorodichi. Leningrad

GENOTYPIC VARIABILITY OF COMMERCIAL VARIETIES OF BREAD WHEAT FOR PARAMETERS OF COMMERCIAL AND INDUSTRIAL QUALITY

J.G. MONTANER* AND M. DI NAPOLI

AACREA. Cátedra de Cereales, Facultad de Agronomía UBA
Lanza del Vasto 562, Tandil, Argentina (7000)
**E-mail: jhmontaner@arnet.com.ar*

Abstract: During the years 2002, 2003 and 2004, three trials were conducted in Azul (Buenos Aires Province) on commercial farms, in order to compare thirty varieties of bread wheat. Single effects and combinations of the application of nitrogen (N) and fungicides (F) were analyzed. The treatments were applied between developmental stages Zadocks 50 to 60. The dose of N was of 34 Kg N ha^{-1} using urea as the source. The fungicides were strobilurine and triazol in the first two years and triazol in the final year. The parameters evaluated were grain yield (GY), test weight (PH), grain protein concentration (GP), grain gluten concentration (GL), dough strength (DS) and farinographic stability (FS). Averaged over years and varieties, the application of N in plots treated with F did not generate significant increases in GY, whereas the quality parameters evaluated tended to increase GP from 0.3 to 0.8%, GL from 0.8 to 3.1% and DS from 6 to 25 10E-4J. The average increase in FS for the N effect was low in all the trials, from −0.4 min to 3.0 min. High variability between years existed in the GY response to F application. In 2002, the response was of +1068 kg ha^{-1}, due to a severe attack of leaf rust (*Puccinia recondita*), whereas in the remaining trials it was low in magnitude: +203 kg ha^{-1} in 2003 and +249 kg ha^{-1} in 2004. The GY response to F was related to PH increase. The fungicide application caused substantial decreases in the quality parameters of some varieties, especially in year 2002, where, averaged over the varieties, the reductions were −0.4% in GP, −1.2% in GL, −77 10E-4J in DS and –6 min in FS. Nevertheless, within each year, great genotypic variability was observed for FS response to F, corresponding to major reductions in varieties with high FS without the application of F. These alterations in the quality parameters might be caused by the prolongation of the grain filling period due to F application, which would expose the phase of deposition of several protein fractions to periods of high temperature, or to some change in the metabolism of the proteins induced by fungicide action. This hypothesis will be tested in future evaluations

Keywords: genotypic variability, Industrial quality

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 519–526

INTRODUCTION

During recent years, wheat sales based upon high baking quality have increased. There have been changes in the local market, where, for example, the industry in Brazil demands high quality products, recognizing their added value. ALEA S.A., Syngenta, ACA, Dreyfus and others companies operate in this market, led by groups focused on wheat quality (INASE). The current work evaluates how genetic differences vary over years in relation to fungicide and nitrogen applications made during the latter stages of crop development, and their relationship with industrial and commercial quality.

MATERIALS AND METHODS

During the years 2002, 2003 and 2004, three trials were conducted in Azul (Buenos Aires Province) on commercial farms, in order to compare different varieties of bread wheat. The number of varieties were 25 in 2002, 24 in 2003 and 36 en 2004. Varieties were selected to cover a large range of industrial characteristics as defined by the Comité de Cereales de Invierno de la Comisión Nacional de Semillas (CONASE) (Table 1).

Two sowing dates were included each year according to ontogeny cycle. Nitrogen fertilizer was applied at sowing at a rate aimed at achieving a soil availability of 150 kg N ha^{-1}, this being the sum of the amount of N-NO3 in the soil (0–60cm of depth) and the quantity of added N. Single effects and their combinations were analyzed for the application of nitrogen (N) and fungicides (F). The moment of N and F application were chosen based upon the Zadocks scale: Z50 to 65 for N and Z37 to 45 for F.

The dose of N was of 34 kgN ha^{-1}, using urea as the source. The fungicides were strobilurine and triazol (Azoxistrobin + Tebuconazole ; Amistar 500 cc + Folicur 500 cc) in the first two years and triazol (Tebuconazole, Folicur 1 l) in the final year. Harvest time was the same for all varieties. Grain samples were sent to the Bahía Blanca Grain Stock Lab. The parameters evaluated were grain yield (GY), test weight (TW), grain protein concentration (GP), grain gluten concentration (GL), dough strength (DS) and farinographic stability (FS).

Table 1. Number of varieties by quality group in each year

Quality Group	Year of trial		
	2002	2003	2004
1	9	7	13
2	11	14	19
3	5	3	4
Total	25	24	36

RESULTS AND DISCUSSION

Environmental differences were apparent between years. Rainfall prior to anthesis (from 15/10 to 15/11) was high in 2002, whereas, in 2003 and 2004, most of the rainfall occurred during the grain filling phase (Fig. 1). There was a higher average temperature through pre-anthesis in 2002 compared with 2003 and 2004. In the latter two years, temperatures were low prior to anthesis, and only in 2003 did this condition continue during the grain filling phase (Fig. 2).

In 2003, mean yields for the N and F treatments were higher than in 2004 ($+136\,kg\,ha^{-1}$) and in 2002 ($+900\,kgha^{-1}$) (Table 2). Other parameters, such as GP and GL, were lower in 2003 than in 2004 and 2002. DS and FS were not related with year.

The analysis of the results according to quality groups showed a decrease in every parameter from group 1 to 3, especially DS and FS (Table 2). This shows that there was a stronger dependence of industrial quality on genetic components than on environmental conditions. Although varieties of group 3 showed lower quality parameters, average grain yield did not exceed those belonging to the other groups due a poor sanitary performance of Nidera Baguette 10 and a low performance of Klein Chajá grown under these experimental conditions.

Main goals in Argentinean wheat programs have been the incorporation of characters aimed at increasing potential yields and improving resistance to biotic and environment stresses. As an example, Nisi et al. (2004) showed that grain yields increased by an average of 45 to $50\,kg\,ha^{-1}\,year^{-1}$ in varieties released to the market in the central wheat growing area over the last seven decades. Even so, during the 90's, high yield along the southeast of Buenos Aires Province was also

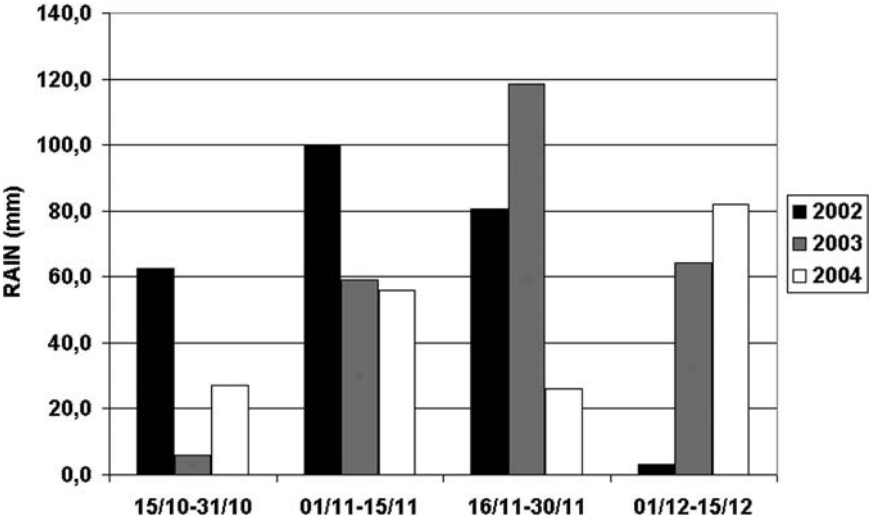


Figure 1. Temporal evolution of Precipitations for each year from pre-anthesis to grain maturity

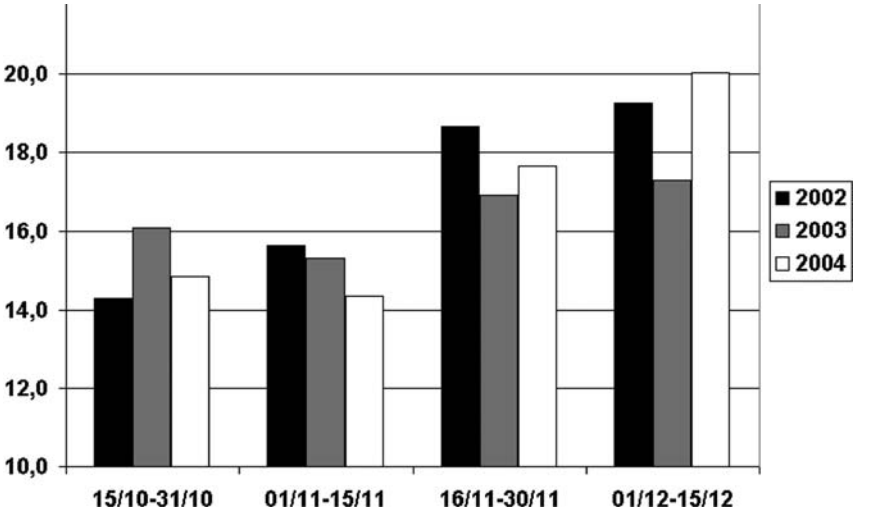


Figure 2. Temporal evolution of Mean temperature for each year from pre-anthesis to grain maturity

related with low quality performance for the baking industry (GM 1998). In the group of varieties tested in the current work, the association of DS and FS with GY within each group was not significant in any case. More than this, varieties of

Table 2. Year means for grain yield and the quality parameters tested. Nitrogen and fungicide treatments

Year	Quality Group	Grain Yield	Test Weight	Protein	Gluten	Dough strength	Farinographic stability
		Kg ha^{-1}	Kg hl^{-1}	%	%	$10E^{-4}J$	min
2002	1	4956	78,7	13,0	33,7	347	13,5
	2	5308	77,4	12,2	31,8	234	8,6
	3	5416	76,1	12,0	32,0	154	4,4
	Mean	5203	77,6	12,4	32,5	259	9,5
2003	1	5791	82,5	12,3	32,5	383	20,7
	2	6256	81,5	11,6	30,4	322	13,7
	3	6115	81,2	11,3	29,2	265	7,3
	Mean	6103	81,7	11,8	30,8	333	15,0
2004	1	5797	78,0	14,1	37,5	397	18,9
	2	6079	77,0	13,3	35,8	320	13,0
	3	5986	77,8	13,0	35,3	269	10,1
	Mean	5967	77,5	13,5	36,4	342	14,8
Means over years	1	5535	79,3	13,3	35,1	378	17,6
	2	5943	78,5	12,5	33,1	299	12,0
	3	5781	77,9	12,2	32,4	220	7,0

group 1 showed a positive association of DS and FS with GY (data not shown). This tendency reflects the improvement achieved in yield and quality programs that also established a genetic base from which to obtain superior genotypes for both characters.

Grain yield did not show a significant response to Nitrogen application, but it appeared that N uptake increased GP (Table 3) by 0.3 to 0.4% on average across years and variety groups. These GP increases are lower than the 1.2% obtained by Sarandon et al. (1986) for N application between Z58 and Z69 in high fertility environments. GP increases depend basically upon the initial N availability, the N source and the environmental conditions that improve N uptake (Porsborg 2005). GP increases were positively correlated with GL increases across the years and variety groups: 2.43% in GL per % in GP ($r=0.58$, $p<0.01$). Nitrogen application increased DS and FS quality parameters. But in contrast to their absolute values, which depended on genotype (Table 2), N responses were more related to environmental conditions, with the lowest increases for both parameters occurring in 2002.

Fungicide application strongly increased average responses in GY in 2002 ($+1068\,kg\,ha^{-1}$), due to the positive environmental conditions prevailing for rust (*Puccinia recondita*) in pre-anthesis (Fig. 1). In 2003 and 2004, the average GY increases were 203 and 249 kg ha^{-1} (Table 4).

Grain yield responses were partially related to increases in TW (Fig. 3), suggesting that the rate or length of the grain filling period was significantly increased by the fungicide.

Table 3. Response to N application in each year for grain yield and the quality parameters tested

Year	Quality Group	Grain Yield	Test Weight	Protein	Gluten	Dough strength	Farinographic stability
		Kg ha^{-1}	Kg hl^{-1}	%	%	$10E^{-4}J$	min
2002	1	−101	0,1	0,3	0,9	12	0,1
	2	−37	0,0	0,3	0,8	23	0,4
	3	−79	0,2	0,2	1,0	6	−0,4
	Mean	−263	0,0	0,3	0,8	5	0,6
2003	1	204	1,3	0,4	1,7	21	2,0
	2	214	2,6	0,8	3,1	25	3,0
	3	104	0,0	0,4	1,6	17	0,9
	Mean	−53	0,4	0,4	1,4	30	1,6
2004	1	30	0,1	0,4	1,5	16	0,8
	2	−69	0,2	0,2	1,4	9	0,2
	3	104	0,0	0,4	1,6	17	0,9
	Mean	−53	0,4	0,4	1,4	30	1,6
Means over years	1	15	0,8	0,4	1,6	18	1,0
	2	75	0,3	0,3	1,4	14	0,8
	3	−49	0,3	0,3	0,8	18	0,8

Table 4. Response to Fungicide application in each year for grain yield and quality parameters tested. Nitrogen treatment

Year	Quality Group	Grain Yield	Test Weight	Protein	Gluten	Dough strength	Farinographic stability
		kg ha^{-1}	kg hl^{-1}	%	%	$10E^{-4}J$	min
2002	1	747	2,8	−0,2	−1,1	−64	−10,1
	2	1232	3,9	−0,4	−1,3	−88	−8,3
	3	1282	4,9	−0,4	−1,3	−74	−4,6
	Mean	1068	3,7	−0,4	−1,2	−77	−8,6
2003	1	37	1,0	0,1	0,0	−34	−5,8
	2	231	0,5	−0,3	−0,4	−16	−2,7
	3	392	0,2	−0,4	−1,3	−32	0,1
	Mean	203	0,7	−0,2	−0,5	−21	−3,2
2004	1	20	−0,5	0,3	1,0	3	−1,0
	2	402	0,5	0,1	0,4	−17	−1,4
	3	265	−0,1	0,0	0,6	−36	−3,1
	Mean	249	0,1	0,2	0,6	−12	−1,4
Means over years	1	241	0,9	0,0	0,1	−27	−4,8
	2	565	1,4	−0,1	−0,3	−33	−3,7
	3	721	2,1	−0,3	−0,7	−51	−3,0

Every quality parameter tested tended to decrease as a response to fungicide application. GP and GL decreased due to a longer sugar synthesis period compared to the period of protein synthesis in the growing grain, hence lowering the protein level in the grain. As a hypothesis, N applications in the latter stages of crop

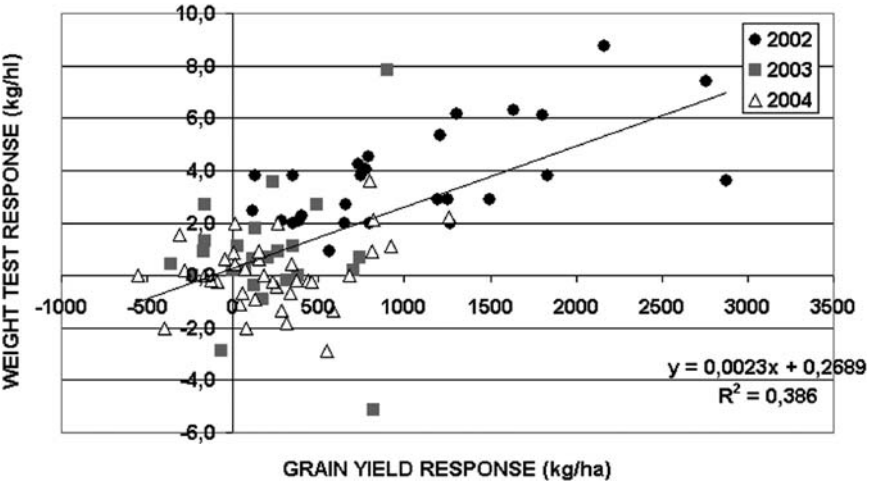


Figure 3. Weight test response plotted against grain yield response to fungicides

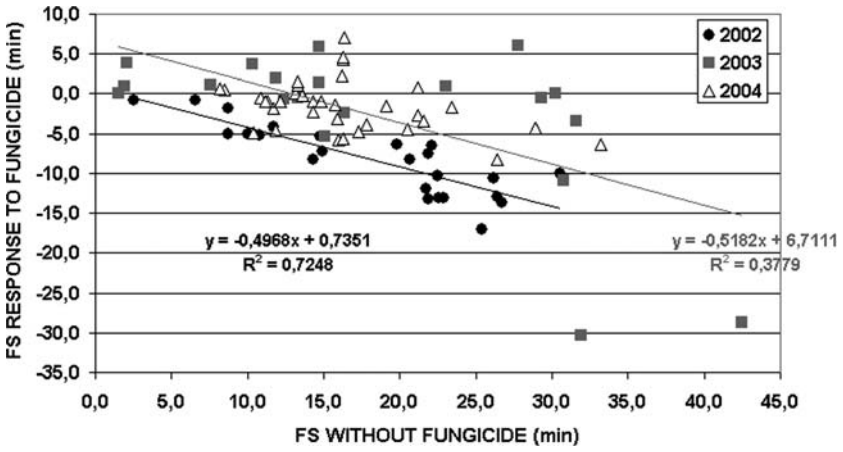


Figure 4. Farinographic stability response to fungicide plotted against farinographic stability without fungicide

development should avoid this. Additionally, fungicide applications decreased DS and FS levels, but were poorly related to GP and GL decreases. FS decreases caused by fungicide applications were higher for varieties with higher FS without fungicide, an effect that was significant in 2002 and 2003 (Fig. 4).

Mechanisms that produce DS and FS decreases are not clear since gliadin deposition precedes that of the glutenins during grain development (Stone and Nicolas 1996). As grain development continues, the gliadin/glutenin ratio should fall, causing an increase in DS (Stone and Nicolas 1994). The contrasting results found in the current work could be due a longer grain filling stage that exposes protein deposition to a higher temperature environment, modifying this relation in the opposite sense. (Blumenthal et al. 1994). This environmental hypothesis should be compared with another that proposes that protein structures change as a physiological response to fungicide addition, without involving interactions with the environment.

REFERENCES

Blumenthal C (1994) The heat-shot response relevant to molecular and structural changes in wheat yield and quality. Aut J Plant Physiol 21:901–909

González Montaner J (1998) Rendimiento y calidad: El equilibrio entre el margen y el mercado. Actas del IV Congreso Nacional de Trigo y II Simposio Nacional de Cereales de siembra Otoño-Invernal. Mar del Plata. Argentina

Nisi J, Bainotti C, Fraschina J, Formica M, Salines J (2004) Avances en el mejoramiento del rendimiento de grano en cultivares de trigo. Actas del VI Congreso Nacional de Trigo y IV Simposio Nacional de Cultivos de Siembra Otoño-Invernal. Bahía Blanca. Argentina, 29–30

Sarandón S, Gianibelli M, Chidichimo H (1986) Fertilización foliar en trigo: Efecto de la dosis y del momento de aplicación sobre el rendimiento y sus componentes, el porcentaje de proteínas y la calidad del grano. Actas del I Congreso Nacional de Trigo. Pergamino. Argentina, 242–258

Porsborg M, González Montaner G, Di Napoli M (2005) Late Application of nitrogen fertilizers in wheat. 7th International wheat conference. Book of Abstracts, Mar del Plata. Argentina, pp 210

Stone PJ, Nicolas ME (1994) Wheat cultivars vary widely in their responses of grain yield and quality to short periods of post-anthesis heat stress. Aust J Plant Phisiol 21:887–900

Stone PJ, Nicolas ME (1996) Varietal differences in mature protein composition of wheat resulted from different rates of polymer accumulation during grain filling. Aust J Plant Phisiol 23:727–737

NIR SPECTROSCOPY AS A TOOL FOR QUALITY SCREENING

D. VÁZQUEZ[1,4,*], P.C. WILLIAMS[2] AND B. WATTS[3]

[1] *INIA, Uruguay*
[2] *PDK Grain, Canada*
[3] *University of Manitoba, Canada*
[4] *R.50, km 11.500, La Estanzuela, Colonia. CP 70000. Uruguay*
[*] *E-mail: dvazquez@inia.org.uy*

Abstract: Simplicity of sample preparation, rapidity of the whole analytical procedure and small sample size requirements make near-infrared spectroscopy (NIRS) a very attractive tool for quality screening in wheat breeding programs. Although good results have been achieved for protein content, limited results have been obtained for other quality components such as protein functionality. The suitability of this technology to measure different quality components was studied using different sets of Uruguayan wheat samples ($n = 73–470$). Reference methods included protein composition, hardness, rheological variables and baking tests. Whole and ground wheat grain, and white flour samples were scanned with a Foss (NIRSystem) Model 6500 spectrophotometer. Differences among several math pretreatments for derivatives, gap and segment (smoothing) were not critical to the quality of the calibration. Results were best when flour was scanned, but were also acceptable using ground wheat and even whole grain. Increasing the size of the scanned sample did not improve results, however duplicating the NIR spectra resulted in better calibrations. Calibration equations obtained were suitable to determine three independent quality components in one simple and fast operation, although with different accuracies. Protein content was predicted with accuracy similar to that of the reference method (standard error of prediction = SEP = 0.16%; coefficient of determination $= r^2 = 0.99$). Hardness calibration was successful only with the ground sample (SEP < 2%; $r^2 = 0.85$). Results obtained for protein functionality variables had lower coefficients of determination but were sufficient for screening breeding lines. Coefficients of determination of calibration sets for the variables alveogram extensibility (L), alveogram baking strength (W), mixogram maximum height, and SDS sedimentation volume were over 0.6

Keywords: NIR spectroscopy, Quality

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 527–533.

INTRODUCTION

In the last two decades the use of NIRS has increased dramatically (Hruschka 2001). The success and acceptance of NIRS is based on two independent factors. One is that no chemicals are needed (Starr et al. 1981). The second is that the technique is very fast, mainly due to the quality of the spectra and the power of the mathematics used in the analyses (Hruschka 2001). In addition, in cereal technology another potential advantage of NIR is that the measurement of grain samples can be nondestructive (Nielsen et al. 2003, Pasikatan and Dowell 2004).

The most important component determined has been protein content. The first useful calibrations date from 1975 (Delwiche et al. 1998, Williams 1975). Since NIRS has proven suitable to predict amino acid content (Fontaine et al. 2002), it may be possible to use it to predict protein quality as well as quantity. This makes the technique attractive to use for the prediction of baking and dough properties in wheat breeding programs (Wrigley 1994). So far there has been limited success in reaching this objective. The main objective of this work was to develop NIRS calibrations valid to be used for high quality selection in a wheat breeding program.

MATERIALS AND METHODS

Two sets of samples were used (set A, $n = 470$ and set B, $n = 73$). Ground samples were obtained using a Perten KT-3100 (Perten Instruments AB, Huddinge, Sweden) grinder. Wheat samples were milled to produce straight-grade flour in a Bühler mill MLU 202 (Bühler AG, Uzwil, Switzerland). Protein ($N \times 5.7$) was determined in both ground wheat (GP) and flour (FP) by the Kjeldahl method (Approved Method 46–12, modified; AACC 2000). Hardness was determined by a modified Particle Size Index (PSI) method. Wet Gluten (WG) and Gluten Index (GI) were determined by the Approved Method 38–12A (AACC 2000). SDS sedimentation tests were run with 1.0 g of sample according to the CIMMYT procedure (Peña et al. 1990) and results were presented as volume expressed in mL (SDSS). Farinograph mixing stability (FarS) and absorption (Abs) were obtained by the Approved Method 54–21 (AACC 2000). Mixograph maximum height (MixH) and mixing time (MixT) were obtained by the AACC Method 54–40A. Alveogram parameters (W, P, L and P/L) were obtained using the standard alveograph procedure (AACC Method 54–30A). Two different types of breads were baked. Pan loaves were prepared according to AACC Method 10–10B. Loaf volume (LV) was used as quality estimator. French-type (Fr) hearth loaves were prepared, based on a typical Uruguayan procedure, and volume (FrV) was measured. All analyses were done on sample set B, while only GP, PSI, Glutomatic determinations, alveogram, mixogram and SDSS were analysed on set A.

A Foss (NIRSystem) Model 6500 (FOSS Inc., Silver Spring, MD, USA) spectrophotometer was used to scan grain, flour and ground wheat of all samples. Both a large rectangular cell and a small ring cup cell were used for grain

scanning. Reflectance spectra recorded were expressed as log (R^{-1}). Averages of two subsamples were used, unless otherwise stated in the text. Calibrations were performed with WinISI software (Infrasoft International Inc, Port Matilda, PA, USA), using the modified PLS regression model. In order to minimize overlapping peaks and large baseline variations, several spectra derivatives and gap values were evaluated. In all cases SNV and detrend scatter correction were performed. Each sample set was separated into two sets (a calibration and a validation set) by the following procedure. Each set was prepared to represent the full range of composition. All samples were sorted by alveogram W. The four samples with lowest W value were included in the calibration set, while the fifth one was in the validation set. The following four were included in the calibration set, the next into the validation set, and so on. Therefore, 80% of the samples were included in the calibration set, and 20% in the validation set. Equations were always developed with the calibration set, and evaluated with the validation set. Coefficient of determination for the validation set (R^2) and standard error of prediction (SEP) were used as estimators of equation success.

RESULTS AND DISCUSSION

Processing spectra using different tools caused some improvements in calibrations results, but they were always minor. Table 1 shows the results obtained with set A samples using nine different math treatments scanning ground samples. Although there were differences among different math treatments, the differences were not critical. For example, GP results were good to very good for all math treatments; the R^2 ranged from 0.965 to 0.975, and the SEP ranged from 0.193 to 0.223 %. At the other extreme, for GI the value of R^2 never was over 0.4. The treatment 2 10 10 2 (derivative, gap, smooth and second smooth) was selected to be used for the rest of the research.

Both GP and PSI showed better results for ground grain than for flour (Table 2). This was expected, since these variables were determined on grain rather than on flour. For the rest of the variables which had been determined on flour samples, the results were generally better when scanning flour than for ground samples. For the best prediction equations (R^2 higher than 0.65), results were always better for flour scans. This was the case for L, WG and SDSS. When prediction was medium to poor (R^2 lower than 0.65) the comparison between flour and ground grain scanning was erratic.

Results for ground samples were better but similar to those for whole grain ones (Table 2), with the exception of predictions for PSI, and GP. PSI calibrations were usable when ground samples were scanned (SEP below 2% and R^2 over 0.800), but using whole grain, the results were poor (SEP over 4% and R^2 below 0.400). When hardness is determined by this method, the actual measurement is on the particle size of the ground sample. Particle size cannot be determined on whole grain. Therefore, NIRS could not predict hardness when scanning whole grain. The difference was also relevant for GP calibration. Results with whole grain were good

Table 1. Results obtained with different math treatments using ground sample (set A)[a]

Math Treatments[b]				Standard error of prediction (SEP)										
				GP	PSI	W	P	L	P/L	WG	GI	MixH	MixT	SDSS
1	2	2	1	0.220	1.80	65.6	21.2	16.3	0.88	2.45	13.7	0.483	1.55	2.56
1	4	4	1	0.213	1.76	65.0	21.1	16.6	0.89	2.41	13.7	0.482	1.62	2.05
1	10	10	1	0.215	1.85	63.3	21.5	16.7	0.90	2.45	12.3	0.483	1.66	2.22
2	2	2	2	0.223	2.14	65.2	22.2	17.1	0.90	2.40	14.6	0.488	1.72	2.83
2	4	4	2	0.197	1.83	65.0	22.3	16.5	0.87	2.44	14.2	0.490	1.66	2.73
2	10	10	2	0.210	1.96	58.2	21.7	16.2	0.91	2.47	12.5	0.495	1.52	2.13
3	4	4	3	0.202	2.33	64.1	21.0	16.5	0.85	2.45	14.2	0.501	1.62	2.74
3	6	6	3	0.193	1.90	64.8	21.1	16.4	0.87	2.47	14.2	0.496	1.62	2.70
3	10	10	3	0.199	2.03	59.9	21.7	16.6	0.91	2.42	13.9	0.493	1.54	2.16
Math Treatments[b]				Coefficient of determination (R^2)										
				GP	PSI	W	P	L	P/L	WG	GI	MixH	MixT	SDSS
1	2	2	1	0.966	0.856	0.380	0.332	0.609	0.373	0.723	0.166	0.657	0.569	0.498
1	4	4	1	0.968	0.863	0.394	0.333	0.599	0.370	0.728	0.171	0.659	0.542	0.685
1	10	10	1	0.968	0.851	0.423	0.310	0.594	0.352	0.728	0.351	0.659	0.527	0.622
2	2	2	2	0.965	0.789	0.386	0.276	0.576	0.356	0.729	0.043	0.644	0.456	0.398
2	4	4	2	0.972	0.852	0.390	0.263	0.603	0.391	0.723	0.081	0.643	0.500	0.440
2	10	10	2	0.969	0.838	0.514	0.298	0.617	0.340	0.721	0.357	0.641	0.583	0.653
3	4	4	3	0.973	0.761	0.407	0.348	0.605	0.425	0.718	0.084	0.625	0.524	0.434
3	6	6	3	0.975	0.857	0.395	0.337	0.605	0.401	0.716	0.085	0.635	0.533	0.443
3	10	10	3	0.972	0.826	0.486	0.301	0.599	0.347	0.728	0.123	0.645	0.582	0.645

[a] GP: grain proteins (%); PSI: Particle Size Index (hardness) (%); W, P, L and P/L: standard alveogram variables, WG: wet gluten (%); GI: Gluten Index (%); MixH: mixogram maximum height (cm); MixT: mixogram mixing time (min); SDSS: sedimentation test volume (ml/g).

[b] Math treatments: derivative, gap, smooth, smooth 2.

($SEP = 0.285,\ R^2 = 0.945$), but they were significantly better with ground samples ($SEP \leq 0.210,\ R^2 \geq 0.969$). The usefulness of whole sample calibrations depends on the objective pursued. If the analysis should be non-destructive or if speed is an issue, then whole grain results could be used when a slightly lower accuracy demand is acceptable. PSI whole grain calibration is not recommended in any case. When the important requirement of the method is accuracy for PSI, samples should be ground.

The rectangular cell is larger than the small cell cup, giving a bigger area for scanning. Therefore, the spectrum collected from a rectangular cell will be more representative than the one obtained with the small one. However, considering only those variables with R^2 higher than 0.6, the use of a bigger area for scanning did not improve calibrations (Table 2). Although the difference between analysis with and without replicate is small (Table 2), in most cases the results obtained with duplicates were better, as expected. In fact, for all variables with R^2 higher than 0.5

Table 2. Results obtained with different sample preparation and size

	Standard error of prediction (SEP)					Coefficient of determination (R^2)				
	Ground[a]		Flour	Whole grain[b]		Ground[a]		Flour	Whole grain[b]	
	One	Two		SRCC	LRC	One	Two		SRCC	LRC
GP	0.224	0.210	0.304	0.253	0.285	0.964	0.969	0.938	0.956	0.945
PSI	1.98	1.96	2.89	3.79	4.24	0.836	0.838	0.618	0.457	0.377
W	64.7	58.2	58.9	60.9	64.2	0.397	0.514	0.498	0.468	0.406
P	20.5	21.7	18.3	21.0	23.7	0.376	0.298	0.504	0.341	0.167
L	16.3	16.2	14.6	16.5	17.4	0.613	0.617	0.688	0.602	0.556
P/L	0.85	0.91	0.80	0.78	0.99	0.418	0.340	0.490	0.527	0.222
WG	2.45	2.47	1.94	2.25	2.65	0.721	0.721	0.821	0.769	0.689
GI	13.7	12.5	13.7	12.5	13.8	0.170	0.357	0.165	0.320	0.148
MixH	0.497	0.495	0.512	0.537	0.564	0.636	0.641	0.622	0.581	0.532
MixT	1.57	1.52	1.44	1.59	1.75	0.564	0.583	0.633	0.546	0.475
SDSS	2.65	2.13	1.74	2.02	2.62	0.466	0.653	0.770	0.688	0.477

GP: grain proteins (%); PSI: Particle Size Index (hardness) (%); W, P, L and P/L: standard alveogram variables; WG: wet gluten (%); GI: Gluten Index (%); MixH: mixogram maximum height (cm); MixT: mixogram mixing time (min); SDSS: sedimentation test volume (ml g^{-1}).

[a] One: results obtained scanning one subsample. Two: results obtained scanning two subsamples

[b] SRCC: small ring cell cup; LRC: large rectangular cell

(GP, PSI, L, WG, MIXH, MIXT and SDSS), duplicating the scanning introduced an improvement to the calibration.

Additional analyses were done on a small set of samples (set B) in order to be able to explore the possibility of NIRS for other variables (Table 3). Calibration results for some variables predicted with set B samples (like FarS, Abs, LV) showed even better results than those measured in set A, indicating potential for further work. It should be remarked that pan loaf volume (LV) prediction had a SEP of 73ml and $R^2 = 0.711$. However, this finding should be considered carefully since validation data were based on only 15 samples.

Among the quality requirements, at least three independent characteristics can be selected: hardness, protein quantity and protein quality (Morris 1998). Hardness can be easily estimated by the PSI method. Protein quantity is mostly determined by Kjeldahl, although wet gluten percentage is a valid estimator as well. The concept protein "quality" or "functionality" (Williams et al. 1988) is ambiguous, and several rheological (W, P, L, P/L, GI, MixH, MixT) or physicochemical (SDSS) parameters could be used to define it. Results confirmed that very good NIRS calibrations could be obtained for protein quantity prediction, as has been previously published (Delwiche et al. 1998, Williams 1975). Calibrations obtained for hardness prediction were also very good when ground samples were scanned. More limited results were obtained for protein quality predictors, but these results still showed accuracy sufficient for screening methods applicable in early generations of wheat breeding programs.

Table 3. Results obtained with sample set B

	SEP[a]	R^{2b}
Grain protein (GP, %)	0.142	0.988
Particle size index, hardness (PSI, %)	2.95	0.601
Alveogram W	71.2	0.637
Alveogram P	12.3	0.779
Alveogram L	19.2	0.438
Alveogram P/L	0.30	0.556
Wet gluten (WG, %)	3.30	0.696
Gluten Index (GI, %)	8.5	0.339
Mixogram maximum height (MixH, cm)	0.542	0.783
Mixogram mixing time (MixT, min)	0.78	0.169
SDS sedimentation volume (SDSS, ml g^{-1})	3.44	0.127
Flour protein (FP, %)	0.324	0.965
Farinogram stability (FarS, min)	2.77	0.872
Farinogram water absorption (Abs, %)	1.40	0.854
French type bread volume (FrV, ml)	94	0.049
Pan loaf volume (LV, ml)	73	0.711

[a] SEP: Standard error of prediction
[b] R^2: coefficient of determination

ACKNOWLEDGEMENTS

This research was partially supported by INIA, Proyecto PDT S/C/OP/07/24 (Programa de Desarrollo Tecnológico (Uruguay), CIMMYT and University of Manitoba (Canada). Technical support from José Hernández, Daniela Ramallo, María García and Patricia González was fully appreciated. Special thanks to Rubén Verges for providing samples. Contribution to the conference was sponsored by PROCISUR.

REFERENCES

American Association of Cereal Chemists (2000) Approved methods of the AACC, 10th edn. Methods 10-10B, 38-12A, 46-12, 54-21, 54-30A and 54-40A. American Association of Cereal Chemists, St. Paul, MN, USA

Delwiche SR, Pierce RO, Chung OK, Seabourn BW (1998) Protein content of wheat by near-infrared spectroscopy of whole grain: collaborative study. J AOAC 81:587–603

Fontaine J, Schirmer B, Horr J (2002) Near-infrared reflectance spectroscopy (NIRS) enables the fast and accurate prediction of essential amino acid contents. 2. Results for wheat, barley, corn, triticale, wheat bran/middlings, rice bran, and sorghum. J Agr Food Chem 50:3902–3911

Hruschka WR (2001) Data analysis: wavelength selection methods. In: Williams PC, Norris KH (eds) Near-infrared technology in the agricultural and food industries. American Association of Cereal Chemists, St.Paul, MN, USA, pp 39–58

Morris CF (1998) Genetics determinants of wheat grain quality. In: Slinkard AE (ed) Proceedings of the 9th International wheat genetics symposium, vol 1. University Extension Press, Univesity of Saskatchewan, Saskatoon, Canada, pp 245–253

Nielsen JP, Pedersen DK, Munck L (2003) Development of nondestructive screening methods for single kernel characterization of wheat. Cereal Chem 80:274–280

Pasikatan MC, Dowell FE (2004) High-speed NIR segregation of high- and low-protein single wheat seeds. Cereal Chem 81:145–150

Peña RJ, Amaya A, Rajaram S, Mujeeb-Kazi A (1990) Variation in quality characteristics associated with some spring 1B/1R translocation wheats. J Cereal Sci 12:105–112

Starr C, Morgan AG, Smith DB (1981) An evaluation of near infrared reflectance analysis in some plant breeding programs. J Agr Sci 97:107–118

Williams PC (1975) Application of near infrared reflectance spectroscopy to analysis of cereal grains and oilseeds. Cereal Chem 52:561–576

Williams PC, Jaby El-Haramein F, Ortiz-Ferreira G, Srivastava JP (1988) Preliminary observations on the determination of wheat strength by near-infrared reflectance. Cereal Chem 65:109–114

Wrigley CW (1994) Developing better strategies to improve grain quality for wheat. Aust J Agr Res 45:1–17

CHANGE IN GRAIN PROTEIN COMPOSITION OF WINTER WHEAT CULTIVARS UNDER DIFFERENT LEVELS OF N AND WATER STRESS

C. SAINT PIERRE*, C.J. PETERSON, A.S. ROSS, J. OHM, M.C. VERHOEVEN, M. LARSON AND B. HOEFER

Oregon State University, Rm 107 Crop Science Bldg. Corvallis, OR, U.S. – 97331

**E-mail: carolina.saint.pierre@oregonstate.edu*

Abstract: Hard white winter (HWW) wheat cultivars in the U.S. must have superior protein quality and consistent processing quality to be successful in the Asian market. Dough rheological properties, baking quality, and end-product attributes are significantly affected by grain protein content and composition. Protein composition, in terms of size and solubility, has been found to be determined by both genetics and environmental conditions. This study investigated the combined influences and interactions of moisture deficit and N management on rheological properties, protein quality, and protein molecular weight distributions of HWW grown in Oregon. Grain was obtained from seven HWW wheat cultivars and two soft white winter (SWW) wheat cultivars grown under line-source irrigation systems in 2002 and 2003. Trials were managed to provide three levels of moisture (100, 80, and 50 % of optimum) over plots receiving two levels of soil nitrogen. The selected cultivars were highly variable for glutenin composition. Protein composition was characterized using size-exclusion HPLC. Resulting chromatograms were divided into three components, which corresponded to HMW and LMW glutenins, gliadins, and non-gluten forming albumins and globulins. Mixograph analyses and SDS sedimentation tests were used to measure variation in protein quality and dough rheological properties. Significant variation in quality was observed among cultivars, irrigation levels, and fertilization rates. Hard wheat cultivars had significantly higher amounts of polymeric proteins (glutenins) than the soft wheat cultivars over both N and water treatments. Concentrations of polymeric proteins were increased under water stress as compared with well-watered conditions for both HWW and SWW cultivars. Moisture stress contributed to higher SDS sedimentation values, although mixing time and mixing tolerance were relatively less affected. As protein content increased, the relative proportion of gliadin proteins also increased, regardless if the protein increase was related to soil N or water stress. Albumin and globulin proteins were the least responsive to changes in protein content. Significant irrigation × N interactions were observed for protein composition and SDS sedimentation volume. AMMI analyses were further used to investigate interactions of water stress and N inputs on

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 535–542

key flour quality attributes. Crop management strategies need to consider interactions between moisture stress and N fertilization to reach desired targets for flour quality and end-product performance

Keywords: protein composition, water stress, nitrogen

INTRODUCTION

Increasing demand for hard white wheat in Asian markets creates both challenges and opportunities for U.S. producers. The challenge is to consistently produce and deliver hard white grain with qualities that can satisfy both bread and noodle applications. Hard white is a relatively new market class for the U.S. To become a reliable grain supplier, large acreage of hard red and soft white wheat will need to be replaced with hard white cultivars.

U.S. quality targets for hard white wheat are grain of minimum 12.5% protein that produces low ash flour with moderate to high starch viscosity, high color stability, and moderately strong and extensible dough. The Great Plains region is considered ideal for production of higher protein hard wheat, as the region has a relatively short grain fill period and high heat stress during grain fill. Hard white production, however, has been stymied by lack of cultivars, costs of segregation, changing disease pressures and unusual weather patterns which have contributed to sprouting. The Pacific Northwest (PNW), in contrast, is characterized by long grain fill duration with less high temperature stress, which is ideal for producing high yields of low protein, soft white grain. However, due to decreasing demand and prices for soft wheat and increased international competition, growers are looking to hard white wheat to better fill the needs of the Asian market. This will be possible only if new cultivars and management strategies are developed that can minimize variability in grain quality that result from diverse environments, highly variable precipitation, and the wide range of management practices used for wheat production in the PNW. This study was conducted to investigate variability in protein content and protein quality in relation to influences and interactions of moisture stress during grain fill and N management in the Pacific Northwest.

MATERIALS AND METHODS

Seven hard white winter (HWW) wheat and two soft white winter (SWW) wheat cultivars were grown in replicated trials at Hermiston and Madras, Oregon, in 2002 and 2003. All plots were irrigated to replace 100 % crop evapotranspiration until one week prior to anthesis. After that date, a line-source sprinkler system was used to impose four irrigation treatments: 100, 80, 50, and less than 30% of measured evapotranspiration. Two levels of soil nitrogen were applied over and

above initial soil N of 28 and 43 kg ha^{-1} for Hermiston and Madras 2003, respectively, and 116 kg ha^{-1} for Madras 2004. All plots received a single fertilization of 170 kg N ha^{-1} in early March (Feekes 4). For the high N level, there was a second application of 170 kg N ha^{-1} in May (Feekes 7), prior to the last uniform irrigation.

Seven diverse hard white wheat cultivars were included in the study. These included 5 experimental lines developed by Oregon State University (with OR prefix) with varying glutenin composition and quality characteristics. NW97S277 (sib of 'Antelope') is a Nebraska selection with good bread-baking properties, but is relatively unadapted to the PNW. IDO591 is an experimental line from the University of Idaho with quality traits that fit both bread and noodles applications. Two regionally-adapted soft wheat cultivars were included in the study. 'Stephens' has been a major soft wheat cultivar grown in the region for over 20 years. 'Eltan' is a unique soft white cultivar that performs well in bread and Asian noodle applications.

Grain was collected from 4 replications at each location. Samples from two replications of three water treatments were used for milling and subsequent flour analyses. Soft and hard wheats were tempered to 14 and 15% moisture, respectively, and milled on a Brabender Quadromat Senior Mill. Grain and flour protein content was measured with a LECO FP-528 Nitrogen/Protein Determinator. SDS sedmentation assays and mixogram analyses were conducted as measures of protein quality and dough rheological properties (AACC Ref. Methods). Mixing tolerance was measured as width of the mixograph curve two minutes after peak dough resistance. Protein composition was characterized using size-exclusion HPLC with a Phenomenex BIOSEP SEC S4000 column (600 × 7.5 mm). Resulting chromatograms were divided into three components which generally correspond to quantities of HMW and LMW glutenins, gliadins, and non-gluten forming albumins and globulins. Polyphenol oxidase activity (PPO) was measured in grain as an indicator of noodle color stability. A colorimetric method was used with L-dopa as a substrate and absorbance at 475 nm. Data were analyzed using Proc Mixed (SAS Institute, Inc., 2001). AMMI analyses and biplots were conducted according to Vargas and Crossa (2000). Data from Madras 2004 and both 2003 trials were used for AMMI analyses of SDS sedimentation and mixograph tolerance, respectively, as interactions of cultivar and treatment were significant in these trials.

RESULTS AND DISCUSSION

Mean grain yield and grain protein of the hard white cultivars over three locations ranged from 8.6 to 10.1 t ha^{-1} and 12 to 14 %, respectively, which is comparable to that for the soft white cultivar Stephens at 9.1 t ha^{-1} and 13 % grain protein. There were no differences in grain yield between low and high N levels, as the lower soil N level was not yield-limiting at any site. Compared with irrigation at 100% of evapotranspiration, the 50% irrigation treatment resulted in water stress and reduced average grain yield by 27%. Grain kernel weight also was significantly

reduced under water stress, from 41 to 38.6 and 35 at the 80% and 50% irrigation treatments, respectively.

In 2003, grain protein increased with late application of N; from 10.4 to 14.1% and 10.9 to 14.4% at Madras and Hermiston sites, respectively. The N response was not evident at Madras in 2004 due to high initial soil N levels. Reducing irrigation from optimum to 50% replacement of water evapotranspiration increased grain protein content from 11.1 to 12.8% and from 12.7 to 14.1% at Madras in 2003 and 2004, respectively. The increase in grain protein was likely a function of yield reduction. Irrigation treatments at Hermiston had a relatively small and non-significant effect on grain protein. SDS sedimentation volumes were highly correlated with grain protein. When an N fertilizer response was significant, an increase in SDS volume also was evident. Water stress increased SDS sedimentation volume at Madras in both years, but not at Hermiston. Mixograph peak time and tolerance were largely influenced by cultivar. Increasing water stress contributed to significantly longer mixograph peak time at two of the locations. Mixograph tolerance was not significantly influenced by main effects of either N or irrigation treatment. However, this may simply be a function of limited df for f-tests. Variation in PPO activity was related to differences in the genotypes. Management practices appear to have a relatively minor effect on PPO activity. N level had no effect on PPO activity and an irrigation treatment effect was significant at only 1 of the 3 locations (Tables 1, 2). Cultivar selection for low PPO seems to be the best strategy to avoid undesirable discoloration in noodles.

Significant variation in concentrations of monomeric (gliadins) and polymeric proteins (glutenins) was related to irrigation treatments, N fertilization rates, and interactions. Variation was most evident when there was significant influence of N and/or water stress on protein content. There was a linear increase in the relative proportion of gliadin and glutenin proteins as flour protein increased (Fig. 1). As flour protein increased, the proportion of monomeric proteins (gliadins) increased more rapidly ($p < 0.01$) than the polymeric proteins (glutenins). Similar results were found by Triboi et al. (2000). The rate of changes in protein composition did not depend on whether the protein increase was related to increased soil N or water stress. Concentrations of albumin and globulin proteins increased with increasing protein content, but there was a slight decrease when considered as a percent of total protein. The results supported previous research (Guttieri et al. 2005) which suggested that the two strategies (N and irrigation management) to elevate grain protein have similar effects on protein quality.

AMMI analyses were used to examine the interactions between cultivars and six treatments ($2\,N \times 3$ irrigation levels) for SDS sedimentation volume and mixograph tolerance (Fig. 2). For SDS sedimentation, the first two AMMI components accounted for 86 percent of the interaction sum of squares between cultivar and treatment. Stephens and OR943576 were clustered in the biplot, suggesting similar response to treatments. These cultivars also had the lowest average SDS sedimentation volume and lower levels of polymeric proteins. The cultivars Eltan, N97S277, and OR850513-19 represent a cluster distinct from Stephens and OR943576,

Table 1. Mean squares from analysis of variance for grain yield, grain protein, thousand kernel weight, PPO activity, SDS sedimentation, mixograph peak time and tolerance, and HPLC area of polymeric and monomeric proteins

Year-Location	Source of variation	Grain			PPO	SDS (cc)	Mixograph		Protein area	
		Yield (t ha^{-1})	Protein (%)	Weight (g)			Peak time (min)	Tolerance (mm)	Polymeric	Monomeric
2003-Hermiston	Variety	6,78 +	5,8 ns	288,3 **	0,357 **	835,0 **	16,55 **	87,42 *	1,06 ns	3,63 ns
	Irrigation	176,49 **	4,1 ns	141,3 *	0,007 ns	12,0 ns	0,55 *	8,90 ns	0,82 ns	1,68 ns
	N	2,90 ns	894,3 **	801,6 *	0,009 ns	6120,1 +	7,32 ns	304,25 ns	137,26 *	326,23 +
	Variety × Irrigation	0,90 ns	0,3 ns	4,1 ns	0,003 ns	4,3 ns	0,20 ns	19,43 *	0,05 ns	0,09 ns
	Variety × N	1,70 ns	2,6 +	11,5 ns	0,006 ns	28,9 +	0,48 ns	35,52 ns	0,35 *	1,00 *
	Irrigation × N	3,26 ns	1,9 ns	8,0 ns	0,004 ns	15,8 ns	0,01 ns	8,68 **	0,50 *	1,32 ns
	Variety × Irrigation × N	0,67 ns	0,6 ns	4,0 ns	0,001 ns	8,6 ns	0,28 +	9,80 *	0,05 ns	0,13 ns
2003-Madras	Variety	26,37 **	7,0 ns	120,6 **	0,424 **	440,4 **	15,31 **	82,47 **	3,58 ns	9,85 +
	Irrigation	456,97 **	106,2 **	1825,4 *	0,004 ns	872,6 **	0,09 ns	9,73 ns	8,67 **	22,10 **
	N	3,E-05 ns	989,3 **	534,1 +	5,E-04 ns	5376,3 **	26,23 +	77,20 ns	127,47 +	343,27 *
	Variety × Irrigation	1,37 ns	1,3 ns	21,3 **	0,005 ns	4,0 ns	0,16 ns	17,35 *	0,22 ns	0,36 ns
	Variety × N	3,11 ns	2,3 ns	5,1 ns	0,008 ns	26,8 ns	0,32 ns	29,60 ns	1,10 +	1,66 +
	Irrigation × N	24,91 *	1,4 ns	246,9 *	0,003 ns	75,4 *	0,39 ns	13,69 ns	0,05 ns	0,85 ns
	Variety × Irrigation × N	0,99 ns	0,8 ns	5,0 ns	0,004 ns	5,1 ns	0,18 ns	6,30 ns	0,34 ns	0,81 ns
2004-Madras	Variety	24,11 **	7,2 +	535,9 **	0,396 **	891,2 **	5,93 **	106,79 **	2,47 +	3,41 ns
	Irrigation	188,25 **	83,3 **	2221,8 **	0,068 **	398,3 *	0,25 *	52,37 ns	6,38 +	20,18 +
	N	0,91 ns	12,0 ns	3,2 ns	3,E-10 ns	2,1 ns	0,23 ns	5,65 ns	2,02 ns	3,39 ns
	Variety × Irrigation	1,99 **	1,3 ns	16,8 **	0,006 ns	9,1 ns	0,14 ns	7,98 ns	0,15 ns	0,24 ns
	Variety × N	0,57 ns	2,5 ns	1,9 ns	0,010 +	9,3 ns	0,16 ns	5,69 ns	0,19 ns	0,44 ns
	Irrigation × N	6,08 ns	1,5 ns	3,1 ns	0,001 ns	0,5 ns	0,02 ns	0,56 ns	0,07 ns	0,29 ns
	Variety × Irrigation × N	0,85 *	0,5 ns	1,9 ns	0,006 ns	6,7 ns	0,17 ns	6,07 ns	0,19 ns	0,24 ns

+, *, ** Significant at $p \leq 0.10$, $p \leq 0.05$, and $p < 0.01$, respectively; ns, not significant, $p > 0.10$

Table 2. Treatment means and standard errors for grain yield, grain protein, thousand kernel weight, PPO activity, SDS sedimentation volume, mixograph peak time and tolerance, and concentrations of polymeric and monomeric proteins

Year-Location			Grain			PPO	SDS (cc)	Mixograph		Protein area	
			Yield (t ha^{-1})	Protein (%)	Weight (g)			Peak time (min)	Tolerance (mm)	Polymeric	Monomeric
2003-Hermiston	Irrigation	Low	7,3	12,4	36	0,37	38,3	3,79	15,67	8,6	11,3
		Medium	10,5	12,8	37	0,37	39,4	3,73	13,53	8,8	11,7
		High	9,4	12,9	38	0,38	38,7	3,55	12,67	8,9	11,7
		SE	0,52	0,4	0,8	0,01	3,2	0,02	0,48	0,5	0,7
	N fertilization	Low	8,77	10,9	38	0,37	31,2	3,95	16,01	7,6	9,8
		High	8,56	14,4	35	0,36	46,3	3,43	11,91	9,9	13,3
		SE	0,41	0,4	0,8	0,01	3,1	0,17	0,60	0,5	0,7
2003-Madras	Irrigation	Low	8,16	12,8	30	0,43	37,9	4,10	13,84	7,80	10,6
		Medium	10,76	11,5	37	0,42	31,0	3,99	14,87	7,16	9,5
		High	10,72	11,1	41	0,41	28,4	4,10	13,54	6,84	9,1
		SE	0,34	0,1	1,8	0,01	0,6	0,17	1,19	0,12	0,2
	Fertilization	Low	8,78	10,4	36	0,42	25,41	4,56	15,62	6,18	7,95
		High	8,78	14,1	33	0,42	39,52	3,56	12,55	8,36	11,52
		SE	0,34	0,1	0,6	0,02	0,51	0,15	0,40	0,18	0,22
2004-Madras	Irrigation	Low	8,25	14,1	39	0,39	36,33	2,50	14,79	9,50	12,50
		Medium	8,77	14,1	42	0,38	33,96	2,43	14,28	9,43	12,29
		High	9,45	12,7	45	0,43	29,76	2,45	10,87	8,74	11,11
		SE	0,44	0,6	0,5	0,01	0,76	0,10	0,89	0,22	0,22
	Fertilization	Low	8,11	13,8	40	0,39	33,21	2,50	14,40	9,09	11,79
		High	7,98	14,2	40	0,39	33,49	2,42	12,22	9,36	12,14
		SE	0,40	0,5	0,6	0,01	1,02	0,11	0,64	0,22	0,19

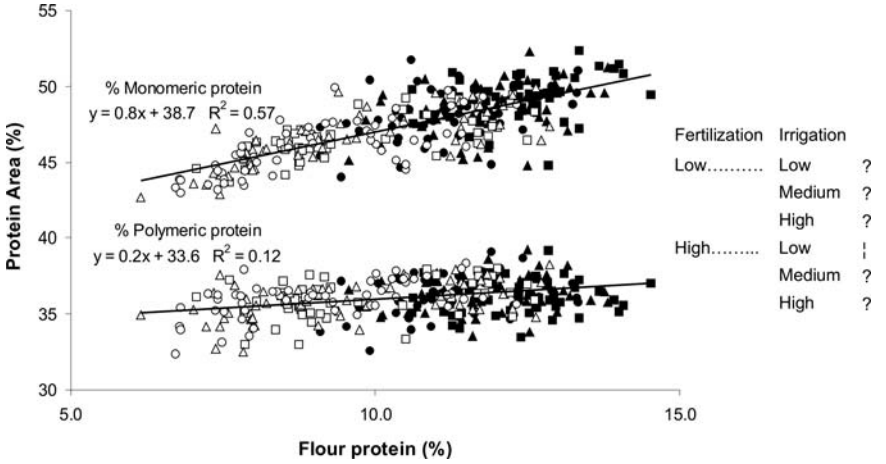


Figure 1. Relationship between % flour protein and monomeric (gliadins) and polymeric (glutenins) proteins from field experiments conducted at Hermiston in 2003 and Madras in 2003 and 2004

suggesting a different pattern of responses to treatments. These cultivars also had higher average SDS sedimentation volumes as compared with other cultivars. For mixograph tolerance (Fig. 2), the first two AMMI components accounted for 85% of the interaction sum of squares. Cultivars with higher average mixing tolerance, such as Eltan, OR942496, and N97S277, tended to cluster, indicating a similar response to treatments. Stephens and OR943576 again had similar response and were distinct from other cultivars in the study.

The long grain fill period, variable production environments, and high average grain yields of the PNW are a challenge for maintaining consistent high grain protein

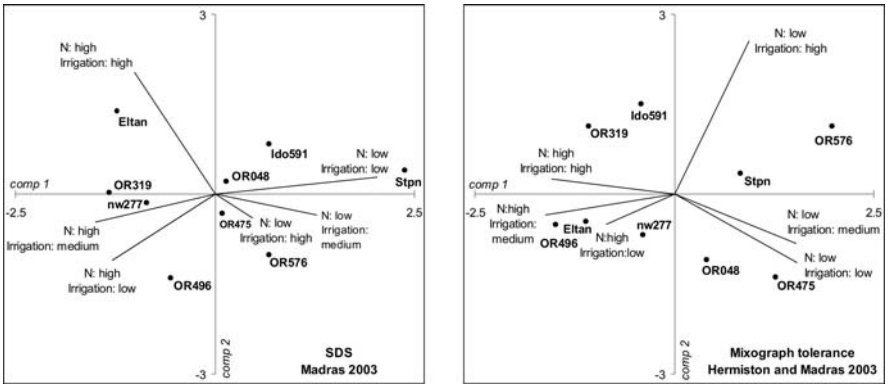


Figure 2. Biplot of first and second AMMI components (axes) for nine varieties (points) and six environments (irrigation-N level combination, vectors) for SDS sedimentation volume and mixograph tolerance

levels. In this study, as expected, N and irrigation treatments had a large influence on protein content. However, these main effects had relatively little direct impact on protein composition or PPO activity. Changes in protein composition were related to general increases in protein concentration, regardless if the result of reducing irrigation or increasing fertilization rate. Significant interactions of cultivar with N and irrigation were observed for protein quality, protein composition, and dough mixing properties. However, cultivars of similar protein quality and composition responded similarly to the N and stress treatments. This reinforces the importance of crop management strategies to reach desired marketing targets for flour quality and end-product performance.

REFERENCES

Guttieri MJ, McLean R, Stark JC, Souza E (2005) Managing irrigation and nitrogen fertility of hard spring wheats for optimum bread and noodle quality. Crop Sci 45:2049–2059

Triboi E, Abad A, Michelena A, Lloveras J, Ollier JL, Daniel C (2000) Environmental effects on the quality of two wheat genotypes: 1. Quantitative and qualitative variation of storage proteins. Eur J Agr 13:47–64

Vargas Hernandez M, Crossa J (2000) The Ammi analysis and graphing the biplot. Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT), Mexico, D.F., Biometric and Statistic Unit, 39pp.

THE INFLUENCE OF DOUGH MIXING TIME ON WHEAT PROTEIN COMPOSITION AND GLUTEN QUALITY FOR FOUR COMMERCIAL FLOUR MIXTURES

R. KUKTAITE[2], H. LARSSON[1,*] AND E. JOHANSSON[2]

[1]*Department of Food Technology, Lund University, PO Box 124, SE-221 00 Lund, Sweden*
[2]*Department of Crop Science, The Swedish University of Agricultural Sciences, PO Box 44, SE-230 53 Alnarp, Sweden*
[*] *E-mail: Helena.Larsson@livsteki.lth.se*

Abstract: The effect of mixing time on wheat protein composition and gluten formation was studied for three commercial flour mixtures (biscuit, standard and strong) and one durum flour. Ultracentrifugation was used to separate the fresh, wet gluten from the wheat-flour dough immediately after mixing. Small deformation dynamic rheological measurements and RP- and SE-HPLC were used to determine the characteristics of the network formed, and the protein composition, respectively

The gluten water content increased due to overmixing for most of the flours. However, no effect of mixing was observed for the storage modulus (G′) of gluten for any of the flours. The value of G′ of gluten was around 3, 3, 4 and 8 for Standard, Biscuit, Strong and Durum flour, respectively. Therefore, the increased water content during prolonged mixing was not related to the effect on G′. The strong flour resulted in the lowest G′ for dough, a high G′ for gluten and no increase in gluten water content with overmixing. The weaker standard flour resulted in the highest gluten water content, which increased considerably with mixing time. The durum flour did not show gluten development and breakdown similar to the other flours. The differences in large UPP, total UPP and large UMP between gluten from the different flours and mixing times originated from the genetic composition of flour proteins

Keywords: protein composition, gluten quality

INTRODUCTION

When flour and water are mixed into dough the wheat flour proteins swell and gluten is developed. In earlier studies we have reported that gluten swells in water to well-defined water content, depending on the gluten quality (wheat cultivar, mixing

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 543–548.

time and additives) more or less independently of the dough water content (Larsson and Eliasson 1996(a), Larsson and Eliasson 1996(b), Georgopoulos et al. 2004, Georgopoulos et al. 2005). Determination of the viscoelastic properties for small oscillating deformations gives a well-defined measure of the cross-link density of gluten that is important for the baking quality of bread. We have prepared gluten by ultracentrifugation of dough in order to obtain fresh gluten, that has experienced the same mixing procedure as the corresponding dough (Georgopoulos et al. 2004). In the present study the effect of mixing time on gluten formation together with the wheat protein composition have been investigated.

Abbreviations:

$G' =$ storage (elasticity) modulus
Large UPP = large unextractable polymeric protein in the total large polymeric protein
Total UPP = total unextractable polymeric protein in the total polymeric protein
Large UMP = large unextractable monomeric protein in the total monomeric protein

MATERIALS AND METHODS

Four commercial wheat flours from Nord Mills AB (Malmö, Sweden) were used in the present study. The protein contents are given in Table 1. Biscuit flour mixture (cv. Ritmo dominating) had HMW-gs composition: $1/2^*, 6+8/7+9$ and $5+10$ (Payne 1987) and LMW-gs composition: *Glu-A3a, Glu-B3g* and *Glu-D3c/Glu D3a* (Gupta and Shepherd 1990). Standard flour (French bread flour, mixture of cv. Tarso and cv. Vinjett) had HMW-gs composition: $2^*/0, 7+9$ and $5+10$, and LMW-gs composition: *Glu-A3a, Glu-B3g/Glu-B3j* and *Glu-D3c*. Strong flour (a flour used for improving weak flour mixtures, imported from Canada) had HMW-gs composition: $2^*, 7+9/7+8$ and $5+10$, and LMW-gs: Glu-*A3a, Glu-B3h* and *Glu-D3a*. Durum flour, a pasta flour imported from Kazakhstan, had HMW-gs composition: $6+8/7+8/20$ and LMW-gs: *Glu-A3b, a, Glu-B2a* and *Glu-B3a* (Nieto-Taladriz et al. 1997).

Flour (10 g) was mixed with distilled water at 25 °C in a mixograph (Reomixer, Bohlin Reologi, Öved, Sweden). Water was added according to the manufacturer's instructions. The dough water contents are given in Table 1. Three mixing times

Table 1. Mixograph data

Flour	Protein content (% dry flour wt)	Dough water content at MAX (% total dough wt)	MAX mixing time (min)
Biscuit	11.2	46.2	5.9
Standard	13.4	48.3	6.2
Strong	16.8	49.5	7.8

were chosen for each flour: HALF (half of the highest mixing resistance in the mixograph), MAX (the highest mixing resistance in the mixograph) and LONG (20 min). Each mixing was repeated at least twice. The same dough was also used for ultracentrifugation to separate the gluten phase.

After 30 min resting the dough was centrifuged for 1h at 100,000 × g in an ultracentrifuge (LE 80K OPTIMA, Beckman, USA) (Larsson and Eliasson 1996). The dough separated into five phases: liquid, gel, gluten, starch and bottom phase. The volume fraction of each of the separated phases was determined. The gluten phase was carefully collected from the rest of the phases and its water content was determined.

Dynamic oscillatory measurements were performed on dough and gluten in a controlled stress rheometer (StressTech Rheologica, Lund, Sweden). The sample (0.5 g) was placed in the plate-plate geometry (diameter 15 mm, gap of 2 mm). The measurements were performed at 25 °C. To prevent water loss, the sample surfaces exposed to air were covered with silicone oil. Before starting any measurements, the sample was held at rest for 30 min to allow the relaxation of stresses generated during sample loading. Dynamic frequency sweep tests of dough and gluten were carried out in the linear viscoelastic region. The values of G′ (elastic modulus) and G″ (viscous modulus) were determined over the frequencies 0.01–10 Hz.

Wheat protein compositions in gluten after different mixing treatments were determined using reversed phase-high performance liquid chromatography (RP-HPLC) and size exclusion (SE)-HPLC according to Kuktaite et al. (2004).

RESULTS AND DISCUSSION

The flours selected for the study varied in protein content from approximately 11 to 17%. Both the time corresponding to the highest mixing resistance in the mixogram (MAX) and the optimal water absorption increased with the flour protein content (Table 1).

The mixed doughs were fractionated by ultracentrifugation to recover the fresh gluten phase. It is shown in Fig. 1 that the water content in the separated gluten phase increased with mixing time for the Standard and Biscuit flours and for the Strong flour up to MAX (the maximum of the mixing resistance). However, mixing the strong flour for 20 min did not increase the water content of gluten further. In earlier studies we have shown that the same water content increased with mixing time for two Swedish spring wheats and one winter wheat (Larsson and Eliasson 1996b).

In Fig. 2 the storage modulus (G′) of dough is shown for the three flours. The value of G′ measures the solid properties of the material, and is an indicator of the network density of the material. Here, in dough the network is formed by the gluten mass and the starch granules. It can be noted that the Strong flour forms dough with less solid properties (lower G′) than the Standard and the Biscuit flours independent of mixing time. This is however a logical consequence of the high protein content, and therefore smaller amount of starch of the Strong flour. It has been shown that

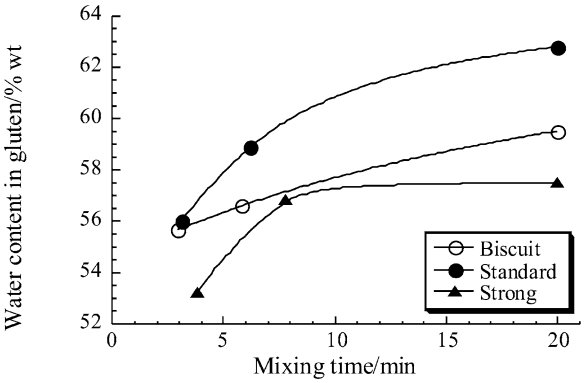


Figure 1. Gluten water content as a function of mixing time

the amount of starch together with the water content are most important for the value of G′ for dough (Hibberd 1970a, Hibberd 1970b, Smith et al. 1970, Larsson and Eliasson 1996a).

On the other hand, the solid properties (G′) of the separated gluten were highest for the Strong flour (Fig. 3). It is remarkable that G′ decreased for the Biscuit and the Strong flours when the mixing time was increased to MAX (the time corresponding to the maximum mixing resistance in the mixograph). None of the gluten showed a further reduction in the solid properties at mixing times longer than MAX (20 min), when the dough was considered to be overmixed. Doughs normally obtain a wet and sticky surface under extended mixing. The wet gluten was confirmed by the elevated water content of gluten shown in Fig. 1 for the Standard and Biscuit flours. However, it seems possible that 20 min was not long enough to overmix the gluten of Strong flour.

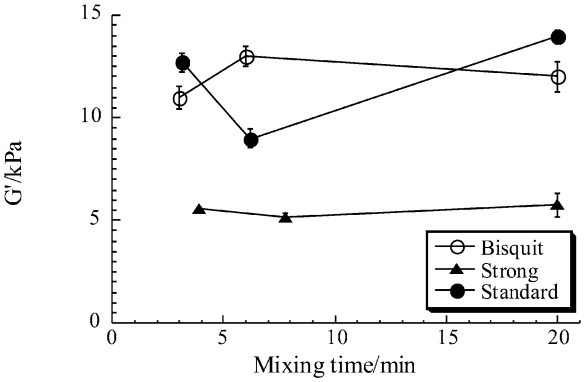


Figure 2. The storage modulus (G′) of dough as a function of mixing time

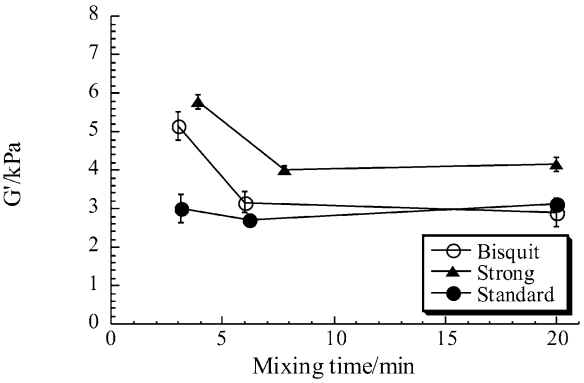


Figure 3. The storage modulus (G′) of gluten as a function of mixing time

The amount of large unextractable polymeric proteins (UPP), total UPP and large unextractable monomeric proteins (UMP) was higher for all flours in the separated gluten at minimum and MAX mixing times compared with the flours. After overmixing, the amount of large UPP, total UPP and large UMP in the gluten decreased to lower levels than in the flours. However, the differences between the flour types were only minor (Kuktaite et al. 2004).

It was concluded that the differences in gluten protein network formation during dough mixing were genetically determined and depended on flour type (Kuktaite et al. 2005).

REFERENCES

Georgopoulos T, Larsson H, Eliasson A-C (2004) A comparison of the rheological properties of wheat flour dough and its gluten prepared by ultracentrifugation. Food Hydrocolloid 18:143–151

Georgopoulos T, Larsson H, Eliasson A-C (2005) The influence of native lipids on the rheological properties of wheat flour dough and gluten. J Texture Studies 37(1):49–62

Gupta RB, Shepherd KW (1990) Two-step one dimensional SDS-PAGE analysis of LMW subunits of glutelin. 1. Variation and genetic control of the subunits in hexaploid wheats. Theor Appl Genetics 80:65–74

Hibberd GE (1970a) Dynamic Viscoelastic Behaviour of Wheat Flour Doughs, Part II: Effects of Water Content in the Linear Region. Rheol Acta 9(4):497–500

Hibberd GE (1970b) Dynamic Viscoelastic Behaviour of Wheat Flour Doughs, Part III: The Influence of the Starch Granules. Rheol Acta 9(4):501–505

Kuktaite R, Larsson H, Johansson E (2004). Variation in protein composition of wheat flour and its relationship to dough mixing behaviour. J Cereal Sci 40:31–39

Kuktaite R, Larsson H, Martilla S, Johansson E (2005). Effect of mixing time on gluten recovered by ultracentrifugation studied by microscopy and rheological measurements. Cereal Chem 82(4):375–384

Larsson H, Eliasson A-C (1996a) Phase separation of wheat flour dough studied by ultracentrifugation and stress relaxation. I. Influence of water content. Cereal Chem 73(1):18–24

Larsson H, Eliasson A-C (1996b) Phase separation of wheat flour dough studied by ultracentrifugation and stress-relaxation II. Influence of mixing time, ascorbic acid and lipids. Cereal Chem 73(1):18–24

Nieto-Taladriz MT, Ruiz M, Martinez MC, Vazquez JF, Carrillo JM (1997) Variation and classification of B low-molecular-weight glutenin subunit alleles in durum wheat. Theor Appl Genetics 95: 1155–1160

Payne PI (1987) Genetics of wheat storage proteins and the effect of allelic variation on bread-making quality. Ann Rev Plant Physiol 38:141–153

Smith JR, Smith TL, Tschoegl NW (1970) Rheological properties of wheat flour doughs, III. Dynamic shear modulus and its dependence on amplitude, frequency, and dough composition. Rheol Acta 9(2):239–252

NITROGEN-SULPHUR FERTILISER INDUCED CHANGES IN STORAGE PROTEIN COMPOSITION IN DURUM WHEAT

S.E. LERNER[1,*], M. COGLIATTI[1], N.R. PONZIO[1], M.L. SEGHEZZO[2], E.R. MOLFESE[2] AND W.J. ROGERS[1,3,†]

[1]*Facultad de Agronomía, Universidad Nacional del Centro de la Provincia de Buenos Aires C.C. 47, (B7300) Azul, Provincia de Buenos Aires, República Argentina*

[2]*Chacra Experimental Integrada Barrow MAGyAI – INTA, C.C. 50, (B7500) Tres Arroyos Provincia de Buenos Aires, República Argentina*

[3]*Researcher of Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) of Argentina*

[*]*E-mail: slerner@faa.unicen.edu.ar,* [†]*rogers@faa.unicen.edu.ar*

Abstract: A set of Argentinean and Chilean cultivars of durum wheat were subjected to the following fertiliser regimes in designed field trials grown over three years: N0S0 (no applied nitrogen or sulphur fertiliser), N1S0 (applied nitrogen fertiliser only), N1S1 (both applied nitrogen and sulphur fertiliser) and N0S1 (applied sulphur fertiliser only), where N was applied as urea and S as potassium sulphate. Two protein extraction protocols were applied to each flour sample obtained from the trial, one for reduced glutenin subunits and one for the gliadins. The resultant extracts were separated by SDS-PAGE, the gels were scanned and the images analysed densitometrically. The data obtained were analysed statistically in order to determine whether the following quantities varied over cultivars and over fertiliser treatments: (i) the relative proportion of total gliadins to total glutenins (Gli:Glu ratio); (ii) the relative proportion of individual ω-gliadin proteins to other gliadin proteins; and (iii) the relative proportion of HMW to LMW subunits of glutenin (HMW:LMW ratio). Such variation would be expected in the light of previous studies in wheat and given that sequence data have shown that these components vary in molecular S-content. It was found that (a) one of the cultivars (cv. Bonerense INTA Cumenay) showed relatively high Gli:Glu ratios compared to the other cultivars; (b) the fertiliser treatment N0S0 gave higher Gli:Glu ratios than the remaining treatments in the two years where significant differences were observed; (c) two of the cultivars (cv. B. I. Cumenay and cv. Chagual INIA) generally gave higher HMW:LMW ratios than the remaining two (cv. Bonaerense INTA Facón and cv. Buck Topacio); (d) the treatments involving N-application (N1S0 and N1S1) generally showed higher HMW:LMW ratios than those without N-application (N0S0 and N0S1); (e) although effects of S-application were observed, these were inconsistent over years; (f) Cv. B. I. Cumenay showed relative stability over fertiliser treatments for

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 549–556.

the quantities evaluated, coherent with its previously observed stability for industrial quality characteristics. The findings hopefully contribute to our understanding of why cultivars differentially respond to fertiliser treatments for quality characteristics

Keywords: nitrogen-sulphur, storage protein

INTRODUCTION

In bread wheat, the relative proportion of HMW glutenin subunits, LMW glutenin subunits and the α-, β-, γ- and ω-gliadins have been shown to influence industrial quality (Singh et al. 1990). The HMW glutenin subunits are encoded by the *Glu-1* loci and the LMW glutenin subunits by the *Glu-3* loci, located on the long arms and short arms, respectively, of the homoeologous group 1 chromosomes. The α-, β-, γ- and ω-gliadins are principally encoded by the *Gli-1* and *Gli-2* loci located on the short arms of the group 1 and 6 chromosomes (Payne et al. 1984). The relative proportion of these proteins has been shown to vary with grain nitrogen (N) and sulphur (S) content, which in turn depend upon N- and S-availability in the soil (Moss et al. 1981, Wrigley et al. 1980, Timms et al. 1981, Zhao et al. 1999). These effects can be broadly explained as follows: the LMW glutenin subunits and the α-, β-, γ-gliadins have been classified as S-rich and the ω-gliadins as S-poor, while the HMW subunits are regarded as having intermediate S-content (Shewry and Tatham 1997). This is in part due to the relative number of cysteine residues in the molecules concerned. The presence of these residues allows the glutenin subunits to form intermolecular disulphide bridges (Shewry and Tatham 1997), which contributes towards the production of large aggregates (unextractable polymeric protein, UPP) that markedly influence gluten functionality, imparting strength and viscoelasticity during dough mixing. It appears that a high relative content of cysteine- carrying glutenin subunits tends to increase the amount of UPP, which consequently enhances the gluten properties mentioned and accounts for the effects on quality of the relative proportion of grain components noted above (Rhazi et al. 2003).

MacRitchie and Gupta (1993) reported that low grain S-content leads to a rise in the proportion of HMW to LMW glutenin subunits, adversely affecting dough properties. Wrigley et al. (1980) showed that low grain S-content results in increased relative amounts of the S-poor ω-gliadins and reduced relative amounts of the S-rich α-, β- and γ-gliadins, also adversely affecting quality. Moss et al. (1981) demonstrated that when flour S-content is low, resistance to extension is increased and extensibility is lowered, leading to unsatisfactory loaf volume and appearance. Furthermore, Zhao et al. (1999) reported that a marked diminution in dough extensibility occurs under such conditions, which is independent of grain protein content. It seems reasonable that these might be the result of these types of changes in the relative proportion of endosperm protein components.

The current work is directed towards examining similar effects in durum wheat, using electrophoretic (SDS-PAGE) densitometry of endosperm storage proteins on

flour samples obtained from field trials of local cultivars grown under distinct N and S fertiliser treatments. It has been shown that different alleles for the glutenin and gliadin components have differential effects on quality parameters in this crop (Ruiz and Carrillo 1995, Vazquez et al. 1996, Brites and Carrillo, 2001, Martinez et al. 2004, 2005). Furthermore, it has been reported that genetically controlled differences in the relative proportion of different gluten components have been shown to influence quality, such as the ratio of HMW and LMW glutenin subunits to intermediate molecular weight glutenin subunits, a fraction containing glutenins, ω-gliadins, albumins and globulins (Fares et al. 1997). It would seem reasonable that the fertiliser treatments employed would also influence the relative proportion of these components in durum wheat.

MATERIALS AND METHODS

Cultivars and Trials

Four cultivars were included in the analysis, three of Argentinean origin, Bonaerense INTA Cumenay (Cumenay), Bonaerense INTA Facón (Facón) and Buck Topacio (Topacio), and one from Chile, Chagual INIA (Chagual). Seed was provided by the respective breeders.

A field trial was grown in each of the years 1998, 1999 and 2000 in Azul in the centre of the Argentinean Pampa (36° 49′53″ S, 59° 53′ 23″ W). In each case, the trials were sown during the first half of August and seedling emergence occurred between two and three weeks later. Each trial included the above four cultivars grown under four fertiliser regimens: N0S0 (no applied N or S fertiliser), N1S0 (applied N only), N1S1 (applied both N and S fertiliser) and N0S1 (applied S only). The amount of N-fertiliser applied as urea to those plots requiring it (N1S0 and N1S1) was adjusted according to soil analyses of four composite samples covering the experimental area of approximately 18m × 24m for each trial and aimed to achieve a grain yield of 5000 kg ha^{-1}, 150 kg ha^{-1} $N-NO_3$, following the model kg ha^{-1} to be applied = 150 kg ha^{-1} $N-NO_3$ – available soil $N-NO_3$ as measured at sowing-time. The dosage was applied on two occasions: 30% 10 days post-emergence and the rest at the tillering stage or Zadocks scale 31 (Zadocks et al. 1979); together with the late application, 40 kg ha-1 of $S-SO_4$ (K_2SO_4) was applied to those plots requiring it (N1S1 and N0S1). Initial soil S amounts were approximately 43 ppm SO_4 in each year. The design was of three randomised complete blocks. Weeds, insects and disease were stringently controlled.

Electrophoresis

HMW and LMW glutenin subunits, alkylated with 4-vinylpyridine and reduced with dithiothreitol, and gliadins were differentially extracted from whole flour samples and separated by SDS-PAGE (10 % acrylamide) according to Singh et al. (1991). The gels were scanned using model HP ScanJet 2200C and the

images analysed densitometrically using Scion Image software. The proportion of total gliadins to total glutenins (Gli:Glu ratio) and of HMW to LMW subunits of glutenin (HMW:LMW ratio) were calculated for each year-cultivar-fertiliser treatment-block combination, and the data were analysed using the statistical programme INFOSTAT (Infostat Group, Faculty of Agronomic Sciences, University of Córdoba, Argentina, http://www.infostat.com.ar).

RESULTS AND DISCUSSION

Gli:Glu Ratio

The three years varied considerably in their behaviour for this characteristic (Table 1). Nonetheless, cv. Cumenay was always one of the two cultivars with the highest Gli:Glu ratio, which may imply, given this cultivar's generally high performance for industrial quality, that it possesses allelic variants for the gliadins favourable in this respect.

In two of the three years, the fertiliser treatments differed significantly for the Gli:Glu ratio, where the treatment N0S0 gave a higher Gli:Glu ratio than the remaining treatments. This implies that, in situations of reduced nutrient availability, gliadin synthesis may be favoured relative to glutenin synthesis, which in turn may be related to their generally lower content of molecular S (Shewry and Tatham 1997).

Table 1. Gli:Glu ratios for cultivar-fertiliser treatment combinations over the three years of trials. Least significant differences (LSD, $P < 0.05$) for cultivar (Cv.) and fertiliser treatment (F.t.) means were 0.402, 0.251 and 0.076 for the years 1998, 1999 and 2000, respectively; LSD for cultivar-fertiliser combinations in the body of the table were 0.804, 0.503 and 0.152, respectively

1998	N0S0	N1S0	N1S1	N0S1	Cv. means
CUMENAY	3,9	2,3	2,23	1,978	2,6
CHAGUAL	0,25	0,32	0,23	0,26	0,27
FACÓN	0,99	1,01	0,88	0,72	0,9
TOPACIO	0,28	0,53	0,47	0,24	0,38
F. t. Means	1,36	1,04	0,96	0,8	
1999	N0S0	N1S0	N1S1	N0S1	
CUMENAY	1,66	1,34	1,24	1,05	1,32
CHAGUAL	1,59	1,15	1,18	0,94	1,21
FACÓN	2,34	1,8	1,64	2,1	1,97
TOPACIO	1,18	0,86	0,81	0,87	0,94
F. t. Means	1,69	1,28	1,22	1,24	
2000	N0S0	N1S0	N1S1	N0S1	
CUMENAY	0,79	0,61	0,49	0,52	0,6
CHAGUAL	0,52	0,53	0,44	0,47	0,49
FACÓN	0,48	0,43	0,46	0,45	0,45
TOPACIO	0,26	0,74	0,69	0,66	0,59
F. t. Means	0,51	0,58	0,52	0,53	

Gliadins

As noted in the Introduction, it would be expected that conditions favouring S-deficiency would provoke an increase in the proportion of S-poor ω-gliadins compared to the gliadins of higher relative mobility. In 1998, this situation was frequently observed, although in 1999 and 2000, the results were less categorical. This may be because S-deficiency is not generally observed in our geographic region (see below).

HMW:LMW Ratio

The cvs. Cumenay and Chagual gave higher HMW:LMW ratios than the remaining cultivars in all three years (Table 2). Since it has been stated that this proportion is related to industrial quality characteristics in wheat (Singh et al. 1990), this is a potentially important observation for the cultivars under study. Nonetheless, previous studies of these cultivars have shown that cv. Cumenay has excellent industrial quality characteristics, whereas cv. Chagual has poor characteristics in this respect (Lerner et al. 2004), although it may be interesting in this context that for the character viscoelasticity of cooked pasta, which is a measure of end-use quality, both cultivars have been reported as being of acceptable performance (Lerner et al. 2006).

Table 2. HMW:LMW ratios for cultivar-fertiliser treatment combinations over the three years of trials. Least significant differences (LSD, $P < 0.05$) for cultivar (Cv.) and fertiliser treatment (F.t.) means were 3.31, 2.92 and 1.15 for the years 1998, 1999 and 2000, respectively; LSD for cultivar-fertiliser combinations in the body of the table were 6.62, 5.85 and 3.30, respectively

1998	N0S0	N1S0	N1S1	N0S1	Cv. means
CUMENAY	26,78	35,85	31,91	24,18	29,68
CHAGUAL	28,98	32,82	28,03	30,57	30,1
FACÓN	19,09	32,75	28,73	21,92	25,62
TOPACIO	20,34	32,61	24,37	20,72	24,51
F. t. Means	23,8	33,51	28,26	24,35	
1999	N0S0	N1S0	N1S1	N0S1	
CUMENAY	22,91	26,38	24,14	25,23	24,67
CHAGUAL	21,96	24,05	31,08	30,77	26,97
FACÓN	11,45	15,42	21,41	14,1	15,59
TOPACIO	14,6	29,48	31,71	19,57	23,84
F. t. means	17,73	23,83	27,08	22,42	
2000	N0S0	N1S0	N1S1	N0S1	
CUMENAY	27,39	28,67	29,67	28,42	28,54
CHAGUAL	18,64	19,93	18,48	18,77	18,96
FACÓN	17,15	18,24	18,03	17,29	17,68
TOPACIO	17,84	17,5	18,56	15,11	17,25
F. t. means	20,26	21,09	21,19	19,9	

Regarding the fertiliser treatments, the combinations that included N-application (N1S0 and N1S1) generally showed higher HMW:LMW ratios than the combinations lacking N-application (N0S0 and N0S1). Again, in the context of industrial quality, it may be interesting that N-application in these cultivars has been shown to result in superior performance for industrial quality characteristics, as well as for the end-use parameter mentioned above.

In 1999, S-application also appeared to affect the HMW:LMW ratio, as it did in 1998 at the higher N-level, but the direction of the change differed over the two years. In the third year, 2000, S-application did not affect the HMW:LMW ratio. This inconsistency over years may be related to the fact that the region in which the study was carried out, in the non-degraded arable cropland of the Argentinean Pampa, is not generally considered to be deficient in soil S, at least in terms of limiting crop yield. Indeed, in the field trials included in the current study, grain yields (results not shown) responded to N-application though not to that of S, which is coherent with the measured soil organic material of 4.8% and relatively few years under cultivation. Therefore it is not surprising that the effect of S-application takes second place behind N in the effects observed in these trials. Nonetheless, S-application has been shown to enhance grain protein content and SDS-sedimentation values in this area (Lerner et al. 2006). S-deficiency tends to be associated with intensive soil use, direct sowing practices, models of continuous agriculture and the diminution of organic material associated with degradation processes (San Martín et al. 1990). It is an incipient though important problem in many areas in Argentina, meaning that studies on the effect of S-deficiency on local cultivars are of considerable importance.

With respect to cultivar × fertiliser treatment interactions, only in 1999 were such interactions statistically significant. In this year, only cv. Cumenay gave consistent results over the fertiliser treatments. The stability of this cultivar in this respect is coherent with its generally observed stability for industrial quality and end-use characteristics, compared to the other cultivars under study here (Lerner et al. 2004; submitted).

In conclusion, the cultivars in the current study varied in the relative proportion of distinct grain protein components, in keeping with previous studies in durum wheat (Pogna et al. 1990, Fares et al. 1993, Galterio et al. 1993). Differences were also observed between fertiliser treatments, particularly related to N-application.

ACKNOWLEDGEMENTS

This work was funded by the Agencia Nacional de Promoción Científica y Tecnológica (National Agency for Scientific and Technological Promotion) of the Secretaría de Ciencia, Técnología e Innovación Productiva, Ministerio de Educación, Ciencia y Tecnología (Secretariat of Science, Technology and Productive Innovation, Ministry of Education, Science and Technology) of Argentina, Project FONCyT-PICT No. 08-03196, and by the Programa Institucional de Investigación y Transferencia Tecnológica de la Facultad de Agronomía

(Institutional Programme of Research and Technological Transfer of the Faculty of Agronomy), Azul, UNCPBA, Argentina, Project No. 03/A118. We are indebted to Negra and Rubia for their constant support.

REFERENCES

Brites C, Carrillo JM (2001) Influence of high molecular weight (HMW) and low molecular weight (LMW) glutenin subunits controlled by Glu-1 and Glu-3 loci on durum wheat quality. Cereal Chem 78:59–63

Fares C, Novembre G, Di Fonzo N, Galterio G, Pogna NE (1997) Relationship between storage protein composition and gluten quality in breeding lines of durum wheat (*Triticum turgidum* spp. *durum*). Agricoltura Mediterranea 127:363–368

Galterio G, Grita L, Brunori, A (1993) Pasta-making quality in Triticum durum. New indices from the ratio among protein components separated by SDS-PAGE. Plant Breed 116:541–545

Lerner SE, Cogliatti M, Ponzio NR, Seghezzo ML, Molfese ER, Rogers WJ (2004) Genetic variation for grain protein components and industrial quality of durum wheat cultivars sown in Argentina. J Cereal Sci 40:161–166

Lerner SE, Seghezzo ML, Molfese ER, Ponzio NR, Cogliatti M, Rogers WJ (2006) N- and S-fertiliser effects on grain composition, industrial quality and end-use in durum wheat. J Cereal Sci 44:2–11

MacRitchie F, Gupta RB (1993) Functionality-composition relationships of wheat flour as a result of variation in sulfur availability. Aust J Agr Res 44:1767–1774

Martinez MC, Ruiz M, Carrillo JM (2004) New B low Mr glutenin subunit alleles at the *Glu-A3, Glu-B2* and *Glu-B3* loci and their relationship with gluten strength in durum wheat. J Cereal Sci 40:101–107

Martinez MC, Ruiz M, Carrillo JM (2005) Effects of different prolamin alleles on durum wheat quality properties. J Cereal Sci 41:123–131

Moss HJ, Wrigley CW, MacRitchie F, Randall PJ (1981) Sulfur and Nitrogen fertilizer effects on wheat. II. Influence on grain quality. Aust J Agr Res 32:213–226

Payne PI, Holt LM, Jackson EA, Law CN (1984) Wheat storage proteins: their genetics and their potential for manipulation by plant breeding. Phil Trans R Soc B, 304:359–371

Pogna NE, Autran JC, Mellini F, Lafiandra D (1990) Chromosome 1B- encoded gliadins and glutenin subunits in durum wheat: genetics and relationship to gluten strength. J Cereal Sci 11:15–34

Rhazi L, Cazalis T, Aussenac T (2003) Sulfhidryl-disulphide changes in storage proteins of developing wheat grains: influence on the SDS-unextractable glutenin polymer formation. J Cereal Sci 38:3–13

Ruiz M, Carrillo JM (1995) Relationships between different prolamin proteins and some quality parameters in durum wheat. Plant Breed 114:40–44

San Martín N, Navarro C, Echeverría H (1990) Sulfur concentration in wheat grains in the south of the Buenos Aires Province. Boletín Técnico INTA N° 99:3–7

Shewry PR, Tatham AS (1997) Mini review: disulphide bonds in wheat gluten proteins. J Cereal Sci 25:207–227

Singh NK, Donovan GR, MacRitchie F (1990) Use of sonication and size-exclusion high-performance liquid chromatography in the study of wheat flour proteins. II. Relative quantity of glutenin as a measure of breadmaking quality. Cereal Chem 67:161–170

Singh NK, Shepherd KW, Cornish GB (1991) A simplified SDS-PAGE procedure for separating LMW subunits of glutenin. J Cereal Sci 14:203–208

Timms MF, Bottomley RC, Ellis JRS, Schofield JD (1981) The baking quality and protein characteristics of a winter wheat grown at different levels of nitrogen fertilisation. J Sci Food Agr 32:648–698

Vazquez JF, Ruiz M, Nieto-Taladriz MT, Albuquerque MM (1996) Effects on gluten strength of low Mr glutenin subunits coded by alleles at the Glu-A3 and Glu-B3 loci in durum wheat. J Cereal Sci 24:125–130

Wrigley CW, du Cros DL, Archer MJ, Downie PG, Roxburg CM (1980) The sulfur content of wheat endosperm proteins and its relevance to grain quality. Aust J Plant Physiol 7:755–766

Zadocks JC, Chang TT, Konzak CF (1979) A decimal code for the growth stages of cereals, with illustrations. Ann Appl Biol 93:221–234

Zhao FJ, Hawkesford MJ, McGrath SP (1999) Mini review: sulphur assimilation and effects on yield and quality of wheat. J Cereal Sci 30:1–17

PHYSIOLOGY OF DETERMINATION OF MAJOR WHEAT YIELD COMPONENTS

G.A. SLAFER

ICREA's Research Professor at the Dept. of Crop and Forest Sciences, University of Lleida, Centre UdL-IRTA, Av. Rovira Roure 191, 25198 Lleida, Spain
E-mail: slafer@pvcf.udl.es

Abstract: I have focused this presentation, within the 7th International Wheat Conference, in the determination of individual grain weight and grain number per unit land area. As the negative relationship between average grain weight and grain number per m^2 seems largely independent of strong competition, understanding the physiological bases of the determination of each of these major components may be important to design strategies to increase yield through either breeding or management. In this presentation I have paid attention to some of the well established and some of the new advances on the physiology of these yield components. Finally I have discussed on how the new advances in the physiology of grain number per m^2 may be useful in practical breeding

Keywords: yield components, grain weight potential, growth habit

INTRODUCTION

Crop yield is the final outcome of complexly interrelated crop growth and development processes strongly affected by genotypic and environmental factors and mainly by their interaction. As yield is normally one of the main traits in which breeding programs and management decisions are focused on, improving our understanding of physiology of crop yield may be of paramount importance for increasing efficiency in breeding and management (*e.g.* Araus et al. 2004). This is because it would be far easier and more certain to manipulate relatively simple attributes putatively related to yield under a wide range of field conditions than to manipulate yield itself, that is why we have been always attempting to find the basis of yield (Slafer 2003).

In the case of breeding, for instance, this improved knowledge may assist directly (e.g. providing elements to adopt selection criteria, or identifying prospective

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 557–565

parents for a cross; *e.g.* Austin 1993) or indirectly by identifying traits that could be trustworthily mapped and then selected for through marker-assisted selection (Slafer et al. 2005), as there is an intrinsic complexity for identifying trustworthy QTLs for yield (Stuber et al. 1999) which may explain the scientific curiosity that while the literature (i) is plenty of papers identifying QTLs for yield, it (ii) is virtually empty of clear success case-stories in which a QTL for yield had been transferred to a different genetic background of that f the mapping population and then grown in a wide range of actual farmer conditions and still produce a yield advantage (Slafer 2003). This may be in part because QTLs for yield frequently have a low resolution; a small individual effect on yield; and are dependent of the GxE interaction (*e.g.* Romagosa et al. 1996, Yin et al. 1999).

In fact, a major aim of most crop physiologists is to contribute to identifying processes most importantly determining yield. In this manuscript I have briefly reviewed the state of the art in the physiology of determination of major wheat yield components.

Yield Components

The yield components approach has been the most popular crop physiological attempt to understand yield from simpler attributes. It consists in simply dividing yield into its two major numerical components (*i.e.* the number of grains per m^2 and average individual grain weight) and then further into sub-components (*e.g.* plants per unit land area, spikes per plant, spikelets per spike, grains per spikelet). This has been (and I believe still is) so popular because it is simple and relatively easy to measure, while it is correct mathematically and eminently sound.

The yield components analysis for identifying simpler traits directly related to yield has, however, a major negative aspect: the components are consistently negatively related among themselves (e.g. Slafer 2003). This drawback makes the yield components approach unsound from a physiological point of view, at least for predicting the effect of manipulating a component on crop yield (*e.g.* Fischer 1984, 1996, Slafer et al. 1996).

As explicitly discussed in Slafer (2003) the generation of sub-components of the final number of grains per unit land area are strongly overlapped and the negative relationships between them could be then due to feedback processes. Unlike the sub-components of the number of grains per m^2, the phases of determination of the two major yield components (number of grains per m^2 and their averaged individual weight) virtually do not overlap and then the negative relationship between them could not be due to feedback. As the negative relationship between these major yield components seems to be largely independent of a strong competition for assimilates (see next section, below), it can be assumed that increasing either the final size of the grains set by the crop or increasing the number of grains fixed by the canopy that will be filled after anthesis would almost unequivocally increase yield.

However, this represents an extremely small step towards understanding yield physiology: these major yield components are almost as complex as yield itself, and therefore it is required that we understand their physiologically sound, simpler determinants.

Grain Weight Determination

Do grains strongly compete for assimilates during grain-filling?

In spite of the fact that the most common (many times implicit) interpretation offered in the literature for the consistently negative relationship between average grain weight and number of grains per unit land area is increased competition among grains when number of grains is increased, direct evidences analysing the degree of source-limitation during post-anthesis for grain growth do hardly support a competitive nature for this negative relationship.

For many different conditions (different researchers working with different genetic and environmental backgrounds, different kind of treatments, etc) in which final grain weight responses to source-sink manipulations during grain-filling was analyzed in wheat it has been concluded that large changes in assimilate availability per grain have only produced very small or negligible changes in grain weight at physiological maturity (Slafer and Savin 1994, Borras et al. 2004). The same seems to be true for other small-grained cereals (e.g. Dreccer et al. 1997, Calderini et al. 2006). This homeostasis in grain weight determination was in close agreement with the fact that radiation use efficiency (RUE) seemed to be limited during post-anthesis by insufficient sink strength. For instance, Matthew Reynolds and colleagues from CIMMYT have suggested that increasing post-anthesis sink size (e.g. number of grains per m^2) would result in increased RUE (Reynolds et al. 2000). This suggestion was (i) in line with evidences from previous studies indicating that semidwarf and modern cultivars have increased their post-anthesis RUE respect to tall and old cultivars (Miralles and Slafer 1997, Calderini et al. 1997); and (ii) confirmed by further studies (*e.g.* Reynolds et al. 2001, 2005).

However, most of these analyses have been done under potential conditions or at least under stresses not particularly occurring in post-anthesis. The few studies analysing responses in field plots of wheat grain weight to source-sink manipulations during post anthesis under stressful conditions (Slafer and Miralles 1992, Cartelle et al. unpublished) and the analyses of the causes behind the negative correlation between grain weight and number of grains per m^2 in a wide range of conditions in a Mediterranean region (Acreche and Slafer 2006) seem to expand the conclusions to stressful conditions during grain filling as well (which is in fact in agreement with studies under controlled conditions in which it has been clear that heat stress during grain filling reduced final grain weight independently of the heat effects on assimilate availability; *e.g.* Jenner 1994). These evidences indicate that the post-anthesis stresses, universally reducing final grain weight in wheat, might actually affect directly on the capacity of the grain to grow, rather than altering

grain weight through the effect the stress may have on the availability of assimilates by accelerating senescence.

All evidences consistently indicate that growing grains do hardly compete for assimilates during the effective phase of grain-filling (after the lag phase). Then, if we can improve potential grain size chances are that actual final grain weight would be increased.

How grain weight potential is determined?

Although there has been a large number of papers reporting grain weight as a consequence of rate and duration of grain-filling, the actual causes of the determination of potential grain size has been far less studied.

Most of the studies designed to understand the determination of grain weight potential have concluded that the period immediately following anthesis and ending at the onset of the rapid grain growth (the lag phase) seems to be of paramount importance in determining final size that the grain may achieve. During this period the number of cells in the endosperm is determined (Stone and Savin 1999) and final grain size seems dependent on this attribute.

Although studies on the physiological determination of final grain weight have focused heavily in the lag phase, there has been a growing body of important evidences suggesting that the period immediately preceding anthesis (say booting-anthesis) could also be a critical timing for the determination of final grain weight. This has been comprehensively discussed in the review by Calderini et al. (2001). To recap it briefly it was found, from experiments with different type of manipulations, different environmental backgrounds and with different genotypes, that final grain weight could be strongly (though hyperbolically) dependent upon the size of the carpels at anthesis, and carpel growth takes place only for few days before anthesis. We still have to improve far more our understanding of processes determining carpel size on the one hand and generation of endosperm cells on the other hand before it can be suggested breeding strategies oriented to improve mechanistically final grain weight.

Grain Number Determination

Critical phase for yield and relative importance of the spike dry weight at anthesis

After it was recognized that the traditional approach of analyzing grain number per unit land area into simpler components (grains per spike, spikes m^{-2}, or even simpler) was of little physiological meaning, an alternative physiological avenue to dissect grain number into simpler traits beyond the use of numerical sub-components was developed and widely described by several physiologists, following the pioneer work by Tony Fischer summarised in his review published in 1985.

This alternative assumes that the number of grains m^{-2} as the consequence of resource accumulation and allocation during a rather short window of phenological time of few weeks immediately before (and perhaps one week after) anthesis.

To reach this conclusion manipulative experiments in which wheat plants were subjected to brief shading periods at different timings were the most common approach. The approach offered no drawbacks and provided a physiologically sound approach to understand the number of grains per m^2 by simpler traits (it should be a trait defined during that relatively short window of phenological time). Consequently, the critical experiments (mainly conducted at CIMMYT and Australia and synthesised in the review by Fischer 1985) were conducted by different researchers in different countries with different cultivars and different background environmental conditions on which the treatments were imposed and the results proven to be universally confirmed (*e.g.* Thorne and Wood 1987, Savin and Slafer 1991, Abbate et al. 1995, 1997 Demotes Mainard and Jeuffroy 2001), which supports the model proposed was physiologically trustworthy.

The physiological model explaining the robustness of that universally proven importance of growth during few weeks before anthesis in determining grain number (and largely yield) is based on the strong relationship found in all cases between the number of grains per m^2 that the crop has at maturity and the dry mater per m^2 allocated into spikes at around anthesis (shown in all the papers quoted above). Then grain number per m^2 might be manipulated by changing spike dry mater at anthesis. Spike dry mater at anthesis seems to be a relatively simple trait: it is the consequence of crop growth and partitioning to the spike during a certain (relatively brief) phenological phase corresponding to that when spikes grow (competitively with the stems elongating then at their maximum rate).

Although the basis for this model were provided by artificial manipulations of crops, the relationship has always being confirmed when breeding and management effects on wheat yield were analysed with this approach (*e.g.* see review by Calderini et al. 1999 for breeding and discussion in Prystupa et al. 2004 for management examples). In the case of breeding the almost universal effect has been the increase in spike dry matter at anthesis by increasing the partitioning to growing spikes during stem elongation while management effects have been the increase in crop growth rate (with no major effects on partitioning) during the same phase. Currently we are analysing whether adaptation and performance of wheat and barley in harsh conditions characterised by strong water and nitrogen stresses across several countries of the Mediterranean basin (Spain, Italy, Jordan, Lebanon, Tunisia and Morocco) may be understood and improved through the use of his physiological approach (project *WatNitMED* of the International Cooperation program of the European Union).

Floret development and grains per m^2

Analyzing the dynamics of the increase and decrease of potential sites to bear grains (by multiplying the curves describing tillering and tiller mortality and floret initiation and floret abortion within a tiller, as elegantly proposed initially by Fischer 1984) it is clear that the final number of grains per m^2 is the product of a large increase in the number of potential sites to bear a grain followed by a dramatic

reduction in these numbers until achieving the number of fertile florets and then grains per m^2 around anthesis.

The process of 'floret death' coincides with the onset of rapid growth of stems and spikes (*e.g.* Kirby 1988), supporting the strong relationship between number of grains per m^2 and spike dry weight at anthesis: if the spikes grow more, more florets may continue with their normal development and become a fertile floret to produce a grain after anthesis. This is not only supported by the fact that normally the increase in number of grains per m^2 is largely independent of differences in maximum number of floret primordia produced (by around the onset of stem elongation; *e.g.* Slafer and Andrade 1993) but also by studies analyzing in detail the fate of floret primordia during their developmental process towards abortion or fertile florets when shading (Gonzalez et al. 2005*b*) and when number of grains per m^2 is increased by the introgression of Rht alleles (Youssefian et al. 1992, Miralles et al. 1998).

May this knowledge be useful in future breeding?

Although a flawless answer to this question has to wait until it happens, there have been some promising developments lately. As it has been elegantly explained by Matthew Reynolds in a recent review, to further increase yield we need improving RUE, and a proper way to achieve this may be by further increasing number of grains per m^2 (Reynolds et al. 2005). It seems that the use of the chromosome translocation containing the *Lr19* gene from *Agropyron elongatum* may be a tool to achieve this objective in the short term. This translocation seemed to have increased number of grains per m^2 without altering biomass until anthesis (though the resulting increase in sink-strength has brought about an increase in RUE and biomass accumulation during grain filling; Reynolds et al. 2005). This has been achieved by a likely effect of this translocation on biomass partitioning towards the growing spikes during the stem elongation phase, resembling what has been the effect of Rht alleles (Youssefian et al. 1992, Miralles et al. 1998).

Another alternative, largely unexplored yet, may be increasing the crop growth accumulated during the stem elongation phase by extending it: lengthening the phase would result in increased spike dry matter at anthesis due to more accumulated growth during this critical phase and similar partitioning, reducing floret primordia degeneration and increasing the number of fertile florets that would become grains after flowering (Slafer et al. 2001). It has been recently found, through a detail screening of developmental patterns in a large number of modern wheats a large variation in duration of stem elongation, even within a similar total time from sowing to anthesis (Whitechurch, Slafer and Miralles, unpublished), a fact also evident for barley (Slafer 2003); in fact in exceptional cases it seems that empirical breeding may have made use of this variability (Abeledo et al. 2001).

The existence of variability indicates that it may be genetically manipulated, but does not provide clues on which developmental processes are involved. The two candidates are differences (i) in intrinsic earliness (Slafer 1996) for the stem

elongation phase (associated with complementary differences in this attribute during previous developmental phases so that time to anthesis would remain unchanged), or (ii) in photoperiod sensitivity during the stem elongation phase (that may be partially independent of sensitivity during previous phases; see Slafer and Rawson 1996). To the best of my knowledge, only likely effects of photoperiodic effects on stem elongation have been so far analysed within this context at least in the literature.

We found firstly in controlled conditions (Miralles et al. 2000) and later in the field (*e.g.* Gonzalez et al. 2003) that exposing only the stem elongation phase to extended photoperiod resulted in the expected reduced duration of the phase accompanied by a parallel reduction in number of grains produced by the plants (controlled conditions) or the whole canopy (in the field). In all cases the model described above did perfectly describe the treatments effects: changes in grains or fertile florets were explained by treatments effects on spike dry weight at anthesis. In addition, there seemed to be the same effect of shading (Gonzalez et al. 2005*d*).

For this approach to be more easily useful, the genetic bases of these responses must be identified. We have made some attempts to relate the responsiveness to photoperiod during stem elongation to particular *Ppd* genes (Whitechurch and Slafer 2002, Gonzalez et al. 2005*d*), but this approach has not provided clear conclusions (Gonzalez et al. 2005*d*).

As an alternative approach, within the European Union project MABDE it has been attempted to identify QTLs related to differences in developmental patterns before and after the onset f stem elongation with two completely different barley mapping populations, one from the US (Marti, Romagosa and Slafer, unpublished) and the other from The Netherlands (Borras, Slafer, Romagosa and van Eeuwijk, unpublished), the latter derived from a cross with parents differing in yield responsiveness to environmental conditions (van Eeuwijk, personal communication). So far results are promising as in both populations and in different environmental conditions we identified independent QTLs from the duration of the phase from sowing to the onset of stem elongation and from then to heading.

ACKNOWLEDGEMENTS

I would like to acknowledge (i) the work developed by several colleagues and PhD students (firstly at the University of Buenos Aires/CONICET and lately at the University of Lleida/Centre UdL-IRTA) who have shared with me the studies I have revisited most heavily throughout this manuscript; mainly Daniel Miralles, Daniel Calderini, Roxana Savin, Gabriela Abeledo, Eileen Whitechurch, Fernanda Gonzalez, Ignacio Romagosa, Gisela Borras, Julia Cartelle, Martin Acreche and Jordi Marti; as well as (ii) the funding received through the years that supported our research (mainly UBACyT, FONCyT and Antorchas Foundation from Argentina, Ministry of Science from Spain and INCO Program from the European Union). I thank Dr. R Savin (University of Lleida, Spain) for suggestions on an early draft of this ms.

REFERENCES

Abbate PE, Andrade FH, Culot JP (1995) The effects of radiation and nitrogen on number of grains in wheat. J Agr Sci (Cambridge) 124:351–360

Abbate PE, Andrade FH, Culot JP, Brindraban PS (1997) Grain yield in wheat: effects of radiation during spike growth period. Field Crop Res 54:245–257

Abeledo LG, Calderini DF, Slafer GA (2001) Genetic improvement of barley yield potential and its physiological determinants in Argentina (1944–1998). Euphytica 130:325–334

Acreche M, Slafer GA (2006) Grain weight response to increases in number of grains in wheat in a Mediterranean area. Field Crop Res 98:52–59

Araus JL, Slafer GA, Reynolds MP, Royo C (2004) Physiology of yield and adaptation in wheat and barley breeding. In: Nguyen HT, Blum A (eds) Physiology and biotechnology integration for plant breeding. Marcel Dekker, Inc, New York, pp 1–49

Austin RB (1993) Augmenting yield-based selection. In: Bosemark NO, Romagosa I (eds) Plant breeding: principles and prospects. Chapman and Hall,, Hayward MD, pp 391–405

Borras L, Slafer GA, Otegui ME (2004) Seed dry weight response to source-sink manipulations in wheat, maize and soybean. A quantitative reappraisal. Field Crop Res 86:131–146

Calderini DF, Dreccer MF, Slafer GA (1997) Consequences of breeding on biomass, radiation interception and radiation use efficiency in wheat. Field Crop Res 52:271–281

Calderini DF, Reynolds MP, Slafer GA (1999) Genetic gains in wheat yield and main physiological changes associated with them during the 20th century. In: Satorre EH, Slafer GA (eds) Wheat: ecology and physiology of yield determination. Food Product Press, New York, pp 351–377

Calderini DF, Savin R, Abeledo LG, Reynolds MP, Slafer GA (2001) The importance of the immediately preceding anthesis period for grain weight determination in wheat. Euphytica 119:199–204

Calderini DF, Reynolds MP, Slafer GA (2006) Source-sink effects on grain weight of bread wheat, durum wheat and triticale at different locations. Aust J Agr Res 57:227–233

Demotes Mainard S, Jeuffroy MH (2001) Partitioning of dry matter and nitrogen to spike throughout the spike growth period in wheat crops subjected to nitrogen deficiency. Field Crop Res 70:153–165

Dreccer MF, Grashoff C, Rabbinge R (1997) Source-sink ratio in barley (*Hordeum vulgare* L.) during grain filling: effects on senescence and grain protein concentration. Field Crop Res 49:269–277

Fischer RA (1984) Wheat. In: Smith WH, Banta JJ (eds) Potential productivity of field crops under different environments. IRRI, Los Baños, pp 129–154

Fischer RA (1985) Number of kernels in wheat crops and the influence of solar radiation and temperature. J Agr Sci (Cambridge) 105:447–461

Fischer RA (1996) Wheat physiology at CIMMYT and raising the yield plateau. In: Reynolds MP, Rajaram S, McNab A (eds) Increasing yield potential in wheat: breaking the barriers. CIMMYT, Mexico, pp 195–203

Gonzalez FG, Slafer GA, Miralles DJ (2003) Grain and floret number in response to photoperiod during stem elongation in fully and slightly vernalized wheats. Field Crop Res 81:17–27

Gonzalez FG, Slafer GA, Miralles DJ (2005*a*) Photoperiod during stem elongation in wheat: is its impact on fertile floret and grain number determination similar to that of radiation? Funct Plant Biol 32:181–188

Gonzalez FG, Slafer GA, Miralles DJ (2005*b*) Floret development and survival in wheat plants exposed to contrasting photoperiod and radiation environments during stem elongation. Funct Plant Biol 32:189–197

Gonzalez FG, Slafer GA, Miralles DJ (2005*c*) Pre-anthesis development and number of fertile florets in wheat as affected by photoperiod sensitivity genes *Ppd-D1* and *Ppd-B1*. Eupytica 146:253–269

Jenner CF (1994) Starch synthesis in the kernel of wheat under high temperature conditions. Aust J Plant Physiol 21:791–806

Kirby EJM (1988) Analysis of leaf, stem and ear growth in wheat from terminal spikelet stage to anthesis. Field Crop Res 18:127–140

Miralles DJ, Slafer GA (1997) Radiation interception and radiation use efficiency of near isogenic wheat lines with different height. Euphytica 97:201–208

Miralles DF, Katz SD, Colloca A, Slafer GA (1998) Floret development in near isogenic wheat lines differing in plant height. Field Crop Res 59:21–30

Miralles DF, Richards RA, Slafer GA (2000) Duration of the stem elongation period influences the number of fertile florets in wheat and barley. Aust J Plant Physiol 27:931–940

Prystupa P, Savin R, Slafer GA (2004) Grain number and its relationship with dry matter, N and P in the spikes at heading in response to NxP fertilization in barley. Field Crop Res 90:245–254

Reynolds MP, van Ginkel M, Ribaut JM (2000) Avenues for genetic modification of radiation use efficiency in wheat. J Exp Bot 51:459–473

Reynolds MP, Calderini DF, Condon AG, Rajaram S (2001) Physiological basis of yield gains in wheat associated with the *LR19* translocation from *Agropyron elongatum*. Euphytica 119:137–141

Reynolds MP, Pellegrineschi A, Skovmand B (2004) Sink-limitation to yield and biomass: a summary of some investigations in spring wheat. Ann Appl Biol 146:39–49

Romagosa I, Ullrich SE, Han F, Hayes PM (1996) Use of the additive main effects and multiplicative interaction model in QTL mapping for adaptation in barley. Theor Appl Genet 93:30–37

Savin R, Slafer GA (1991) Shading effects on the yield of an Argentinean wheat cultivar. J Agr Sci (Cambridge) 116:1–7

Slafer GA (1996) Differences in phasic development rate amongst wheat cultivars independent of responses to photoperiod and vernalization. A viewpoint of the intrinsic earliness hypothesis. J Agr Sci (Cambridge) 126:403–419

Slafer GA (2003) Genetic basis of yield as viewed from a crop physiologist's perspective. Ann Appl Biol 142:117–128

Slafer GA, Andrade FH (1993) Physiological attributes related to the generation of grain yield in bread wheat cultivars released at different eras. Field Crop Res 31:351–367

Slafer GA, Miralles DJ (1992) Green area duration during the grain filling period of wheat as affected by sowing date, temperature and sink strength. J Agron Crop Sci 168:191–200

Slafer GA, Rawson HM (1996) Responses to photoperiod change with phenophase and temperature during wheat development. Field Crop Res 46:1–13

Slafer GA, Savin R (1994) Sink-source relationships and grain mass at different positions within the spike in wheat. Field Crop Res 37:39–49

Slafer GA, Calderini DF, Miralles DJ (1996) Yield components and compensation in wheat: opportunities for further increasing yield potential. In: Reynolds MP, Rajaram S, McNab A (eds) Increasing yield potential in wheat: breaking the barriers. CIMMYT, Mexico DF, pp 101–133

Slafer GA, Araus JL, Royo C, García del Moral LF (2005) Promising ecophysiological traits for genetic improvement of cereal yields in Mediterranean environments. Ann Appl Biol 146:61–70

Stone PJ, Savin R (1999) Grain quality and its physiological determinants. In: Satorre EH, Slafer GA (eds) Wheat: ecology and physiology of yield determination food product press, New York, pp 85–120

Stuber CW, Polacco M, Lynn Senior M (1999) Synergy of empirical breeding, marker-assisted selection, and genomics to increase crop yield potential. Crop Sci 39:1571–1583

Thorne GN, Wood DW (1987) Effects of radiation and temperature on tiller survival, grain number and grain yield in winter wheat. Ann Bot 59:413–426

Whitechurch EM, Slafer GA (2002) Contrasting *Ppd* genes in wheat affect sensitivity to photoperiod in different phases. Field Crop Res 73:95–105

Yin X, Stam P, Dourleijn CJ, Kropff MJ (1999) AFLP mapping of quantitative trait loci for yield-determining physiological characters in spring barley. Theor Appl Genet 99:244–253

Youssefian S, Kirby EJM, Gale MD (1992) Pleiotropic effects of the G.A. insensitive Rht dwarfing gene in wheat. 2. Effects on leaf, stem, ear and floret growth. Field Crop Res 28:191–210

INFLUENCE OF FOLIAR DISEASES AND THEIR CONTROL BY FUNGICIDES ON GRAIN YIELD AND QUALITY IN WHEAT

M.J. GOODING

Crops Research Unit, School of Agriculture Policy and Development, The University of Reading, Earley Gate, PO Box 237, Reading RG6 6AR
E-mail: m.j.gooding@reading.ac.uk

Abstract: Twenty-eight field experiments on sandy-loam soils in the UK (1982–2003) are reviewed by relating the extension of the green area duration of the flag leaf (GLADF) by fungicides to effects on yield and quality of winter wheat. Over all experiments mean grain yield = 8.85 t ha^{-1} at 85% DM. With regards quality, mean values were: thousand grain weight (TGW) = 44.5 g; specific weight (SWT) = 76.9 kg hl^{-1}; crude protein concentration (CP (N × 5.7)) = 12.5% DM; Hagberg falling number (HFN) = 285 s; and sodium dodecyl sulphate (SDS)-sedimentation volume = 69 ml. For each day (d) that fungicides increased GLADF there were associated average increases in yield (0.144 t ha^{-1} d^{-1}, se = 0.0049, df = 333), TGW (0.56 gd^{-1}, se = 0.017) and SWT (0.22 kg hl^{-1}d^{-1}, se = 0.011). Some curvature was evident in all these relationships. When GLADF was delayed beyond 700 °Cd after anthesis, as was possible in cool wet seasons, responses were curtailed, or less reliable. Despite this apparent terminal sink limitation, fungicide effects on sink size, eg endosperm cell numbers or maximum water mass per grain, were not prerequisites for large effects on grain yield, TGW or SWT. Fungicide effects on CP were variable. Although the average response of CP was negative (−0.029%DM/d; se = 0.00338), this depended on cultivar and disease controlled. Controlling biotrophs such as rusts (*Puccinia* spp.) tended to increase CP, whereas controlling a more necrotrophic pathogen (*Septoria tritici*) usually reduced CP. Irrespective of pathogen controlled, delaying senescence of the flag leaf was associated with increased nitrogen yields in the grain (averaging 2.24 kg N ha^{-1}d^{-1}, se = 0.0848) due to both increased N uptake into the above ground crop, and also more efficient remobilisation of N from leaf laminas. When sulphur availability appeared to be adequate, fungicide x cultivar interactions were similar on S as for CP, although N:S ratios tended to decline (i.e. improve for bread making) when *S. tritici* was controlled. On average, SDS-sedimentation volume declined (−0.18 ml/d, se = 0.027) with increased GLADF, broadly commensurate with the average effect on CP. Hagberg falling number decreased as fungicide increased GLADF (−2.73 s/d, se = 0.178), indicating an increase in *alpha*-amylase activity

Keywords: grain yield, Quality

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 567–581

INTRODUCTION

Fungicides are important inputs to winter wheat production in temperate areas because these regions combine high yield potential with high infection pressures; both deriving from mild temperatures with adequate moisture, large applications of nitrogenous fertilizers and rotations dominated by cereals (Christen 2001). The UK typifies this situation with average rain fed wheat yields of 8 t ha^{-1} on 2 million ha receiving about 190 kg N ha^{-1} as fertilizer. Additionally in the UK, cultivars have been released with high yield potential but also with susceptibility to *Septoria* spp. and *Puccinia* spp. Farmers have appeared ready to adopt these cultivars to achieve high yields, whilst accepting the need to apply robust fungicide programmes. Furthermore, the recommended listing regime has increased the importance of yields in trials receiving an intensive fungicide programme for marketing and recommending cultivars (Angus 2001). Consequently, in 2002 about 1000 t of fungicidal active ingredient was applied to the UK wheat crop (not including seed treatments) (Garthwaite et al. 2002). Nearly all the wheat area received at least one fungicide spray (98.1%), but typically three sprays are applied (average = 2.8), containing, on average a total of 4.3 products and 5.9 different active ingredients. Thirty seven percent (by mass) of the active ingredient applied was a triazole or closely related ergosterol biosynthesis inhibitor (EBI), while 31% was a strobilurin or closely related inhibitor of mitochondrial respiration (MRI). Protecting the flag leaf is particularly important to maintain yields, and a fungicide application at flag leaf emergence (GS 37–39; Zadoks et al. 1974) often gives the largest yield response in the UK (Clare et al. 1993). Additional timings are typically at the start of stem extension (GS 30–31) and at, or after ear emergence (GS 55–65).

Here the results from twenty-eight field experiments are combined by relating the effects of EBIs and/or MRIs, usually applied between GS 37 and 59, on the green area duration of the flag leaf, to the fungicide effects on grain yield and quality. This approach reduces the need to explore whether fungicide effects arise purely by controlling recognised pathogens (Ruske et al. 2003*a*); or by additional effects on minor pathogens, not easily recognised in the field (Bertelsen et al. 2001); or through direct physiological effects on the plant (Grossman and Retzlaff 1997). The flag leaf is studied as it provides more photosynthate for the grain than any other leaf (Lupton 1972) because: it is held above other leaves during a period of increasing radiation receipts; it can persist during grain filling and is in close vascular proximity to the ear; and its health and duration are good indicators of the previous and current health of other plant organs. Others have found the end of grain filling to be associated with the senescence of the flag leaf (Hanft and Wych 1982).

Quality of harvested grain is assessed for criteria commonly used when trading wheat. Routine measurements include: 1) thousand grain weight (TGW) and 2) specific (or hectolitre) weight because well filled, plump grain yield more flour and energy compared with shrivelled grain (Gooding and Davies 1997); 3) grain protein concentration (N × 5.7) due to its impact on dough rheology and hence suitability for numerous wheat-based foods including various breads, cookies and extruded products; 4) SDS-sedimentation volume (Axford et al. 1979) whereby high molecular weight proteins, particularly the glutenins that impart viscosity and

elasticity to a dough, remain expanded in a layer of sediment consisting mainly of swollen gluten and occluded starch (Peña et al. 2002); and 5) Hagberg falling number (HFN) because of its inverse relationship with *alpha*-amylase activity which, when in excess, causes crumb discoloration, poor crumb structure, and stickiness of doughs (Chamberlain et al. 1982). In subsets of experiments fungicide effects on grain filling, nitrogen uptake and partitioning, grain sulphur content, and loaf quality are also assessed.

MATERIALS AND METHODS

Field experiments were conducted at three sites in England between 1982 and 2003, mostly on sandy loam soil, and all using commercial rates of nitrogen fertilizer. All experiments involved complete factorial combinations of fungicide treatments x a range of commercially recommended cultivars. Further details of experimental designs and conditions are available for: Series 1, 1982–1985 (Lawson 1989) where fungicide treatments were different rates and timings of propiconazole applied at and after GS39; Series 2, 1984–1987 (Gooding et al. 1991), with propiconazole plus tridemorph applied at GS39 and again at 59; Series 3, 1997–2000 (Dimmock and Gooding 2002*a*), including flusilazole, famaxadone and azoxystrobin fungicide treatments at GS 39 and GS 59; Series 4, 1999–2002 (Ruske et al. 2003*b*), including azoxystrobin, picoxystrobin and epoxiconazole treatments from GS 31 to GS 59; and Series 5, 2000–2003 (Pepler et al. 2005*a*) including azoxystrobin and epoxiconazole treatments from GS 31 to GS 67. Fungicide treatment plots were at least 2×5 m (usually 2×10 m), replicated in at least three (usually four) randomised blocks. Fungicide treatments were applied via nozzles mounted on a 2m boom. A spray of medium droplet size (Matthews 2000) was achieved by delivering the active ingredient in 220 l ha^{-1} water under 200–250 Pa pressure through either flat fan (Series 1–3) or air bubble jet (Series 4 and 5) nozzles.

In all experiments green area and disease status of the flag leaf was assessed at approximately weekly intervals when at least seven (usually ten) leaves per fungicide treatment plot were visually scored for disease and the area of the flag leaf that was green. A modified Gompertz curve (Gooding et al. 2000) was fitted to the green area x time (or thermal time) curves for each plot. In this modification, the Gompertz time scalar (m) is the time taken between when the flag leaf emerged (GS 39) to when the green area of the leaf had declined to 37% and is taken as green leaf area duration of the flag leaf (GLADF). Grain filling with dry matter and nitrogen was assessed (Series 2, 4 and 5) by harvesting ten ears per plot every 7 to 14 days after anthesis. Ears were dried at 80 °C for 48 h before chaff was separated from the grain either by hand (early development samples) or by a laboratory threshing machine. Grain moisture and dry matter contents during grain filling were also assessed (Series 3 and 5) every seven to ten days when medial grains (basal floret in the fourth spikelet from the base of one side of the ear) and apical grains (grain in the basal floret in the penultimate spikelet) were dissected from each of seven to ten ears per fungicide treatment plot. Nitrogen content and partitioning during grain filling was assessed (Series 5) from fifteen (2001 and

2003) or thirty (2002) randomly harvested ear-bearing stems every seven to ten days in each fungicide treatment plot. Samples were separated into flag leaf laminas, penultimate leaf laminas, other leaf laminas, stems plus leaf sheaths, chaff and grain. Grain in the medial positions in Series 5 were assessed for endosperm cell number. Endosperms were: dissected from grains and fixed in acetic acid/alcohol (25:75); stored in 70% ethanol; treated with cellulase and amylase; stained; resuspended; and nuclei counted with a haemocytometer (Singh and Jenner 1982).

The grain and vegetative samples were milled with mills appropriate to the material in each experiment: Perten Instruments 3100 for grain; and either Tema, Model T100 or Retsch, Model SM1 for vegetative components. Assessments of nitrogen content were either by: a semi-automated Kjeldhal method (Series 1–3; Tecator Ltd, Bristol); by an oxidative combustion method (Series 4 and 5; Association of Official Analytical Chemists 1990) using an automated Dumas type combustion analyser (LECO FP-528); or with a VG602 mass spectrometer after first combusting the material in a linked Roboprep C and N analyser (N partitioning in Series 5; Europa Scientific Ltd).

In all experiments yield and quality was determined from combine-harvested grain. Assessments of quality always included: thousand grain weight; grain specific weight with a chondrometer (Gooding and Davies 1997); crude protein content ($N \times 5.7$); Hagberg falling number to IS 3039 using a Perten Instruments Falling Number 1500 machine; and SDS-sedimentation volume to BS 4317: Part 19. Grain sulphur content was assessed with X-ray florescence spectrometry (Gooding et al. 1991) in Series 2, and following oxidative combustion with a LECO SC-144DR analyser in Series 5. Dough rheology and baking tests were conducted on grain from Series 2 (Gooding et al. 1991) and 4 (Ruske et al. 2004).

Appropriate factorial and split plot analyses of variance were applied to the data using Genstat 6 (VSN International Ltd. 2002). To understand effects of fungicides over a large number of seasons and cultivars, the fungicide treatment means on yield and quality were regressed against effects on GLADF, after also fitting effects for season x experiment x cultivar x other main treatment factors (such as late season nitrogen treatments). For presentation purposes, these regressions are fitted for residuals after removing effects of season x experiment x other main treatment factors. The effect of adding a quadratic term to the regression assessed curvature. When the quadratic effect was significant ($P < 0.05$), an exponential model was fitted to the data using the FITCURVE directive in Genstat and variance accounted for calculated as r^2_{adj}.

RESULTS AND DISCUSSION

The principal foliar disease controlled on the flag leaf was *Septoria tritici* Rob. in Desm. (perfect stage *Mycosphaerella graminicola*), but yellow rust (*Puccinia striiformis*), brown rust (*P. recondita*) and powdery mildew (*Erysiphe graminis*) also occurred on susceptible cultivars in some seasons.

The average grain yield of 8.85 t ha^{-1} @ 85% dm was higher than the national average (currently about 8 t ha^{-1}) but not dissimilar to many commercial crops in the UK. Over all experiments, fungicide effects on grain yield were closely associated with fungicide effects on GLADF (Fig. 1*a*). Fungicide effects on both measures were greatest on susceptible cultivars in cool, wet seasons when infection pressures were high, and least in dry, hot summers (Gooding et al. 2000). On average, grain yield was increased by 0.144 t ha^{-1} (se = 0.0049, df = 333) for each day that the flag leaf remained green. This response tended to be lower on a cultivar susceptible to brown rust, compared with cultivars particularly susceptible to *S. tritici* (Ruske et al. 2003*a*, Pepler et al. 2005*b*).

There was no consistent effect of fungicide on grain numbers, so the yield effect is mirrored in the data for TGW (Fig. 1*b*) which was increased, on average, by 0.56g (se = 0.017) for each day that fungicides maintained GLADF. Green leaf area duration of the flag leaf, as extended by fungicide use, was closely associated with the duration of grain filling (Fig. 2, Dimmock and Gooding 2002*a*), although rate of grain filling could also be increased by fungicide application (Gooding et al. 1994, Pepler et al. 2006). However, it was occasionally possible, when relatively cool temperatures and low soil moisture deficits were conducive to long lived canopies when disease was controlled with powerful fungicide programmes, for GLADF to be extended beyond the end of grain filling (Dimmock and Gooding 2002*a*, Pepler et al. 2005*a*). In such situations, there was a limit to the association between GLADF and grain yield and TGW, i.e. over a range of cultivars and seasons, there appeared little point to extending GLADF beyond 700 °Cd after anthesis (Fig. 3*a*, *b*; Pepler et al. 2005*a*). This apparent sink limitation contributed

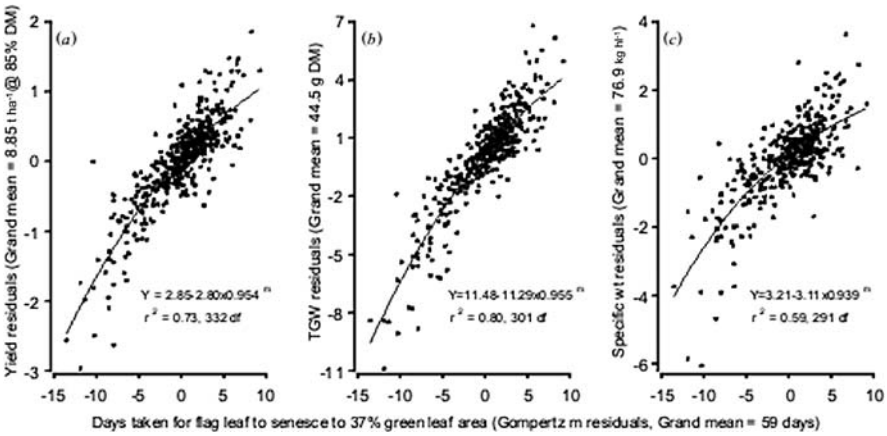


Figure 1. Relationships between fungicide effects on green area duration of the flag leaf and effects on (*a*) grain yield, (*b*) thousand grain weight, and (*c*) grain specific weight. Points are fungicide treatment means after removal of effects of year x experiment x cultivar x other treatment factors. Presented degrees of freedom (df) account for removal of effects, rather than n-1

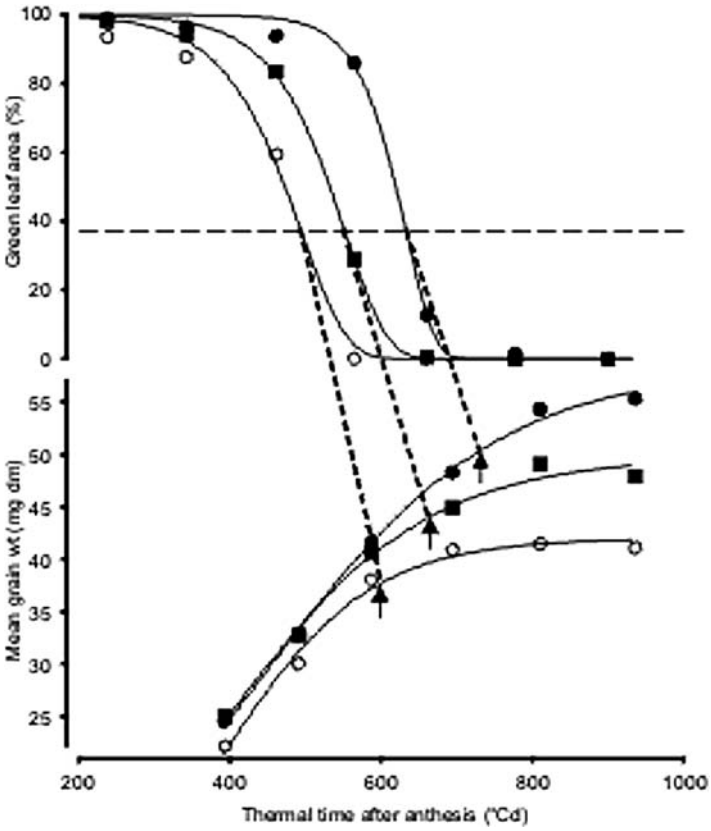


Figure 2. The effect of fungicide on green leaf area duration of the flag leaf and filling of medial grain of winter wheat cv. Consort. ○ = nil fungicide, ■ = flusilazole, ● = azoxystrobin at GS 39 and 59. Arrows = 90% of final grain weight. Dashed horizontal line = 37% green leaf area. Data from Dimmock and Gooding (2002*a*)

to slight, but statistically significant curvature in the overall association between yield and TGW, and GLADF (Fig. 1*a*, *b*).

Although sink limitation was apparent, there was no evidence that fungicides needed to increase sink size as a prerequisite for large increases in mean grain weight. Final grain size has been correlated by others with the maximum water mass per grain and/or the number of endosperm cells, both as attained during the two to three weeks after anthesis (Brocklehurst 1977, Schnyder and Baum 1992). However, we recorded large increases in final grain weight with fungicide-use, even when application had failed to significantly increase maximum water content or endosperm cell numbers (Fig. 4).

The fungicide response of the bulk density of wheat, i.e. specific weight broadly followed that of TGW. i.e. as fungicide delayed senescence, or prevented premature

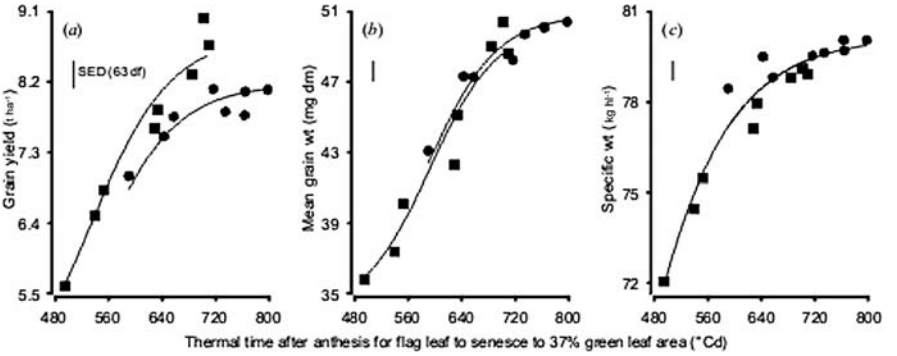


Figure 3. Relationships between fungicide effects on green area duration of the flag leaf (modified Gompertz *m*) and effects on (*a*) grain yield, (*b*) thousand grain weight, and (*c*) grain specific weight of winter wheat cvs Consort (■) and Hereward (•). Points are different means for different fungicide treatments applied at GS 39 and again at 59. Fitted curves are logistic (constant omitted). Data from Dimmock and Gooding (2002*a*)

senescence, specific weight increased at an average rate of 0.22 kg hl^{-1} d^{-1} (se = 0.011). As with TGW, however, the response deviated significantly from linearity (Fig. 1*c*) and was unreliable as GLADF was extended beyond 700 °Cd after anthesis (Fig. 3*c*; Dimmock and Gooding 2002*a*, Pepler et al. 2005a). Packing density is known to decline as grain become shrivelled (Bayles 1977), as may occur in response to late-season disease infection (Gooding et al. 1994). However, many factors, as well as grain shrivelling, can influence specific weight, including: the density of individual grain (and therefore grain protein concentration), the shape of filled grain, and grain surface texture (Bayles 1977). It is unsurprising, therefore, that only 59% of variation in fungicide effects on specific weight can be accounted for by effects on GLADF (Fig. 1*c*), compared with 80% for TGW (Fig. 1*a*).

The yield of nitrogen in the grain increased as fungicide delayed senescence at an average rate of 2.24 kg N ha^{-1} d^{-1} (se = 0.085; Fig. 5*a*). This occurred because fungicide use both increased net nitrogen accumulation in the above ground parts (Fig. 6*g*), and net remobilization of nitrogen from vegetative tissues to the grain. Over three experiments and three cultivars within Series 5 (Gooding et al. 2006), fungicide increased total N in the above ground crop biomass from 207 kg ha^{-1} (no fungicide at or after GS 39) to 222 kg ha^{-1} (63 + 125 g ha^{-1} of epoxiconazole + azoxystrobin at GS 39 and again at GS 59). Net remobilization of vegetative N ($Remobilized\ N_{(at\ harvest)} = (Total\ N_{(at\ anthesis)} - Vegetative\ N_{(at\ harvest)}/Total\ N_{(at\ anthesis)}$; Cox et al. 1986) was also increased from 0.68 to 0.72 (se = 0.007). These combined effects meant that grain N yield increased from 155 to 177 kg N ha^{-1} (se = 2.5), and nitrogen harvest index increased from 75 to 80% (se = 0.4). Similar effects were found for Series 4 (Ruske et al. 2003*a*) and occurred whether *S. tritici* or brown rust, or a mixture of the two, was the principal threat. The ability of fungicides to maintain N uptake must depend on

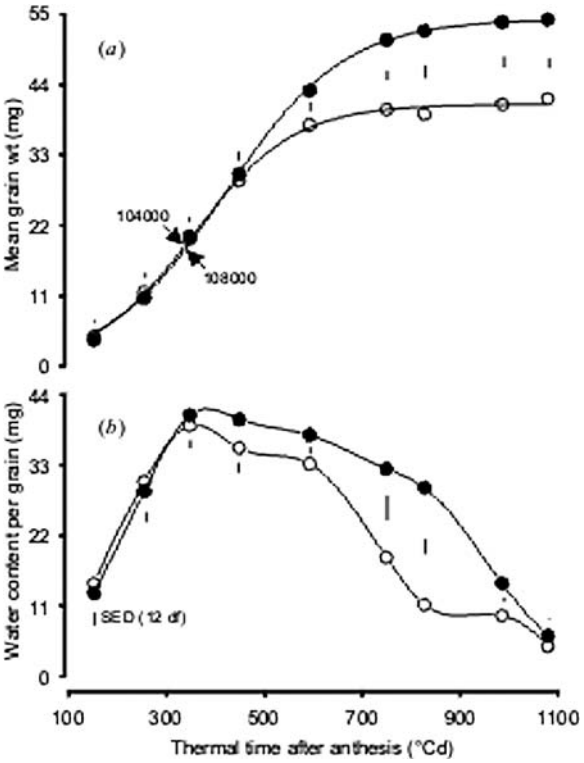


Figure 4. The effect of fungicide on (*a*) grain filling and (*b*) water content of medial grain of Malacca winter wheat. Arrowed numerals are endosperm cell numbers and maximum water content. ○ = nil fungicide, ● = epoxiconazole (63 g a.i. ha^{-1}) at GS 31 + 59 and azoxystrobin at GS 39 (250g) + GS 59 (125g). Data from (Pepler et al. 2006)

the availability of N late in to the season. In the UK, even wheat crops not given nitrogen fertilizer (Sylvester Bradley et al. 2001), or crops given nitrogen before the end of April, as was the case here, can maintain an average rate of N uptake of about 1–1.5 kg N $ha^{-1}d^{-1}$ during grain filling. Net remobilization of vegetative N was increased because fully senesced laminas, previously infected with brown rust or *S. tritici* have retained more nitrogen than fully senesced leaves where disease had been controlled with fungicide (Fig. 6*a-d*, Gooding et al. 2006). This late movement of nitrogen from the more healthy leaves contributes to increases in grain protein concentration during the latter stages of grain filling (Ruske et al. 2003*a*). Estimates of end of grain filling with N can thus be later than estimates of the end of grain filling with dry matter and this difference is more marked where disease has been controlled (Ruske et al. 2003*a*). Nonetheless, it is still possible to extend flag leaf life beyond the end of grain filling with N (Pepler et al. 2005*b*), and this accounts for the slight curvature in response shown in Fig. 5*a*. The increase in grain yields of both dry matter and nitrogen means that fungicide effects on the ratio of the

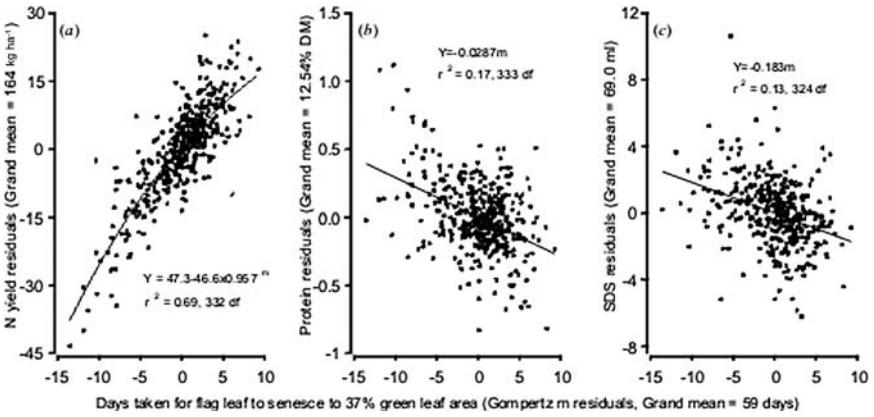


Figure 5. Relationships between fungicide effects on green area duration of the flag leaf and effects on (*a*) grain nitrogen yield, (*b*) grain protein concentration, and (*c*) SDS-sedimentation volume. Points are fungicide treatment means after removal of effects of year x experiment x cultivar x other treatment factors. Presented degrees of freedom (df) account for removal of effects, rather than n-1

two, as expressed in terms of crude protein concentration (N% dm × 5.7), is not dramatically affected by disease control. In one experiment (Ruske et al. 2003*a*), fungicide-use increased yield by 3 t ha^{-1} without any dilution of protein.

A review (Dimmock and Gooding 2002*b*) reveals that when classic biotrophs such as brown rust, yellow rust, stem rust and powdery mildew are controlled, the concentration of protein often increases, i.e. the pathogen has a more damaging effect on the accumulation and partitoning of nitrogen to the grain, than it does on the accumulation and partitioning of dry matter. In contrast, most reports of the effect of controlling more necrotrophic pathogens, such as the *Septoria* spp. find that fungicide use is associated with a reduction in protein concentration. This difference between infection strategies on protein concentration is supported by the experiments described here (Tables 1 and 2), although it is difficult to separate comparative pathogen effects from other confounding influences of climate and cultivar in field studies. In 2001, brown rust was the prevalent disease on untreated cv. Consort (Table 1) and fungicide-use controlled the pathogen and simultaneously increased grain yield and protein concentration. In 2002, the same fungicide programme on the same cultivar diluted protein as yield increased in response to *S. tritici* control. In another experiment repeated in 2001 and 2002 (Table 2), fungicide-use on the cultivar most susceptible to the biotrophs, cv. Shamrock, concentrated protein, whereas for other cultivars on which only *S. tritici* was recorded, fungicide-use diluted protein. The response in Fig. 5*b* is negative because *S. tritici* was the prevalent foliar pathogen in most seasons.

Fungicide effects on grain sulphur concentration generally followed effects on grain nitrogen concentration, although the N:S ratio did tend to decline as *S. tritici* was controlled (Tables 1 and 2, Gooding et al. 1991, Ruske et al. 2004, Pepler et al. 2005*b*). Sulphur concentration is an important determinant of protein quality

Table 1. The effect of fungicide[a] on areas of disease symptoms, grain yield and grain quality of cv. Consort winter wheat in successive years

Year	Fungicide[a]	Disease (% flag leaf area on last assessment date)		Grain yield	Protein	N:S	SDS-sediment vol.	Hagberg falling number
		S. tritici	Brown rust	(t ha^{-1} @ 85% DM)	(% DM)	ratio	(ml)	(s)
2001	−	4.4	8.3	9.9	11.5	14.7	41.3	272
	+	2.2	0.0	11.3	12.0	15.1	43.3	234
SED (28 df)		0.35		0.30	0.20	0.40	1.65	20.5
2002	−	15.7	1.7	7.8	11.8	14.3	60.7	314
	+	2.6	0.0	10.8	11.0	13.8	55.7	254
SED (28 df)		0.96		0.34	0.24	0.27	2.49	23.1

[a] − = no fungicide at or after GS 39; + = 63 + 125 g ha^{-1} epoxiconazole + azoxystrobin at GS 39 and again at GS 59.

Table 2. The effect of fungicide (F[a]) and cultivar on areas of disease symptoms, grain yield and grain quality of winter wheat in successive years

Year	Cultivar	F[a]	Disease (% flag leaf area on last assessment date)			Grain yield	Protein	N:S	SDS sediment	Hagberg falling number
			S. tritici	Brown rust	Powd. mildew	(t ha^{-1} @ 85% DM)	(% DM)	ratio	(ml)	(s)
2001	Shamrock	−	2.9	6.6	0.0	10.6	12.1	13.6	61.8	288
		+	2.5	0.0	0.0	11.6	12.6	13.9	61.8	281
	Savannah	−	9.3	0.0	0.0	11.1	11.4	14.2	35.2	253
		+	3.0	0.0	0.0	11.9	11.0	14.1	33.3	244
SED (60 df)			1.03			0.25	0.17	0.27	1.37	13.0
2002	Shamrock	−	5.8	1.5	1.8	8.9	11.7	14.5	77.0	321
		+	0.7	0.0	0.2	10.4	12.1	15.0	75.0	250
	Savannah	−	16.5	0.0	0.0	9.4	10.6	12.5	45.8	322
		+	1.1	0.0	0.0	12.4	10.2	12.0	43.2	280
	Malacca	−	16.9	0.0	0.0	9.1	12.3	15.5	75.8	387
		+	1.7	0.0	0.0	11.2	11.8	14.6	76.7	370
SED (24 df)			1.74			0.24	0.16	0.28	1.26	17.3

[a] − = no fungicide at or after GS 39; + = 63 + 125 g ha^{-1} epoxiconazole + azoxystrobin at GS 39 and again at GS 59.

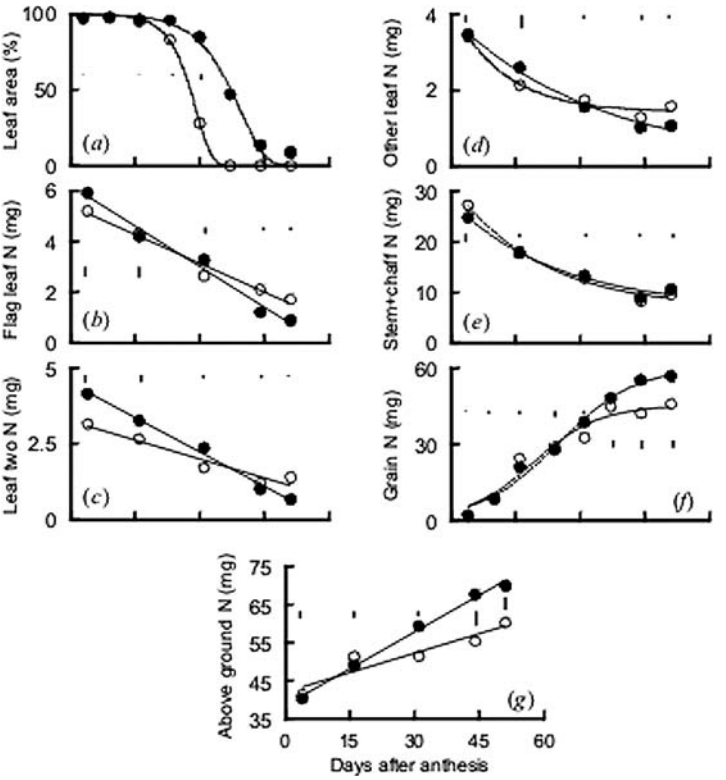


Figure 6. The effect of fungicide treatment on the green leaf area of the flag leaf and the amounts of nitrogen per ear bearing stem in above ground components of cv. Consort winter wheat in 2001. ○ = no fungicide at or after GS 39; ● = 63 + 125 g ha^{-1} of epoxiconazole + azoxystrobin at GS 39 and again at GS 59. Vertical bars are SE. Data from Gooding et al. (2006)

because it provides the inter-and intra-chain disulphide bonds that help maintain gluten functionality. In the UK, therefore, loaf quality can be more closely correlated with sulphur concentration than with crude protein concentration (Zhao et al. 1999). The overall reduction SDS-sedimentation volume (Fig. 5*c*) was commensurate with the overall reduction in protein concentration (Fig. 5*b*).

This observation, together with the sulphur results and the quality of loaves in test baking (Gooding et al. 1991, Ruske et al. 2004) suggests that protein quality was not greatly affected by fungicide application. It is acknowledged, however, that grain analyses mostly indicated that sulphur availability was sufficient, and that a different result may have been obtained in deficient circumstances.

Fungicides tended to reduce HFN (Fig. 7, Tables 1 and 2), an affect indicating increased alpha-amylase activity as demonstrated by Salmon and Cook (1987). Effects of fungicides on HFN and *alpha*-amylase have been correlated with both delayed drying of the grain and grain size, and plausible mechanisms have been proposed to

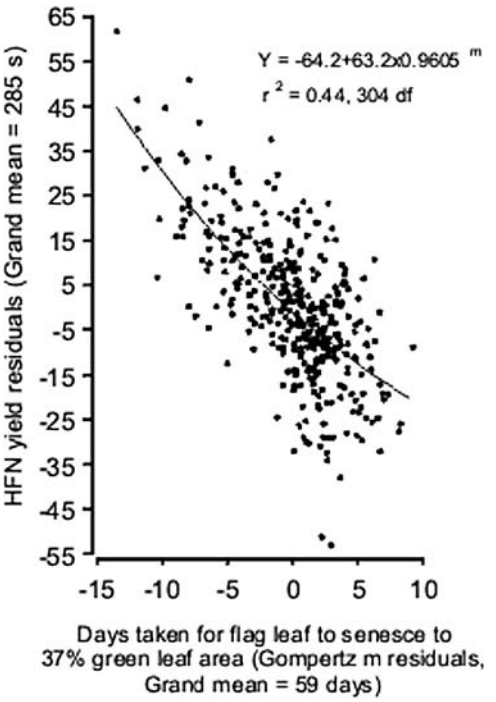


Figure 7. Relationship between fungicide effects on green area duration of the flag leaf and effects on Hagberg falling number (HFN). Points are fungicide treatment means after removal of effects of year x experiment x cultivar x other treatment factors

explain both associations (Dimmock and Gooding 2002*c*). Disease control can reduce HFN irrespective of whether fungicide increases or reduces grain protein concentration, and also irrespective of pathogen infection strategy (Tables 1 and 2). The average effect of fungicides was to reduce HFN by 2.73 s (se = 0.178) for each day that flag leaf life was extend. However, the curvature evident, and statistically justified in Fig. 7 is consistent with the effect being more related to grain dry matter, rather than flag leaf senescence. As with grain weight, initial studies have indicated that fungicides are less likely to reduce HFN as green area duration is extended beyond 700 °Cd after anthesis (Pepler 2004). Other treatment effects on HFN, such as genotype and nitrogen, have been linked to the size of the endosperm cavity, and associated disruption of the aleurone layer (Kindred et al. 2005), and it is possible that disease control might also contribute to this disruption as grain sizes are increased.

REFERENCES

Angus WJ (2001) United Kingdom wheat pool. In: Bonjean AP, Angus WJ (es) The World Wheat Book, A History of Wheat Breeding. Lavoisier Publishing Inc Paris.Intercept, pp 103–126

Association of Official Analytical Chemists (1990) , Protein (crude) in animal feed Dumas method 968.06. In:Official methods of analysis (15th edn.). AOAC, Arlington, Virginia, pp 71–72

Axford DWE, McDermott EE, Redman DG (1979) Note on the sodium dodecyl sulphate test of breadmaking quality: comparison with Pelshenke and Zeleny tests. Cereal Chem 56:582–584

Bayles RA (1977) Poorly filled grain in the cereal crop I. The assessment of poor grain filling. J Natl Inst Agr Bot 14:232–240

Bertelsen JR, de Neergaard E, Smedegaard-Petersen V (2001) Fungicidal effects of azoxystrobin and epoxiconazole on phyllosphere fungi, senescence and yield of winter wheat. Plant Pathol 50:190–205

Brocklehurst PA (1977) Factors controlling grain weight in wheat. Nature 266:348–349

Chamberlain N, Collins TH, McDermott EE (1982) The influence of loaf properties in the UK. In: Proceedings of the 7th World Cereal and Bread Congress, International Association for Cereal Science and Technology, Vienna, pp 841–845

Christen O (2001) Charactistics and problems of cropping systems in Europe. In: Srinivasan A (ed) Sustainable Food Production in Cropping Systems of Temperate Regions. Hokkaido: National Agricultural Experiment Station, pp 15–26

Clare RW, Spink JH, Laverick RM, Bailey J (1993) Factors affecting the quality of milling wheats produced in a high yield situation. Aspect Appl Biol 36, Cereal Quality III, 397–406

Cox MC, Qualset CO, Rains DW (1986) Genetic-variation for nitrogen assimilation and translocation in wheat. III. Nitrogen translocation in relation to grain-yield and protein. Crop Sci 26:737–740

Dimmock JPRE, Gooding MJ (2002*a*) The effects of fungicides on rate and duration of grain filling in winter wheat in relation to maintenance of flag leaf green area. J Agr Sci 138:1–16

Dimmock JPRE, Gooding MJ (2002*b*) The influence of foliar diseases, and their control by fungicides, on the protein concentration in wheat grain: a review. J Agr Sci 138:349–366

Dimmock JPRE, Gooding MJ (2002*c*) Effects of fungicides on Hagberg falling number and blackpoint in winter wheat. Crop Protect 21:475–487

Garthwaite DG, Thomas MR, Dawson A, Stoddart H (2002) Pesticide usage survey report 187 arable crops in Great Britain 2002. Department of environment, food and rural affairs, London

Gooding MJ, Davies WP (1997) Wheat production and utilization: systems, quality and the environment. CAB International, Wallingford, UK, p 355

Gooding MJ, Kettlewell PS, Hocking TJ (1991) Effect of urea alone or with fungicide on the yield and breadmaking quality of wheat when sprayed at flag leaf and ear emergence. J Agr Sci 117:149–155

Gooding MJ, Smith SP, Davies WP, Kettlewell PS (1994) Effect of late-season. applications of propiconazole and tridemorph on disease levels, senescence, grain development and breadmaking quality of winter wheat. Crop Protect 13:362–370

Gooding MJ, Dimmock JPRE, France J, Jones SA (2000) Green leaf area decline of flag leaves, the influence of fungicides and relationships with wheat mean grain weight and grain yield. Ann Appl Biol 136:77–84

Gooding MJ, Gregory PJ, Ford KE, Pepler S (2006) Fungicide and cultivar affect post-anthesis patterns of nitrogen uptake, remobilization and utilization efficiency in wheat. J Agr Sci (In press)

Grossmann K, Retzlaff G (1997) Bioregulatory effects of the fungicidal strobilurin kresoxim- methyl in wheat (Triticum aestivum). Pestic Sci 50:11–20

Hanft JM, Wych RD (1982) Visual indicators of physiological maturity of hard red spring wheat. Crop Sci 22:584–588

Kindred DR, Gooding MJ, Ellis RH (2005) Nitrogen fertilizer and seed rate effects on Hagberg falling number of hybrid wheats and their parents are associated with α–amylase activity, grain cavity size and dormancy. J Sci Food Agr 85:727–742

Lawson JA (1989) The Effect of propiconazole on the physiology and pathology of winter wheat. PhD Thesis. Harper Adams University College

Lupton FGH (1972) Further experiments on photosynthesis and translocation in wheat. Ann Appl Biol 71:69–71

Matthews GA (2000) Pesticide application methods, 3rd edn. Blackwell Science, Oxford

Peña R, Trethowen R, Pfieffer WH, van Ginkel M (2002) Quality (end-use) improvement in wheat: Compositional, genetic and environmental factors. J Crop Prod 5:1–37

Pepler S (2004) Flag leaf life extension by fungicide-use and the formation of yield and quality in wheat grains. PhD Thesis. The University of Reading

Pepler S, Gooding MJ, Ford KE, Ellis RH (2005*a*) A temporal limit to the association between flag leaf life extension by fungicides and wheat yields. Eur J Agron 22:365–376

Pepler S, Gooding MJ, Ford KE, Ellis RH, Jones SA (2005*b*) Delaying senescence of wheat with fungicides has interacting effects with cultivar on sulphur concentration but not with nitrogen: sulphur ratios. Eur J Agron 22:407–418

Pepler S, Gooding MJ, Ellis RH (2006) Modelling simultaneously water content and dry matter dynamics of wheat grains. Field Crop Res (In press)

Ruske RE, Gooding MJ, Jones SA (2003*a*) The effects of triazole and strobilurin fungicide programmes on nitrogen uptake, partitioning and grain N accumulation in winter wheat cultivars. J Agr Sci 140:395–407

Ruske RE, Gooding MJ, Jones SA (2003*b*) The effects of adding picoxystrobin, azoxystrobin and nitrogen to a triazole programme on disease control, flag leaf senescence, yield and grain quality of winter wheat. Crop Protect 22:975–987

Ruske RE, Gooding MJ, Dobraszczyk BJ (2004) Effects of triazole and strobilurin fungicide programmes, with and without late-season nitrogen fertiliser on the baking quality of Malacca winter wheat. J Cereal Sci 40:1–8

Salmon SE, Cook RJ (1987) Effects of fungicides on the milling and baking quality of wheat. Aspect Appl Biol 15, Cereal Quality, pp 373–384

Schnyder H, Baum U (1992) Growth of the grain of wheat (Triticum aestivum L.). The relationship between water content and dry matter accumulation. Eur J Agron 1:51–57

Singh BK, Jenner CF (1982) A modified method for the determination of cell number in wheat endosperm. Plant Sci Lett 26:273–278

Sylvester-Bradley R, Stokes DT, Scott RK (2001) Dynamics of nitrogen capture without fertilizer: the baseline for fertilizing winter wheat in the UK. J Agr Sci 136:15–33

VSN International Ltd (2002) Genstat Release 6.1. Reference Manual. VSN International, Oxford

Zadoks JC, Chang TT, Konzak CF (1974) A decimal code for the growth stages of cereals. Weed Res 14:415–421

Zhao F, Salmon SE, Withers PJA, Evans EJ, Monaghan JM, Shewry PR, McGrath SP (1999) Responses of breadmaking quality to sulphur in three wheat varieties. J Cereal Sci 79:1865–1874

GENETIC IMPROVEMENT OF WHEAT YIELD POTENTIAL IN NORTH CHINA

Y. ZHOU[1], Z.H. HE[1,2,*], X.M. CHEN[1], D.S. WANG[1], J. YAN[1], X.C. XIA[1] AND Y. ZHANG[1]

[1] *Institute of Crop Science/National Wheat Improvement Center, Chinese Academy of Agricultural Sciences (CAAS)*

[2] *CIMMYT, c/o CAAS, Zhongguancun South Street 12, 100081, Beijing, China*

[*] *E-mail:zhhe@public3.bta.net.cn*

Abstract: During 2001–03 seasons, three trials including 11 varieties from Hebei Province, 15 varieties from Shandong Province, and 11 varieties from Henan Province were sown in Shijiazhuang, Jinan, and Zhengzhou, respectively. All tested genotypes are the major varieties from 1970s to present. A randomized block design with three replicates was employed. Measurements consisted of heading date, plant height, spike number m^{-2}, thousand kernel weight, kernel number and kernel weight per spike, yield, biomass, and harvest index. In addition, SDS-PAGE and SSR markers were used to detect the presence of 1B/1R translocation, and distribution of dwarfing genes, respectively. Results indicate that annual genetic gain in yield potential is 0.54% (35.4 kg ha^{-1} $year^{-1}$, $P < 0.05$), 0.48% (32.0 kg $ha^{-1}year^{-1}$, $P < 0.01$), and 1.05% (72.1 kg ha^{-1} $year^{-1}$, $P < 0.01$), in Hebei, Shandong, and Henan, respectively. In all three trials, yield is positively and significantly associated with kernel weight per spike ($r = 0.77$, 0.73, and 0.75, in Shijiazhuang, Jinan, and Zhengzhou, respectively, the same order below, $P < 0.05$), biomass ($r = 0.72$, 0.70, 0.80, $P < 0.05$) and harvest index ($r = 0.86$, 0.67, 0.76, $P < 0.05$). Significant and negative association is also observed between yield and plant height in Shijiazhuang and Zhengzhou, but not in Jinan. However, positive and significant association between yield and kernel number per spike is presented in Shijiazhuang and Jinan, but not in Zhengzhou. Use of dwarfing genes and 1B/1R translocation are the major factors for yield improvement. Frequencies of *Rht-D1b*, *Rht-B1b*, and *Rht8c* are 67.6%, 16.2%, and 43.2%, respectively

Keywords: yield potential, harvest index, 1B/1R translocation

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 583–589.

INTRODUCTION

China is the largest wheat producer in the world, with annual acreage of more than 25 million hectare and production about 94 million ton. Improvement of yield potential has been the major breeding objective in north China where facultative wheat is grown and contributes more than 60% of national wheat production. Genetic improvement of wheat yield potential have been documented in Britain (Austin et al. 1980), France (Brancourt-Hulmel et al. 2003), United States (Donmez et al. 2001), Canada (McCaig and DePauw 1995), Australia (Siddique et al. 1989), and Mexico (Ortiz Monasterio et al. 1997). The yield increase is mainly associated with the reduction of plant height, which results in the increase of harvest index (HI), and the increase of kernels m^{-2}. They provide valuable information for determining strategies in breeding programs. However, such information is not available for Chinese wheats. Therefore, major varieties from three leading wheat producing Provinces in north China from 1970s to present, were used to characterize the genetic gain in wheat yield potential and associated traits.

MATERIALS AND METHODS

From 2001–03 seasons, three trials including 11 varieties from Hebei Province, 15 varieties from Shandong Province, and 11 varieties from Henan Province, were sown in Shijiazhuang (lat. 38°04′N, long. 114°26′E, 81.8 masl), Jinan (lat. 36°41′N, long. 116°59′E, 51.6 masl) and Zhengzhou (lat. 34°43′N, long.113°39′E, 110.4 masl) respectively. All tested genotypes are the major varieties from 1970s to present, with detailed information in Table 1. A randomized block design with three replicates was used, each plot consisted of six rows of 7.0 m length. Seeding rates were determined according to local practices, about 270 plants m^{-2} in Shijiazhuang and 180 plants m^{-2} in both Jinan and Zhengzhou, respectively.

The soil types at the three locations are all sandy clay, high in organic matter and slightly alkaline (pH8.0, 8.1, 8.2). Prior to planting, ammonium phosphate (ca170 kg ha^{-1} of P, 67 kg ha^{-1} of N), urea (ca13 kg ha^{-1} of N) and potassium chloride (ca 75 kg ha^{-1} of K) were broadcasted and incorporated. A further 120 kg ha^{-1} of N was top-dressed at shooting stage (Zadoks stage 30). Irrigations were provided at stages of tillering, stem elongation, booting, and grain filling. Nets with a mesh size of approximately 20 by 20 cm, through which the young crop plants grew, were used to prevent lodging. Fungicides and pesticides were applied at shooting, booting and grain filling stages to prevent diseases and pests. Weeds were controlled by hand. Heading date and plant height was recorded, respectively. After physiological maturity, two 0.5-m-long rows in the middle of each plot, as samples, were cut with sickle at the soil level, the remain four central rows were hand harvested, threshed, dried and weighted to give grain yield. The subsumples were used to record biomass, harvest index, spikes m^{-2}, kernels per spike, and kernels m^{-2}. Data was analyzed by SAS (SAS Institute Inc. 1988). Genetic gain of yield and yield related traits were calculated based on the method used by Ortiz Monasterio et al. (1997).

Table 1. Varieties used in this study and the presence of dwarf gene and 1B/1R translocation

Variety	Year released	Dwarf gene	1B/1R*	Yield (t ha^{-1})**
Trial I				
Shijiazhuang 54	1964	*Rht8c*	N	5.90c
Jimai 3	1973	*Rht8c*	N	5.29d
Jimai 7	1977	*Rht-D1b*	N	5.18d
Jimai 24	1983	*Rht-D1b*	Y	6.53ab
Jimai 26	1982	*Rht-D1b*	Y	6.49ab
Jimai 30	1984	*Rht-B1b+Rht-D1b*	Y	6.20bc
Jimai 36	1988	*Rht-D1b*	Y	6.66a
Jimai 38	1993	*Rht-B1b*	N	6.51ab
Heng 4041	1994	*Rht-B1b*	Y	6.60ab
Henong 326	1994	–	Y	6.21bc
Shi 4185	1995	*Rht-B1b+Rht-D1b*	Y	6.58ab
Trial II				
Jinan 2	1960	*Rht8c*	N	6.10def
Taishan 1	1969	*Rht8c*	N	6.41cde
Taishan 4	1970	*Rht-D1b*	N	5.60ef
Yannong 15	1976	*Rht8c*	N	6.15def
Jinan 13	1977	*Rht-D1b*	N	7.10abc
Shannongfu 63	1978	*Rht-D1b*	N	5.95ef
Lumai 1	1981	*Rht-D1b*	Y	5.96ef
Lumai 7	1981	*Rht8c+Rht-D1b*	Y	7.40a
Lumai 14	1986	*Rht-B1b*	Y	6.48bcde
Jinan 16	1987	*Rht-D1b*	Y	6.95abc
Lumai 21	1991	*Rht8c+Rht-D1b*	N	6.84abcd
Lumai 22	1991	*Rht8c+Rht-D1b*	N	7.12abc
Jinan 17	1993	*Rht-D1b*	N	6.67abcde
Jimai 19	1999	*Rht-D1b*	N	7.28ab
95(6)161	2000	*Rht8c+Rht-D1b*	Y	7.13abc
Trial III				
Bonong 7023	1970	*Rht8c*	N	5.99f
Zhengyin 1	1972	*Rht8c*	N	6.36ef
Bainong 3217	1975	*Rht-D1b*	N	6.51e
Xian 8	1978	*Rht8c+Rht-D1b*	N	6.63de
Yumai 2	1979	*Rht-D1b*	N	7.46ab
Yumai 18	1982	*Rht8c+Rht-D1b*	N	7.18bc
Yumai 17	1986	*Rht8c+Rht-B1b*	Y	6.95cd
Yumai 13	1987	*Rht-D1b*	Y	7.50ab
Yumai 21	1988	*Rht8c+Rht-D1b*	Y	7.67a
Yumai 34	1988	*Rht-D1b*	N	7.77a
Yumai 49	1993	*Rht8c+Rht-D1b*	N	7.59a

* N and Y indicate the non and presence of 1B/1R translocation.

** Different letters indicate significant difference at 5% probablity level.

– All three dwarf genes were not observed in Henong 326.

The presence of 1B/1R translocation was examined by SDS-PAGE and SCAR (Fransis et al. 1995), and distribution of *Rht8c* (Korzun et al. 1998), *Rht-D1b*, and *Rht-B1b* were also detected by SSR (Ellis et al. 2002).

RESULTS AND DISCUSSION

Average yield, presence of dwarfing genes and 1B/1R translocation of the tested genotypes in three trials are presented in Table 1. The genetic progresses are showed in Figs. 1, 2, and 3, for Provinces of Hebei, Shandong, and Henan, respectively. The annual genetic gain in yield is 0.54% ($35.4\,kg^{-1}ha^{-1}year$, $P < 0.05$), 0.48% ($32.0\,kg\ ha^{-1}\ year^{-1}$, $P < 0.01$) and 1.05% ($72.1\,kg\ ha^{-1}\ year^{-1}$, $P < 0.01$) in Provinces of Hebei, Shandong and Henan, respectively. Significant yield increase is observed in Hebei Province after 1980. It is associated with the use of 1B/1R translocation since all varieties except for Jimai 38 released after 1983 confer 1B/1R translocation. Yield potential has continued to increase in Shandong Province. Lumai 7 with late maturity released in 1981, has the highest yield, which is significant higher than Taishan 1, Yannong 15, Jinan 2, Lumai 1, Shannongfu 63, and Taishan 4. This could also be linked with use of 1B/1R translocation since Lumai 1, Lumai 7, Lumai 14, and Jinan 16 all confer 1B/1R translocation. However, Lumai 21, Lumai 22, Jinan 17, and Jimai 19 released from 1996 to 2001, do not confer 1B/1R translocation due to the shift of breeding objectives from yield to processing quality. 95(6) 161, a high yielding advanced line, was not officially released. Jinan 17 was released in 1993 due to its outstanding breadmaking quality. However, Jimai 19, the most popular variety in Shandong Province at present, is a landmark variety since it ranks the second position in yield, but also shows outstanding noodle quality. Yield potential has also continued to increase in Henan Province. Yumai 34, Yumai 21, and Yumai 49, released after 1994, show the highest yield performance. Yield of Yumai 13 and Yumai 2 are not significant different from that of Yumai 34, Yumai 21, and Yumai 49, but significant higher than other varieties released before 1990 except for Yumai 18. Therefore, Yumai 2, released in 1983, is a landmark variety for yield improvement in Henan Province. It is also observed that 1B/1R translocation is present in Yumai 13, Yumai 17, and Yumai 21. Yumai 34 confer excellent pan bread and noodle quality, and Yumai 49 is outstandingg for noodle quality. Therefore, 1B/1R translocation has contributed to wheat yield improvement in north China.

Increase of thousand kernel weight in three Provinces is not significant. The yield improvement in Hebei Province is characterized with significant increase of kernels per spike (1.03% annually, $P < 0.01$) and kernel weight per spike (0.78% annually, $P < 0.01$). In Shandong Province, it is characterized with significant decrease of spike number m^{-2} (−0.74% annually, $P < 0.05$), and significant increase of kernels per spike (0.54% annually, $P < 0.05$) and kernel weight per spike (0.78% annually, $P < 0.01$). However, only significant increase for spike number m^{-2} (0.59% annually, $P < 0.05$) is observed in Henan Province.

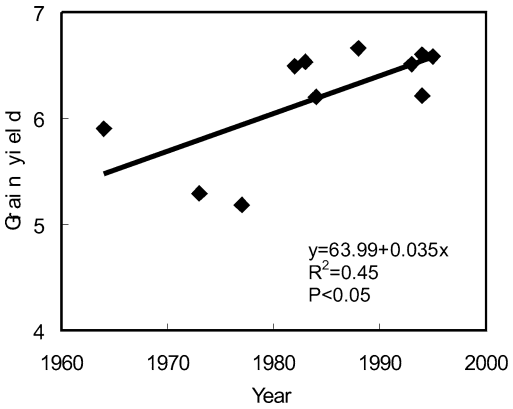


Figure 1. Genetic progress of 11 varieties in Hebei Province

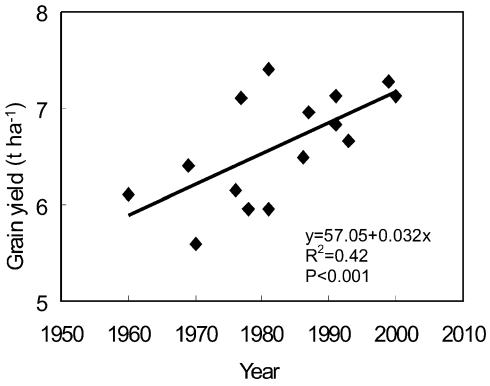


Figure 2. Genetic progress of 15 varieties in Shandong Province

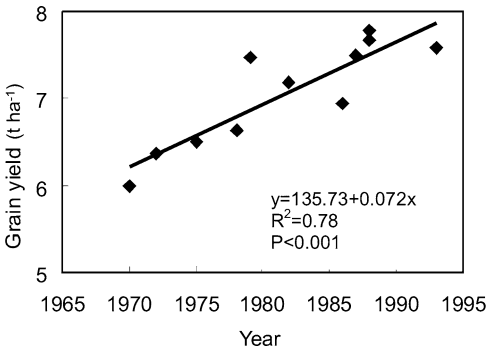


Figure 3. Genetic progress of 11 varieties in Henan Province

Plant height has reduced significantly in all three Provinces from 90–100 cm in 1970s to 75–85 cm at present. *Rht 8c* alone is presented in most varieties in late 1960s and early 1970s, but *Rht-D1b* alone or in combination with *Rht 8c* is commonly presented in varieties released after mid 1970s. Both *Rht-B1b* and *Rht-D1b* are presented in Jimai 30 and Shi 4185. In the tested 37 genotypes, frequencies of *Rht-D1b*, *Rht-B1b*, and *Rht8c* are 67.6%, 16.2%, and 43.2%, respectively. The change of biomass is not significant in Hebei and Shandong, but significant increase in Henan Province (0.54% annually, $P < 0.01$). However, harvest index shows significant increase in all three trials, i.e., 0.47% ($P < 0.01$), 0.46% ($P < 0.01$), and 0.50% ($P < 0.05$) annually in Hebei, Shandong, and Henan.On average, harvest index increases from 34–44% in mid 1960s-1970s to 45–50% in 1980s and afterwards.

In all three trials, yield is positively and significantly associated with kernel weight per spike ($r = 0.77$, 0.73, and 0.75, in Hebei, Shandong, and Henan, respectively, the same order below, $P < 0.05$), biomass ($r = 0.72$, 0.70, 0.80, $P < 0.05$) and harvest index ($r = 0.86$, 0.67, 0.76, $P < 0.05$). Significant and negative association is also observed between yield and plant height in Hebei and Henan, but not in Shandong. However, positive and significant association between yield and kernel number per spike are presented in Hebei and Shandong, but not in Henan.

ACKNOWLEDGEMENTS

The authors are grateful to Ms. Li Xingpu, Ms. Lan Suque, Mr. Huang Chengyan, Mr. Liu Jianjun, Ms. Sui Xinxia, Dr. Xu Weigang, Dr. Hu lin, Mr. Shen Tianmin, Mr. Xu Caizhi, and Mr. Lu Pingan for their invaluable assistance in field trials, and to Dr. Xia Lanqin and Dr. Zhang Xiaoke for their support in laboratory operation.

REFERENCES

Austin RB, Bingham J, Blackwell RD, Evens LT, Ford MA, Morgan CL, Taylor M (1980) Genetic improvement in winter wheat yields since 1900 and associated physiological changes. J Agri Sci (Cambridge) 94:675–689

Brancourt Hulmel M, Doussinault G, Lecomte C, Berard P, Le Buanec B, Trottet M (2003) Genetic improvement of Agronomic traits of winter wheat cultivars released in France from 1946 to 1992. Crop Sci 43:37–45

Donmez E, Sears RG, Shroyer JP, Paulsen GM (2001) Genetic gain in yield attributes of winter wheat in the Great Plains. Crop Sci 41:1412–1419

Ellis MH, Spielmeyer KR, Gale GJ, Rechards RA (2002) Perfect markers for the *Rht-B1b* and *Rht-D1b* dwarfing genes in wheat. Theor Appl Genet 105:1038–1042

Fransis HA, Leitch AR, Koebner RMD (1995) Conversion of a RAPD-generated PCR product, containing a novel dispersed repetitive element, into a fast robust assay for the presence of rye chromatin in wheat. Theor Appl Genet 90:636–642

Korzun V, Roder MS, Ganal MW, Worland J (1998) Genetic analysis of the dwarfing gene (*Rht8*) in wheat. Part I. Molecular mapping of *Rht8* on the short arm of chromosome 2D of bread wheat (*Triticum aestivum* L.). Theor Appl Genet 96:1104–1109

McCaig TN, DePauw RM (1995) Breeding hard red spring wheat in western Canada: historical trends in yield and related variables. Can J Plant Sci 75:387–393

Ortiz Monasterio R, Sayre KD, Rajaram S, McMahon M (1997) Genetic progress in wheat yield and nitrogen use efficiency under four nitrogen rates. Crop Sci 37:898–904

SAS Institute (1988) SAS user's guide: statistics. SAS Institute, Cary, NC

Siddique KHM, Belford RK, Perry MW, Tennant D (1989) Growth, development and light interception of old and modern wheat cultivars in a Mediterranean-type environment. Aust J Agri Res 40:473–487

PHYSIOLOGICAL PROCESSES ASSOCIATED WITH WINTER WHEAT YIELD POTENTIAL PROGRESS

M.J. FOULKES[1,*], J.W. SNAPE[2], V.J. SHEARMAN[1] AND R. SYLVESTER-BRADLEY[3]

[1]*Division of Agricultural & Environmental Sciences, University of Nottingham, Sutton Bonington Campus, Leicestershire, LE12 5RD, UK*

[2]*John Innes Centre, Crop Genetics Department, Colney Lane, Norwich, NR4 7UH, UK*

[3]*ADAS Boxworth, Cambridge CB3 8NN, UK*

[*] *E-mail: John.Foulkes@nottingham.ac.uk*

Abstract: Knowledge of the changes in physiological traits associated with genetic gains in yield potential is essential to improve understanding of yield-limiting factors and to inform future breeding strategies. The growth and development of eight representative UK wheat (*Triticum aestivum* L.) cultivars introduced from 1972 to 1995 was examined in field experiments in 1997–1999. Significant genetic changes over time and positive correlations with grain yield were found for pre-anthesis radiation-use efficiency (0.012 g MJ^{-1} yr^{-1}; RUE) and water soluble carbohydrate (WSC) content of stems and leaf sheaths at anthesis (4.6 g m^{-2} yr^{-1}). Results suggested that yield of modern UK cultivars although still sink limited might be closer to source-limitation than their predecessors. Therefore, breeders may eventually need to take further steps to increase source size post-anthesis alongside improvements in grain sink size. In this respect, greater accumulation of stem carbohydrate reserve may be beneficial, providing this is not competitive with ear growth. Results further suggested that the 1BL.1RS wheat-rye translocation may be associated with greater harvest biomass in modern cultivars. The relationship between the amount of stem WSC measured shortly after flowering and grain yield was further tested in two doubled haploid (DH) populations, Rialto x Spark and Beaver x Soissons in 2001 and 2002. There was a positive linear relationship between stem WSC and grain yield in both populations. The effects of 1BR.1RS were further examined in two DH populations, Beaver (1BL.1RS) x Soissons (1B) and Drake-sib (1BL.1RS) x Welton (1B), in 1998–2002. 1BL.1RS increased harvest biomass in both populations, although grain yield was only increased in the Beaver x Soissons population. In the Drake-sib x Welton population, the physiological basis of the increased harvest biomass was investigated, and found to be associated with greater pre-anthesis biomass. There was no change in green area index or light extinction coefficient in the pre-anthesis period indicating 1BL.1RS may have conferred an increase in RUE

Keywords: yield potential, harvest biomass

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 591–597.

INTRODUCTION

Knowledge of the changes in physiological traits associated with genetic gains in yield potential is essential to improve understanding of yield-limiting factors and to inform future breeding strategies. The genetic gain in yield potential in high output wheat systems worldwide since the mid 1960s has been approximately 1% per year, e.g. Mexico (Sayre et al. 1997) and the UK (Austin et al. 1989). Studies of the physiological basis of these genetic gains have generally shown grains m^{-2} (usually due to grains ear^{-1}) and Harvest Index (HI) to be most closely correlated with grain yield; in part contributed by the semi-dwarf cultivars, introduced in the 1960's and 1970's. In temperate North Western Europe, an upper limit for HI may soon be reached, estimated to be about 0.6 for winter wheat, and UK winter wheats have already achieved close to this, e.g. 0.61 for Consort (Spink et al. 2000). Thus future gains in yield may increasingly depend upon achieving greater harvest biomass, whilst maintaining HI.

During the 1990s biomass increases were reported attributed to introductions of alien chromatin into wheat germplasm, e.g. the 1BL.1RS translocation in the Great Plains (Carver and Rayburn 1994), and the 7DL.7Ag wheat-*Agropyron elongatum* translocation in Mexico (Reynolds et al. 2001). Current harvest indices for wheat in the UK are of the order of 0.5, hence possibilities for improvements in ear partitioning probably still exist, e.g. by lengthening the sub-phase of rapid ear growth from the terminal spikelet stage to anthesis (Slafer et al. 2005). However, future increases in anthesis ear biomass might increasingly depend on the ability of breeders to increase anthesis above-ground biomass. Assuming no change in the root: shoot DM ratio, this will require improved pre-anthesis radiation interception and/or radiation-use efficiency (above-ground DM production per unit radiation interception). Prospects for increasing interception may be limited in high output environments, since interception during the rapid ear-growth phase is already close to maximal in modern cultivars (e.g. Foulkes et al. 2001). RUE therefore seems the more promising target.

The objectives of the current paper are to: (i) examine changes in physiological traits associated with genetic gains in yield potential in UK winter wheat in recent years, (ii) test associations between specific traits (harvest biomass and stem WSC reserves) and between the 1BL.1RS translocation and yield potential in DH populations derived from contrasting parents and (iii) consider future prospects for combinations of traits to raise yield potential in UK wheat.

MATERIALS AND METHODS

The growth and development of eight representative UK wheat (*Triticum aestivum* L.) cultivars introduced from 1972 to 1995 was examined in field experiments at Sutton Bonington in 1996/7–1998/9, as described by Shearman et al. (2005). Changes in grain yield, number of grains per square meter, HI and harvest AGDM as well as developmental rates, green area production, radiation capture

and radiation-use efficiency were quantified. The effects of the 1BL.1RS translocation on yield potential and the relationship between stem WSC reserves and yield potential were investigated using three DH populations, Drake-sib (1BL.1RS) x Welton (1B), Rialto (1BL.1RS) x Spark (1B) and Beaver (1BL.1RS) x Soissons (1B), in field experiments at Sutton Bonington and ADAS Gleadthorpe, Nottinghamshire during 1997/8 to 2001/2, as described by Foulkes et al. (2002).

RESULTS AND DISCUSSION

Trends in Yield Potential and Traits with Year of Release

The growth and development of eight representative cultivars introduced from 1972 to 1995 (one tall *rht-D1b* cultivar, Maris Huntsman, and seven *Rht-D1b*, formerly *Rht2*, semidwarf cultivars) was examined (Table 1). The three most recently introduced cultivars contained the 1BL.1RS translocation, whereas others did not. A linear genetic gain in grain yield of 0.12 t ha^{-1} yr^{-1} (1.2% yr^{-1}) was positively correlated with both harvest index and above-ground biomass; progress in harvest index was most apparent during the earlier phase of the 23-year period, whereas biomass contributed most since about 1983. There was a linear increase over time of 217 grains m^{-2} yr^{-1}, but no change in grain weight. Significant genetic changes over time and correlations with grain yield were also found for pre-anthesis radiation-use efficiency (0.012 g MJ^{-1} yr^{-1}) and WSC content of stems and leaf sheaths at anthesis (4.6 g m^{-2} yr^{-1}). It is suggested that the 1BL.1RS translocation may be associated with greater pre-anthesis RUE and harvest biomass in modern cultivars.

Table 1. Grain yield, above-ground dry matter (AGDM), radiation-use efficiency (RUE_{PAR}) from GS31 to GS61, stem WSC at GS61 + 75 °Cd and flag leaf area (FLA) for eight wheat cultivars. Values represent means across 1997 and 1998; except for FLA measured in 1999, after Shearman et al. (2005)

Cultivar (year of release)	Grain t ha^{-1}	AGDM t ha^{-1}	HI	RUE_{PAR} g MJ^{-1}	Stem WSC g m^{-2}	Flag leaf GS39 cm^2
Maris	8.75	17.85	0.423	2.33	244	38.2
Avalon (1980)	9.43	17.29	0.467	2.34	255	39.7
Norman (1981)	10.23	17.98	0.484	2.43	268	33.4
Galahad (1983)	9.56	17.05	0.477	2.50	294	29.7
Riband (1989)	10.64	18.35	0.495	2.41	257	24.7
Haven (1990)	11.40	19.31	0.503	2.63	391	25.7
Brigadier	11.23	18.97	0.504	2.47	284	24.4
Rialto (1995)	11.31	20.14	0.478	2.64	364	22.1
Mean	10.32	18.37	0.479	2.47	295	29.7
SED, DF	0.428 28	0.541	0.0210	0.090	19.20, 28	2.38, 14

Effects of Stem WSC Reserves and Presence of 1BL.1RS in DH Populations on Yield Potential

The relationship between the amount of stem WSC measured shortly after flowering and grain yield was tested in two DH populations, Rialto x Spark in 2001 and Beaver x Soissons in 2002, in experiments located at ADAS Gleadthorpe, Nottinghamshire. Lines differed in the range 1.71–4.26 t ha^{-1} in the Beaver x Soissons population and 1.52–4.23 t ha^{-1} in the Rialto x Spark population ($P < 0.001$). There was a positive linear relationship between stem WSC and grain yield in both populations ($P < 0.10$, $P < 0.001$, respectively; Fig. 1).

These results confirmed the importance of the contribution of stem reserves to grain yield potential in modern UK winter wheat cultivars even under favourable post-anthesis conditions. Unlike in semi-arid environments, the remobilization of reserves appears to be a constitutive trait in UK conditions. Although stem reserves were not measured at harvest in the work reported here, Foulkes et al. (1998) showed these amounts to be consistently very low (< 0.25 t ha^{-1}) for 17 cultivars tested in the UK environment.

The effect of the 1BR.1RS translocation was examined in two DH populations, Beaver (1BL.1RS) x Soissons (1B) at ADAS Gleadthorpe, Nottinghamshire and Drake-sib (1BL.1RS) x Welton (1B) at Sutton Bonington, Nottinghamshire. In the Beaver x Soissons population, 1BL.1RS increased harvest biomass by 0.66 t ha^{-1} in 2001 ($P < 0.05$; Table 2) resulting in increased yield ($P < 0.01$), but effects of 1BL.1RS were neutral in 2002. In the Drake-sib x Welton population, 1BR.1RS on average increased harvest biomass by 55 g m^{-2} ($P < 0.001$), but this did not result in increased grain yield (Table 3). The physiological basis of the increased harvest biomass was investigated, and found to be associated with greater pre-anthesis biomass ($P < 0.01$; Table 3). The greater pre-anthesis biomass was produced from a similar GAI (Table 3) and light extinction coefficient at GS55 (data not shown) consistent with the suggestion above that pre-anthesis RUE may be increased in the presence of 1BL.1RS. Effects of 1BL.1RS on flowering were small (1 day's delay to GS61 with 1BL.1RS, data not shown) and could not account for the differences in pre-anthesis growth observed.

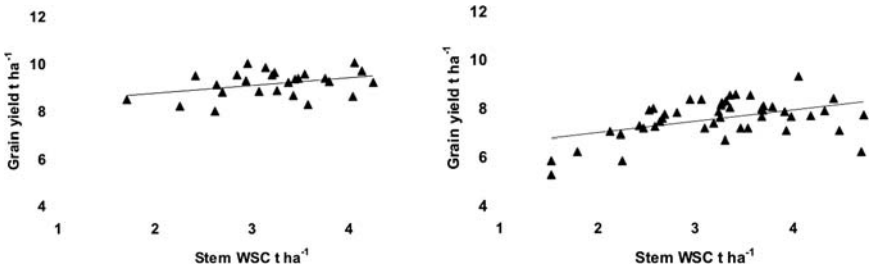


Figure 1. Linear regression of WSC in stems and leaf sheaths at GS61 + 75 °Cd (base temp. 0 °Cd) on grain yield (t ha^{-1} 85% DM) in a) Rialto x Spark lines in 2001 ($R^2 = 0.13$, $y = 0.32x–8.10$, $P < 0.10$) and b) Beaver x Soissons lines in 2002 ($R^2 = 0.22$, $y = 0.47x + 6.03$, $P < 0.001$)

Table 2. Gain yield (t ha^{-1} 85% DM) and above-ground DM (t ha^{-1} 100% DM) for groups[a] of 1BL.1RS and 1B DH lines derived from Beaver (1BL.1RS) x Soissons (1B) at Gleadthorpe, Nottinghamshire

	2001		2002		Year	±1BL.1RS	Year x ± 1BL.1RS
	1BL.1RS	1B	1BL.1RS	1B			
AGDM 100% DM t ha^{-1}	16.02	15.36	12.91	13.04	*	NS	*
Grain yield t ha^{-1} 85% DM	9.10	8.87	7.48	7.63	*	NS	**

[a] 2001: 16 1BL.1RS and 18 1B lines; 2002: 24 1BL.1RS and 22 1B lines

Traits for Raising Yield Potential in UK Winter Wheat in Future Years

More uniform rooting with depth

The genetic gains in above-ground biomass, assuming no change in root:shoot ratio or water-use efficiency, could result in yield being increasingly limited by ability to access water and/or N in future years.Therefore some attention should be focused on optimising rooting systems for most efficient below-ground resource capture. We have recently developed a quantitative model (King et al. 2003) examining the effects of altering rooting traits, including cumulative root length density distribution with depth, on water and N uptake. A parameter β describing cumulative root distribution with depth had a profound effect on N capture during grain filling, with more uniform root distribution with depth conferring greater resource capture. It may be that distributing roots more evenly with depth is part of an ideotype for maximizing yield potential in the UK, and traits favouring this pattern of rooting should be investigated.

Table 3. Grain yield (t ha^{-1} 100% DM) and above-ground DM (t ha^{-1} 100% DM) at harvest, and ear number, green area index and above-ground DM at ear emergence (GS55) for groups[a] of 1BL.1RS and 1B DH lines derived from Drake-sib (1BL.1RS) x Welton (1B) cross. Values at harvest represent means across 6 experiments (2 expts in each of 1998, 1999 and 2000). Values at GS55 represent means across 3 of the 6 experiments (1 expt in each of 1998, 1999 and 2000) at Sutton Bonington

1BL.1RS group	GS55			Harvest	
	Ears m^{-2}	Green area Index	AGDM g m^{-2}	AGDM g m^{-2}	Grain DM t ha^{-1}
1BL.1RS	648	7.43	1081	2206	10.57
1B	635	7.27	1047	2151	10.62
1BL.1RS VR, df = 1	1.58 NS	0.99 NS	7.03**	14.6***	1.3 NS

[a] 10 1BL.1RS and 10 1B lines

Extending the stem elongation period

Several studies point to the potential advantages of increasing the proportion of thermal time to anthesis accounted for by the stem elongation period to increase ear partitioning and ear biomass (Sylvester Bradley et al. 2005, Slafer et al. 2005). Delaying flowering significantly in the UK could increase grain losses associated with late harvesting, so advancing GS31 whilst maintaining GS61 is the most realistic target. An extended stem elongation period should simultaneously favour greater ear biomass, stem WSC and crown root growth at anthesis. To explore the dominant climatic influences on UK wheat yields, we have related annual deviations from the linear trend in national average winter wheat yields between 1978 and 2002 to weather data for each cropping year (September to August) (Sylvester Bradley et al. 2005). The parameter showing the best correlation with yield was the photo-thermal quotient during April. Since onset of stem elongation typically occurs in UK winter wheat around the beginning of April, this confirms the critical importance of the period of stem elongation in determining attainable yield in the UK environment. Further studies in collaboration with UK breeders seem justified to establish the potential value of this trait in future breeding programmes.

Increasing pre-anthesis RUE

In our investigation on the set of eight historic UK cultivars, we found higher RUE to be associated with smaller flag leaf size (Table 1) and increased flag leaf specific weight (data not shown). Smaller flag leaf area in modern cultivars may have reduced saturating light intensities at the top of the canopy and improved distribution of light to lower leaves. Greater flag leaf specific dry weight may indicate increased photosynthetic tissues per unit leaf area and hence improved RUE. Selection for these leaf traits may offer scope for further improvement in pre-anthesis RUE. Estimating RUE in breeders' plots is infeasible, so further work seems justified to identify surrogates or 'smart-screens' for RUE, including genetic markers, and to develop appropriate protocols for their use in breeding programs in the UK environment.

Post-anthesis traits (culm leaf longevity/grain weight potential)

In the UK environment, we have failed to show a positive association between flag-leaf green area duration and yield under favourable post-flowering conditions (Verma et al. 2004). This appears to confirm that yield potential is still predominantly sink-limited under optimal conditions. Increasing grain weight potential could possibly exploit the apparent genetic differences in stay green. In the UK and worldwide, there has been little genetic change over time in grain weight potential. This may be partly because there is still a poor understanding of the causes determining this trait. Grain weight potential appears to be source-limited, both in the periods immediately prior to anthesis (Calderini and Reynolds 2000) and post-anthesis (Fischer and HillResLambers 1978), although the extent of genetic diversity in this source limitation has not been well characterised. Synthetic hexaploids developed by CIMMYT provide a source of genetic variation for final grain weight.

Further studies to define available genetic variation and elucidate the genetic control of grain weight potential would be useful in establishing the potential value of this trait in future breeding programmes.

ACKNOWLEDGEMENTS

We thank UK government Department of Environment, Food and Rural Affairs for funding projects OC9602, CC0370, AR9008 and IS0210 and the PhD studentship of Vicky Shearman.

REFERENCES

Austin RB, Ford MA, Morgan CL (1989) Genetic improvement in the yield of winter: a further evaluation. J Agr Sci (Cambridge) 112:295–301

Calderini DF, Reynolds MP (2000) Changes in grain weight as a consequence of degraining treatments at pre- and post-anthesis in synthetic hexaploids (*Triticum durum x T. tauschii*). Aust J Plant Physiol 27:183-191

Carver BF, Rayburn AL (1994) Comparison of related wheat stocks possessing 1B or 1RS.1BL chromosomes: agronomic performance. Crop Sci 34:1505–1510

Fischer RA, HillResLambers D (1978) Effect of environment and cultivar on source limitation to grain weight in spring wheats. Aust J Agr Res 29:443–458

Foulkes MJ, Spink J, Scott K, Clare R (1998) Varietal typing trials and NIAB additional character assessments. Home-Grown Cereals Authority Final Report 174 (V), HGCA, London

Foulkes MJ, Sylvester Bradley R, Scott RK (2001) The ability of wheat cultivars to withstand drought: resource capture. J Agr Sci (Cambridge) 137:1–16

Foulkes MJ, Sylvester Bradley R, Snape J (2002) Maintaining wheat performance through improved exploitation of drought resistance traits. Final report CE0370, HMSO, London, pp 20

King J, Gay A, Sylvester Bradley R, Bingham I, Foulkes MJ, Gregory P, Robinson R (2003) Modelling cereal root systems for water and nitrogen capture: towards and economic optimum. Ann Bot 91:383–390

Reynolds MP, Calderini DF, Condon AG, Rajaram S (2001) Physiological basis of yield gains in wheat associated with the LR19 translocation from *Agropyron elongatum*. Euphytica 119:137–141

Sayre KD, Rajaram S, Fischer RA (1997) Yield potential progress in short bread wheats in northwest Mexico. Crop Sci 37:36–42

Shearman VJ, Sylvester Bradley R, Scott RK, Foulkes MJ (2005) Physiological changes associated with wheat yield progress in the UK. Crop Sci 45:175–178

Slafer GA, Araus JL, Royo C, Garcia Del Moral L (2005) Promising eco-physiological traits for genetic improvement of cereal yields in Mediterranean environments. Ann Appl Biol 146:61–70

Spink JH, Foulkes MJ, Gay A, Bryson R, Berry P, Sylvester Bradley R, Clare RW, Scott RK (2000) Reducing winter wheat production costs through crop intelligence. Home-grown cereals authority project report no. 235, Home-Grown Cereals Authority, London

Sylvester Bradley R, Foulkes MJ, Reynolds MP (2005) Future wheat yields: evidence, theory and conjecture. In: Sylvester Bradley R, Wiseman J (eds) Yields of farmed species – constraints and opportunities. Nottingham University Press, Nottingham, pp 151–169

Verma V, Foulkes MJ, Caligari P, Sylvester Bradley R, Snape J (2004) Mapping quantitative trait loci for flag leaf senescence as a yield determinant in winter wheat under optimal and drought-stressed environments. Euphytica 135:255–263

VARIABILITY ON PHOTOPERIOD RESPONSES IN ARGENTINEAN WHEAT CULTIVARS DIFFERING IN LENGTH OF CROP CYCLE

D.J. MIRALLES[1,2,*] M.V. SPINEDI[1], L.G. ABELEDO[1] AND D. ABELLEYRA[1]

[1] *University of Buenos Aires, Dep. Plant Production. Av. San Martin 4453 (C1417 DSE), Buenos Aires, Argentina*

[2] *CONICET, (Consejo Nacional de Investigaciones Cientificas y Técnicas)*

[*] *E-mail: miralles@agro.uba.ar*

Abstract: Twenty facultative Argentinean commercial wheat cultivars classified as early and late maturity were grown during 2004 and exposed to different sowing dates from June to September under field conditions with the aim of establish how the duration of different pre-and post-flowering phases are affected when plants are exposed to a wide range of temperature and photoperiod regimes. The changes in the duration of the emergence-anthesis phase, measured in thermal time, between early and late maturity groups were mainly associated with differences in the photoperiod sensitivity (in average 74 and 165 °C d h^{-1} for early and late maturity groups, respectively) without significant differences in the optimum photoperiod (13.4 hs) and/or intrinsic earliness (in average 829 and 907 °C d for early and late maturity groups, respectively). Similar responses were observed in the two pre-anthesis phases analyzed. Because of only three cultivars evidenced vernalization requirements this trait seems not appear as an important attribute to be considered by breeding into the Argentinean commercial cultivars, with the aim of lengthening to the pre anthesis phases. The duration of the post-anthesis phase was less variable not only among cultivars, also between early and late maturity groups with an average duration of 450 °C d. The data obtained from this study allowed the construction of thermo-photoperiodic models and include this information into friendly software named CRONOTRIGO© (www.agro.uba.ar/catedras/cerealicultura/servicios.htm) for predicting the timing of phenological events as the beginning of stem elongation, flowering and physiological maturity

Keywords: early and late maturity, crop phenology, sowing date, wheat

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 599–609.

INTRODUCTION

Crop cycle in cereals is associated with a sequence of phenological events controlled by environmental factors, mainly temperature (including vernalization) and photoperiod (Slafer and Rawson 1994, Miralles and Slafer 1999). Although previous evidences demonstrated that photoperiod affects the rate of development primarily during the vegetative phases (Riddell et al. 1958), others works (Miralles and Richards 2000, Whitechurch and Slafer 2001) confirmed some suggestion of the literature indicating that photoperiod might be also important not only in the early (Slafer and Rawson 1994) also during the late reproductive phases (i.e during the spike growth period) when the number of grains is determined (Fischer 1993).

Cultivars with different photoperiod or vernalization sensitivity during different developmental phases may reduce or enlarge the duration of those phases exposing the critical periods for yield determination to different environmental conditions modifying thereby the yield components that are being determined in each particular phase (Miralles and Slafer 1999).

Previous evidences manipulating photoperiod artificially, carried out under controlled (Miralles et al. 2000) and field conditions (Gonzalez et al. 2005a) during the stem elongation period, determined that as photoperiod was shortened, duration of spike growth was lengthened with the consequence of an increased spike dry weight, which was positively associated with the number of grains per unit land. Following with this idea, and supported by the previous evidences, those cultivars with strong photoperiod sensitivity (or longer earliness per ser) during the stem elongation phase could enlarge this phase with beneficial effects on the number of grains. Management practices as sowing dates could expose the vegetative and reproductive phases to different environmental conditions enlarging or shortening the pre-anthesis phases and thereby promote or penalize, respectively, the yield.

In this scenario, it is clear that the duration of developmental phases has an important impact on yield, and it is necessary understand the genotype x environmental interaction for determining how the duration of the phases is altered by management practices, for instance different sowing dates. Additionally understand how and at what extend both genotype and environment affect the timing of phenological events is of paramount importance for both (i) calculating the risk of climatic conditions during phenological phases (eg. frost damage on heading) and (ii) organizing in advance management practices (eg. fertilization during tillering). In Argentina breeding companies offer more than 30 different commercial wheat varieties that can be classified as early (short cycle duration) and late maturity (long cycle duration) types, i.e. same flowering time but different duration of the crop cycle, but there is not information for farmer and/or technicians about the thermo-photoperiod responses to those cultivars.

This study was designed to establish how the duration of pre-and post-flowering phases is affected in early and late facultative commercial wheat cultivars available in the Argentinean market by different sowing dates, under field conditions, which combine a wide range of temperature and photoperiod regimes. The data obtained from this study allowed the construction of thermo-photoperiodic models and

include this information into friendly software for predicting the timing of phenological events as the beginning of stem elongation, flowering and physiological maturity.

MATERIALS AND METHODS

The experiment was carried out during the 2004 growing season in the experimental field of Department of Plant Production, Faculty of Agronomy, University of Buenos Aires (35° 35′ S, 59° 29′W, 25 m above sea level). The soil was classified as Vertic Argiudol (USDA Taxonomy). Plots were fertilized with urea to achieve 150 kg N ha^{-1} in the top 0–40 cm soil layer. Pests and weeds were periodically controlled, and rainfall was complemented by irrigation throughout crop cycle to avoid water stress.

The study consisted on a factorial combination of 20 commercial facultative wheat cultivars differing on the length of the whole cycle (10 classified as early maturity and 10 classified as late maturity- see Table 1) and six sowing dates (8 June, 26 June, 19 July, 9 August, 18 August and 18 September).

Within sowing dates, treatments (cultivars) were arranged in a randomized block design with two replicates per treatment. Cultivars were randomly sown in plots of 2 m long and 0,175 m apart where each genotype constituted a line within the plot. Density was 300 pl m^{-2} at emergence.

The number of leaves emerged on the main stem was registered twice weekly from seedling emergence to anthesis. Phyllochron was calculated as the ratio between the cumulative thermal time from emergence to fully flag leaf expanded and the final leaf number recorded in the crop. For phyllochron calculation a base of 0 °C was used. Phenology was registered by external observations recording the developmental stages of emergence (E), first visible node (1st Node), flag leaf emergence (FL), heading (H) and anthesis (An). Each stage was recorded when 50% of the plants reached the stage.

Table 1. Early and Late Cultivars included in the study

Commercial cultivars	
Early maturity	Late maturity
ACA 801	ACA 303
Baguette Premiun 13	Baguette 10
Buck Bigua	BIOINTA 2001
BIOINTA 1000	BIOINTA 3002
Klein Chaja	Klein Escorpion
Klein Don Enrique	Klein Escudo
Klein Flecha	Buck Guapo
Pro INTA Gaucho	Buck Guatimozin
Onix	INIA Tijetera
Klein Proteo	Klein Martillo

From anthesis, spikes were collected twice weekly and basal grains of central spikelets weighted until constant weight to determine the physiological maturity. Bi-linear models, by optimization techniques, were used to fit the relationship between dry grain weight and time (thermal and calendar) from anthesis.

Photo-thermal models were built for different phenological phases by fitting the duration of the phase measured in thermal units and the average photoperiod of the corresponding phase. Optimum photoperiod (i.e the photoperiod at which the rate of development for each phase reaches the maximum value) and intrinsic earliness (i.e. the minimum thermal time required at optimum photoperiod) was also determined. Base temperature for thermal time calculation in all phenological phases was 5 °C.

RESULTS

Results showed that 3 out of 20 cultivars evidenced vernalization requirements. Cultivars with vernalization requirements were Baguette 10 and Guapo, within the late maturity and BioINTA 1000 within the early maturity group.

All cultivars showed photoperiod sensitivity during the pre anthesis phases, i.e emergence-anthesis. However, cultivars classified as early and late maturity showed significant differences ($p < 0.05$) in photoperiod sensitivity being the early maturity cultivars less sensitive than those classified as late maturity. Averaging across cultivars photoperiod sensitivity for the emergence-anthesis phase were -74 and -165 °C d h^{-1} for early and late maturity groups, respectively (Fig. 1). Thermo-photoperiodic responses were different not only between groups but also among cultivars within each group of maturity. Thus, photoperiod sensitivity ranged

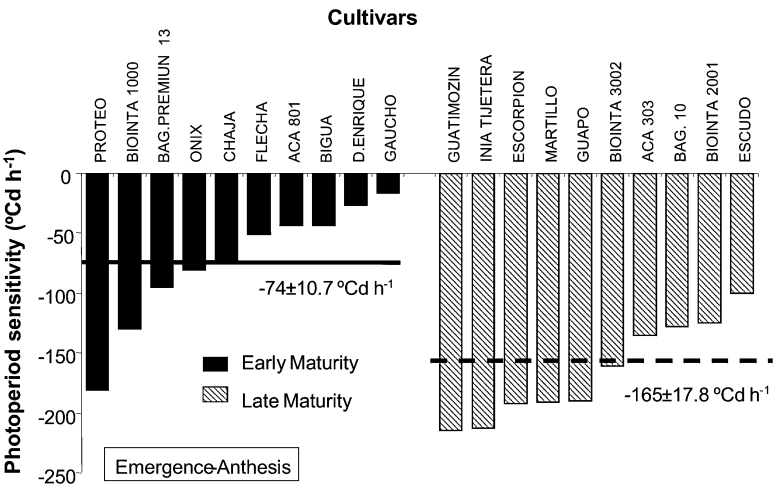


Figure 1. Photoperiod sensitivity for the emergence anthesis phase for early (black bars) and late (shadow bars) maturity cultivars. Bold and tagged lines indicate the average of photoperiod sensitivity for early and late maturity cultivars, respectively

between −20 (Gaucho) and −180 °C d h^{-1} (Proteo) for the early maturity, while the late maturity group ranged from −100 (Escudo) to −230 °C d h^{-1} (Guatimozin and Tijetera) (Fig. 1).

The same trend was also observed in the mostly vegetative and early reproductive phase (emergence-1st. Node). With the exception of Baguette 13, in average early maturity cultivars shortened the duration of the emergence-1st Node phase at a rate of −18 °C d h^{-1} while the average for photoperiod sensitivity in the late maturity group was −36 °C d h^{-1}. The lowest photoperiod sensitivity in both groups was close to −6 °C d h^{-1}in Gaucho and Escudo, for early and late maturity, respectively (Fig. 2). Both cultivars also recorded the lowest photoperiod sensitivity in the emergence-anthesis phase (as was indicated above).

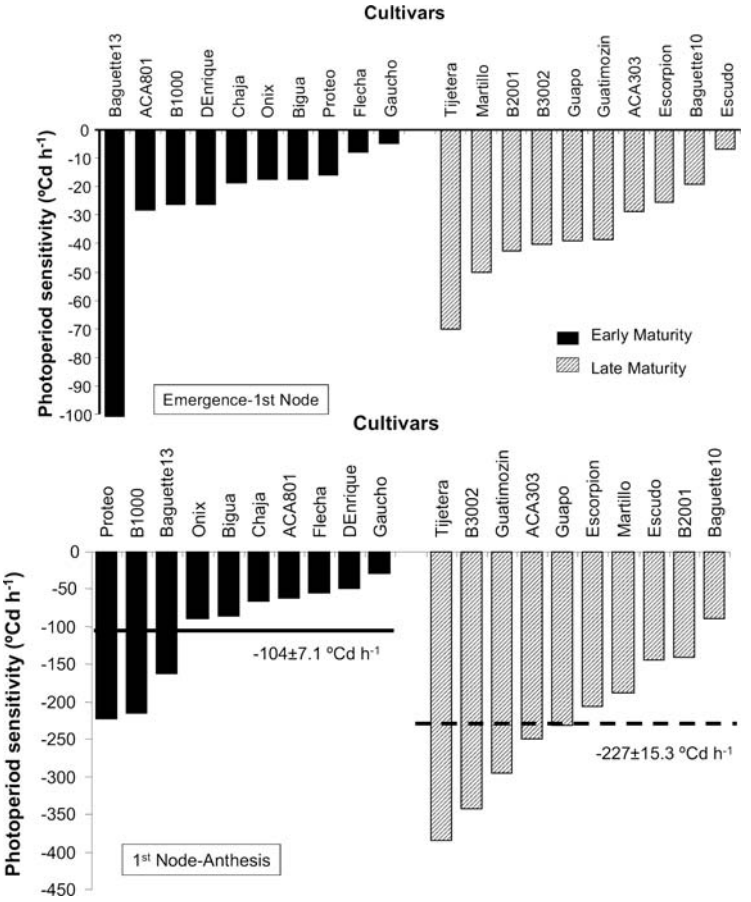


Figure 2. Photoperiod sensitivity for (a) the emergence -1st Node and (b) 1st Node-Anthesis phase for early (black bars) and late (shadow bars) maturity cultivars. Bold and tagged lines indicate the average of photoperiod sensitivity for early and late maturity cultivars, respectively

For the emergence-1st node period, within the early maturity group with the exception of Baguette 13 ($-104\,°C\ d\ h^{-1}$) most of the cultivars showed values of photoperiod sensitivity from -20 to $-30\,°C\ d\ h^{-1}$. With the exception of the cultivar Escudo, the late maturity cultivars recorded higher photoperiod sensitivity than the early group. A similar response was observed in the late reproductive phase from 1st Node to anthesis where the early maturity showed lower photoperiod sensitivity ($-105\,°C\ d\ h^{-1}$) than those of late maturity ($-227\,°C\ d\ h^{-1}$). Although the trend between both maturity groups was the same for both pre-anthesis phases, the ranking of the cultivars within each group, in terms of photoperiod sensitivity, was not exactly the same when both phases are compared. It is also important highlight that the late reproductive phase showed greater photoperiod sensitivity than did the preceding phase (emergence-1st Node) (Fig. 2).

Intrinsic earliness (i.e. the duration of phase when the maximum rate of development is reached) for the emergence-anthesis phase did not show significant differences ($p < 0.05$) between groups, i.e. 829 and 907 °C d, for early and late maturity, respectively. In the same way, not significant differences ($p > 0.10$) were detected in this trait between both maturity groups for the different pre-anthesis phases considered in this study. However, there was a slight trend in the late maturity group to increase the duration of the pre-anthesis phases respect to those cultivars classified as early maturity. Averaging across cultivars, and in both groups, intrinsic earliness of the emerge-1st Node phase represented more than 60% of the emergence-anthesis phase (Fig. 3).

Intrinsic earliness of the emergence-anthesis phase ranged between 760 and 1040 °C d and between 800 and 1160 °C d, for early and late maturity group, respectively. Similar trend was observed in the previous phases. Thus, intrinsic earliness for the emergence-1st Node ranged from 480 to 765 °C d in the early and

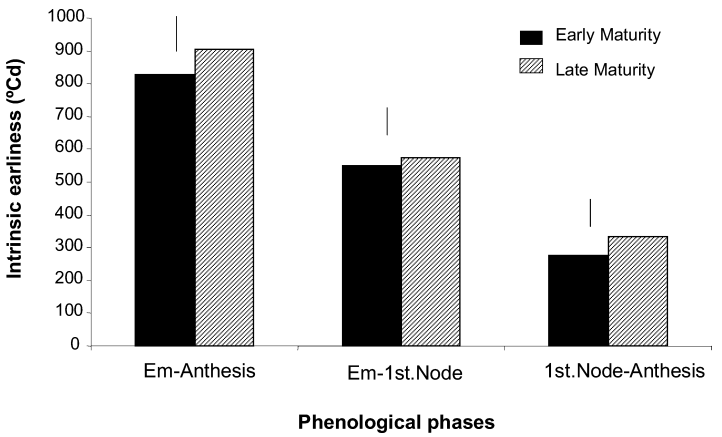


Figure 3. Intrinsic earliness of different phenological phases in early (black bars) and late (shadow bars) maturity cultivars. Intrinsic earliness was calculated as the average across cultivars with each group. Vertical bars indicate the standard deviation of means ($p < 0.05$)

from 500 to 685 °C d into the late maturity cultivars. Finally, the intrinsic values for the late reproductive phase ranged from 225 to 340 °C d and from 280 to 475 °C d for the early and late maturity groups, respectively.

All cultivars, i.e. those classified as early as well as late maturity, showed a reduction (measured in thermal units) of the emergence-anthesis phase up to 13.4 hs photoperiod. Thereby, this value could be considered as optimum photoperiod, i.e. when the rate of development reaches the maximum value, for this phase. Not significant differences were found among cultivars for the optimum photoperiod during the pre-anthesis phases although the late reproductive phase registered greater values for the optimum photoperiod i.e. 14.4 hs (Fig. 4).

As was described above the higher photoperiod sensitivity of the late maturity cultivars generally determined a longer emergence-anthesis phase than those classified as early maturity. In the late maturity group there was an evident compensation between both pre-anthesis phases. Thus, the longer the mostly vegetative phase (i.e. emergence-1st Node) the shorter the late reproductive phase (i.e. stem elongation period) (Fig. 5). This compensation was less evident in the early maturity cultivars.

Within each maturity group important variations were detected in the duration of the vegetative and reproductive phases, for the same flowering time. For instance, early maturity cultivars as ACA 303 and INIA Tijetera reached flowering time at

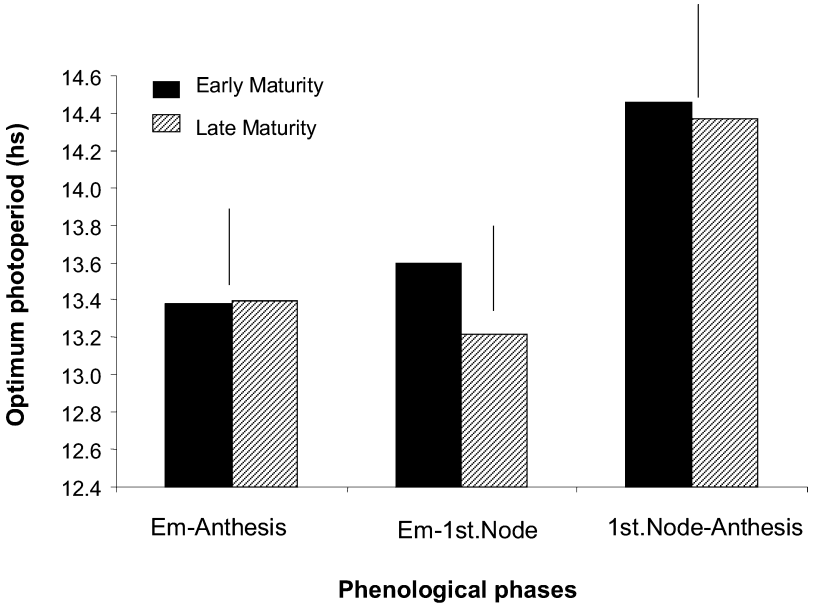


Figure 4. Optimum photoperiod (calculated as the average across cultivars within early and late maturity groups) in early (black bars) and late (shadow bars) maturity cultivars. Vertical bars indicate the standard deviation of means ($p < 0.05$)

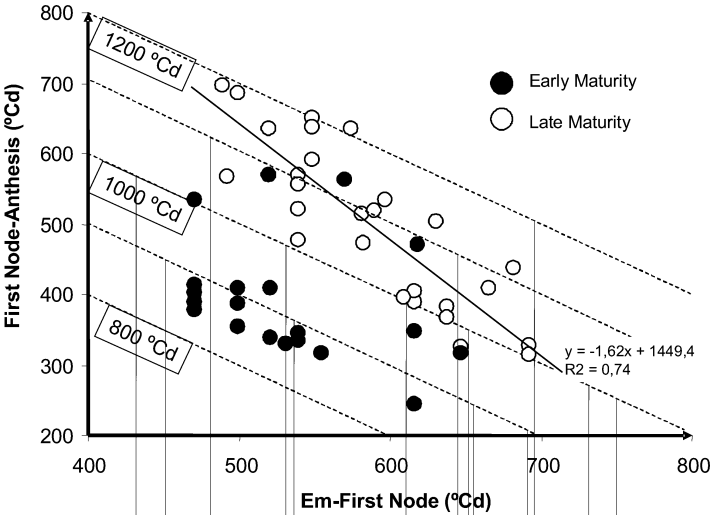


Figure 5. Duration of phase from 1st Node-anthesis against of the duration of phase from emergence to 1st Node for 20 triticale cultivars grown under the three first sowing dates. The lines into the figure represent iso-thermal times from emergence to anthesis. Cultivars into of the lines or on the same line have similar to anthesis duration

1100 °C d with a combination of shorter vegetative phase and longer reproductive phase than Guapo and Baguette 10 that registered the same time at flowering but with longer reproductive duration of the vegetative phase and shorter duration for the reproductive phase (i.e 1st Node-anthesis). The same behavior was evident in the early maturity, where the same duration of the emergence-anthesis phase (i.e. 900 °C d) in the cultivars Bigua and Flecha was reached by a combination of shorter vegetative and longer reproductive phases duration while in ACA 801 and Baguette 13 that degree days duration was a combination of longer vegetative and shorter reproductive phase. Grain filling period (i.e. the period between anthesis and physiological maturity) did not show important variation among cultivars neither among sowing dates. The average duration for both groups and sowing dates was ca. 450 °C d.

No-significant differences ($p>0.10$) were found in phyllochron (ranging from 94 to 112 °C d) between early and late maturity cultivars. However, a trend to a slightly reduced phyllochron (i.e per 10 days of delay in sowing dates phyllochron was shortened 3 °C d) as sowing was delayed was evident in all cultivars up to middle of August (Fig. 6). Similar that was observed in phyllochron the final leaf number was slightly reduced due to delays in sowing date. Averaging across cultivars the final number of leaves was reduced at a rate of 0.15 leaves per 10 days of delay in sowing date. Therefore, the differences in anthesis time among sowing dates was mainly associated with both changes in phyllochron as well as changes in the final number of leaves.

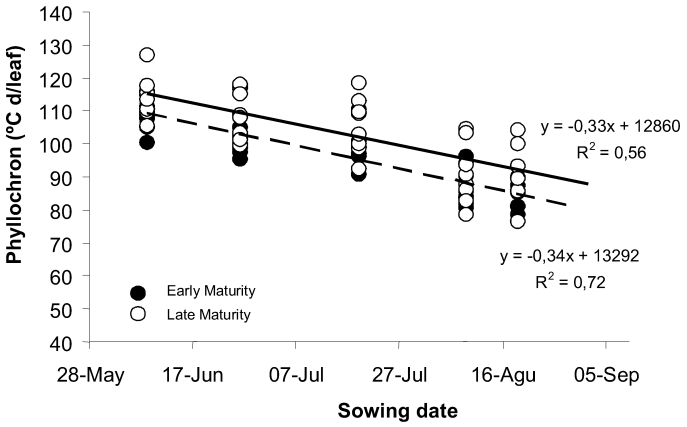


Figure 6. Relationship between phyllochron and date of sowing (considering only the first five sowing dates). Tagged and bold lines correspond the fitting for the early and late maturity groups, respectively

DISCUSSION

The result of this study demonstrated that the differences in the duration of the emergence-anthesis phase, measured in thermal time, between early and late maturity groups were mainly associated with different photoperiod sensitivity more than changes in the optimum photoperiod and/or intrinsic earliness. Since only three cultivars evidenced vernalization requirements this trait does not appear as an important attribute to be introgresed by breeding into the Argentinean commercial cultivars for conferring lengthening to the pre anthesis phases.

Slafer and Rawson (1996) showed that sensitivity to photoperiod was stronger in the winter than in the spring wheat cultivars. It is possible that the Argentinean commercial cultivars characterized as late maturity for the agronomical conditions of Argentina are associated with one or more sensitive Ppd alleles into the backgrounds conferring stronger photoperiod sensitivity than those classified as early maturity. This aspect is of remarkable interest to be analyzed because different Ppd alleles combination into the background could modify the relative duration of the pre anthesis phases without altering the flowering time (Whitechurch and Slafer 2002, Gonzalez et al. 2005b). The positive association between the length of the late of reproductive phase and the numbers of grains per unit area evidenced in previous studies under controlled (Miralles et al. 2000) or field conditions (Gonzalez et al. 2005a, 2005b) was not clearly found in this study (data not shown).

Conversely that was found in previous reports (Rawson 1971), where optimum photoperiod for the emergence-anthesis phase varied among cultivars between 15 and 21 hs, for the 20 cultivars analyzed in this study not important variations were registered in this trait. Moreover, the average optimum photoperiod among cultivars was shorter (13.4 hs) than those reported by Rawson (1971). This response is similar

than that was found in maize where a narrow variation, i.e. between 12 and 13 hs, was found in the optimum photoperiod among hybrids (Kiniry et al. 1983).

Although in average both groups (early and late maturity) did not register significant differences in intrinsic earliness, the variations among cultivars within each group (ca. 300 °C d) evidenced that this trait could be taken account for cultivar selection in high latitudes with longer photoperiods.

In spite of several evidences showed variability in phyllochron when plants were grown in different sowing dates (Hotsonyame and Hunt 1997, Jamieson et al. 1995, Miralles et al. 2001) the results of this study did not evidence important variation in phyllochron throughout different sowing dates (at least those included within the agronomic range used by farmers in the Argentinean field conditions).

Taking account the parameters of the thermo-photoperiodic model for both maturity groups it is possible build a general model that is represented in Fig. 7 where both, early and late maturity groups, have the same optimum photoperiod and similar intrinsic earliness (although slightly greater in the late maturity cultivars) and the main difference between groups is the photoperiod sensitivity. The late maturity cultivars registered (in average) almost the double of sensitivity than that of the early maturity group. This, it is possible speculate that the main differences between groups would be evidenced in early more than in late sowing dates because as sowing is delayed the differences between groups will be reduced.

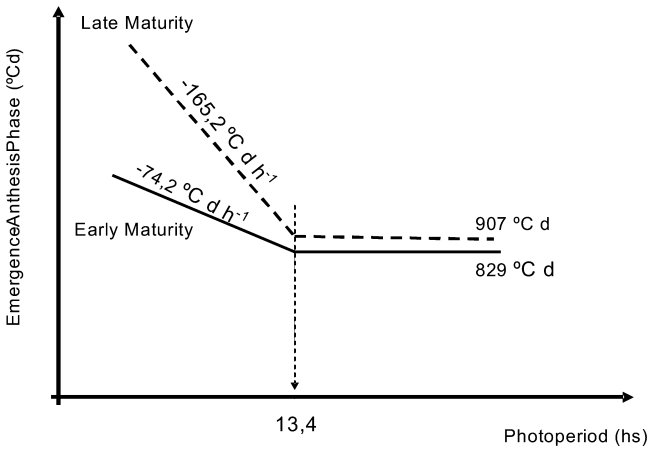


Figure 7. Schematic thermo-photoperiod response of early and late maturity groups indicating photoperiod sensitivity, optimum photoperiod and intrinsic earliness

REFERENCES

Fischer RA (1993) Irrigated spring wheat and timing and amount of nitrogen fertilizer. II. Physiology of grain and yield response. Field Crop Res 33:57–80

Gonzalez FG, Slafer GA, Miralles DJ (2005a) Floret development and survival in wheat plants exposed to contrasting photperiod and radiation environments during stem elongation. Funct Plant Biol 32:189–197

Gonzalez FG, Slafer GA, Miralles DJ (2005b) Pre-anthesis development and number of fertile florets in wheat as affected by photoperiod sensitivity genes *Ppd-D1* and *Ppd-B*. Euphytica 146:253–269

Hotsonyame GK, Hunt LA (1997) Sowing date and photoperiod effects on leaf appearance in field-grown wheat. Can J Plant Sci 77:23–31

Jamieson PD, Brooking IR, Porter JR, Wilson DR (1995) Prediction of leaf appearance in wheat: a question of temperature. Field Crop Res 41:35–44

Kiniry JR, Ritchie JT, Musser RL (1983) Dynamic nature of photoperiod response in maiza. Agron J 75:700–703

Miralles DJ, Slafer GA (1999) Wheat development. In: Satorre EH, Slafer GA (eds) Wheat: Ecology and physiology of yield determination. Food Product Press Ed New York pp 13–14.

Miralles DJ, Richards RA (2000) Responses of leaf and tiller emergence and primordium initiation in wheat and barley to interchanged photoperiod. Ann Bot 85:655–663

Miralles DJ, Richards RA, Slafer GA (2000) Duration of stem elongation period influences the number of fertile florets in wheat and barley. Aust J Plant Physiol 27:931–940

Miralles DJ, Ferro BC, Slafer GA (2001) Developmental responses to sowing date in wheat, barley and rapeseed. Field Crop Res 71:211–223

Rawson HM (1971) An upper limit for spikelet number per ear in wheat as controlled by photoperiod. Aust J Agr Res 22:537–546

Riddell JA, Gries GA, Stearns FW (1958) Development of spring wheat: I. The effect of photoperiod. Agron J 50:735–739

Slafer GA, Rawson HM (1994) Sensitivity of wheat phasic development to major environmental factors: a re-examination of some assumptions made by physiologists and modellers. Aust J Plant Physiol 21:393–426

Slafer GA, Rawson HM (1996) Responses to photoperiod change with phenophase and temperature during wheat development. Field Crop Res 46:1–13

Whitechurch EM, Slafer GA (2001) Response to photoperiod before and after jointing in wheat substitution lines. Euphytica 118:47–51

Whitechurch EM, Slafer GA (2002) Contrasting Ppd alleles in wheat: effects on sensitivity to photoperiod in different phases. Field Crop Res 73:95–105

ACCLIMATION OF PHOTOSYNTHESIS AND STOMATAL CONDUCTANCE TO ELEVATED CO_2 IN CANOPY LEAVES OF WHEAT AT TWO NITROGEN SUPPLIES

A. DEL POZO[1,*], P. PÉREZ[2], R. MORCUENDE[2], D. GUTIÉRREZ[2], A. ALONSO[2] AND R. MARTíNEZ CARRASCO[2]

[1] *Facultad de Ciencias Agrarias, Universidad de Talca, Casilla 747, Talca, Chile*
[2] *Instituto de Recursos Naturales y Agrobiología de Salamanca, CSIC, Apartado 257, 37071 Salamanca, Spain*
[*] *E-mail: adelpozo@utalca.cl*

Abstract: The great majority of the studies on photosynthetic acclimation have focused on upper-sunlit leaves and little attention has been paid to the acclimatory responses of lower-shaded canopy leaves. In this study we assessed the acclimatory responses of photosynthesis and stomatal conductance (gs), as well as chlorophyll content (Chl) and ribulose-1,5-biphosphate carboxylase/oxygenase (Rubisco) activity in flag and lower canopy leaves of wheat growing in polyethylene tunnels set at ambient (360μmol mol^{-1}) and elevated (700μmol mol^{-1}) CO_2, and under two levels of N supply. The rate of photosynthesis, gs, transpiration (E), Chl and Rubisco activity of sunlit upper- (flag) and shaded lower-canopy leaves were significantly ($P < 0.05$) lower in elevated relative to ambient CO_2-grown plants. All the parameters were lower in N deficient plants, except gs, and all declined with leaf position at both growth CO_2. The acclimatory responses of An and gs to elevated CO_2, evaluated as the ratio of An (or gs) measured at 700μmol mol^{-1} of elevated to ambient CO_2-grown plants, were enhanced in N deficient plants; with N supply the acclimatory responses were less pronounced in lower canopy leaves relative to flag leaf. It is concluded that in elevated CO_2, photosynthetic capacity and stomatal conductance were reduced in sunlit upper- and shaded lower-canopy leaves, and that N supply reduced acclimation, particularly in lower- canopy, shaded leaves

Keywords: stomatal conductance, nitrogen

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 611–616.

INTRODUCTION

CO_2 concentration in the atmosphere is increasing, therefore information about long-term (months) responses of photosynthesis (An) and stomatal conductance (gs) to elevated CO_2 is necessary to foresee the performance of wheat in a future environment. In general, when plants are exposed to elevated CO_2 often show a decline of the photosynthetic capacity of leaves (acclimation) and in gs (Drake et al. 1997, Del Pozo et al. 2005). The loss of photosynthetic capacity in elevated CO_2 is attributed to a reduction in the amount and activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) (Drake et al. 1997). But, studies on photosynthetic acclimation have focused mainly on upper-sunlit leaves and little attention has been paid to the acclimatory responses of lower-shaded canopy leaves.

Acclimation of sunlit leaves seems to be less pronounced on plants well supplied with N (Nakano et al. 1997, Rogers et al. 1998), suggesting that N availability has an important role. Nitrogen nutrition not only increases the amount of nitrogen in the whole canopy but also affect the distribution of N among the different leaves within the canopy, being more uniform at higher N nutrition (Del Pozo 1994, Dreccer et al. 2000). Accordingly, N nutrition could mitigate the CO_2-acclimation particularly in lower-shaded leaves.

In this derivative study (Del Pozo et al. 2006) we assessed the acclimatory responses of photosynthesis and stomatal conductance (gs), as well as chlorophyll content (Chl) and ribulose-1,5-biphosphate carboxylase/oxygenase (Rubisco) activity in flag and lower canopy leaves of a wheat crop growing in polyethylene tunnels set at ambient (360μmol mol^{-1}) and elevated (700μmol mol^{-1}) CO_2, and under two levels of N supply.

MATERIALS AND METHODS

Site and Experimental Setup

The experimental site was located at the IRNASA Muñovela Farm at Salamanca (41 °N, 800 m.a.s.1.), Spain. Climate in Salamanca corresponds to a Mediterranean type; long term average of the minimum temperatures in the coldest month (January) is 0 °C and of the maximum temperatures in the warmest month (July) is 27.2 °C. Mean annual rainfall is 506 mm. The soil was clay sand, alkaline (pH 7.7), with normal levels of P, K and Ca (22, 140 and 2800 ppm, respectively).

Spring wheat cv. Alcalá was sown at a rate of 180 kg ha^{-1} and 0.13 m between rows, on 11 February 2003. The crop was sown after turnip and no fertilizer was applied before sowing. The crop was watered weekly through a drip irrigation system and provided 198 mm between February and June, which is the average rainfall in the area during the period of the experiment. Weeds were controlled chemically.

Two tunnels of 9.9 m long, 2.2 m wide and 1.7 m high were placed after the emergence of the crop. One tunnel was kept at ambient (360μmol mol^{-1}) and the other at elevated (700μmol mol^{-1})CO_2 concentration during light hours. Temperature in the two extreme modules of the tunnels was set at ambient (T) and 4 °C warmer (T + 4). Additionally, two levels of nitrogen supply were established by adding 70 kg ha^{-1} and

none to each longitudinal half of the tunnels the on 30 April of 2003. More details of functioning and control systems are in Del Pozo et al. (2005).

Leaf gas exchange measurements and plant sampling were conducted in the middle module of each tunnel where temperature was homogeneous within the module. Therefore only the effect of growth CO_2 and nitrogen were the treatments in this experiment.

Gas Exchange Measurements

Rate of photosynthesis (An) and stomatal conductance (gs) measured at 350 and 700μmol mol^{-1} were determined in attached flag and lower canopy leaves using a portable open system infra-red gas analyser (CIRAS-2, PP Systems), after ear emergence. Measurements were performed in 1.7 cm^2 leaf areas with 300 ml min^{-1} air flow rate, at a photon flux density (PFD) of 1500μmol $m^{-2}s^{-1}$ and leaf temperatures of 25 °C. VPD was maintained at 1.6 ± 0.23 kPa. For measurements each plot was divided in four sampling sectors (replicates).

The acclimatory or long-term response of An to elevated CO_2 of leaves from different positions in the canopy were quantified as ratios of An according to Bunce (2001), that is An(e,E)/An(a,E), where a and e refer to ambient (360μmol mol^{-1}) and elevated (700μmol mol^{-1}) growth conditions, respectively, and A and E refer to ambient (360μmol mol^{-1}) and elevated (700μmol mol^{-1}) measurement conditions. Similar ratios were calculated for gs.

After leaf gas exchange measurements, leaves of four plants from each sampling sector were harvested and immediately transferred to liquid nitrogen for determination of chlorophyll content and Rubisco activity. Four plants from each sector were also harvested for leaf area and dry matter determination.

Chlorophyll Content and Rubisco Activity

The projected area of a sub sample of leaves from different positions in the canopy was measured by image analysis, and then weighed and ground in liquid nitrogen. The chlorophyll was extracted with acetone (80%) and the absorbance recorded at 664 y 646 nm.

For Rubisco activity, samples of the frozen leaves were ground in a mortar with liquid nitrogen and extracted according to Perez et al. (2005). Activity was assayed by adding extract to a mixture of 100 mM Bicine (pH 8.2), 20 mM $MgCl_2$, 10 mM $NaHCO_3$, 10 mM KCl, 1 mM Ribulose-1,5-biphosphate (RuBP), 0.2 mM NADH, 5 mM ATP, 5 mM creatine phosphate, 52 units ml^{-1} phosphocreatine kinase, 12 units ml^{-1} phosphoglycerate kinase, 11 units ml^{-1} glyceraldehyde 3-phosphate dehydrogenase and recording the decrease in absorbance at 340 nm minus 400 nm for 40–60 s, at a stoichiometry of 2:1 between NADH oxidation and RuBP carboxylation. To assay total Rubisco activity, an aliquot of the extract was incubated with $NaHCO_3$ and MgCl for 10 min at room temperature before the addition of coupling enzymes and NADH; the reaction was commenced by adding RuBP. The activation state was estimated as initial activity, as percentage of total activity.

Statistical Analysis

Differences between treatments were determined through analysis of variance using a nested design according to Snedecor and Cochran (1967), with nitrogen as a stratum included in CO_2, and replicates as a stratum included in that for nitrogen. The analyses were performed with GenStat 6.2 (2002).

RESULTS AND DISCUSSION

An and gs measured at 700μmol mol^{-1} during anthesis significantly ($P < 0.05$) decreased with leaf position in elevated and ambient CO_2-grown plants (Fig. 1). The acclimatory responses of An and gs (measured at 700μmol mol^{-1}) in flag and lower canopy leaves to elevated CO_2 were more pronounced in N deficient plants; with N supply the acclimatory responses were reduced in lower canopy leaves

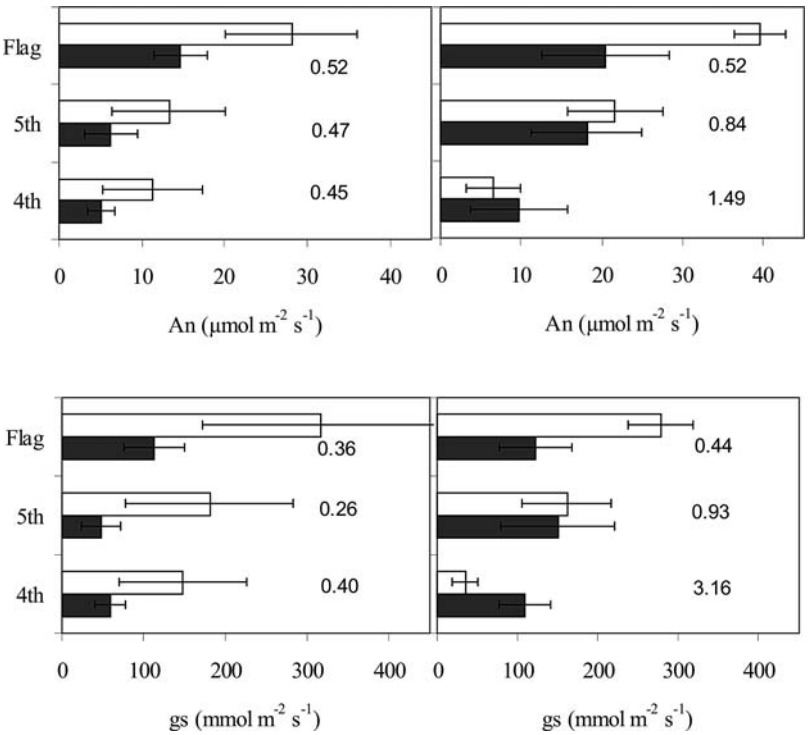


Figure 1. Mean values (± S.E.) of rate of photosynthesis (An), A and B, and stomatal conductance (gs), C and D, measured at 700μmol $mol^{-1}CO_2$ for flag, 5th and 4th leaves at anthesis in wheat grown in the field either at elevated (closed bars) or ambient (open bars) CO_2, and at low (A and C) or high (B and D) nitrogen supply, in 2003. Values for the acclimatory responses of An and gs measured at anthesis are also shown. PPFD was 1500μmol $m^{-2}s^{-1}$ and temperature was 25 °C

reaching values greater than one in the 4th leaf, indicating a stronger reduction in An and gs at ambient than at elevated growth CO_2 (Fig. 1).

Chlorophyll content and Rubisco activity of flag and lower canopy leaves were significantly ($P < 0.05$) lower in elevated relative to ambient CO_2-grown plants (Fig. 2). N deficient plants presented lower ($P < 0.05$) chlorophyll content and Rubisco activity at both growth CO_2 levels. Chlorophyll content and Rubisco activity declined with leaf position in both growth CO_2.

Clearly a down-regulation or decline in photosynthetic capacity in plants grown at elevated CO_2 occurred in upper-sunlit (flag) and in lower-shaded canopy leaves. The loss of photosynthetic capacity in elevated CO_2-grown plants as well as the gradient in An within the leaf canopy were closely related to chlorophyll concentration and Rubisco activity of leaves. In contrast with previous reports (Osborne et al. 1998, Adam et al. 2000) we found that in plants with adequate N supply the acclimatory responses were less pronounced in lower canopy leaves relative to flag leaf. But,

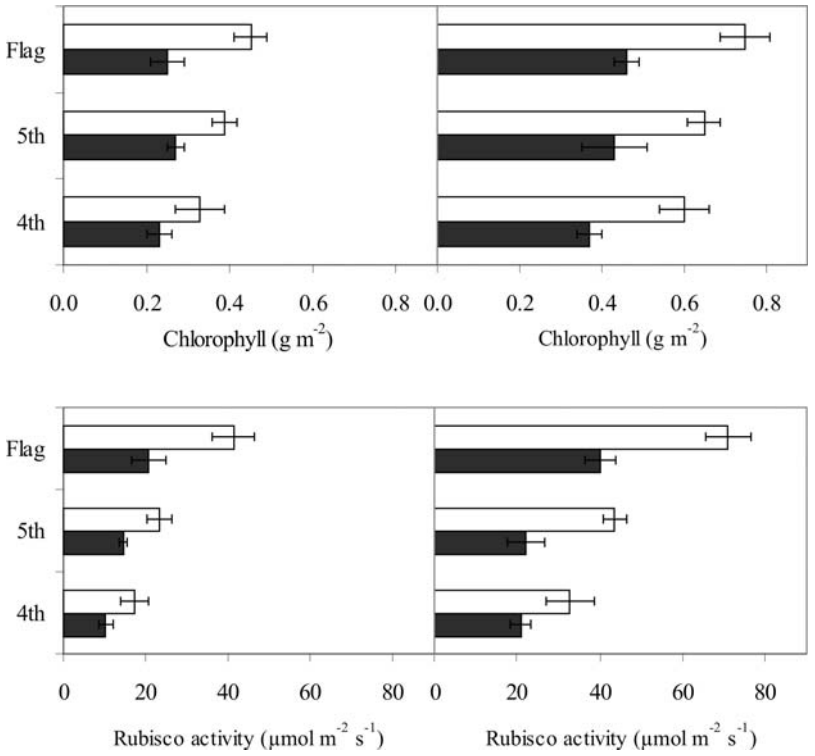


Figure 2. Mean values (±S.E.) of chlorophyll concentration (Chl, g m^{-2}), A and B, and total Rubisco activity (µmol $m^{-2}s^{-1}$), C and D, for flag, 5th and 4th leaves of wheat grown in the field either at elevated (closed bars) or ambient (open bars) CO_2, and at low (A and C) or high (B and D) nitrogen supply, in 2003

in N stressed plants we found a greater acclimation in lower canopy leaves than in the flag leaf.

In wheat nitrogen nutrition has profound effects not only in the total amount of N in the whole canopy but also in the distribution of N among the different leaves within the canopy (Del Pozo 1994; Dreccer et al. 2000). In this experiment chlorophyll concentration per unit leaf area and Rubisco activity of flag and lower canopy leaves were increased with N supply, both parameters closely related with N concentration per unit of leaf area (Osborne et al. 1998). As was expected, the degree of CO_2-acclimation depended on N supply, the acclimatory responses of all leaves being more pronounced in N stressed plants. Much lower acclimatory responses were found by Adam et al. (2000) in wheat, particularly at low N supply, compared to the present study.

REFERENCES

Adam NR, Wall GW, Kimball BA, Pinter PJ, LaMorte RL, Hunsaker DJ, Adamsem FJ, Thompson T, Matthias AD, Leavitt SW, Webber A (2000) Acclimation response of spring wheat in a free-air CO_2 enrichment (FACE) atmosphere with the variable soil nitrogen regimes. 1. Leaf position and phenology determine acclimation response. Photosynth Res 66:65–77

Bunce JA (2001) Direct and acclimatory responses of stomatal conductance to elevated carbon dioxide in four herbaceous crop species in the field. Global Change Biol 7:323–331

Del Pozo A (1994) Leaf area index and relative irradiance within the canopy of wheat and faba bean. In: Borin M, Sattin M (eds) Proc 3rd European society for agronomy, Albano-Padova, Italy, pp 108–109

Del Pozo A, Pérez P, Morcuende R, Alonso A, Martínez Carrasco R (2005) Acclimatory responses of stomatal conductance and photosynthesis to elevated CO_2 and temperature in a wheat crop growing at two levels of N supply, in a mediterranean environment. Plant Sci 169:908–916

Del Pozo A, Pérez P, Gutierrez D, Alonso A, Morcuende R, Martínez Carrasco R (2006) Gas exchange acclimation to elevated CO_2 in upper-sunlit and lower-shaded canopy leaves in relation to nitrogen acquisition and partitioning in wheat grown in field chambers. Environ Exp Bot doi:10.1016/j.envexpbot.2006.04.009

Drake BG, González Meler MA, Long SP (1997) More efficient plants: a consequence of rising atmospheric CO_2? Annu Rev Plant Physiol Plant Mol Biol 48:609–639

Dreccer MF, van Oijen M, Schapendonk AHCM, Pot CS, Rabbinge R (2000) Dynamics of vertical leaf nitrogen distribution in a vegetative wheat canopy. Impact on canopy photosynthesis. Ann Bot 86:821–831

GenStat 6.2 2002 Statistic. Lawes Agricultural Trust, Rothamsted

Nakano H, Makino A, Mae T (1997) The effect of elevated partial pressures of CO_2 on the relationship between photosynthetic capacity and N content in rice leaves. Plant Physiol 115:191–198

Osborne C, LaRoche J, García R, Kimball BA, Wall GW, Pinter PJ, LaMorte RL, Hendrey GR, Long S (1998) Does leaf position within a canopy affect acclimation of photosynthesis to elevated CO_2? Plant Physiol 177:1037–1045

Perez P, Morcuende R, Martín de Molino R, Martínez Carrasco R (2005) Diurnal changes of Rubisco in response to elevated CO_2, temperature and nitrogen in wheat grown under temperature gradient tunnels. Environ Exp Bot 53:13–27

Rogers A, Fischer B, Bryant J, Frehner M, Blum H, Raines A, Long S (1998) Acclimation of photosynthesis to elevated CO_2 under low-nitrogen nutrition is affected by the capacity for assimilate utilization. Perennial ryegrass under free air CO_2 enrichment. Plant Physiol 118:683–689

Snedecor G, Cochran W (1967) Statistical Methods. The Iowa State University Press, Ames, Iowa, USA

USING STOMATAL APERTURE-RELATED TRAITS TO SELECT FOR HIGH YIELD POTENTIAL IN BREAD WHEAT

A.G. CONDON[1,*], M.P. REYNOLDS[2], G.J. REBETZKE[1], M. VAN GINKEL[2], R.A. RICHARDS[1] AND G.D. FARQUHAR[3]

[1] *CSIRO Plant Industry, Canberra, ACT 2600, Australia*
[2] *CIMMYT, AP 6–641, 06600 Mexico D.F., Mexico*
[3] *Research School of Biological Sciences, Australian National University, Canberra, 2600, Australia*
[*] *E-mail: tony.condon@csiro.au*

Abstract: More efficient wheat breeding methods are needed to meet demand for wheat from expected population growth in developing countries. This paper reports results from recent studies conducted at CIMMYT aimed at assessing the use of stomatal aperture-related traits (SATs) as indirect selection criteria for high yield-potential in bread wheat. Two classes of SATs were assessed: the instantaneous trait leaf porosity (POR), which is a close surrogate for stomatal conductance, and the integrative traits $\Delta^{13}C$ of leaf and grain and $\delta^{18}O$ of grain. For 3 out of the 4 populations of breeding lines tested in 2001–02, the results indicated strong prospects for using SATs in screening for high yield potential. Grain $\Delta^{13}C$, leaf $\Delta^{13}C$ and POR each showed promise, with moderate to high heritability, moderate to strong genetic correlations with yield, and yield gains from retrospective selection of about 40–50g m^{-2} at trial mean yields of 520g m^{-2}. Grain $\delta^{18}O$ showed less promise. Heritability of this trait was moderately high, but grain $\delta^{18}O$ was not strongly correlated with yield and retrospective gains from selection were small. For one population, none of the SATs showed any convincing association with yield. The reasons for this need further investigation as SATs are evaluated over additional seasons

Keywords: stomatal aperture, yield potential

INTRODUCTION

Since the initial impact of the Green Revolution, improvement in genetic yield potential of wheat stands at *ca*. 0.9% per year. This is about half what will be required to meet expected demand for wheat in developing countries, and indicates an urgent need to develop more efficient breeding methods for yield improvement.

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 617–624.

Traits related to stomatal conductance may prove useful for improving selection for yield potential. Research during the 1990's, aimed at understanding the physio logical basis of historic gains in yield potential at CIMMYT, showed a consistent correlation between the historic increase in yield potential among CIMMYT semi-dwarf bread wheats and changes in stomatal aperture-related traits (SATs). In summary, more-recent, higher yield-potential CIMMYT wheats had greater stomatal conductance and, therefore, cooler canopies than older, lower yield-potential releases, (Fischer et al. 1998). These differences in instantaneous physiological parameters were also reflected in the long-term stable-isotope composition of C and O measured in plant dry matter. More-recent CIMMYT releases showed greater discrimination against $\Delta^{13}C$ (higher $\Delta^{13}C$) and a lower $\delta^{18}O$ composition (lower $\delta^{18}O$) (Fischer et al. 1998, Barbour et al. 2000), both consistent with the observed historic changes in stomatal conductance. Earlier, Condon et al. (1987) had demonstrated positive associations between yield and $\Delta^{13}C$ under well-watered conditions in Australia and Reynolds et al. (1994) had shown cooler canopies to be associated with higher yield under warm, irrigated conditions in Mexico.

This paper reports results from recent studies conducted at CIMMYT aimed at assessing the utility of SATs as indirect selection criteria for high yield-potential in bread wheat. Two classes of SATs were assessed: the instantaneous trait leaf porosity (POR), which is a close surrogate for stomatal conductance, and the integrative traits $\Delta^{13}C$ of leaf and grain and $\delta^{18}O$ of grain.

MATERIALS AND METHODS

Germplasm

Large sets ($n = 48–64$) of random, F_3-derived F_5 lines from 4 crosses were grown. Two populations were from crosses already known to be varying for SATs among the progeny: Siete Cerros/Seri (Cross 1) and Quarrion/3*Genaro (Cross 2). Two populations were from crosses among elite parents selected from breeders' crossing blocks on the basis of measurements of SATs: Ures/Jun//Kaus/3/SW89.3243 (Cross 3) and SSeri1/SW89.3243 (Cross 4).

Trial Management

The 4 populations were sown in 2-replicate trials at CIMMYT's field station at Obregon, NW Mexico, using lattice designs with repeated checks. Populations were sown in $6m^2$ yield plots and in small plots ($1.5m \times 2$ rows) simulating breeders' early-generation observation plots. Sowing was in mid-late October 2001, anthesis occurred towards the end of February and plots were harvested after grain maturity in late April. Yield plots were also sown in 2002 and 2003 to obtain a more robust estimate of yield potential, i.e. yield averaged over 3 seasons. All sowings received 5–6 irrigations, each of *ca*. 100mm. Weeds were controlled by early herbicide application and then by hand. Pests and diseases were controlled with foliar sprays when necessary.

Measurements

Grain yield was measured by machine-harvesting yield plots grown in all 3 seasons and the 2-row plots grown in 2001–02. Data on stomatal aperture traits (SATs) was collected on 2-row plots in 2001–02 to simulate the use of SATs in breeders' observation plots.

Leaf porosity (POR) of six sun-lit flag-leaves per plot was measured using a Thermoline viscous-flow porometer. Raw data from the porometer (counts) was inverted (1/counts) to generate POR data linearly related to stomatal conductance over the range of counts measured on irrigated wheat plants (Rebetzke et al. 2001). Single sets of data were collected from each plot once in the 2 weeks before anthesis and once in the 2 weeks after anthesis. POR data was collected 3–12d after irrigation and on cloud-free days without high wind between 1000h and 1500h.

Recently-expanded leaf material was sampled in early January 2002, near the time of full ground cover, for stable isotope analysis. Subsamples of grain for isotope analysis were taken after machine-harvest in April 2002. Analysis of carbon isotope composition ($\Delta^{13}C$) of ground, dried (70 °C) leaf and grain samples was done using a Europa 'ANCA' sample preparation system and a Europa '20–20' ratio mass spectrometer. Values of carbon isotope discrimination ($\Delta^{13}C$) were calculated assuming a $\Delta^{13}C$ of air of $-8‰$. Grain samples only were analysed for oxygen isotope composition $\delta^{18}O$) using the same mass spectrometer after pyrolysis of dry matter at very high temperature and separation of CO from N_2 using gas chromatography (Barbour et al. 2000).

Statistical Analysis of Data

Data were analysed using mixed models (REML) after checking for normality and error variance homogeneity. Data transformation was not required. Estimates of heritability (h^2), and of phenotypic (r_p) and genetic (r_g) correlations of yield and SATs, were calculated from components of variance and co-variance.

RESULTS

Variation in Yield and SATs

The mean grain yield in these trials of 520g m^{-2} was very similar for all 3 yield estimates, i.e. 3-year-mean plot yield, 2001–02 plot yield and 2001–02 two-row yield (Table 1). Average yields were similar for the 4 populations and all 3 yield estimates showed large, highly significant genotypic variation. Depending on cross and yield estimate, yield varied over a range of *ca.* 120–300g m^{-2} among lines. Average values for SATs measured on 2-row plots in 2001–02 were similar for the 4 populations (Table 1). Within each population there was substantial genotypic variation for the stable isotopes and for POR. The stable isotopes showed the highest heritability (h^2), similar to or higher than h^2 of yield estimates (Table 1). Heritability of POR was similar to h^2 of yield.

Table 1. Summary of variation in grain yield and SATs among $F_{3:5}$ lines from four crosses

Cross	Trait value[1]	Grain yield (g m^{-2})			Stable isotopes (‰)			POR	
		3-year yield 2001–04	Plot yield 2001–02	2-row yield 2001–02	Leaf $\Delta^{13}C$	Grain $\Delta^{13}C$	Grain $\delta^{18}O$	Pre-anthesis	Post-anthesis
Cross 1	mean	500	539	493	18.7	17.9	31.6	8.7	6.2
(n = 64)	max	584	642	610	19.6	18.9	33.1	10.1	8.7
	min	342	342	343	17.7	16.3	30.6	6.5	1.2
	lsd	53	91	84	0.6	0.5	0.9	1.2	1.8
	h^2	0.69	0.62	0.61	0.50	0.78	0.52	0.54	0.80
Cross 2	mean	542	532	557	18.5	18.0	31.0	8.4	3.9
(n = 48)	max	595	612	645	19.2	18.6	31.9	10.2	6.8
	min	472	392	462	17.7	17.1	29.8	5.4	1.4
	lsd	30	57	84	0.5	0.4	0.7	1.8	2.4
	h^2	0.78	0.63	0.39	0.56	0.80	0.60	0.33	0.43
Cross 3	mean	523	513	517	18.3	17.9	31.9	7.1	3.9
(n = 48)	max	583	589	598	19.1	18.7	32.9	8.5	6.8
	min	435	335	386	17.6	17.0	30.6	5.6	2.2
	lsd	30	66	87	0.5	0.4	0.7	1.3	2.1
	h^2	0.77	0.59	0.41	0.44	0.69	0.56	0.27	0.31
Cross 4	mean	511	499	507	18.1	17.8	32.3	8.0	4.2
(n = 48)	max	634	581	597	19.2	18.4	33.7	9.1	7.0
	min	453	390	369	17.4	16.4	30.8	6.9	1.2
	lsd	43	74	89	0.8	0.4	0.9	1.1	1.6
	h^2	0.64	0.34	0.43	0.36	0.71	0.63	0.37	0.59
All	mean	519	521	519	18.4	17.9	31.7	8.1	4.6
	h^2	0.72	0.55	0.46	0.47	0.75	0.58	0.38	0.53

[1] For each cross, the mean, maximum and minimum values are shown for each yield estimate and SAT, as is the lsd ($P < 0.05$) and heritability (h^2).

Associations of SATs with Yield

Phenotypic correlations (r_p) of plot yield with SATs are shown in Table 2 in comparison with r_p of plot yield with 2-row yield. Correlations tended to be greater with 3-year plot yield than with plot yield measured in 2001–02. The strength of correlations of plot yield with SATs and with 2-row yield varied with population. Correlations were strongest for Cross 1 and weakest for Cross 4.

For Crosses 1, 2 and 3, correlations were always in the direction expected, i.e. positive for $\Delta^{13}C$ and POR, negative for $\delta^{18}O$. The results for Cross 4 were inconsistent with the other 3 crosses. For Cross 4, phenotypic correlations of SATs with plot yield were small and variable in direction. Even the phenotypic correlations of 2-row yield with plot yield were small (0.0 with plot yield in 2001–02; 0.2 with 3-year plot yield). The very poor correlation of 2-row yield with 3-year plot yield indicates that there may have been unobserved problems with the 2-row plots of Cross 4 that caused more 'random' variation in 2-row yield and perhaps more 'random' variation in SATs.

As expected, genetic correlations (r_g) of SATs with yield were larger than values of r_p, (Table 3). Values of r_g were in most cases greater for 3-year plot yield than for 2001–02 plot yield. Genetic correlations of 2-row yield with plot yield were consistently high (0.7–1.0), except for Cross 4 (*ca*. 0.5). Values of r_g for plot yield with leaf $\Delta^{13}C$, grain $\Delta^{13}C$ and POR were moderate to high in most cases, except for Cross 4. Value of r_g for $\delta^{18}O$ were lower. For Crosses 1, 2 and 3, genetic correlations of plot yield with $\Delta^{13}C$, $\delta^{18}O$ and POR were usually in the direction anticipated. For Cross 4, the direction of r_g was more variable and the values were low.

Calculations from retrospective selection of the top and bottom 25% of lines based on SATs generated average gains in plot yield of up to 40g m^{-2} (Table 4). Gains in plot yield in 2001–02 were similar to gains in 3-year yield. Average yield

Table 2. Phenotypic correlations (r_p) of SATs measured on 2-row plots with grain yield measured on large plots. Correlations are shown with grain yield in the year SATs were measured and with 3-year average grain yield

Yield estimate	Cross	2-row yield	Leaf $\Delta^{13}C$	Grain $\Delta^{13}C$	Grain $\delta^{18}O$	POR Pre-anth	POR Post-anth
2001–02	Cross 1	0.63	0.42	0.56	−0.16	0.38	0.48
	Cross 2	0.42	0.19	0.20	−0.16	0.07	0.04
	Cross 3	0.39	0.03	0.16	−0.02	0.19	0.17
	Cross 4	0.00	−0.12	−0.05	0.02	−0.19	−0.13
	Average	0.36	0.13	0.22	−0.08	0.11	0.14
2001–04	Cross 1	0.62	0.40	0.60	−0.19	0.45	0.50
	Cross 2	0.59	0.38	0.48	−0.20	0.13	0.21
	Cross 3	0.52	0.19	0.40	−0.11	0.26	0.23
	Cross 4	0.20	−0.06	0.07	−0.09	−0.21	−0.14
	Average	0.48	0.23	0.39	−0.15	0.15	0.27

Table 3. Genetic correlations (rg) of SATs measured on 2-row plots with grain yield measured on large plots. Correlations are shown with grain yield in the year SATs were measured and with 3-year average grain yield

Yield estimate	Cross	2-row yield	Leaf $\Delta^{13}C$	Grain $\Delta^{13}C$	Grain $\delta^{18}O$	POR Pre-anthesis	POR Post-anthesis
2001–02	Cross 1	0.90	0.80	0.84	−0.22	0.53	0.73
	Cross 2	1.00	0.35	0.33	−0.12	0.48	0.32
	Cross 3	0.86	0.34	0.44	0.11	−0.07	0.46
	Cross 4	0.48	−0.07	−0.01	0.22	0.21	0.14
	Average	0.81	0.36	0.40	0.00	0.29	0.41
2001–04	Cross 1	0.71	0.69	0.76	−0.15	0.68	0.71
	Cross 2	0.85	0.54	0.62	−0.26	0.41	0.40
	Cross 3	0.93	0.44	0.54	−0.12	0.16	0.36
	Cross 4	0.55	0.11	0.00	0.07	0.01	0.01
	Average	0.76	0.45	0.48	−0.12	0.32	0.37

Table 4. Yield gains in large plots from retrospective divergent selection based on SATs measured on 2-row plots. Yield gains (g m^{-2}) were calculated for a selection intensity of 25%, i.e [average yield of top 25% of lines based on indirect traits] minus [average yield of bottom 25% of lines based on indirect traits]. Yield responses to retrospective selection for SATs

Yield estimate	Cross	2-row yield	Leaf $\Delta^{13}C$	Grain $\Delta^{13}C$	Grain $\delta^{18}O$	POR Pre-anthesis	POR Post-anthesis
2001–02	Cross 1	95	93	93	20	54	78
	Cross 2	77	28	33	19	12	29
	Cross 3	44	14	28	−6	13	25
	Cross 4	19	−1	−12	−25	16	7
	Average	59	34	36	2	24	35
2001–04	Cross 1	74	61	81	14	56	62
	Cross 2	58	40	39	13	10	26
	Cross 3	39	19	34	10	21	17
	Cross 4	31	9	8	−13	4	−6
	Average	51	32	41	6	23	25

gains from retrospective selection were positive based on all SATs. They were highest for grain $\Delta^{13}C$ and leaf $\Delta^{13}C$, a little lower for POR (post-anthesis then pre-anthesis) and lowest for grain $\delta^{18}O$. Yield gains from retrospective selection were poorest, sometimes negative, for all SATs applied to Cross 4. Averaged over the other 3 crosses, retrospective selection based on grain $\Delta^{13}C$ gave yield gains of 50g m^{-2}. This is about 10% of the average trial yield. Selection based on leaf $\Delta^{13}C$ and POR gave yield gains of *ca.* 40g m^{-2}, averaged over Crosses 1, 2 and 3.

DISCUSSION

For 3 out of the 4 populations, the results of this study indicated strong prospects for using SATs in screening for high yield potential. Grain $\Delta^{13}C$, leaf $\Delta^{13}C$ and POR showed the most promise, with moderate to high heritability, moderate to strong genetic correlations with yield, and yield gains from retrospective selection of about 40–50g m^{-2}. Grain $\delta^{18}O$, which reflects the effect of changes in stomatal conductance on canopy temperature, showed less promise. Heritability of this trait was moderately high, but grain $\delta^{18}O$ was not strongly correlated with yield and retrospective gains from selection were small. It is possible that a more direct measure of canopy temperature may prove more effective.

For one population (Cross 4), none of the SATs showed any convincing association with yield. The reasons for this need further investigation. It may reflect poor growth of plants in the 2-row plots relative to growth in yield plots, since for this population the correlations between 2-row yield and plot yield were much weaker than for the other 3 populations. Factors resulting in poor growth in the 2-row plots may have also resulted in variation in stomatal conductance and related traits that was not reflected in yield measured in large plots, either in 2001–02 or subsequent years.

The results of this study indicate that SATs may have potential to cut costs associated with yield testing by, for instance, being used to cull the number of lines taken to the yield-plot stages of a breeding program or reducing the number of years of testing in yield plots. Freeing up resources from the yield-testing activity may permit early-generation testing of a greater number of progeny from more crosses. Measuring SATs is not without cost, of course. Each involves provision of labour, some equipment and there are substantial analysis costs for $\Delta^{13}C$. While this additional cost is a disadvantage for $\Delta^{13}C$, the collection of stable isotope samples is not dependent on the sunny, stable weather conditions that are required for collection of POR data. Nor is there a strong requirement for operator training. Samples are dried, ground and sent to a lab for analysis.

ACKNOWLEDGEMENTS

Financial support for this research was provided by the Australian Centre for International Agricultural Research (ACIAR), the Grains Research and Development Corporation (GRDC) and the International Atomic Energy Agency (IAEA).

REFERENCES

Barbour MM, Fischer RA, Sayre KD, Farquhar GD (2000) Oxygen isotope ratio of leaf and grain material correlates with stomatal conductance and grain yield in irrigated wheat. Aust J Plant Physiol 27:625–637

Condon AG, Richards RA, Farquhar GD (1987) Carbon isotope discrimination is positively correlated with grain yield and dry matter production in field-grown wheat. Crop Sci 27:996–1001

Fischer RA, Rees D, Sayre KD, Lu ZM, Condon AG, Larqué Saavedra A (1998) Wheat yield progress is associated with higher stomatal conductance, higher photosynthetic rate and cooler canopies. Crop Sci 38:1467–1475

Rebetzke GJ, Condon AG, Richards RA, Read JJ (2001) Phenotypic variation and sampling for leaf conductance in wheat (*Triticum aestivum*) breeding populations. Euphytica 121:335–341

Reynolds MP, Balota M, Delgado MIB, Amani I, Fischer RA (1994) Physiological and morphological traits associated with spring wheat yield under hot, irrigated conditions. Aust J Plant Physiol 21:717–730

STRATEGIC RESEARCH TO ENHANCE THE YIELD POTENTIAL THROUGH REDESIGNING OF WHEAT PLANT ARCHITECTURE

S.S. SINGH*, J.B. SHARMA, D.N. SHARMA AND NANAK CHAND
Indian Agricultural Research Institute, New Delhi 110012, India
**E-mail: singhss@rediffmail.com*

Abstract: In India wheat is the second most important crop occupying 27.3 million hectare area with production and productivity of 72 million tons and 2.64 tons per hectare, respectively. To cope up the ever increasing demand which will be 109 million tons by the year 2020, the present level of productivity has to be increased to 4.4 tons per hectare. The only approach for achieving quantum jump in productivity is to restructure the wheat plant architecture which can yield up to 8 tons/hectare. The Indian Agricultural Research Institute, New Delhi, India has developed new plant type (NPT) wheat, utilizing a local germplasm SFW and released wheat and genetic stocks, which has high 1000 grain weight (45–50g), high number of grain per spike (90–100), higher biomass, thick, broad, semi erect and dark green leaves, thick stem, plant height 85–100 cm and good root system. Efforts are on to increase the productive tillers in these wheats along with diverse genes for resistance to diseases in order to break the yield barrier

Keywords: yield potential, architecture of wheat plant

INTRODUCTION

The wheat is one of the important staple foods of the Indian and also 35 percent of world population and it is widely cultivated in the world. During the last four decades India has witnessed a continuous increase in wheat production from one ton per hectare in early 1960's to nearly 2.7 tons per hectare in 2005. This spectacular progress in wheat production led to a status of second largest wheat producing nation after China. The change in wheat plant architecture from tall to semi dwarf types has brought a significant change in wheat production. However, due to continuous increase in population has put pressure on the demand of wheat not only in India but

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 625–631.

also in the world. The global wheat demand by the year 2020 would be between 840 and 1050 million tons and India would require 109 million tons to feed the expected 1.3 billion citizens of the country in the same year. The genetic gain in wheat, stagnating around one percent over one decade has become a serious challenge for wheat breeders. The Indian Agricultural Research Institute, New Delhi in India initiated strategic research in1994 for altering plant architecture of wheat to break the productivity barrier.

MATERIALS AND METHODS

Initially the materials involved as parental line in the successful development of New Plant Type (NPT) of wheat were local germplasm, SFW (Sirsa Farm Wheat) and two released wheats (Vaishali and Vidisha). SFW has very long spike with high spikelet number but unfilled middle spikelets, long and shriveled grains, few tillers per plant and high susceptibility to diseases. The other parents involved have bold, lustrous grains and carrying tightly linked alien genes *Lr24/Sr24* for leaf rust and stem rust resistance derived from *Agropyron elongatum*. In second step the objective was to redesign the architecture with increased tillering capability ($400\,m^{-2}$) keeping number of grains around 80 per spike, grain weight around 42–45g per1000 grains and diverse genes for resistance to diseases and also the grain quality in these NPT lines. To achieve the objectives over 100 selected commercially released wheats and elite germplasm e.g. DBW14, DL1329, HD2329, HD2790, HD2808, HW1085, HW2045, HUW468, Kundan, LOK45, NW1012, NW1014, PBW343, PBW502, RAJ3765, UP2338 etc. were used in the hybridization programme involving NPT wheats as the first parent.

A variant of pedigree method of selection was specially designed to handle segregating populations for the development of NPT wheats. The emphasis was laid on planting a very high population of early generation material in order to pickup the transgressive segregants combining all the traits envisaged to develop new plant architecture. The early generation material was planted like commercial planting and the bold ear heads with maximum spikelets were picked up from each family. All the segregating generations were subjected to artificial epiphytotic of leaf rust pathotypes 77–5 and 104–2 which are highly virulent and prevalent in the Indo-Gangetic plains (20 million hectare area out of total wheat area of 27 million hectares).

For studying the correlation between grain yield and different yield components the NPT genotypes were evaluated in a randomized block design experiment with 4 replications and a plot size of 5.5m × 1.38m along with check varieties PBW343(Attila), HD2329 and UP2338. For physiological analysis, the response of NPT wheats at various levels of nitrogen (100kg, 150kg, 200kg) was observed. A large number of main shoots were tagged and data was collected on yield attributes. An assay of nitrate reductase activity was restricted to the laminae of the main shoot of the tagged plant only. Nitrate-reductase activity was assayed in vivo as suggested by Hagman and Hucklesby (1971) and Nair and Abrol (1977). The reduced nitrogen content in grain of

the final harvest was determined by the alkaline-phenol-sodium hypocloride method. Biomass, grain yield and number of ear per m^2 were sampled randomly.

For post-anthesis stem reserve mobilization studies the main shoot was tagged at the time of anthesis and was harvested for sampling on the day of anthesis and on 2nd, 10th, 16th, and 25th day after anthesis (DAA) in moistened polythene bags to avoid any moisture loss. Separated peduncle portion were gently packed in NMR sample tubes (18 mm diameter) up to sample height restricted to 30mm within the homogeneous field of the magnet. The proton relaxation time (*T1*) was measured by Saturation Recovery method using software loaded in EDM 511, which gives a single component with three parameters fit by a 20 MHz Burker minispec pc20 NMR system. The water content was measured in the same sample after measuring *T1* by oven drying to constant weight at 85 °C.

The shuttle breeding approach has been adopted for growing alternate segregating generations, at IARI, New Delhi and Dalang Maidan in Lahaul valley in north India located at an altitude of 3500 m and the Wellington centre of IARI in Nilgiri hills in south India located at an altitude of 1700 m during the off season in order to select for wider adaptability and resistance to broad spectrum pathotypes of rusts.

RESULTS AND DISCUSSION

In order to achieve quantum jump in wheat production and productivity, the designing of new plant architecture of wheat was initiated at India Agricultural Research Institute, New Delhi in the year 1994, keeping in view the stagnation of genetic gain around one percent which was not sufficient to cope up with the demand of wheat in the country. The change in architecture from tall to semidwarf brought green revolution in 1960's not only in India but also in the neighboring countries. However, the same need was again felt to break the genetic barrier in wheat. In this endeavor, one local germplasm SFW was made as the basic material in order to improve upon it in enhancing the yield potential. This germplasm has many negative traits related to yielding ability except the one, the size of the spike, which is unusually long with more number of spikelets. The other parental lines including released wheats and genetic stocks for various agronomic traits and genes for resistance to diseases were carefully selected and used in hybridizing with SFW. Initially only two wheat varieties released nationally Vaishali and Vidisha were successful in giving the transgressive segregants when crossed with SFW, leading to successfully designing of new plant architecture. Some of the selected lines developed with new plant type are DL1266-1, DL1266-2, DL1266-5, DL1266-6, DL1267-2, DL1267-3, DL1267-3, DL1270-1, DL1270-2 and DL1270-5. DL1266-5 was registered, as a representative of new plant type in wheat, at National Bureau of Plant Genetic Resources, New Delhi in India. This genotype possess a high 1000 grain weight (45–50g), high number of grain per spike (90–100), higher biomass, thick, broad, semi erect and dark green leaves, thick stem, plant height 85–100 cm and good root system. This NPT line is also resistant to leaf rust and has around 13 percent grain protein content. The correlation analysis suggested that the negative

correlation between yield components has been broken leading to a positive correlation between grain weight and grain number per spike with optimum productive tillering capacity (Singh et al. 2000, 2001).

Physiological and Biochemical Analysis of the NPT Genotypes

The biomass in PBW343 showed increasing trend with the increase in nitrogen levels from 100 to 150 and 200 kg N hectare^{-1}, whereas in DL1266-2 and DL1266-5 the maximum biomass was achieved at 150 kg N hectare^{-1}. This indicates that the new wheat are more efficient nitrogen utilizer at lower levels, although the grain yield m^{-2} was not significantly higher at different nitrogen levels and between the three genotypes (Table 1). The over all harvest index was superior in DL1266-2 and DL1266-5 as compared to PBW343, particularly at low fertility levels. It was concluded from the harvest index data that NPT wheat are more efficient mobilizers of the assimilates to the developing grain sink. The number of productive tillers m^{-2} is always lower in the NPT genotypes as is evident from number of spikes m^{-2}. This is indicative of the fact that DL1266-2 and DL1266-5 also are nutrient efficient, as they produce limited number of productive and synchronous tillers with thick stems and compact, long spikes (Singh et al. 2002). The superior harvest index also supports this fact and indicates a superior assimilate reserve accumulation and its mobilization to the developing grain sink so that a very high number and improved grain weight result in significantly higher grain weight spike^{-1}. Such improved ideotypes predicted earlier also are expected to be nutrient-efficient based on the comparative study of pre and post-green revolution plant types (Pandey et al. 1983). Analysis of grain weight spike^{-1} confirms the superiority of NPT genotypes over the check at all three fertility levels. It may be concluded that NPT wheat have developed a superior spike architecture that ensures improved transportation of assimilates to the grain (Fig. 1). As for the analysis of grain-yield components, the grain number spike^{-1}, grain weight spike^{-1} and 1,000-grain weight show remarkable superiority even at lower fertility levels in these genotypes compared to the check varieties. In NPT genotypes not only grain weight per spike has increased but also grain number per spike due to increased number of florets at distal location in the spike over the best check PBW343 (Fig. 2).In post-anthesis stem reserve mobilization studies *T1* parameter showed an increase after 10th day of anthesis in spite of a sharp decline in the moisture percentage suggesting rapid mobilization of stem reserves from stem in this genotype after 10th day of anthesis under normal sowings. This suggests efficient post-anthesis mobilization of stem reserve to sink in DL1266-5 (Pandey et al. 2002).

STMS-Based DNA Fingerprinting of NPT Lines

With the objective of precise differentiation and identification of NPT genotypes seven lines DL1266-1, DL1266-2, DL1266-5, DL1266-6, DL1266-10, DL1266-16, and DL1266-17 along with two other unrelated breeding lines, DL1337-1 and

Table 1. Influence of different nitrogen fertility levels on yield components of DL 1266-5.

Character	Genotype	Nitrogen levels (kg ha^{-1})			
		100	150	200	Mean
Biomass m^{-2}(g)	DL1266-2	1280	1585	1566	1477
	DL1266-5	1541	1741	1632	1638
	PBW343©	1491	1665	1784	1647
Grain yield m^{-2}(g)	DL1266-2	589	691	653	644
	DL1266-5	681	790	729	733
	PBW343©	650	759	700	703
No. of spikes m^{-2}	DL1266-2	261	285	310	285
	DL1266-5	308	335	331	325
	PBW343©	456	462	496	471
Grain wt $spike^{-1}$	DL1266-2	4.66	4.92	4.80	4.79
	DL1266-5	5.70	5.79	5.32	5.60
	PBW343©	2.69	2.71	2.86	2.75
Grains $spike^{-1}$(g)	DL1266-2	76	85	89	83.33
	DL1266-5	100	100	98	99.33
	PBW343©	58	60	66	61.33
1000 Grain wt. (g)	DL1266-2	58.99	57.73	54.39	57.00
	DL1266-5	56.77	57.84	54.06	56.22
	PBW343©	46.81	45.75	43.26	45.27
Protein percent	DL1266-2	12.06	13.10	14.41	13.19
	DL1266-5	12.17	13.01	13.70	12.96
	PBW343©	11.27	12.06	11.76	11.70
NR activity	DL1266-2	2595	2392	2497	2489
(mμ moles g^{-1}	DL1266-5	2090	2595	2317	2334
fresh wt h^{r-1}	PBW343©	1758	2190	2115	2021

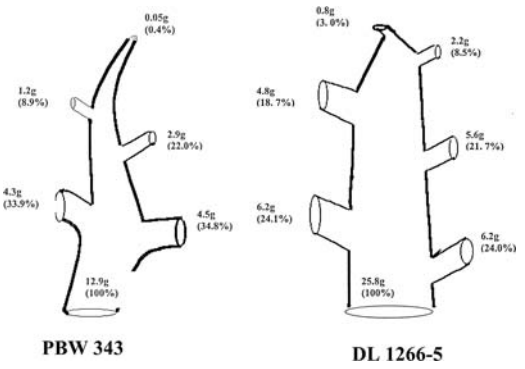


Figure 1. Comparative grain weight distribution in two types of wheat spikes per five spikes

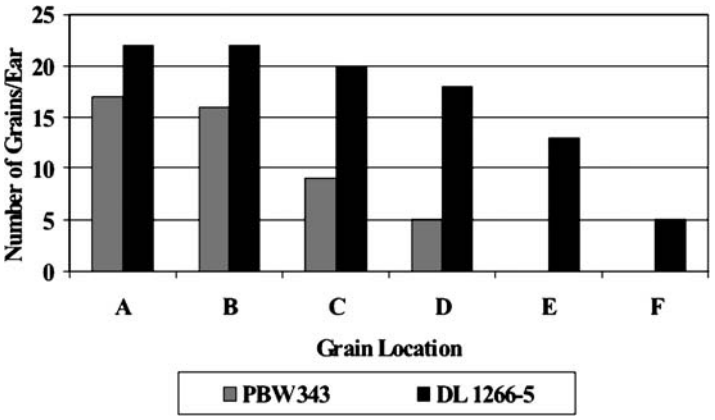


Figure 2. Comparative grain number per spike at different location in DL 1266-5 (NPT) and PBW 343

DL1396-11, two parent lines, SFW and Vaishali and two commercial varieties, PBW343 and HD2329 were used and 33 sequence tagged microsatellite site markers, which are genetically located on different chromosomes of wheat genome, were employed. These markers in combination differentiated the new lines from 137 wheat varieties released for commercial cultivation in India. Fourteen markers detected polymorphism among the thirteen genotypes included in this study, of which eight markers in combination differentiated the seven new wheat lines from each other as well as from their parents. Inheritance of parent-specific alleles revealed the direction of selection during breeding. Grouping of lines based on cluster analysis reflected the nature of their relationship with the parent varieties. A graphic presentation of the genetic constitution of the new plant type lines was developed, which can be used as bar-coded molecular tags for identification of the respective seed samples (Mohapatra et al. 2002).

The success in the development of DL1266-1, DL1266-2, and DL1266-5 with positive combination of grain weight and grain number per spike has given a new direction and opened up the new challenges to further enhance the yield potential of NPT wheats to the tune of around 8 tonnes per hectare when the present most popular wheat PBW343 has yield potential of 6 tonnes per hectare and being grown in around 6 million hectare area of the total 27 million hectare wheat area. In this endeavour these genotypes has been crossed with indigenous and exotic germplasm with the objective to increase the number of productive tillers per plant (around $400\,m^{-2}$) while marginally decreasing the spike size in order to get a combination of an average of 80 grains per spike and 1000 grain weight between 42–45g.

In the beginning of the programme the NPT genotypes were incorporated *Lr24/Sr24*, which provide very high level of resistance to leaf and stem rust not only in India but whole of Indian subcontinent. However, since 2001 the emphasis was laid on to develop NPT derivatives with diverse resistance for all the three rust along with increased yield potential. For diversification of rust resistance many

indigenous and exotic germplasm were used carrying genes for resistance to rusts like *Lr9, Lr19, Lr32, Lr34, Lr41, Lr42, Sr26, Sr31, Sr36, Yr18 etc.*

ACKNOWLEDGEMENTS

Authors are grateful to Indian Council of Agricultural Research, New Delhi and Indian Agricultural Research Institute, New Delhi for providing facilities to work on this line. First author is thankful to Local Organizing Committee of the 7th International Wheat Conference, National Institute for Agricultural Technology, Argentina for providing grants to present this work in person. First author is indebted to Dr. S. Rajaram, ICRISAT, Syria for his constant encouragement for pursuing this research work and also providing part of the financial support for attending the conference.

REFERENCES

Hagman RH, Hucklesby DP (1971) Nitrate reductase from higher plants. In: San Pietro A (ed) Methods in enzymology. Academic Press, New York, NY. pp 491–503

Mohapatra T, Krishanpal, Singh SS, Swain SC, Sharma RK, Singh NK (2002) STMS based DNA fingerprints of the new plant type wheat lines. Curr Sci (Bangalore) 84(8):1125–1129

Nair TVR, Abrol YP (1977) Studies on nitrate reducing system in developing wheat ears. Crop Sci 17:438–442

Pandey PC, Singh SP, Mishra BK, Shukla LM (1983) A comparative study on three wheat cultivars in relation to nutritional stress in developing tillers. Trop Plant Sci Res 1(3):265–266

Pandey PC, Kaim MRS, Singh SS, Singh GP, Joshi DK, Verma APS (2002) Post-anthesis stem reserve mobilization in new plant type wheat. Wheat Inf Serv 95:41–42

Singh SS, Sharma JB, Chand N, Sharma DN (2000) Breaking yield barriers in wheat-designing a new plant type. Indian Wheat Newsletter, DWR, vol 6(2):2

Singh SS, Sharma JB, Chand N, Sharma DN (2001) Breaking yield barriers in wheat-new plant type designed. Wheat Inf Serv 93:22–26

Singh SS, Singh GP, Sharma JB, Chand N, Sharma DN, Singh JB, Pandey PC, Kaim MRS, Mohapatra T, Singh KP (2002) Development of new plant type (NPT) wheats with increased yield potential: Methodology & response to various levels of fertility. Ann Wheat Newsletter 48:8186

EFFECTS OF ABIOTIC STRESS ON SINK AND SOURCE AFFECTING GRAIN YIELD AND QUALITY OF DURUM WHEAT: A MODEL EVALUATION

T.C. PONSIOEN[1], X. YIN[1], J.H.J. SPIERTZ[1,*] AND C. ROYO[2]

[1]*Crop and Weed Ecology Group, Plant Sciences, Wageningen University, P.O. Box 430, 6700 AK Wageningen, The Netherlands*

[2]*Centre UdL-IRTA, Alcalde Rovira Roure 177, 25198 Lleida, Spain*

[*]*E-mail: huub.spiertz@wur.nl*

Abstract: Heat and drought affect grain yield and quality of wheat through sink development and source capacity. Improving grain yield and quality requires an optimization of dynamic interactions of both storage and photosynthetic processes. Seed nitrogen accumulation and the resulting quality traits can be simulated using ecophysiological models. Differences in stress tolerance between genotypes are reflected in individual grain weights and grain yields per culm rather than in rate of leaf photosynthesis. Narrowing the gap between genetic potential and phenotypic expression requires knowledge about the physiological mechanism of increasing sink strength and source capacity

A new model to study genotype-by-environment interaction (GECROS) is used to integrate effects of stresses on sink-source processes and on grain yield and quality. GECROS models each process with a consistent level of detail and deals with interactive aspects and feedback mechanisms of crop growth. This applies to photosynthesis-transpiration-coupling via stomatal conductance, carbon-nitrogen interaction on leaf area index, functional balance between shoot and root activities, and the interplay between supply and demand affecting reserve formation and remobilization

An evaluation of abiotic stress effects on durum wheat in a Mediterranean climate, aiming at a time-resolved simulation of sink-source interactions during stress periods at different stages of development, will be presented

Keywords: grain yield, quality, abiotic stress

INTRODUCTION

Grain yield and quality of wheat are affected by abiotic factors through its effects on sink development and source capacity. The main processes related to sink development are spike development, grain set, grain filling duration, and biosynthesis

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 633–639.

of starch and proteins during grain filling. Narrowing the gap between genetic potential and phenotypic expression, particularly in multiple-stress environments requires knowledge about the physiological mechanism of increasing sink strength and source capacity (Reynolds et al. 2005). Adaptation mechanisms and stress tolerance of wheat genotypes are extremely important for yield stability and grain quality. The importance of nitrogen availability in the soil and nitrogen accumulation and (re)distribution in the crop is emphasised by the often found inverse relation between yield and grain protein concentration. The grain protein concentration is considered as an important quality factor (Gras et al. 2001).

There have been several recent developments in modelling sink-source relations, yield and yield quality under abiotic stresses in wheat (e.g.: Longhui Li et al. 2005, Moreno Sotomayor and Weiss 2004, Martre et al. 2003, Asseng et al. 2002, Jamieson and Semenov 2000). In order to reduce the triviality in modelling sink-source interactions for different genotypes, abiotic stresses and management options, the generic crop growth model GECROS (Genotype-by-Environment interaction on CROp growth Simulator) was developed (Yin and Van Laar 2005). The model algorithms were developed using concepts, which are applicable to a large range of genotypes, environments and nitrogen and water management options. For the Netherlands there are detailed data on winter wheat available for model evaluation (Groot and Verberne 1991). For these conditions, GECROS adequately simulates the crop's biomass and nitrogen accumulation and partitioning at three different N application levels. However, under the temperate climate conditions such as in the Netherlands, the crop is hardly exposed to heat and drought stress. Therefore a dataset of experiments with durum wheat genotypes in a Mediterranean environment under irrigated and rainfed conditions is used to evaluate GECROS for terminal heat and drought stress.

MATERIALS AND METHODS

The GECROS Model

The model incorporates the Farquhar et al. (1980) type of algorithms for calculating potential leaf photosynthesis. When water stress occurs, leaf photosynthesis is reduced according to stomatal regulation based on the actual transpiration. The concept of the two-leaf model is adopted, in which the canopy is divided into sunlit and shaded fractions (De Pury and Farquhar 1997, Wang and Leuning 1998), to calculate canopy photosynthesis and transpiration. For both fractions, the photosynthetically active nitrogen is calculated by a base value of leaf nitrogen (below which photosynthesis is zero) and a leaf nitrogen extinction coefficient for describing an exponential profile in the canopy for vertical decline in nitrogen. GECROS includes several types of respiration: (i) growth respiration, which is based on Penning de Vries et al. (1989), (ii) respiration for ammonium and nitrate uptake and nitrate reduction; uptake of other ions, phloem loading, and (iii) residual maintenance respiration. Nitrogen demand is the maximum of the deficiency driven

and the growth-activity driven demand. The deficiency driven demand is the amount of nitrogen required to restore the nitrogen concentration in the plant to a critical minimum concentration. The growth-activity driven demand is based on the optimum nitrogen/carbon ratio for maximising the relative carbon gain. The actual nitrogen uptake is limited by the maximum nitrogen uptake rate, which is an input parameter.

Root-shoot partitioning for carbon and nitrogen responds to environmental conditions, based on the root-shoot functional balance theory (e.g. Charles Edwards 1976). Intra-shoot carbon partitioning to the stems (including sheaths) and to the grains are determined according to their expected daily carbon demands, which are described by the differential form of a sigmoid function for asymmetric determinate growth (Yin et al. 2003). The remaining shoot-carbon goes either to the leaves, or to the carbon reserve pool in the stems when the leaf area index (LAI) becomes nitrogen limited. The LAI is calculated according to the principles described by Yin et al. (2000 and 2003), as either the carbon or the nitrogen limited leaf area index. The carbon reserves, if any, become available to the grains, when current photosynthesis does not satisfy the carbon demand by grains.

The intra-shoot nitrogen partitioning is based on a pre-defined maximum grain nitrogen concentration of a genotype and a minimum nitrogen concentration in the stems. If the nitrogen requirements for the grains and stems are met from the current nitrogen uptake, the remaining shoot nitrogen goes to the leaves, which include the photosynthetically active part of the stems, sheaths and ears. If the requirements for the grains are not met, remobilisation of nitrogen first from the reserves and then from the leaves and the roots takes place, until the reserves are depleted and the nitrogen concentrations in the leaves and roots reach their minimum values. This remobilisation stimulates leaf and root senescence. If the grain nitrogen requirements are not met by shoot nitrogen and remobilisation, the grain nitrogen concentration declines.

In GECROS, development stages are defined as 0 at seedling emergence, 1 at start of grain filling and 2 at physiological grain maturity. The intervals from stage 0 to 1 and 1 to 2 depend on the genotype specific number of days at optimum daily average temperature. A flexible bell-shaped non-linear function (Yin et al. 1995) is used to describe temperature response of development rate, which has a value of zero when the daily average temperature is below the base temperature or above the ceiling temperature and one when it is equal to the optimum temperature. In case of photoperiod-sensitive genotypes, development rate is also affected by day-length during the photoperiod sensitive phase of the interval from stage 0 to 1.

Data Inputs

Required daily weather data inputs are: global radiation (kJ $m^{-2}d^{-1}$), minimum and maximum air temperature (°C), rainfall (mm d^{-1}), vapour pressure (kPa), and wind speed (m s^{-1}). Latitude of the location is required for calculating day length. The most important soil data are: clay content, minimum soil water content, soil water

content at field capacity and maximum soil water content. Management data include the sowing date, seeding rate and daily supply of water and nitrogen available for crop uptake. Genotype specific input data are: pre-emergence duration, duration between emergence and anthesis, photoperiod-sensitivity and post-anthesis duration, maximum plant height, specific leaf area, stem weight at anthesis, maximum rooting depth, maximum daily nitrogen uptake, potential mature seed weight and maximum seed nitrogen concentration. Crop specific parameters concerning photosynthesis, phenology, morphology, biomass composition and nitrogen content are provided for wheat and a range of other crops. Provided input parameters can be varied for sensitivity analysis.

Datasets were used from IRTA (Centre UdL-IRTA at Lleida), Northern Spain. The dataset contains two seasons (1996/97 and 1997/98) and two different water regimes at different locations in the Lleida province (rainfed and irrigated). For detailed descriptions of the experiments see Villegas et al. (2001) and Rharrabti et al. (2003). Plots were sown on 3 December 1996 (irrigated and rainfed), 23 November 1997 (irrigated) and 17 November 1997 (rainfed). The pre-emergence duration was estimated to 12 days considering the mild temperatures. In the experiments 25 durum wheat varieties were used. There were no significant differences found between genotypes for specific leaf area ($0.022\,m^{-2}g^{-1}$ in the irrigated and $0.017\,m^{-2}g^{-1}$ in the rainfed experiments), grain weight (0.048 g per grain), grain nitrogen concentration ($0.021\,g\,g^{-1}$ in the irrigated and $0.026\,g\,g^{-1}$ in the rainfed experiments), the optimal number of days from emergence to anthesis (40 days) and from anthesis to maturity (27 days). The initial nitrogen in the soil was estimated by the amount of nitrogen in the grains. The minimum and maximum water content and water content at field capacity for the irrigated sites were estimated based on the texture description. For the rainfed sites, the values were adapted in a way that takes the difference in biomass accumulation and yield between the different years into account. The maximum grain N concentration was taken from the value of the rainfed experiment, i.e. 2.6%. It was assumed that 60% of the amount of grain nitrogen came from remobilisation of nitrogen from the vegetative parts.

RESULTS AND DISCUSSION

The applicability of the GECROS crop growth model for simulating wheat growth and yield in Mediterranean conditions is confirmed by this evaluation. The differences between simulated and measured biomass and grain yields turned out to be small (Fig. 1). Grain nitrogen concentrations were lower in the irrigated than rainfed experiments, because post-anthesis nitrogen uptake and remobilised nitrogen were not sufficient to match the higher dry mass of the grains. Under rainfed conditions, grain filling was incomplete and therefore grain nitrogen concentration is equal or only slightly lower than the input value.

In most cases leaf development (LAI) was considerably overestimated in the simulations. Leaf senescence was too fast for irrigated and too slow for rainfed conditions. In GECROS simulated LAI refers to all photosynthetically active green

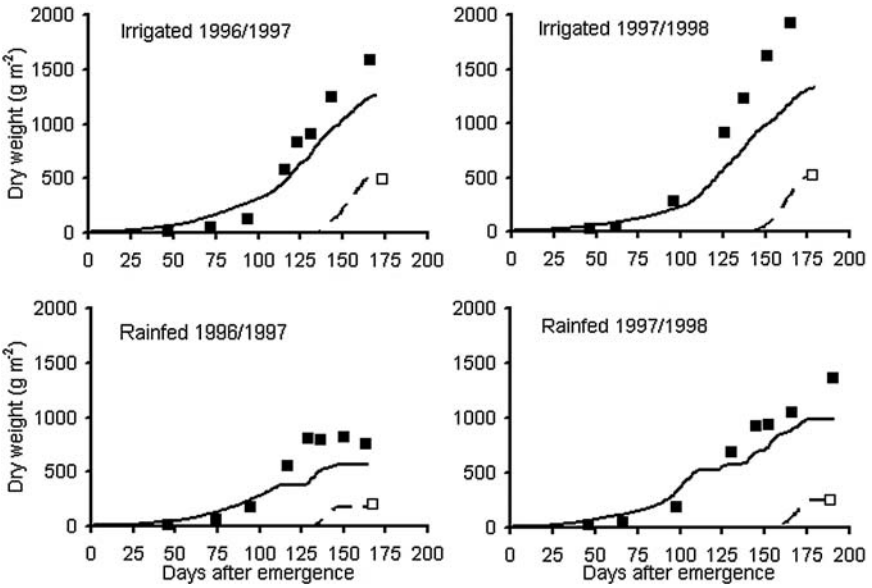


Figure 1. Simulated biomass (full lines), observed biomass (full symbols), simulated grain dry weight (interrupted lines), and observed grain dry weight (open symbols)

surface area of the crop, whereas measured LAI in the experiment refers to green area of leaves. Therefore, leaf senescence simulated by the model also includes the yellowing of green areas of stems, sheaths and ears. Differences between observed and simulated results can also be caused by sampling errors. Grain yield data measured in the experiments are more reliable, because larger plots were harvested, while at intermediate harvests measurements were carried on small samples and units are converted to plot level. Nevertheless, some concepts in the model could be re-examined. Here, we limit the discussion to the effect of temperature on sink development and nitrogen accumulation in the leaves, stems and grains.

The leaf nitrogen pool in GECROS includes the photosynthetically active parts of the stem, even though the amount of nitrogen in the leaves and the stems can be almost equal in size and show a different pattern of remobilisation (e.g. Groot and Verberne 1991). The fast simulated leaf senescence could be reduced if this pool would be divided and nitrogen remobilisation from the photosynthetically active parts of the stem to the grains would be simulated in a way that corresponds to the observed pattern. There is some discussion about the values of the optimum and ceiling temperatures for carbohydrate and protein accumulation (Dupont and Altenbach 2003, Wardlaw et al. 2002, Jenner 1994). In APSIM-NWheat (Asseng et al. 2002), two separate empirical correction factors are included for describing the relation between carbon and nitrogen accumulation in the grains.

Grain dry matter accumulation in GECROS is related to temperature through the development rate and grain nitrogen accumulation is directly coupled to grain

dry matter accumulation. The grain nitrogen concentration will decline when N uptake and N remobilisation is insufficient to meet the requirement for grain protein biosynthesis. This applies to the evaluated circumstances, but might overestimate grain yield under terminal heat stress, when a large pool of carbon reserves is still available. The question is whether the rate of grain filling should be able to compensate for the reduced duration of the grain filling period under heat stress.

With the concept used in GECROS for grain nitrogen accumulation, the fraction of grain nitrogen from redistribution of nitrogen is a very sensitive input parameter, not only affecting the final nitrogen concentration, but also the harvest index and the number of grains per square meter. CERES-Wheat (Moreno Sotomayor and Weiss 2004) based the number of grains on a fraction of the stem biomass accumulation during certain development stages and an empirical factor related to maximum temperature during certain development stages. The way GECROS calculates the number of grains, based on the nitrogen available at anthesis for remobilisation to the grains, is robust and performs well under a wide range of conditions, but it does not simulate the actual development of the spikes and grain set and its temporal vulnerability towards heat and radiation.

In conclusion, GECROS has proven to be a valid tool for simulating sink-source relations in wheat under heat and drought stress. It introduces new concepts describing carbon and nitrogen accumulation in the different parts of the crop and their interactions. The interrelationships between different processes describing direct and indirect effects of environmental conditions and management decisions makes the model robust and reduces the triviality compared to other models using many correction factors and coefficients. Hence, only a few crucial input parameters are needed for describing the differences between genotypes in tolerance to abiotic stresses. However, some concepts need a more comprehensive evaluation (using different heat affected environments and levels of water and nitrogen availability). We are planning multi-environmental experiments to evaluate some GECROS concepts and improve its algorithms. The improved model will offer opportunities to perform reliable scenario studies on effects of specific genotypic traits to improve grain yield and quality for a range of environments and management options.

REFERENCES

Asseng S, Bar-Tal A, Bowden JW, Keating BA, van Herwaarden A, Palta JA, Huth NI, Probert ME (2002) Simulation of grain protein content with APSIM-Nwheat. Eur J Agron 16:25–42

Charles-Edwards DA (1976) Shoot and root activities during steady-state plant growth. Ann Bot 40(168):767–772

De Pury DGG, Farquhar GD (1997) Simple scaling of photosynthesis from leaves to canopies without the errors of big-leaf models. Plant Cell Environ 20(5):537–557

Dupont FM, Altenbach SB (2003) Molecular and biochemical impacts of environmental factors on wheat grain development and protein synthesis. J Cereal Sci 38:133–146

Farquhar GD, von Caemmerer S, Berry JA (1980) A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. Planta 149:78–90

Gras PW, Anderssen RS, Keentok M, Békés F, Appels R (2001) Gluten protein functionality in wheat flour processing: a review. Aust J Agr Res 52:1311–1323

Groot JJR, Verberne ELJ (1991) Response of wheat to nitrogen fertilization, a data set to validate simulation models for nitrogen dynamics in crop and soil. Fert Res 27:349–383

Jamieson PD, Semenov MA (2000) Modelling nitrogen uptake and redistribution in wheat. Field Crop Res 68:21–29

Jenner CF (1994) High temperature and starch synthesis in wheat. Aust J Plant Physiol 21:791–806

Longhui L, Qiang Y, Youfei Z, Jing W, Quanxiao F (2005) Simulating the response of photosynthate partitioning during vegetative growth in winter wheat to environmental factors. Field Crop Res (in press) doi:10.1016/j.fcr.2005.06.003

Martre P, Porter JR, Jamieson PD, Triboï E (2003) Modelling grain nitrogen accumulation and protein composition to understand sink/source regulations of nitrogen remobilization for wheat. Plant Physiol 133:1959–1967

Moreno-Sotomayor A, Weiss A (2004) Improvements in the simulation of kernel number and grain yield in CERES-Wheat. Field Crop Res 88:157–169

Penning de Vries FWT, Jansen DM, Ten Berge HFM, Bakema A (1989) Simulation of ecophysiological processes of growth in several annual crops. IRRI, Los Baños and Pudoc, Wageningen, p 271

Reynolds MP, Pellegrineschi A, Skovmand B (2005) Sink-limitation to yield and biomass: a summary of some investigations in spring wheat. Ann Appl Biol 146:39–49

Rharrabtia Y, Royo C, Villegas D, Aparicio N, García del Moral LF (2003) Durum wheat quality in Mediterranean environments I. Quality expression under different zones, latitudes and water regimes across Spain. Field Crop Res 80:123–131

Villegas D, Aparicio N, Blanco R, Royo C (2001) Biomass accumulation and main stem elongation of durum wheat grown under Mediterranean conditions. Ann Bot 88:617–627

Wang YP, Leuning R (1998) A two-leaf model for canopy conductance, photosynthesis and partitioning of available energy I: - model description and comparison with a multi-layered model. Agr Forest Meteorol 91(1):89–111

Wardlaw IF, Blumenthal C, Larroque O, Wrigley CW (2002) Contrasting effects of chronic heat stress and heat shock on kernel weight and flour quality in wheat. Funct Plant Biol 29:25–34

Yin X, Kropff MJ, McLaren G, Visperas RM (1995) A nonlinear model for crop development as a function of temperature. Agr Forest Meteorol 77:1–16

Yin X, Schapendonk AHCM, Kropff MJ, van Oijen M, Bindraban PS (2000) A generic equation for nitrogen-limited leaf area index and its application in crop growth models for predicting leaf senescence. Ann Bot 85:579–585

Yin X, Goudriaan J, Lantinga EA, Vos J, Spiertz H (2003) A flexible sigmoid function of determinate growth. Ann Bot 91:361–371

Yin X, Van Laar HH (2005) Crop systems dynamics: an ecophysiological simulation model for genotype-by environment interactions. Wageningen Academic Publishers, The Netherlands. 155pp

EFFECTS OF SOME MANAGEMENT PRACTICES AND WEATHER CONDITIONS ON *TRITICUM AESTIVUM* FARINOGRAPHIC STABILITY IN MIRAMAR, ARGENTINA

F. GUTHEIM

Chacra Experimental Miramar, Ministerio de Asuntos Agrarios de la Prov. de Buenos Aires
E-mail: chexpmir@yahoo.com

Abstract: Variability in farinographic stability of wheat flour was analyzed on samples obtained from yield trials with commercial cultivars in Miramar. Year, planting dates and control of foliar diseases were used as sources of variation. Regression analyses were studied to determine better production practices, to maximize farinographic stability levels in wheat grown under field conditions

Keywords: farinographic stability, management practice

INTRODUCTION

Dough properties and baking performance of wheat are strongly dependent on both genotype and environment (Peterson et al. 1992, Johansson and Svensson 1998, Johansson and Svensson 1999). While genotype (G) is recognized as a major farinographic stability determinant factor, environment (E) and the interaction G x E (GE) are less known effects. One problem is to produce flour with good industrial quality, but at the same time it is necessary for farmers have stability in quality terms year by year for avoiding penalties in the price of wheat at the time of reception of the grains by the industry.

Farinographic stability of wheat flour is of paramount importance for Argentine wheat exportation, mainly to the Brazilian market. Mills are searching for wheat with more than 20 minutes of stability. The aim of this work is to show how management practices as planting date and foliar fungicides application affect flour farinographic stability.

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 641–648.

MATERIALS AND METHODS

Data were obtained from field yield trials conducted in Chacra Experimental Miramar (38°10'S, 58°0'W), Buenos Aires, Argentina, during 2002–2004 seasons. Different wheat cultivars were sown in four planting dates (from June 10 to mid-August, spaced *ca*. 20 days among them) under non-irrigated field conditions. During the first and third planting dates, treatments with fungicides (triazol plus strobilurin) were applied at Zadoks (1974) code 49 to prevent foliar diseases. A control treatment without fungicides application was also included. In second and fourth planting dates no fungicides treatments were applied. When fungicides were used, treatments were arranged in a split plot design where fungicides represented the main plot and cultivars the sub-plot. This study although analyses the performance of different cultivars (Table 1) will put more emphasis in the behavior of Buck Poncho (a traditional, Argentine, good quality wheat cultivar).

Experiments were fertilized with nitrogen and phosphorus based fertilizers. Insects were controlled.

Daily maximum and minimum temperature, rainfall and relative humidity were registered from thermo-hygrometers. Meteorological data were registered not far 400 m from experimental sites. Solar radiation was obtained from EEA INTA Balcarce meteorological station, distant 40 kilometers from experimental sites. The Table 2 shows 10-days means of meteorological data for each year and the climatic mean for each one.

Phenological data recorded include crop emergence, heading (Zadoks 55) and physiological maturity (CIMMYT 1989). Harvest was made with a combine to measure grain yield (g m^{-2}). Grain protein (%), test weight (kg hl^{-1}) and weight per grain (mg) were recorded from a mixed sample of grain prepared with grains obtained from the four or five repetitions of each experiment. Stability of wheat flour was analyzed as mentioned in standard protocol IRAM 15855:2000 using a Brabender farinograph. Stability of wheat flour is defined as the difference in time between the point at which the highest part of the curve raised, at first time, the 500 farinographic units and the point at which it is left. It is expressed in minutes.

Regressions between variables were analyzed. Stability values obtained without fungicides were used to obtained relationships with climatic and crop variables (such as test weight, individual grain, and others).

RESULTS AND DISCUSSION

Phenological data, yield and grain characteristics for different planting dates and fungicides treatments are shown in Table 2. Fungicides did not affect the timing of different phenological events. Yield was the highest in 2003, following by 2004 and 2002 growing seasons, respectively. The optimum sowing date in terms of yield was between the 2nd and 3rd sowing dates (Table 3).

Farinographic stability was associated with weight per grain in three of nine genotypes (which are regularly included in the experimental net carried out in

Table 1. Dates of sowing and varieties utilized for each year and planting date

Planting dates	1st	2nd	3rd	4th	1st	2nd	3rd	4th	1st	2nd	3rd	4th
Year	2002	2002	2002	2002	2003	2003	2003	2003	2004	2004	2004	2004
Sowing date	13-Jun	01-Jul	19-Jul	12-Ago	13-Jun	02-Jul	21-Jul	14-Ago	15-Jun	01-Jul	20-Jul	11-Ago
ACA302	–	–	x	–	–	x	x	–	–	–	x	–
ACA303	x	–	–	–	–	–	–	–	–	–	–	–
ACA304	–	–	–	–	–	–	–	–	x	–	–	–
B. Arriero	–	–	–	–	x	x	–	–	x	–	–	–
B. Biguá	–	–	–	–	–	–	x	x	–	–	x	–
B. Brasil	–	–	x	–	–	–	–	–	–	–	x	–
B. Guapo	x	x	–	–	–	–	–	–	x	–	–	–
B. Guatimozín	–	–	–	–	–	–	–	–	x	–	–	–
B. Mejorpan	–	–	–	–	–	–	–	–	–	–	x	–
B. Panadero	–	–	x	–	–	–	–	–	–	–	x	–
B. Pingo	–	–	x	–	–	–	–	–	–	–	–	–
B. Poncho	**–**	**x**	**x**	**–**	**x**	**x**	**x**	–	**x**	**x**	**x**	**x**
B. Pronto	–	–	x	x	–	–	–	–	–	–	x	–
B. Sureño	–	x	x	–	–	–	–	–	x	–	–	–
Biointa 3000	–	–	–	–	–	–	–	–	x	–	–	–
Biointa B2001	–	–	–	–	–	x	x	–	–	–	–	–
Biointa B2001	–	–	–	–	–	–	–	–	–	–	x	–
Caudillo	x	–	–	–	–	–	–	–	–	–	–	–
INIA Churrinche	–	–	–	–	–	–	–	–	–	–	x	–
K. Jabalí	–	–	–	–	–	–	–	–	x	–	–	–
K. Proteo	–	–	–	–	–	x	x		–	–	–	–
K. Proteo	–	–	–	–	–	–	–	–	–	–	x	–
K. Sagitario	–	–	–	–	x	x	–	–	–	–		–
Onix	–	–	–	–	–	–	–	–	–	–	x	–

Table 2. Means of daily maximum and minimum air temperature, relative humidity and radiation and accumulate precipitation on a 10-days basis for each growing season. Temperature, relative humidity and rainfall were registered at CE Miramar. Radiation was registered at EEA Balcarce. AVG: Means 1971–2004

	Daily maximum temperature °C				Daily minimum temperature °C				Rainfall mm				Relative humidity %				Radiation MJ m^{-2} d^{-1}		
Period	AVG	2002	2003	2004	AVG	2002	2003	2004	AVG	2002	2003	2004	AVG	2002	2003	2004	2002	2003	2004
June 1–10	12,9	13,4	12,7	14,8	4,1	3,3	2,3	2,6	22,1	26,0	0,0	0,0	78,2	77,7	70,8	77,3	6,0	6,5	5,7
June 11–20	12,4	11,7	15,6	15,2	4,1	0,4	4,4	4,6	21,1	0,5	23,0	10,0	79,8	75,3	78,1	70,6	7,8	5,5	5,8
June 21–30	12,1	8,6	12,2	15,7	3,5	-0,8	4,9	3,4	16,9	12,0	8,5	16,0	79,8	88,2	86,0	82,6	5,9	4,6	6,1
July 1–10	11,6	9,1	11,5	11,4	2,8	-0,6	0,0	1,4	15,9	27,0	21,5	16,5	79,8	84,1	82,5	71,2	6,0	5,8	5,9
July 11–20	11,8	13,3	11,5	10,7	3,0	3,6	1,9	0,9	19,7	0,0	25,0	8,5	79,0	85,2	77,9	80,0	6,2	6,7	6,8
July 21–31	12,4	13,0	13,1	14,0	3,2	3,1	1,3	5,8	22,3	8,5	61,5	53,0	78,4	77,1	78,5	88,0	7,1	6,6	6,1
August 1–10	12,7	12,4	11,9	11,8	3,1	2,5	3,6	4,1	16,5	6,0	75,5	19,0	77,7	83,8	85,9	88,9	7,0	7,0	7,2
August 11–20	13,6	13,5	15,4	12,6	3,5	3,5	3,4	4,4	19,4	63,5	3,0	38,5	77,6	82,0	73,6	90,0	6,8	8,4	6,9
August 21–31	14,5	15,7	11,4	13,3	3,9	7,1	-0,8	2,6	24,8	32,5	5,5	36,5	74,8	88,7	76,0	76,2	7,2	10,6	10,1
September 1–10	15,0	12,3	17,6	14,7	3,7	2,3	5,5	4,0	19,5	6,0	0,0	47,0	74,1	81,0	71,1	75,3	9,9	9,7	10,3
September 11–20	15,4	14,3	12,1	16,9	4,3	4,4	1,2	3,1	17,4	46,0	19,5	0,0	73,8	86,6	71,0	66,8	11,4	9,9	14,5
September 21–3	16,7	19,3	16,5	16,9	5,0	2,4	3,1	1,7	17,6	19,5	15,0	0,0	74,2	77,1	74,2	68,2	16,8	13,1	14,9
October 1–10	17,0	20,4	17,2	15,3	5,9	5,8	4,6	7,2	28,8	8,5	36,5	15,5	75,1	67,8	71,2	87,2	15,5	13,4	11,8
October 11–20	19,0	20,2	19,7	18,6	7,0	9,4	7,1	6,0	34,2	91,0	49,5	23,5	74,1	83,7	76,4	74,1	14,2	15,8	16,3
October 21–31	19,7	20,9	23,1	21,7	7,4	6,9	6,1	4,8	32,4	103,5	11,5	7,0	71,9	68,8	63,0	54,3	20,6	21,1	19,6
November 1–10	20,3	19,7	23,1	18,9	8,1	9,6	7,9	8,9	34,1	31,5	5,5	74,0	72,1	81,2	66,0	76,0	16,2	21,3	17,7
November 11–20	21,3	23,6	18,9	17,6	8,9	9,6	8,0	8,6	30,7	40,5	61,0	48,5	71,4	76,3	77,1	81,7	17,5	18,2	17,7
November 21–30	23,4	24,1	23,2	25,4	9,9	9,5	8,3	11,7	21,8	7,6	17,5	0,0	66,6	63,1	61,6	73,0	23,8	22,0	19,8
December 1–10	24,2	25,4	22,4	24,8	10,4	10,8	8,6	12,6	28,4	7,0	154,5	31,5	65,9	57,8	70,5	73,7	24,1	20,1	19,7
December 11–20	24,6	25,7	26,1	26,4	11,2	12,3	9,8	12,1	31,4	38,5	5,5	16,5	66,0	64,7	56,3	58,6	23,4	23,4	23,4
December 21–31	26,3	27,1	24,9	27,8	13,0	13,0	12,1	14,5	33,9	0,0	69,5	21,0	65,5	58,1	68,7	65,8	22,6	21,6	22,4

Table 3. Mean phenology, grain yield and other variables registered during each growing season and planting date in CE Miramar experiments

Growing season	Planting dates	Without fungicides						With fungicides					
		Heading	Maturity	(mg)	Test weight	Grain protein	Yield (Kg ha^{-1})	Heading	Maturity	(mg)	Test weight	Grain protein	Yield (Kg ha^{-1})
2002	1	30-Oct	14-Dic	35, 7	83, 2	12,5	4902	30-Oct	15-Dic	39, 3	84, 3	12,7	5720
	2	31-Oct	14-Dic	36, 7	83, 4	12,7	5250						
	3	30-Oct	14-Dic	34, 7	83, 7	12,1	4701	30-Oct	15-Dic	38, 6	84, 7	12,5	5782
	4	08-Nov	17-Dic	35, 3	82, 3	12,3	4792						
2003	1	31-Oct	17-Dic	40, 3	82, 2	11,9	6157	31-Oct	17-Dic	42, 0	82, 7	12,0	6719
	2	02-Nov	20-Dic	40, 1	82, 5	12,4	5868						
	3	02-Nov	20-Dic	42, 7	83, 0	12,4	6034	02-Nov	20-Dic	44, 5	83, 6	12,4	6542
	4	10-Nov	26-Dic	42, 1	82, 8	12,5	5825						
2004	1	30-Oct	09-Dic	37, 0	79, 3	11,9	5306	30-Oct	09-Dic	39, 9	80, 6	12,1	5930
	2	02-Nov	12-Dic	37, 6	80, 4	12,6	5661						
	3	01-Nov	11-Dic	37, 1	81, 1	12,2	5767	01-Nov	11-Dic	39, 4	82, 1	12,5	6141
	4	07-Nov	16-Dic	34, 5	81, 8	12,3	5323						

Miramar) with more than 3 observations (Fig. 1). B. Poncho, with 9 observations, produced a lineal positive regression ($r^2 = 0.54$, $n = 9$, $p = 0.03$). ACA302 and B. Pronto values of farinographic stability could be associated with weight per grain also ($r^2 = 0.91$, $n = 4$, $p = 0.04$ and $r^2 = 0.99$, $n = 3$, $p = 0.05$, respectively). For other varieties evaluated, no strong relationships could be obtained. The Fig. 1 shows five varieties with high relationship between both variables. Thus, the general trend was the heavier individual grain weight the higher farinographic stability. In addition, weight per grain was positive related to low temperatures and well water supply, suggesting that good conditions during grain filling improve not only grain weight but also farinographic stability. Different slopes could be observed in Fig. 1, which suggests genotype interaction.

The case of B. Poncho cultivar could help to understand how planting date could affect farinographic stability. Fig. 2 shows evolution of values for each planting date and the differences between the three years of evaluation. Earlier sowing dates showed higher stability values, with no differences between first and second sowing dates, while third sowing date shows a variable result. Earlier sowing dates have a longer grain filling period, and frequently it determines better grain filling conditions. Mean farinographic stability for all cultivars shows less variation between planting dates than the farinographic stability registered with B. Poncho. At each planting date cultivars were different but they were sown according to the cycle (long cycle in 1st and 2nd planting dates, intermediate in 2nd and 3rd and short cycle in 3rd and 4th planting dates). Cultivars show a wide range for farinographic stability, according to inherent quality characteristics (Table 3).

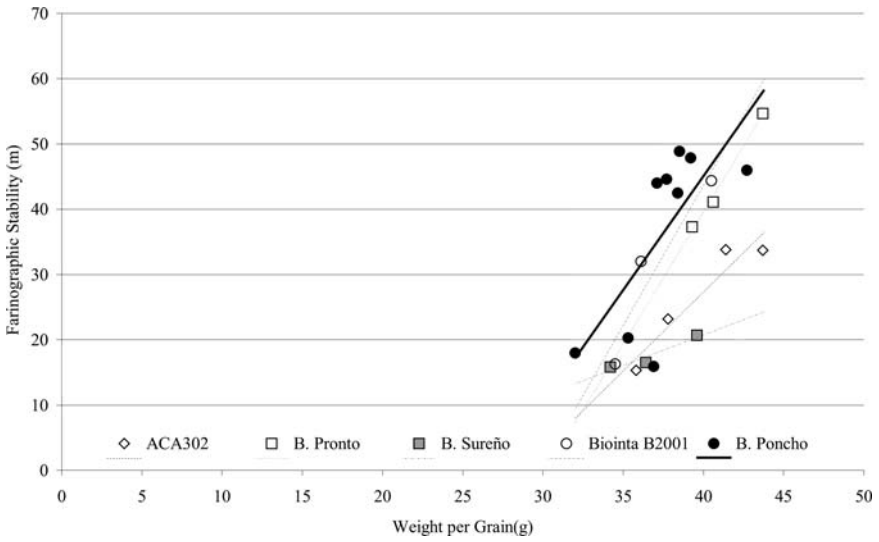


Figure 1. Relationship between farinographic stability and weight per grain. Lines show lineal regression adjustment for each cultivar

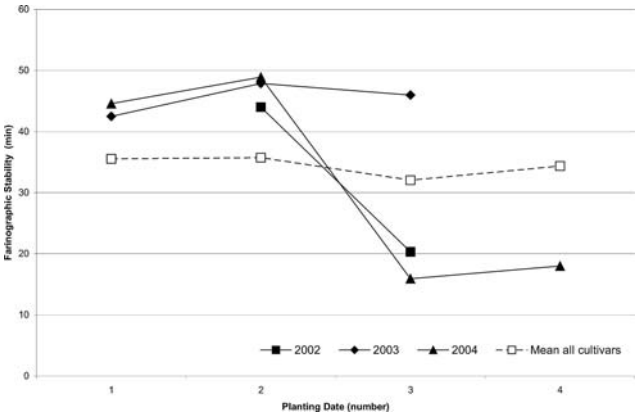


Figure 2. Farinographic stability affected by planting date for B. Poncho cultivar (without fungicide application)

The control of foliar diseases by fungicides before heading increased farinographic stability ($r^2 = 0.58$; $n = 34$; $p < 0.001$). Slope of relationship between farinographic stability with and without fungicide treatment of all cultivars was lower than 1 (fig.3). Regression adjustment for B. Poncho ($r^2 = 0.58$; $n = 5$; $p = 0.14$) produced a similar trend. Main difference between regressions refers to a higher intercept value for B. Poncho. Probably, fungicides control promotes better grain filling and, as said before, it has relationship with farinographic stability. In

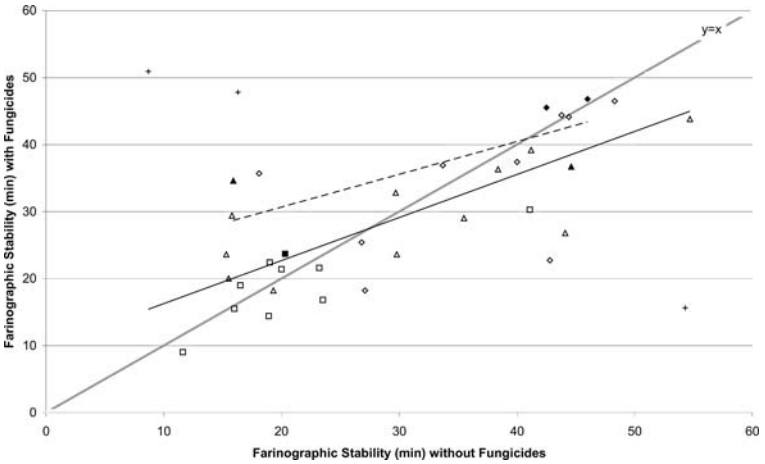


Figure 3. Relationship between farinographic stability obtained with (y) and without (x) foliar fungicides applied before heading. Entire line is the regression for all cultivars, Cut line is the regression for B. Poncho cultivar. Black points refer to B. Poncho, + points were not considered on regression lines. Squares: 2002, diamonds: 2003, and triangles: 2004 growing seasons.

conclusion, the available data show that management practices that improve grain filling could increase farinographic stability.

ACKNOWLEDGEMENTS

The author would like to thanks Ing. Miguel Corti Maderna from ALEA S.A. Company for farinograph analyses and Dr. Pablo E. Abbate for his helpful comments.

REFERENCES

CIMMYT (1989) Guía para el registro de datos

Instituto Argentino de Normalización (2000) Harina de trigo. Características físicas de las masas. Determinación de la absorción de agua y de las propiedades reológicas, utilizando un farinógrafo. Norma IRAM 15855:2000

Johansson E, Svensson G (1998) Variation in bread-making quality: Effects of weather parameters on protein concentration and quality in some Swedish wheat cultivars grown during the period 1975–1996. J Sci Food Agric 78:109–118

Johansson E, Svensson G (1999) Influences of yearly weather variation and fertilizer rate on bread-making quality in Swedish grown wheats containing HMW glutenin subunits 2 + 12 or 5 + 10 cultivated during the period 1990–1996. J Agric Sci 132:13–22

Peterson CJ, Graybosch RA, Baenzinger PS, Grombacher AW (1992) Genotype and environment effects on quality characteristics of hard red winter wheat. Crop Sci 32:98–103

Zadoks JC, Chang TT, Konzak CF (1974) A decimal code for the growth stages of cereals. Weed Res 14:415–421

GRAIN WEIGHT AND GRAIN QUALITY OF WHEAT IN RESPONSE TO ENHANCED ULTRA-VIOLET (UV-B) RADIATION AT LATTER STAGES OF CROP DEVELOPMENT

D.F. CALDERINI[1,*], S. HESS[2], C.R. JOBET[3], AND J.A. ZÚÑIGA[3]

[1]*Instituto de Producción y Sanidad Vegetal, Facultad de Ciencias Agrarias, Universidad Austral de Chile*

[2]*Instituto de Química, Facultad de Ciencias. Universidad Austral de Chile*

[3]*INIA Carillanca, Chile*

[*]*E-mail:danielcalderini@uach.cl*

Abstract: The increase of UV-B radiation may be a challenge for wheat production systems in Southern Chile. Previous reports had shown that increased UV-B radiation decreased wheat yield by affecting both grain number and grain weight. The objective of the present study was to evaluate the response of grain yield, yield components and grain quality to higher UV-B radiation during key periods for yield component determination. The experiment was carried out at field conditions in the Universidad Austral de Chile (40° South latitude). Treatments consisted on 2 spring wheat cultivars exposed to 2 periods of supplemented UV-B radiation (280–320 nm): between (*i*) booting and anthesis and (*ii*) anthesis and physiological maturity. Ultra-violet radiation was enhanced 3.5 kJ m^{-2} during 5 hours per day by UV-B lamps (Q panel UV-313, Philips). At harvest, plants were sampled to quantify above-gound biomass, grain yield, grain number and thousand grain weight. In addition, protein and gluten concentration of grains were measured. Grain yield was not affected ($p > 0.05$) by UV-B increase either at pre- nor at post-anthesis treatments. Similar results were found for each yield component. In addition, grain protein and gluten concentration showed similar values between enhanced UV-B and control treatments. Therefore, these results do not support that expected increases of UV-B radiation in Southern Chile could compromise wheat production systems, at least if increases of UV-B radiation take place at latter stages of the crop cycle

Keywords: radiation, grain weight

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 649–654.

INTRODUCTION

Ozone depletion in the stratosphere has increased UV-B radiation at high latitudes in the South Hemisphere (Kerr and McElroy 1993). Biological consequences of this are not sufficiently known but different reports have shown negative effects on both natural and agricultural ecosystems (e.g., Caldwell 1968, Ballare et al. 2001, Kakani et al. 2003). Although different results have been found on yield of grain crops (Kakani et al. 2003) Li et al. (1998) reported that wheat yield was decreased between 18 and 57% assuming stratospheric ozone depletion between 12 and 25%. Therefore, this could be an important challenge for wheat production systems in Southern Chile where this crop shows the highest harvested area. Even though the relevance of the possible effect of increased UV-B radiation there are few studies evaluating the effect of enhanced UV-B radiation on wheat in Chile.

Li et al. (1998) found that enhanced UV-B radiation decreased both grain number m^{-2} (up to 50%) and thousand grain weight (up to 30%) when solar radiation was supplemented with UV-B radiation between 3 leaf stage and ripening. Tacking into account that the most probable scenario for UV-B increase will occur during spring and summer time in Southern Chile (Lovengreen et al. 2000) it is expected that key phenological phases for grain number and grain weight determination (from jointing to ripening) could be exposed to higher UV-B radiation than previous phases. In addition to grain yield, grain quality is also clearly important in wheat production systems. However, little is known about the effect of higher UV-B radiation on quality traits as grain protein and gluten content.

Milling industry at Southern Chile is progressively more interested in increasing industrial traits as test weight and milling efficiency, which are associated with both grain size and grain weight. Regarding the relevance of grain weight for grain yield, milling efficiency and related traits, the aim of the present study was to evaluate the response of grain yield, grain weight and grain quality to enhanced UV-B radiation.

Reports published over the last years (Wardlaw 1994, Calderini et al. 1999) found that thermal conditions before anthesis affect grain weight in wheat. Moreover, the period immediately before anthesis (between booting and anthesis) has been proposed as relevant for grain weight determination in addition to the grain filling period (Calderini et al. 2001). Tacking this into account, the present study evaluated the impact of enhanced UV-B radiation on grain weight both at pre- and post-anthesis periods.

MATERIALS AND METHODS

One experiment was carried out at field conditions at the Universidad Austral de Chile (Valdivia, 40° South latitude) in the 2004–2005 growing season. Treatments consisted on 2 spring wheat cultivars (Huañil and Pandora) exposed to 2 periods of supplemented UV-B radiation (280–320 nm): between (*i*) booting and anthesis and (*ii*) 10 days after anthesis and physiological maturity. Plots were arranged in a split-plot design with three replicates, where the main plot was assigned to the period of

supplemented UV-B and the sub-plots to cultivars. UV was enhanced at 3.5 kJ m^{-2} during 5 hours per day by UV-B lamps, Q panel UV-313 (Philips). Lamps were set in plastic structures at 20 cm above the canopy and kept at this distance during the whole treatment period. Controls at both treatment periods (pre- and post-antesis) consisted on similar plastic structures without UV-B lamps. Sowing date was the optimal for spring wheat cultivars and sowing rate was 320 plant m^{-2}. Plots were 1 m long and 5 rows, 0.15 m apart. They were fertilized with 200 kg N ha^{-1} and 60 kg P_2O_5 ha^{-1}. Diseases and insects were prevented or controlled by spraying recommended pesticides as required. Weeds were periodically removed by hand. Plots were irrigated, complementing rainfall, from planting to maturity, to avoid water stress.

At harvest, plants in 0.5m of the central row were sampled to quantify biomass, grain yield, grain number and one thousand grain weight. In addition, 10 spikes were randomly sampled at harvest in each plot for recording individual weight of grains corresponding to each grain position within the two central spikelets (G_1, G_2, G_3 and G_4 from the rachis). These grains were weighted after oven drying at 60 °C for 48 h with an electronic balance (Mettler, Germany).

Nitrogen concentration and gluten of grains from 0.5m samples were measured as follow: 35 g seed samples were tempered to 15% moisture for 18h and milled on a Brabender Quadraplex laboratory mill. Flour protein was determined by Kjeldahl procedure using a conversion factor of 5.7 of the total nitrogen to percent protein. Wet gluten, dry gluten content and gluten index were determined in a Glutomatic System using ICC methods.

The effect of UV-B treatments and cultivars on key response variables were assessed with analysis of variance. Regression analyses were used to assess the degree of association between variables.

RESULTS AND DISCUSSION

The increase of UV-B radiation at pre-anthesis did not affect anthesis date in Huañil (30/11) and Pandora (29/11). Physiological maturity was reached at similar dates in controls and pre- and post-anthesis treatments. These results do not agree with previous reports on wheat (Li et al. 1998) where enhanced UV-B radiation delayed anthesis and physiological maturity dates. However, the difference between reported results and the present study could be due to the moment at which enhanced UV-B treatments were initiated (at 3 leaves stage and booting, respectively). In previous experiments (Li et al. 1998) the grain filling duration was not affected by supplemented UV-B radiation. In addition, it is increasingly accepted that projected UV-B levels will not outstandingly modify crop phenology in crop production systems (Kakani et al. 2003).

Grain yield was not affected by enhanced UV-B radiation (Table II). Similar yields ($p > 0.05$) were found when the crop underwent higher UV-B radiation at pre- and post-anthesis in Pandora. On the other hand, Huañil showed lower grain yield ($p < 0.05$) in the pre-anthesis treatment (both in control and enhanced UV-B

Table 1. Grain yield, above-ground biomass, harvest index (HI), grain number and thousand grain weight of wheat cultivars under UV-B treatments at pre- and post-anthesis. Same letter within each crop phase are not statistically different at $P < 0.05$

Crop phase	Genotype	UV-B treatment	Grain yield (g m^{-2})	Biomass (g m^{-2})	HI (%)	Grain number (m^{-2})	Grain weight (g)
Pre-anthesis	Huañil	Control	590.7 b	1359.1 b	43.4 a	11,853 b	49.7 a
		UV-B	577.7 b	1336.1 b	43.3 a	11,689 b	49.3 a
	Pandora	Control	854.1 a	1718.6 a	49.6 a	16,489 a	51.7 a
		UV-B	839.6 a	1668.2 a	50.4 a	16,582 a	50.7 a
CV (%)			10.3	9.4	3.2	8.0	3.9
Post-anthesis	Huañil	Control	846.3 a	1806.2 a	46.7 a	17,165 a	49.1 a
		UV-B	816.9 a	1783.5 a	45.7 a	17,124 a	47.7 a
	Pandora	Control	878.0 a	1794.2 a	48.9 a	16,569 a	54.0 a
		UV-B	867.2 a	1800.2 a	48.1 a	17,618 a	49.3 a
CV (%)			17.6	16.3	2.5	16.5	11.2

radiation). This could be attributed to the effect of lower solar radiation (due to solar radiation interception by structures holding up the lamps) as similar decrease was found in controls at pre-anthesis (Table 1). In addition, the negative effect of solar radiation interception by structures on grain yield at the pre-anthesis treatment found in Huañil is supported by the fact that grain number was significantly lower in this cultivar while thousand grain weight was unchanged (Table 1). Other crop variables at harvest as biomass and yield components, were also unaffected by increased UV-B radiation.

Although cultivar variability could be expected in wheat response to enhanced UV-B radiation (Li et al. 2000) the lack of UV-B effects found in the present study is in agreement with other reports (Temura et al. 1990, Hakala et al. 2002).

A further insight on grain weight was carried out through the evaluation of grains set at particular positions within the spike to avoid confounding results due to compensatory effects between grain number and thousand grain weight. Individual grain weight did not show significant ($p > 0.05$) effects of UV-B treatments both at pre- and post-anthesis (Table 2). However, grain weight at enhanced UV-B treatments consistently showed lower values than controls. Averaged across UV-B treatments, grain weight of supplemented UV-B plots decreased 5% respect to controls showing similar effects at pre- (4.8%) and post-anthesis (5.2%) treatments. In addition, the relationship between grain weight in controls and under increased UV-B treatments showed a linear and significant relationship ($b = 0.98$; $r = 0.98$, $n = 16$) but the intercept was lower than zero ($a = -1.6$). Nevertheless, the effect of enhanced UV-B radiation suggested from detailed analyses of individual grain position, if any, had negligible impact in terms of the outcome of grain weight and grain yield of the crop.

Grain quality was evaluated as protein and gluten concentration of grains and gluten index. Although a decrease in chlorophyll content has been shown when

Table 2. Grain weight at different positions within the central spikelets of the spikes of wheat cultivars under UV-B treatments at pre- and post-anthesis. Same letter within each crop phase are not statistically different at $P < 0.05$

Crop phase	Genotype	UV-B treatment	Individual grain weight (mg)			
			G_1	G_2	G_3	G_4
Pre-anthesis	Huañil	Control	58.7 a	60.7 a	55.9 a	43.0 a
		UV-B	57.5 a	59.2 a	52.8 a	42.6 a
	Pandora	Control	59.3 a	61.2 a	58.8 a	47.3 a
		UV-B	56.5 a	58.4 a	54.7 a	41.9 a
CV (%)			4.7	4.4	6.0	9.3
Post-anthesis	Huañil	Control	61.5 a	63.6 a	58.1 a	45.1 a
		UV-B	57.5 a	58.3 a	55.2 a	42.3 a
	Pandora	Control	58.7 a	61.8 a	58.7 a	46.7 a
		UV-B	56.7 a	60.4 a	56.7 a	43.8 a
CV (%)			3.6	3.9	3.5	5.6

crops grow under enhanced UV-B radiation in different studies (see Kakani et al. 2003), which could affect nitrogen economy of plants, increased UV-B radiation did not affect protein concentration of grains both at pre- and post-antesis treatments (Table 3). In agreement with protein concentration, wet and dry gluten concentrations were not affected by UV-B treatments. On the contrary, gluten index showed significant differences ($p < 0.05$) between UV-B treatments at pre-anthesis (Table 3). However, this effect was found only in one genotype (Huañil).

Results obtained in the present study do not support that expected increases of UV-B radiation in Southern Chile could compromise wheat production systems, at least if increases of UV radiation were reached at latter stages of the crop cycle.

Table 3. Protein, wet gluten and dry gluten relative contain (%), and gluten index of wheat cultivars under UV-B treatments at pre- and post-anthesis. Same letter within each crop phase are not statistically different at $P < 0.05$

Crop phase	Genotype	UV-B treatment	Protein concentration	Gluten (wet)	Gluten (dry)	Gluten index
Pre-anthesis	Huañil	Control	11.6 a	38.1 a	13.2 a	89.0 a
		UV-B	11.7 a	39.1 a	13.3 a	79.3 b
	Pandora	Control	10.9 a	32.3 c	11.2 b	94.3 a
		UV-B	10.4 a	31.8 c	10.9 b	91.3 a
CV (%)			7.0	6.7	6.1	5.4
Post-anthesis	Huañil	Control	9.8 a	31.7 a	11.1 a	89.7 a
		UV-B	10.8 a	33.9 a	11.9 a	91.3 a
	Pandora	Control	10.2 a	30.6 a	10.8 a	95.0 a
		UV-B	10.4 a	31.7 a	10.8 a	90.0 a
CV (%)			9.7	7.3	7.1	4.8

ACKNOWLEDGEMENTS

We especially thank Magda Lobnik and Luis Vargas for technical assistance, and the personal of the Tissue Culture Laboratory for providing facilities to carry out measurements. This work was supported by the Chilean Technical and Scientific Research Council (CONICYT), Project FONDECYT 1040125.

REFERENCES

Ballare CL, Rousseaux MC, Searles PS, Zaller JG, Giordano CV, Robson TM, Caldwell MM, Sala OE, Scopel AL (2001) Impacts of solar ultraviolet-B radiation on terrestrial ecosystems of Tierra del Fuego (southern Argentina) - an overview of recent progress. J Photochem Photobiol 62:67–77

Calderini DF, Abeledo LG, Savin R, Slafer GA (1999) Carpel size and temperature in pre-anthesis modify potential grain weight in wheat. J Agr Sci 132:453–460

Calderini DF, Savin R, Abeledo LG, Reynolds M, Slafer GA (2001) The importance of the period immediately preceding anthesis for grain weight determination in wheat. Euphytica 119:199–204

Caldwell MM (1968) Solar ultraviolet radiation as an ecological factor for alpine plants. Ecol Monogr 38:243–268

Hakala K, Jauhiainen L, Hoskela T, Kayhko P, Vorne V (2002) Sensitivity of crops to increased ultraviolet radiation in northern growing conditions. J Agron Crop Sci 188:8–18

Lovengreen C, Fuenzalida H, Villanueva L (2000) Ultraviolet solar radiation at Valdivia, Chile (39.8oS). Atmos Environ 34:4051–4061

Kakani VG, Reddy KR, Zhao D, Sailaja K (2003) Field crops responses to ultraviolet-B radiation: a review. Agr Forest Meteorol 120:191–218

Kerr JB, McElroy CT (1993) Evidence for large upward trends of ultraviolet-B radiation linked to ozone depletion. Science 262:1032–1034

Li Y, Yue M, Wang XL (1998) Effects of enhanced ultraviolet-B radiation on crop structure, growth and yield components of spring wheat under field conditions. Field Crop Res 57:253–263

Li YA, Zu YQ, Chen HY, Chen JJ, Yang JL, Hu ZD (2000) Intraespecific responses in crop growth and yield of 20 wheat cultivars to enhanced ultraviolet-B radiation under field conditions. Field Crop Res 67:2533

Teramura AH, Sullivan JH, Ziska LH (1990) Interaction of elevated ultraviolet-B radiation and CO_2 on productivity and photosynthetic characteristics in wheat, rice, and soybean. Plant Physiol 94:470–475

Wardlaw IF (1994) The effect of high temperature on kernel development in wheat: variability related to pre-heading and post-anthesis conditions. Aust J Plant Physiol 21:731–739

EFFECT OF WATER STRESS AND POTASSIUM CHLORIDE ON BIOLOGICAL AND GRAIN YIELD OF DIFFERENT WHEAT CULTIVARS

M. MOUSSAVI-NIK[1,2,*], H.R. MOBASSER[2] AND A. MHERABAN[2]

[1] *Birjand University, Birjand, Iran, 97175-331*
Mailing address: Faculty of Agriculture, Birjand University, Birjand, Iran, 97175-331
[2] *Islamic of Azade university, Zahadan, Iran, University of Azade, Zahadan, Iran, 98135-978*
[*] *E-mail: mohsen_372001@yahoo.com.au*

Abstract: Water stress is the main restriction in arid and semi-arid for agriculture around of the world. Although all cropland in Sistan and Baluchestan province of Iran are cultivated under water irrigation, frequently at the end of growth season, i.e during the beginning of the grain filling period, crops are exposed to water deficiency. To determine the effect of water deficiency and application potassium chloride (KCL) on biological and grain yield of two wheat cultivars, Falate and Hermiand were cultivated under field conditions at Sistan. The experiment was carried out during 2003–2004 growing season at RCI of Zabol under three irrigation levels (full irrigation, stop irrigation after pollination and after milky stage of grain) and three levels of KCl solution (control-without application of KCL solution, application of KCL solution at appearance of first spikelet 10.5–1 Fix scale and at milky stage of grain 10.5–5 Fix scale). A split-split plot design was used where the main plot was irrigation, sub plot cultivars and the KCL application as a sub-sub plot. Three replicates per treatments were used. The results showed that biological yield was significantly affected by water stress in both wheat cultivars, but treatments, had no significantly effect on number of grain per spike. Harvest index and thousand-grain weight were significantly effected by water stress and wheat cultivars. In addition, grain yield was only affected by water stress. Potassium chloride treatment had no effect on biological and grain yield

Keywords: wheat, water stress, harvest index, biological yield, potassium chloride, grain yield

INTRODUCTION

Arid and semi arid environment besides other factors may induce water stress during the crop cycle reducing yield crop (Ashraf et al. 1995). A large portion of geographical area of Iran could not be taken under cultivation due to lack of

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 655–658

water availability for crops. Reduction in yield and yield components due to water stress has been reported in both durum and bread wheat (Sinha et al. 1986). In spite water stress is recognized as an important factor that affects wheat growth and yield (Ashraf and Nagvi 1995, Ashraf 1998), differences among cultivars were found in response to soil moisture restrictions (Rascio et al. 1992, Iqbal et al. 1999). The objective of this study was to determine the relative tolerance of bread wheat cultivars to water stress occurring at different plant growth stages, to recommend suitable time for stop irrigation and application of KCL to avoid water stress in Sistan area.

MATERIALS AND METHODS

Two cultivars of bread-wheat (*Tritcium aestivum*. L), **Hirmand** and **Falate**, obtained from wheat section Zabol Agricultural Research Institute (ZARI), Sistan, Iran were sown under field conditions in the field Research area, university of Azade Islamic Zahadan. Sowing date was on November 2003 and harvest was on March 2004. Low rainfall (79 mm per annum), low humidity, clear sunny and sometime windy days were the characteristic feature of this growing season. Seed were hand drilled and each cultivar was sown in six rows of 5 meter with row spacing of 20 cm. The water treatments resumed after pollination, as normal irrigation (W_0), stop irrigation after pollination (W_1) and stop irrigation at milky stage of grain (W_2). Treatments of potassium chloride (KCL) application were (i) control (without fertilizer), (ii) spray when the first spikelet emerged (Fix scale 10.5 -1) and (iii) spray at grain filling stage (Fix scale 10.5-5). The rate of KCL was used 20 kg ha^{-1} with 1% concentration. The experiment was conducted in a randomized complete block design (RCBD) with three replicates per treatment where irrigation regimes constituted the main plots and application of KCL in sub-sub plot. At maturity the plants were harvested and biological yield, yield component (number of spike per square meter, number of grain per spike and thousand of grain weight), grain yield and harvest index were measured. These parameters were compared by Duncan's new multiple range test (DMRT), at 5% probability (Steel and Torrie 1980).

RESULTS AND DISCUSSION

Plant produced their maximum biomass under adequate water supply, whereas water stress causes a marked decrease in plant production (Clarke et al. 1991, Ashraf 1998). Hence, in addition to other factors, dry matter production can be used a selection criterion for water stress. Wheat production responded differently to water treatments. Stop Irrigation at pollination stage (W_1) caused a significant reduction ($P < 0.05$) in biological yield (BY) of both wheat cultivars (Table 1).

A decrease in aerial biomass observed in both wheat cultivars due to water stress might be associated with a decrease in relative turgidity and dehydration of protoplasm, which is associated with a loss of turgor and reduced expansion of cell (Arnon 1972a). In addition, water treatment W_2 significantly effected BY compared

Table 1. Effect of different water stress treatments on yield and its components. Data are the average of both cultivars. W0, W1 and W2 are water treatments

Treat	BY (t ha^{-1})	No. of spike m^{-2}	No. of grain spike^{-1}	1000 grain weight (g)	HI%	Grain yield (t ha^{-1})
W0	8.23a	244a	16a	37.94a	35a	2.82a
W1	4.87c	133b	14a	21.44b	17b	0.84c
W2	7.19b	218a	15a	35.78a	39a	2.28b

Table 2. Effect of wheat cultivars on 1000-grain weight

Cultivar	1000 grain weight (g)
Hirmand	33.28a
Falate	30.18b

to control. In the present study, number of spikes and 1000-grain weight were also affected by water stress (Table 1). Water treatment W_1 decreased ca. 50 the number of spikes per unit area and similar effect was found on thousand of grain weight (Table 1). The decrease in grain weight may be due to disturbed nutrient uptake efficiency and photosynthetic translocation within the plant (Iqbal et al. 1999) that produced shriveled grain due to hastened maturity (Arnon 1972b). Since the number of grain per spike was not affected by water treatments, yield reduction was a consequence of a diminished number of spikes per unit area and grain weight. Cultivar Hirmand produced higher thousand-grain weight than Falate cultivar, however, wheat cultivars were significantly affected on thousand-grain weight ($P < 0.05$) (Table 2). Thereby, grain yield was also affected, under water restriction environments, even in tolerant genotypes (Leinhos and Bergmann 1995, Far and Allam 1995).

Times of KCL application was not effect on BY, yield component and grain yield. Based on these results, it is inferred that irrigation at pollinating stage of plant is more an important in Sistan conditions.

ACKNOWLEDGEMENTS

We are grateful to Mr. Akbaeri and M. Safaei for their help throughout this experiment.

REFERENCES

Arnon I (1972a) Crop production in dry regions. In: Polunin N (ed) vol 1. Leonard Hill Book, London, pp 203–211

Arnon I (1972b) Crop production in dry regions. In: Polunin N (ed) vol 2. Leonard Hill Book, London, pp 11–19

Ashraf MY, Azime AR, Nagvi SSM, Khan AH (1995) Alpha-amylase, protease activities and associated changes under water stress condition in wheat seedling. Pakistan J Sci Ind Res 38:430–434

Ashraf MY, Nagvi SSM (1995) Studies on water uptake, germination and seedling growth of wheat genotypes under PEG-6000 induced water stress. Pakistan J Sci Ind Res 38:103–133

Ashraf MY (1998) Yield and yield components response of wheat (*Tritcium aestivum L.*) genotypesa groen under different soil water deficit conditions. Acta Agronomica Hungarica 46:45–51

Clarke JM, Richards RA, Condon AL (1991) Effect of drrought stress on residual transpiration and its relationship with water use of wheat. Can J Plant Sci 71:695–699

Far IA, Allam AY (1995) Responses of some wheat cultivars to sowing methods and drought at different stages of growth. Aussiut J Agric Sci 26:267 –yield potential and moisture stress. Vortage pur pflanzanzuchtung, 6:187–191

Iqbal M, Ahmad K, Sadiq M, Ashraf MY (1999) Yield and yield component of durum wheat as influenced by water stress at various growh stages. Pakistan J Biol Sci 2:11–14

Leinhos V, Bergmann H (1995) Changes in the yield, ligin content and protein patterns of barley (*Hordeum vulgare* cv. Alexis) induced by drought stress. Angewandte Botanik 69:206–210

Sinha SK, Aggarwal PK, Chaturvedi GS, Singh AK, Kailasnathan K (1986) Performance of wheat and triticale cultivars in avariable soil water environment. I. Grain yield stability. Field Crop Res 13:289–299

Steel RGD, Torrie JH (1980) Principles and procedures of Statistics. McGraw Hill Book Co., New Yark

Rascio A, Platani C, DiFonza N, Wittemer G (1992) Bound water in durum wheat under drought stress. Plant Physiol 98:908–912

REGULATION OF FLOWERING TIME IN WHEAT

J. DUBCOVSKY*, A. LOUKOIANOV AND M.D. BONAFEDE
University of California, Dept. Plant Sciences, Davis, CA956161, USA
* *E-mail: jdubcovsky@ucdavis.edu*

Abstract: Vernalization is the requirement of a long exposure to cold temperatures to induce flowering. In wheat and barley, the *VRN-1* and *VRN-2* genes are mainly responsible for this requirement. The alleles for winter growth habit are the ancestral forms of these genes in the Triticeae, and independent mutations in both genes have resulted in the recurrent generation of spring forms in the temperate cereals. The *VRN-1* gene is a meristem identity gene (≅ *APETALA1*) and is dominant for spring growth habit. Reduction of *VRN-1* transcript levels by RNA interference (RNAi) result in a delay in flowering of 2–3 weeks, suggesting that the transcript level of *VRN-1* is critical for the determination of flowering time in wheat

The spring wheat and barley lines characterized so far show deletions in the promoter or the first intron of *VRN-1* suggesting that they are important regulatory regions. Mutations in any of these regions in a single *VRN-1* copy in polyploid wheat are sufficient to determine a spring growth habit. We propose that these mutations interfere with the recognition of a repressor coded or regulated by *VRN-2*. Analysis of the allele frequencies of the different combinations of dominant *Vrn-A1, -B1*, and *-D1* in common spring wheat varieties from California and Argentina suggests that some of these combinations might have an adaptative value

The *VRN-2* gene is a Zinc-finger CCT transcription factor and is dominant for the winter growth habit. This gene is down-regulated by vernalization, releasing the transcription of the *VRN-1* genes and promoting flowering. Mutations in the CCT domain or deletions of the *VRN-2* gene are associated with a spring growth habit in both barley and diploid wheat. Reduction of *VRN-2* transcript levels by RNAi in winter wheat variety Jagger resulted in the increase of *VRN-1* transcript levels and in a significant acceleration of flowering time

We have recently proposed the existence a feedback regulatory loop between *VRN-1* and *VRN-2*, which is responsible for the transcription of recessive *vrn-1* alleles several days after the initiation of the transcription of the dominant *Vrn-1* alleles, in plants showing different combinations of dominant and recessive alleles. We found here that the induction of the recessive alleles is mediated by the down-regulation of *VRN-2* after the initiation of transcription of the dominant *Vrn-1* allele. Once *VRN-2* is repressed, the recessive *vrn-1* alleles can be transcribed. Three experiments supporting this hypothesis are discussed

Keywords: vernalization genes, growth habit, Triticeae

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 659–665

INTRODUCTION

One critical trait in the adaptation of temperate grasses to cold winters is the requirement of long exposures to low temperatures (vernalization) to accelerate flowering. Vernalization requirement in temperate grasses is mainly controlled by allelic variation at the vernalization genes *VRN-1* and *VRN-2* (Jensen et al. 2005, von Zitzewitz et al. 2005, Yan et al. 2004b, Yan et al. 2003), which are different from the Arabidopsis genes with the same name (Gendall et al. 2001, Levy et al. 2002). The central repressors in the vernalization pathways of Arabidopsis (*FLC*, Michaels and Amasino 1999) and the temperate cereals (*VRN-2*, Yan et al. 2004b) belong to different gene classes and have no clear homologues in the other species suggesting an independent evolution of their vernalization pathways (Yan et al. 2004b).

The wheat *VRN-2* gene is a Zinc finger - CCT domain transcription factor (*ZCCT-1*) down-regulated by vernalization (Yan et al. 2004b). Non-functional mutations (*vrn-2a* allele) or complete deletions of this gene (*vrn-2b* allele) are associated with a recessive spring growth habit in diploid wheat (*Triticum monococcum* L.) and barley (*Hordeum vulgare* L.) (Dubcovsky et al. 2005, Yan et al. 2004b). Down-regulation of *VRN-2* by vernalization is followed by the up-regulation of the meristem identity gene *VRN-1* (similar to Arabidopsis *AP1*, Yan et al. 2003) in winter varieties from both species. These two genes show strong epistatic interactions suggesting that they are part of the same regulatory pathway (Dubcovsky et al. 2005, Takahashi and Yasuda 1971, Tranquilli and Dubcovsky 2000). In spring lines homozygous for the non-functional *vrn-2* alleles, allelic differences in *VRN-1* have no effect on flowering time. Conversely, mutations in the promoter (Yan et al. 2004a; Yan et al. 2003) or the first intron (Fu et al. 2005) of the *VRN1* gene eliminate or reduce the effect of *VRN-2* and eliminate the vernalization requirement (Dubcovsky et al. 2005, Tranquilli and Dubcovsky 2000). The vernalization requirement is also eliminated, or greatly reduced, in transgenic winter plants of hexaploid wheat 'Jagger' (*T. aestivum* L.) when *VRN-2* transcript levels are reduced by RNA interference (RNAi::*VRN-2*) (Yan et al. 2004b).

Based on these results Yan et al. (2003, 2004) proposed a model in which *VRN-2* represses directly or indirectly the transcription of *VRN-1*. Down-regulation of *VRN-2* by vernalization releases *VRN-1* from this repression resulting in the induction of flowering. This model predicts that only the dominant *Vrn-1* allele will be transcribed in lines carrying simultaneously recessive and dominant alleles. We have recently confirmed this prediction in young isogenic lines of hexaploid wheat variety 'Triple Dirk' carrying different combinations of dominant and recessive *VRN-A1*, *VRN-B1*, and *VRN-D1* alleles, and also in heterozygous *VRN-1* diploid wheat plants (Loukoianov et al. 2005). Using the Triple Dirk near-Isogenic Lines (NIL) (Pugsley 1971, Pugsley 1972) we showed that in leaves of young plants only the dominant *Vrn-A1* allele is transcribed in Triple Dirk D (TDD: *Vrn-A1vrn-B1 vrn-D1*), only the *Vrn-B1* allele is transcribed in TDB (*vrn-A1 Vrn-B1vrn-D1*), and only the *Vrn-D1* allele in TDE (*vrn-A1 vrn-B1 Vrn-D1*).

However, a few weeks later, transcripts from the recessive alleles were also detected in both the polyploid and heterozygous diploid spring plants suggesting

the existence of a feedback regulatory loop that releases the recessive *vrn-1* alleles after the initiation of transcription of the dominant *Vrn-1* alleles (Loukoianov et al. 2005). In this paper we present three lines of evidence suggesting that this regulatory loop is mediated by the down-regulation of the *VRN-2* repressor after the initiation of the transcription of the dominant *VRN-1* alleles.

MATERIALS AND METHODS

For the first experiment, RNA samples were collected from the leaves of diploid wheat T. monococcum winter accession G3116 (*vrn-A^m1*), spring accession PI 266844 (*Vrn-A^m1*, carrying a 1-bp deletion in the promoter CArG box), and F2 heterozygous plants from the cross between these two accessions (Dubcovsky and Yan 2003, Yan et al. 2004b). These two accessions differ in an SNP in exon five that was used to develop a Cleavage Amplification Polymorphic Sequence (CAPS) marker to select heterozygous F2 plants and to identify the parental source of the transcripts from the dominant *vrn-A^m1* and recessive *vrn-A^m1* alleles in the heterozygous plants. The Quantitative PCR experiments were done using TaqMan systems developed before (Yan et al. 2004b, Yan et al. 2003).

The second experiment was performed using Near-Isogenic Lines (NIL) developed in hexaploid wheat variety 'Triple Dirk' (Pugsley 1971, Pugsley 1972). The winter isogenic line Triple Dirk C (TDC) has all three recessive *vrn-1* alleles, whereas the three spring Triple Dirk lines have one different dominant *Vrn-1* allele each (TDD:*Vrn-A1 vrn-B1 vrn-D1;* TDB:*vrn-A1 Vrn-B1 vrn-D1*, and TDE: *vrn-A1 vrn-B1 Vrn-D1)*. In this experiment we used two SYBR GREEN® quantitative PCR systems to measure *VRN-1* and *VRN-2*. Quantification of the *VRN-1* transcripts was done by a combination of the conserved primers Ex4-5_F1 (TCAGATCCAGGAA-GAACCAA) and Ex8_R1 (TTGATGTGGCT(A/C)ACCATC CA) which amplify all three *VRN1* copies. The quantification of the *VRN-A2* transcript levels was done with primers ZCCT-A1-F (GACCCATGGCTCACCTA GTG) and ZCCT-A1-R (TTGCTTCATTGCTAATAGTGTTGGT). We currently do not know the sequence of the *VRN-B2* and *VRN-D2* genes and, therefore, we are not sure if our *VRN-A2* primers amplify only the *VRN-A2* transcripts or a combination of the three different homoeoalleles present in hexaploid wheat.

In the third experiment we used transgenic Bobwhite plants (HWS common wheat) with reduced levels of endogenous *VRN1* caused by RNA interference (RNAi) (Loukoianov et al. 2005). Positive transgenic plants were confirmed by PCR of genomic DNA using primers Rs_S_F/R and Ri_AntiS_F/R designed from the vector sequence flanking the sense and antisense insertions (Yan et al. 2004b). We excluded the MADS-box and K-box domains from the cloned region to avoid interference with other MADS-box genes. Transcription levels of the endogenous *VRN-1* were investigated using a SYBR GREEN ® quantitative PCR system using the conserved primers Ex4-5_F1 and Ex8_R1 AP1_ Ex3 -F3 described above. The quantification of the *VRN-A2* transcript levels was done

with primers ZCCT-A1-F and ZCCT-A1-R described above. Twelve transgenic plants and 12 controls at the third leaf stage were used in this experiment. Primers for the ACTIN endogenous controls were U211_SYBR_Actin_F: ACCTTCAGTTGCCCAGCAAT and U212_SYBR_Actin_R: CAGAGTCGAGCACAATACCAGTTG.

Quantitative PCR experiments were performed in an ABI7700 using ACTIN as endogenous controls (Yan et al. 2003). The 2-ΔΔCT method (Livak and Schmittgen 2001) was used to normalize and calibrate the *VRN-1* CT values relative to the *ACTIN* endogenous controls. For the statistical analysis of the experiments comparing *VRN-1* and *VRN-2* transcript levels among different genotypes, we used a log transformation of the 2-ΔΔCT values to correct the lack of homogeneity of variances in the untransformed data detected by Levene's test (SAS Institute Inc. 2003).

RESULTS AND DISCUSSION

Diploid Wheat Experiment

For the diploid wheat experiment, we selected eight homozygous $vrn-A^m1$, seven homozygous $Vrn-A^m1$, and eleven heterozygous $vrn-A^m1$ $Vrn-A^m1$ F_2 plants from the cross G3116 × PI266844. First leaves from unvernalized plants carrying the dominant $Vrn-A^m1$ alleles showed, as expected, significantly higher transcript levels of $VRN-A^m1$ than the plants carrying the recessive $vrn-A^m1$ alleles ($P < 0.0001$, Fig. 1A). The $VRN-A^m1$ transcript levels in the heterozygous plants were closer to those in the homozygous $Vrn-A^m1$ lines than to the ones observed in the homozygous $vrn-A^m1$ lines (Fig. 1A).

The *VRN-2* transcript showed the opposite trend, with significantly lower levels of *VRN-2* transcripts in the homozygous $Vrn-A^m1$ plants than in the homozygous $vrn-A^m1$ plants ($P = 0.0001$). The heterozygous plants showed intermediate levels of *VRN-2* transcripts that were not significantly different from the average of the two homozygous classes (Fig. 1B).

The results from the *T. monococcum* F_2 plants segregating for the $VRN-A^m1$ locus but homozygous for the dominant *Vrn-2* winter allele provide support for the hypothesis that the late induction of the recessive *vrn1* alleles is mediated by the down-regulation of *VRN-2*. A lower level of *VRN-2* transcripts was observed in the first leaves of the F_2 plants carrying one or two copies of the dominant $VRN-A^m1$ allele relative to the plants homozygous for the recessive $vrn-A^m1$ allele. All plants were grown in the same greenhouse under environmental conditions that generally do not result in down-regulation of *VRN-2* transcripts (no vernalization, 16 h light). The plants homozygous for the recessive $vrn-A^m1$ allele served as controls for the normal transcript levels of *VRN-2* under our experimental conditions. We conclude that the initiation of the $Vrn-A^m1$ transcription was likely the trigger leading to the observed decline in $VRN-A^m2$ transcripts. The repression of the $VRN-A^m2$ transcripts preceded the transcription of the $vrn-A^m1$ alleles, providing a likely explanation to the later transcription of the recessive $vrn-A^m1$ alleles.

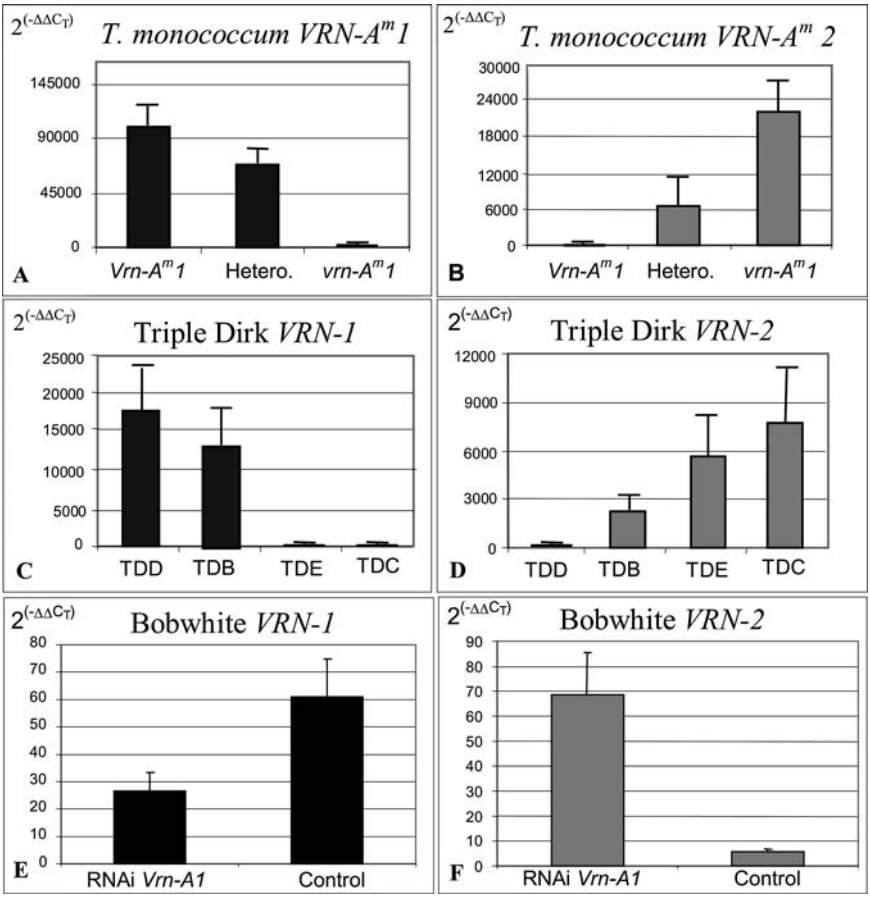


Figure 1. Comparison of *VRN-1* (A, C, & E) and *VRN-2* (B, D, & F) transcript levels in the first leaves of unvernalized plants grown under 16 h light by Quantitative PCR. A & B) F_2 plants from the cross G3116 × PI 266844. Results are averages of 8 homozygous $Vrn-A^m1$, 11 heterozygous $vrn-A^m1$ $Vrn-A^m1$, and 7 homozygous $vrn-A^m1$ plants. C & D) Isogenic Triple Dirk lines TDD ($Vrn\text{-}A1 vrn-B1 vrn-D1$), TDB ($vrn-A1$ $Vrn\text{-}B1$ $vrn-D1$), TDE ($vrn-A1$ $vrn-B1$ $Vrn\text{-}D1$) and TDC ($vrn-A1 vrn-B1 vrn-D1$). E&F) Transgenic RNAi Bobwhite plants with reduced levels of *VRN-1* transcripts compared to the non-transgenic control. Results are averages of 12 plants. The $2^{-\Delta\Delta C}{}_T$ method (Livak and Schmittgen 2001) was used to normalize and calibrate the C_T values relative to the *ACTIN* endogenous control. Error bars represent one SE of the mean

Triple Dirk Experiments

The isogenic Triple Dirk lines also showed opposite transcript levels of *VRN-1* and *VRN-2* in the first leaves of unvernalized plants. The overall *VRN-1* transcript level in TDD detected by the conserved primers (Ex4-5_F1 and Ex8_R1) was significantly higher ($P = 0.001$) than in TDB or TDE, which did not differ significantly

between each other ($P = 0.40$, Fig. 1C). On the contrary, the *VRN-2* transcript levels in TDD were significantly lower ($P = 0.0009$) than in TDB or TDE, which did not differ significantly between each other ($P = 0.25$, Fig. 1D).

The first leaves of Triple Dirk isogenic line TDD which showed the highest levels of *VRN-1* transcripts, also showed the lowest levels of *VRN-2* transcripts. This suggests that transcription of the dominant *Vrn-A1* allele (directly or indirectly) represses *VRN-2*, and that the elimination of this flowering repressor then allows the initiation of the transcription of the recessive *vrn-B1* and *vrn-D1* alleles observed several days later.

RNAi transgenic Bobwhite experiments: The transgenic Bobwhite plants showed the expected reduction of the endogenous levels of *VRN-1* relative to the non-transgenic controls ($P = 0.04$). The transcript levels of the endogenous*VRN-1* in the transgenic plants were less than half of the level observed in the non-transgenic controls (Fig. 1E).

The *VRN-2* transcript levels showed the opposite trend. A significant increase of the *VRN-2* transcript levels ($P < 0.0001$) was observed in the transgenic plants relative to the non-transgenic controls. *VRN-2* transcript levels in the RNAi plants with reduced *VRN-1* levels were more than ten fold higher than in the control plants (Fig. 1F).

Conclusions

Taken together, these results provide a strong support to the hypothesis that the transcription of *VRN-2* is repressed after the initiation of the transcription of the dominant *VRN-1* alleles, resulting in the release of the recessive *vrn-1* from the *VRN-2* repression. The induction of the recessive alleles after the transcription of the dominant *Vrn-1* alleles might contribute to increase the overall accumulation of *VRN-1* transcripts to levels that trigger the apex transition from the vegetative to the reproductive stage. One possible function of this feedback regulatory loop might be to coordinate the transcription of dominant and recessive alleles contributing to an earlier attainment of the inductive *VRN-1* transcript threshold, and triggering an irreversible flowering response.

ACKNOWLEDGEMENTS

This research was supported by the United States Department of Agriculture CSREES NRI competitive grant 2003-00929.

REFERENCES

Dubcovsky J, Yan L (2003) Allelic variation in the promoter of *Ap1*, the candidate gene for *Vrn-1*. In: Pogna N (ed) X int wheat genet symp. Paestum, Italy, 1:243–246

Dubcovsky J, Chen C, Yan L (2005) Molecular characterization of the allelic variation at the *VRN-H2* vernalization locus in barley. Mol Breeding 15:395–407

Fu D, Szucs P, Yan L, Helguera M, Skinner J, Hayes P, Dubcovsky J (2005) Large deletions in the first intron of the *VRN-1* vernalization gene are associated with spring growth habit in barley and polyploid wheat. Mol Gen Genomics 273:54–65

Gendall AR, Levy YY, Wilson A, Dean C (2001) The VERNALIZATION 2 gene mediates the epigenetic regulation of vernalization in Arabidopsis. Cell 107:525–535

Jensen LB, Andersen JR, Frei U, Xing YZ, Taylor C, Holm PB, Lubberstedt TL (2005) QTL mapping of vernalization response in perennial ryegrass (*Lolium perenne* L.) reveals co-location with an orthologue of wheat *VRN1*. Theor Appl Genet 110:527–536

Levy YY, Mesnage S, Mylne JS, Gendall AR, Dean C (2002) Multiple roles of Arabidopsis *VRN1* in vernalization and flowering time control. Science 297:243–246

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2 to the (-Delta Delta Ct) method. Methods 25:402–408

Loukoianov A, Yan L, Blechl A, Sanchez A, Dubcovsky J (2005) Regulation of *VRN-1* vernalization genes in normal and transgenic polyploid wheat. Plant Physiol 138:2364–2373

Michaels SD, Amasino RM (1999) Flowering locus C encodes a novel MADS domain protein that acts as a repressor of flowering. Plant Cell 11:949–956

Pugsley AT (1971) A genetic analysis of the spring-winter habit of growth in wheat. Aust J Agric Res 22:21–31

Pugsley AT (1972) Additional genes inhibiting winter habit in wheat. Euphytica 21:547–552

Takahashi R, Yasuda S, (1971) Genetics of earliness and growth habit in barley. In: Nilan RA (ed) Proceedings of the 2nd int barley genet symp. Washington State University Press, Washington, pp 388–408

Tranquilli GE, Dubcovsky J (2000) Epistatic interactions between vernalization genes $Vrn-A^m1$ and $Vrn-A^m2$ in diploid wheat. J Hered 91:304–306

VonZitzewitz J, Szûcs P, Dubcovsky J, Yan L, Francia E, Pecchioni N, Casas A, Chen THH, Hayes PM, Skinner JS (2005) Molecular and structural characterization of barley vernalization genes. Pl Mol Biol 59:449–467

Yan L, Loukoianov A, Tranquilli G, Helguera M, Fahima T, Dubcovsky J (2003) Positional cloning of wheat vernalization gene *VRN1*. Proc Natl Acad Sci USA 100:6263–6268

Yan L, Helguera M, Kato K, Fukuyama S, Sherman J, Dubcovsky J (2004a) Allelic variation at the *VRN-1* promoter region in polyploid wheat. Theor Appl Genet 109:1677–1686

Yan L, Loukoianov A, Blechl A, Tranquilli G, Ramakrishna W, SanMiguel P, Bennetzen JL, Echenique V, Dubcovsky J (2004b) The wheat *VRN2* gene is a flowering repressor down-regulated by vernalization. Science 303:1640–1644

MOLECULAR BREEDING FOR MULTIPLE PEST RESISTANCE IN WHEAT

D.J SOMERS[1,*], C. MCCARTNEY[1], R. DEPAUW[2], J. THOMAS[1], S. FOX[1], G. FEDAK[3], G. HUMPHREYS[1], J. GILBERT[1], B. MCCALLUM[1] AND T. BANKS[1]

[1] *Agriculture and Agri-Food Canada – Cereal Research Centre, 195 Dafoe Rd, Winnipeg, MB Canada*

[2] *Agriculture and Agri-Food Canada – Semi-arid Prairie Agriculture Research Centre, 1 Airport Rd, Swift Current, SK, Canada*

[3] *Agriculture and Agri-Food Canada – Eastern Cereals and Oilseeds Research Centre, 960 Carling Ave, Ottawa, ON, Canada*

[*] *E-mail: SomersD@agr.gc.ca*

Abstract: The development of wheat genetic maps based on microsatellite (SSR) markers is giving researchers an unprecedented view into genome organization and breeding population structure. When this DNA-based technology is coupled with phenotypic data, QTL analysis and trait mapping are facilitated. This ultimately enables design of experiments to assemble complex genotypes that pyramid multiple traits

Our research team worked to pyramid multiple pest resistance together into elite Canadian wheat lines through marker-assisted backcrossing. This program included six *Fusarium* resistance QTL, *Lr21* and *Sm1* (wheat midge resistance). Further, the strategy made use of high throughput genotyping and whole genome analysis which lead to an accelerated recovery of the elite genetic background

Disease nursery results testing 120 homozygous BC_2F_3 lines carrying varying numbers of FHB resistance QTL clearly showed the introgression of FHB resistance QTL markedly reduced infection symptoms and DON levels by 45–80%

This report will include the strategy and technology used to introgress multiple pest resistance in wheat and field results for disease and performance evaluation. The report will also introduce how molecular breeding of wheat can be augmented with association mapping studies and how bioinformatics supports and accelerates molecular breeding research

Keywords: molecular breeding, diseases resistance, pest resistance, gene introgression

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 667–676.

INTRODUCTION

Molecular breeding is the science of using DNA fingerprinting or genotyping of plants to determine the allelic makeup of a plant on which we can predict its phenotype. If plant phenotypes can be predicted reasonably, then selections can be made to retain plants with specific genotypes and thus the potential to express a desirable phenotype. Molecular breeding can circumvent more cumbersome, established pedigree breeding strategies and even generate plant genotypes unachievable by conventional methods (Young 1999).

The implementation of molecular breeding techniques requires certain genetic resources, the most important being a high-density genetic map of the crop species as well as access to genetic markers; these are the basic tools in molecular breeding. With these tools in hand, molecular breeding can 1) accelerate elite line production, 2) establish novel combinations of disease resistance genes/alleles, 3) assemble complex genotypes across the genome and 4) create gene pyramids for more durable or multiple pest resistance.

This strategy is particularly effective for diseases where plant phenotyping is expensive, highly variable and time consuming, such as *Fusarium* infection in wheat (Gilbert and Tekauz 2000). There is now extensive research on QTL analysis of *Fusarium* head blight (FHB) resistance, particularly from Sumai 3 (Bai et al. 1999, Anderson et al. 2001, Buerstmayr et al. 2002, Yang et al. 2003, Yang et al. 2005, Zhou et al. 2002). Other sources of FHB resistance are derived from Wangshuibai, Frontana, Wuhan and Nyubai (Zhou et al. 2002, Buerstmayr et al. 2002, Somers et al. 2003, Steiner et al. 2004, Han et al. 2005). When the knowledge of FHB resistance QTL mapping is combined with a high density microsatellite map of wheat (Somers et al. 2004) and various high throughput genotyping technologies, the prospects of implementing molecular breeding for FHB resistance in wheat can be realized.

The following research brings together many aspects of molecular breeding for assembling complex genotypes. The research includes the pyramiding of six FHB resistance genes plus resistance genes for orange blossom wheat midge (*Sm1*) (Thomas et al. 2005) and leaf rust (*Lr21*) (Spielmeyer et al. 2000).

MATERIALS AND METHODS

Plant Material

The germplasm is divided into two groups, elite parents and FHB resistance donor parents. The elite parents were spring wheat and included 98B69*L47, BW301, Prodigy, and Kanata (BW263). The donor parents were HC374 (Wuhan/Nyubai), HC736 (AC Foremost//Biggar/Sumai 3), and 98B08*A111 (BW252//AC Domain*2/Sumai 3). Each of the donor parents was a doubled haploid (DH) line carrying specific FHB resistance QTL (Somers et al. 2003, Yang et al. 2003) and has demonstrated FHB resistance based on several years of field and greenhouse testing.

Crossing Scheme

Four crossing streams were used to facilitate assembling the desired gene/QTL combinations (Fig. 1) with each stream introgressing different FHB resistance QTL into different elite parents. Each crossing stream followed an identical scheme of donor × elite followed by two backcrosses to the elite parent and one selfing generation to derive BC_2F_2plants. Stream 3 was delayed one generation due to dormancy and BC_2F_1 plants were used for stream intercrossing while BC_2F_2 plants were used for intercrossing streams 1, 2 and 4. Streams 1, 2, and 4 used the same elite parent as was used in the initial cross, stream 3 began with HC376 × 98B69*L47 and used Prodigy as the recurrent parent in the subsequent two backcrosses (Fig. 1).

Genotyping and Selection

Genotyping was performed on an ABI 3100 capillary electrophoresis instrument (Applied Biosystems, Foster City, California) and used M13-tailed microsatellite markers as described in Somers et al. (2004). The list of codominant, polymorphic markers used for selection of each chromosomal segment carrying FHB resistance QTL is indicated in Table 1. The parents used in the project were screened with 356 microsatellite markers (gwm – Roder et al. 1998, GDM – Pestova et al. 2000, wmc – Somers et al. 2004, and BARC – http://www.ScabUSA.org) to

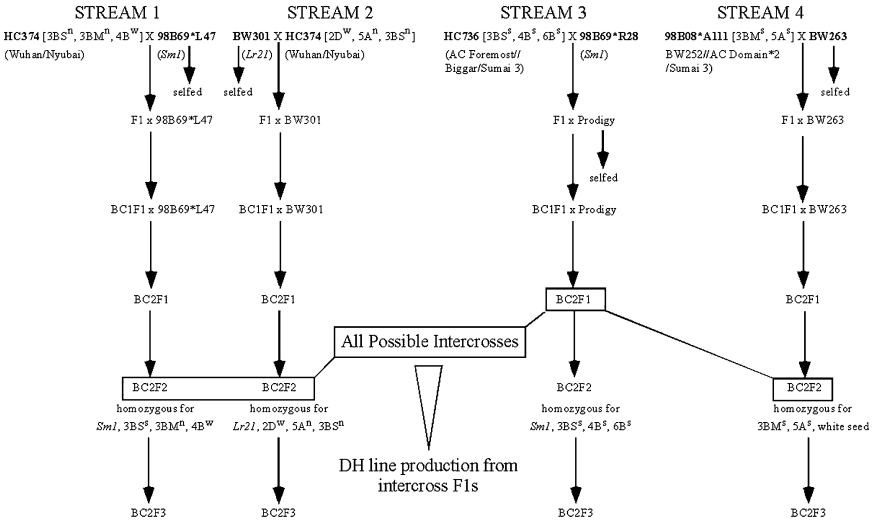


Figure 1. A schematic diagram showing the crosses and essential genetic contributions each donor and elite parent makes to the multiple pest resistance project. The work is divided into four streams, each introgressing 2–4 pest resistance genes and is ended by intercrossing the streams to form novel gene pyramids for phenotypic evaluation

Table 1. List of microsatellite markers used for selection of FHB resistance QTL intervals. The allele size of the FHB resistant donor is shown in parentheses

Crossing Stream	FHB[a] QTL	FHB resistance type[b]	Microsatellite markers[c]			Distance[d] (cM)	Reference
			Distal	Centre	Proximal		
1	3BS	II	gwm533(140)	gwm493(197)	wmc808(159)	33	Somers et al. 2003
	3BSc	I	wmc625(121)	gwm566(124)	wmc418(263)	13	Somers et al. 2003
	4B	II	wmc710(89)	wmc238(220)	gwm149(154)	20	Somers et al. 2003
2	2DL	I	wmc245(152)	gwm608(133)	gwm301(214)	40	Somers et al. 2003
	3BS	II	gwm533(140)	gwm493(197)	wmc808(159)	33	Somers et al. 2003
	5AS	I, II	gwm293(199)	wmc705(170)	gwm154(97)	18	Bürstmayr et al. 2002
3	3BS	II	gwm389(134)	gwm533(141)	gwm493(194)	11	Anderson et al. 2001
	4B	II	wmc710(89)	wmc238(226)	gwm149(154)	20	Somers et al. 2003
	6B	I, II	wmc104(135)	wmc397(157)	gwm219(177)	45	Yang et al. 2003
4	3BSc	I	wmc625(113)	gmw566(124)	wmc418(267)	13	Somers et al. 2003
	5AS	I, II	gwm293(199)	wmc705(167)	gwm154(103)	18	Buerstmayr et al. 2002

[a] FHB resistance QTL 3BS is near the telomere, 3BSc is near the centromere.

[b] Type I = resistance to initial infection based on field observations; Type II = resistance to fungal spread based on single floret injection experiments.

[c] Marker order on the chromosome is based on Somers et al. (2004) and sizes include a 19bp M13 tail.

[d] Distance between distal and proximal markers is based on Somers et al. (2004).

identify microsatellites across the genome in each cross that were codominant and polymorphic to be used for recurrent parent genome selection. The segregating populations for each stream in the BC_1F_1 generation consisted of 968 - 1,152 seeds. Each seed was cut laterally in half to provide the endosperm and embryo halves for further analysis. The endosperm half-seeds were ground to a powder for DNA extraction.

Three polymorphic markers for each FHB resistance QTL were identified; one in the centre of the QTL and two flanking markers (Table 1). Each BC_1F_1 half-seed was first genotyped with the centre FHB resistance QTL marker. Half-seeds that were heterozygous for the centre marker were then genotyped with the flanking markers. Half-seeds that were heterozygous across the three marker interval were then genotyped with the suite of background genome markers polymorphic for that cross. Half-seed embryos that had the most fixation of the elite genome were germinated to be advanced as females in the crossing scheme. This same process was performed at the BC_2F_1 and BC_2F_2 generations in all four streams with the exception that genome analysis was omitted on BC_2F_2 plants. Stream 1, 2 and 4 BC_2F_2 plants were selfed to create BC_2F_3 families.

Background genome loci that were fixed for elite alleles at BC_1 were not re-genotyped at BC_2 and thus fewer markers were used in the BC_2 generation. All possible stream intercrosses were made and included multiple intercrosses between the same streams in order to sample all of the different BC_1F_1 plants. Intercross F_1 seed was then used to develop large DH populations. Intercross F_1 seed involving stream 3 was screened with appropriate FHB resistance markers to select seeds fixed for FHB resistance QTL where possible, before DH line production.

Phenotyping

The BC_2F_3 material, intercross DH progeny plus checks were grown in three replicated rows in an epiphytotic field nursery at the Eastern Cereal and Oilseed Research Centre in Ottawa, Ont in 2004 and 2005. The Fusarium inoculum was produced as described by Sung and Cook (1981). When plants in each row reached the stage of 50% anthesis they were inoculated using a backpack sprayer with a macroconidial suspension of *F. graminearum* at a concentration of 50,000 spores mL^{-1}, 60 mL per row. Fusarium infected corn inoculum was also placed between the rows before anthesis. The plots were sprinkler irrigated for 1-h intervals from 11:00–12:00 and 15:00–16:00 daily for 3 days after each inoculation to maintain high humidity in the nursery. Twenty-one days after the initial inoculation each row was rated on a scale of 1–10 for incidence of disease in the row and severity of infection on spikes. Days to heading and plant height were also recorded for each row. The rows were harvested at maturity and a one-gram sample of seed was milled for DON analysis utilizing the ELISA method (Sinha and Savard 1996).

RESULTS AND DISCUSSION

Assembling Complex Genotypes

There were two types of populations developed from the project. The first type (pedigreed) included BC2F2 populations advanced and evaluated as BC2F3 families that contained a suite of FHB resistance QTL ranging from zero QTL up to three QTL. The QTL combinations are restricted to those initiated in the original cross. The second type of population were doubled haploid (DH), derived from the stream intercross F1s which again ranged in the number and type of FHB resistance QTL, but can include lines with novel QTL pyramids from different streams (Fig. 1).

Table 1 lists the markers used for selection of FHB resistance QTL in each stream. These markers were chosen since they were polymorphic in the cross and were codominant in nature, to enable identification of heterozygotes. Other markers flanking these intervals can be used in different crosses. In each cycle of backcrossing, the centre marker was used first to reduce the population size, followed by the flanking markers to select the final population for whole genome genotyping. This approach reduced the amount of genotyping (datapoints) by focusing on plants carrying one FHB resistance donor allele in the interval. Typically approximately 60 plants carrying the FHB resistance alleles at three loci were selected from an initial 1,100 plants. The size of the selected intervals depended on the cross and ranged from 11 to 45 cM based on the microsatellite consensus map (Somers et al. 2004) (Table 1).

The elite genetic background was restored through whole genome genotyping to the extent possible. Underpinning this effort is the wheat microsatellite consensus map (Somers et al. 2004) which provided a reasonable measure of genetic distance between markers used to genotype the genetic background. Approximately 70% of the genetic background could be genotyped in each cross. This was limited largely by a lack of polymorphic markers near the telomeres in the four crosses. Whole genome genotyping used between 1 and 2 markers/chromosome arm and depended on the availability of robust, codominant markers in the cross. Of the 70% of the genome that could be genotyped, we achieved an average fixation of elite alleles at $> 88\%$ of the loci in all four streams. This provided a high degree of plant uniformity in the BC2F3 families as was observed in the FHB nurseries.

It was important to the wheat breeding program to demonstrate that MAS could keep a similar pace in crossing and selection as would be expected by conventional breeding combined with phenotyping. This project passed through five generations in each of four crossing streams and took 25 months to complete. This included all genotyping for both pedigreed and DH populations. The expected advantage of the MAS strategy was an efficient restoration of the elite genetic background and enrichment of populations with lines carrying effective or novel FHB resistance QTL combinations.

Multiple Pest Resistance in Populations

Lr21 maps on chromosome 1B and *Sm1* (wheat midge resistance) maps on chromosome 2BS. *Lr21* was fixed in the stream 2 progeny easily since it was present in the recurrent parent (BW301). *Sm1* was fixed easily in stream 1 since the recurrent parent (98B69*L47) carries the resistance allele. The presence of resistance alleles for *Sm1* was monitored with microsatellites in stream 3, as Prodigy does not carry the resistance allele (Fig. 1). The derived BC2F3 families were fixed for *Lr21* and *Sm1* where possible and these resistance genes were pyramided with FHB resistance QTL to provide multiple pest resistance in the populations.

The BC2F2 populations in each stream afforded the opportunity to genotype and select individuals fixed for various FHB resistance QTL, ranging from zero to three FHB resistance QTL. By advancing these plants to F3 families, we were able to evaluate the FHB resistance provided by single and multiple QTL combinations. The experimental BC2F3 families are most appropriately compared to ND2710 and FHB37 (both Sumai 3 derivatives) since these FHB resistant checks are more similar in adaptation and performance to Canadian wheat than would be HC374. The data collected over two years in the Ottawa FHB nursery showed that FHB resistance QTL in general have an additive effect. Both QTL on 3BS and 4B in stream 1 provided added resistance and the combination of these QTL showed the best FHB resistance in stream 1. Similarly in stream 2, FHB resistance QTL on 2D and 5A both provided added resistance and together, even further reductions in FHB symptoms. Conversely, FHB resistance QTL 3BS in stream 2 did not further reduce FHB infection when combined with the QTL from 2D and 5A. Mixed results were observed for the FHB resistance QTL on 3BSc in stream 1, where there was an additive effect when combined with the 4B QTL, but not with the 3BS QTL. Similarly, the 3BSc QTL did not show an additive effect when combined with the 5A QTL in stream 4. In summary, it appears the most essential QTL to combine will be located on 2D, 3BS, 4B and 5A, with less emphasis put on the 3BSc QTL.

The best FHB resistance QTL combinations reduced the FHB symptoms between 45–80% relative to the "NONE" and check entries across all streams. The least improvements were observed in stream 4, where there appears to be natural resistance in this white wheat stream compared to the other red wheat streams (Fig. 2).

A more controlled genetic comparison is presented in Fig. 3, where two BC_2F_2 sister plants, derived from a common BC_2F_1 are compared. The harvested BC_2F_4 seed from the field grown and inoculated BC_2F_3 plants (x 3 reps) are shown and the difference in the amount of bleaching and Fusarium damaged kernels in the seed samples is very evident. The seed samples are genetically, highly similar with the exception of the presence of three FHB resistance QTL (*) on 2D, 3BS and 5A in family 2–2614.

The evaluation of these populations will continue in the future and selections will be made for improved plant agronomy and seed quality. The project has developed approximately 3,500 DH populations from the stream intercross F1s (Fig. 1) and these lines are currently under field evaluation and seed increase. It is expected that substantially improved FHB resistance will be identified within the DH populations

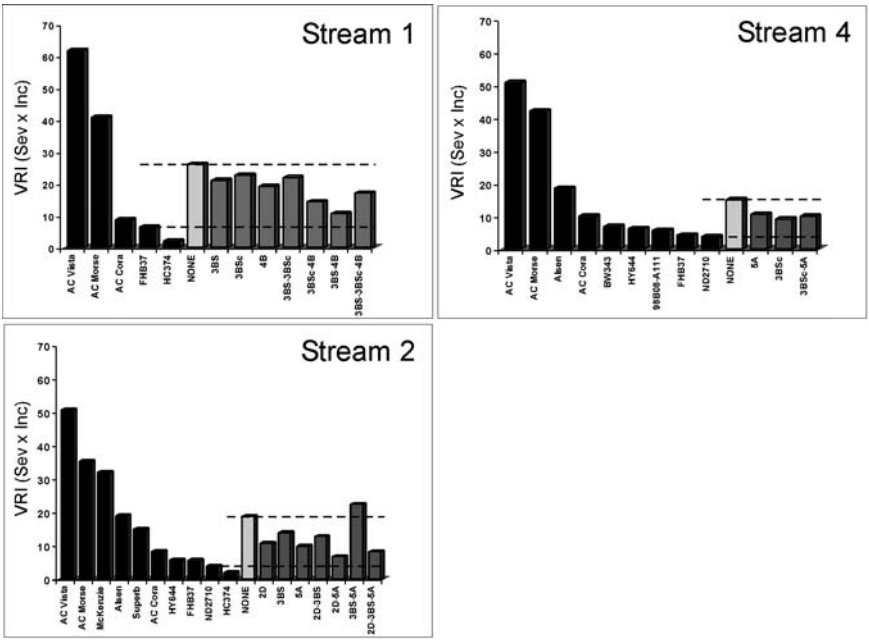


Figure 2. Three histograms from streams 1, 2 and 4 showing the level of FHB symptoms in experimental BC_2F_3 lines derived from the crossing strategy. The visual rating index (VRI) is based on 2 years x 3 reps/year of data for each entry. The values for experimental BC_2F_3 lines (light grey shades, right) are the average of a minimum of 3 entries/QTL combination. Dashed lines compare the "NONE" QTL entries and experimental lines to check entries such as FHB37 and ND2710

and lines with acceptable performance and seed quality will be advanced to yield trials and further. The project succeeded in pyramiding multiple pest resistance into elite Canadian genetic backgrounds over a very short period of time and strategies are now being considered how to improve the process and expand the approach to other market classes.

Further Studies

Genetic materials from the project were also used to examine other aspects of Fusarium resistance in wheat. Most notably, the BC_2F_1 population from stream 1 was used to identify plants useful for fine mapping the FHB resistance genes in a qualitative manner. For example, BC_2F_1 plants were selected to be heterozygous at just on FHB locus such as 3BS, 2D, 4B or 5A. In each of these plants, all other known FHB resistance loci were homozygous for susceptible alleles. Thus these plants could be used to raise large populations segregating for a single FHB resistance gene which facilitated a more qualitative measurement of disease spread or incidence. So far, we have placed the 3BS FHB resistance gene into a 2 cM interval.

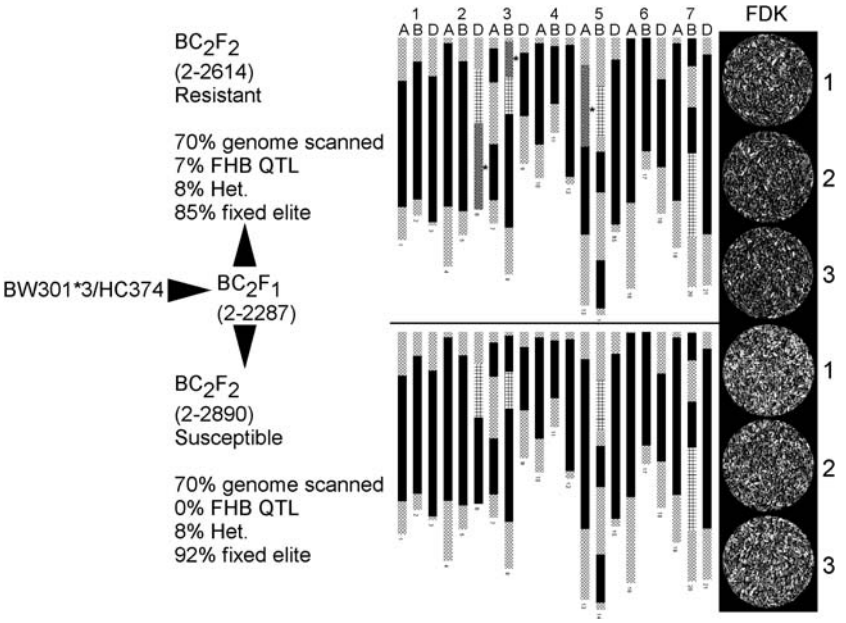


Figure 3. Presented are graphical genotypes of a pair of sister BC_2F_2 plants differing in FHB resistance as measured by Fusarium damaged kernels (FDK). The plants are highly similar genetically, with the exception of three FHB resistance QTL (*) on 2D, 3BS and 5A in plant 2–2614. Black areas are homozygous recurrent parent, telomere shaded areas were not genotyped due to a lack of polymorphic markers, (*) indicates the interval carrying the FHB resistance QTL and interstitial shaded areas are heterozygous. The harvested BC2F4 seed samples from 2004 (3 replications) show the substantial amount of bleached, FDK in the susceptible 2–2890 plant

In another study, a set of 800 intercross DH lines from the project were grown in New Zealand (2004) and Canada (2005). The DH lines were selected for plant type, plant height, lodging, and time to maturity in New Zealand, and response to leaf rust, stem rust, common bunt, and FHB in nurseries near Swift Current, SK, and Carman, MB. All DH lines were analyzed with microsatellite markers at FHB resistance QTL on chromosomes 2D, 3BS, 3BSc, 4B, and 5AS. The microsatellite allele frequencies did not change significantly between the original 800 DH lines and the selected population. In general, selection for field performance and response to leaf and stem rust and common bunt did not adversely affect allele variation at FHB resistance QTL in the populations evaluated, except for 5AS. This suggests, FHB resistance derived from these loci can likely be combined with existing alleles controlling plant agronomics, and disease resistance without significant penalty.

ACKNOWLEDGEMENTS

The authors wish to thank the AAFC technical staff including Debbie Stern, Wenguang Cao, and Devin Dahlman for production of BC seed and plant maintenance. We also thank the molecular breeding technical staff including Leslie

Bezte, Monika Eng, Debbie Miranda, Yanfen Zheng, Allison Brown-Hoeppner, Mira Popovic, and Zlatko Popovic for the genotypic analysis. This project was supported by the Western Grains Research Foundation Wheat Check-off fund and the Agriculture and Agri-Food Canada Matching Investment Initiative.

REFERENCES

Anderson JA, Stack RW, Liu S, Waldron BL, Field AD, Coyne C, Moreno-Sevilla B, Mitchell Fetch J, Song QJ, Cregan PB, Frohberg RC (2001) DNA markers for Fusarium head blight resistance QTL in two wheat populations. Theor Appl Genet 102:1164–1168

Bai G, Kolb FL, Shaner G, Domier LL (1999) Amplified fragment length polymorphism markers linked to a major quantitative trait locus controlling scab resistance in wheat. Phytopathology 89:343–348

Buerstmayr H, Lemmens M, Hartl L, Doldi L, Steiner B, Stierschneider M, Ruckenbauer P (2002) Molecular mapping of QTL for Fusarium head blight resistance in spring wheat. I. Resistance to fungal spread (type II resistance). Theor Appl Genet 104:84–91

Gilbert J, Tekauz A (2000) Review: recent developments in research on Fusarium head blight in Canada. Can J Plant Path 22:1–8

Han FP, Fedak G, Ouellet T, Dan H, Somers DJ (2005) Mapping of genes expressed in *Fusarium graminearum*-infected heads of wheat cultivar 'Frontana'. Genome 48:88–96

Pestsova E, Ganal MW, Roder MS (2000) Isolation and mapping of microsatellite markers specific for the D genome of bread wheat. Genome 43:689–697

Roder MS, Korzun V, Wendehake K, Plaschke J, Tixier M-H, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007–2023

Sinha R, Savard M (1996) Comparisons of immunoassays and gas chromatography for the detection of the mycotoxin deoxynivalenol in grain samples. Can J Plant Path 18:233–236

Somers DJ, Fedak G, Savard M (2003) Molecular mapping of novel genes controlling Fusarium head blight resistance and deoxynivalenol accumulation in spring wheat. Genome 46:555–564

Somers DJ, Isaac P, Edwards K (2004) A high density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theor Appl Genet 109(6):1105–1114

Spielmeyer W, Huang L, Bariana H, Laroche A, Gill BS, Lagudah E (2000) NBS-LRR sequence family is associated with leaf and stripe rust resistance on the end of homoeologous chromosome group 1S of wheat. Theor Appl Genet 101:1139–1144

Steiner B, Lemmens M, Griesser M, Scholz U, Schondelmaier J, Buerstmayr H (2004) Molecular mapping of resistance to *Fusarium* head blight in the spring wheat cultivar Frontana. Theor Appl Genet 109:215–224

Sung JM, Cook RF (1981) Effect of water potential on reproduction and spore germination of Fusarium graminearum. Phytopathology 71:499–504

Thomas J, Fineberg N, Penner G, McCartney C, Aung T, Wise I, McCallum B (2005) Chromosome location and markers of *Sm1*: a gene of wheat that conditions antibiotic resistance to orange wheat blossom midge. Mol Breed 15(2):183–192

Yang Z-P, Gilbert J, Somers DJ, Fedak G, Procunier JD, McKenzie R (2003) Marker-assisted selection of Fusarium head blight resistance genes in two doubled-haploid populations of wheat. Mol Breed 12(4):309–317

Yang Z-P, Gilbert J, Fedak G, Somers DJ (2005) Genetic characterization of QTL associated with resistance to Fusarium head blight in a doubled-haploid spring wheat population. Genome 48(2):187–196

Young ND (1999) A cautiously optimisitc vision for marker-assisted selection. Mol Breed 5(6):505–510

Zhou WC, Kolb FL, Bai G, Shaner G, Domier LL (2002) Genetic analysis of scab resistance QTL in wheat with microsatellite and AFLP markers. Genome 45:719–727

REFLECTIONS AND OPPORTUNITIES: GENE DISCOVERY IN THE COMPLEX WHEAT GENOME

J.W. SNAPE* AND G. MOORE

John Innes Centre, Colney Lane, Norwich, Norfolk, NR4 7UH, U.K.
**E-mail: john.snape@bbsrc.ac.uk*

Abstract: Despite more than 80 years of wheat genetic studies, only several hundred genes out of the probable 120,000 coding sequences that are presumed present in the wheat genome have been mapped to chromosomes. Many fewer have been associated with traits, and very many fewer have been cloned and their mechanisms and mode of action elucidated. This past failure has been due to the complexity of the hexaploid wheat genome of more that 16,000 million base pairs, but also the paucity of molecular tools and investment relative to other agricultural species such as rice or maize. Fortunately, in recent years, the pace of gene discovery has greatly accelerated in wheat because of the development of molecular tools, including molecular marker based genetic maps, libraries of sequence, particularly ESTs, and microarrays. Most recently a BAC library covering the whole wheat genome in the model variety Chinese Spring has been constructed

QTL and major gene discovery by forward genetics is now routine and a catalogue of mapped genes for both simple and complex traits is being accumulated. This is leading to plant breeding applications through marker-assisted selection and detailed genetical understanding in terms of trait architecture and genetic diversity. However, the knowledge of mechanisms by which mapped genes work is rare since cloning genes in the complex wheat genome is still a major undertaking both in terms of time and resources. Gene isolation by chromosome walking is the most direct, but also the most difficult route. But the feasibility of this has now been demonstrated for key genes, such as the *Ph1* locus controlling homoeologous chromosome pairing. However, the resource implications indicate that only a few key genes are important enough for the investment, and short cuts through other approaches are generally necessary. Most commonly this involves isolating orthologues from model species, usually *Arabidopsis* and rice. This is possible, but examples show that it does not always work or is meaningful to do. Because of this, there is increasing interest in sequencing the whole genome, although a daunting undertaking in terms of cost and effort. However, sequencing other model species, such as the small grass, *Brachypodium*, can provide a useful substitute for wheat gene content, as well as providing closely linked markers for chromosome walking. Moreover, a comparison of the corresponding regions between rice and Brachypodium will enable wheat genes to be clearly identified and annotated

Keywords: genetic analysis, marker assisted selection, gene cloning, wheat genome sequencing

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 677–684.

INTRODUCTION

Despite more than 80 years of wheat genetic studies, only several hundred genes out of the probable 120,000 coding sequences (3 genomes circa 40 K genes) that are presumed present in the wheat genome have been mapped to chromosomes. Many fewer have been associated with traits, and very many fewer have been cloned and their mechanisms and mode of action elucidated. This past failure has been due to the complexity of the hexaploid wheat genome of more that 16,000 million base pairs, 80% of which are repetitive sequences, but also the paucity of molecular tools and investment relative to other agricultural species such as rice or maize. Fortunately, in recent years, the pace of gene discovery has greatly accelerated in wheat because of the development of much better molecular tools and resources. We are now on the verge of a revolution where the power of genetic analysis and the ability to clone genes is greatly accelerating, due to the availability of comprehensive genomic resources, but also in part due to the transfer of technologies and information between models, particularly *Arabidopsis*, but more importantly rice. Additionally, new genomic resources are becoming available, such as in the small genome grass, *Brachypodium distychion*, and this will complement and enlarge on the toolkit available to wheat geneticists. Ultimately, we will have the full wheat genome sequence. This paper discusses these advances and the issues to be faced in progress towards a comprehensive genetic understanding of this most important world food crop.

GENOMIC RESOURCES IN WHEAT

Basic to gene identification and discovery in any species is the availability of genomic resources. Until the last five years, wheat was almost an orphan crop in this respect. But investment has increased, particularly in the USA, Europe and Australia, such that now we have a range of tools and resources equivalent to most other crop species (excluding rice). These include a large number of molecular markers, particularly simple sequence repeat primer pairs, where around 2000 are now available. These have been the main tools leading to good molecular marker based genetic maps. However, more markers are still needed, particularly for physical mapping and gene cloning. We have been applying a marker technique called Sequence Tagged Microsatellite Profiling (STMP), which is a rapid technique for developing SSR markers (Hayden and Sharp 2001). This technique has the advantage of using one common primer for several different markers, allowing it to be used economically in a high-through-put automated format. Using this technique, we have developed a further 250 markers in wheat which we have mapped in the wheat reference 'ITMI' population (Hayden et al. 2006). Similarly, the Diversity Arrays Technology (DarT) (Jaccouds et al. 2001) is now taking off in wheat with greatly reduced costs for mapping, and this complements the other marker systems. Nevertheless, there is still a major issue in developing assays for the most abundant marker system in plants, namely single nucleotide polymorphisms

(SNP), and progress towards large numbers of SNP markers is wilfully inadequate in wheat at the present time. Also, to date, SNP discovery in wheat has been disappointing. Despite the large EST resources in wheat, very few 'true' SNPs have been found, presumably this is due to the fact that most of the data is derived from a single variety, Chinese Spring. The quality of the sequence has also led to a lot of sequencing errors being present in EST contigues. However, perseverance has to continue to greatly increase this resource.

In addition to molecular markers, further genomic resources in wheat include the libraries of sequences, particularly ESTs, and many thousand (> 650 K) have been developed, and now a significant number of unigene ESTs have also been mapped to the Chinese Spring deletion set (Sorrells et al. 2003) and mapped syntenically with rice. This is fast becoming a very important resource for further marker development and gene cloning. Indeed the number of wheat ESTs available now exceeds that of any other species, and many of these have been arranged into contigues. There are also now both proprietary and publicly available microarray resources for studying gene expression, such as an Affymetrix chip. Most recently, a BAC library covering the whole wheat genome in the model variety Chinese Spring was constructed for developing a physical map of the wheat genome (Allouis et al. 2003), and this will provide the basis, ultimately, for the complete wheat genome sequence. Additionally, techniques for developing single chromosome BAC libraries via chromosome sorting are being developed (Safar et al. 2004).

PROGRESS IN GENETIC ANALYSIS

Both QTL and major gene discovery by forward genetics is now routine in wheat because of the development of several good molecular marker based genetic maps, usually based on F1 derived doubled haploid mapping populations developed using the maize cross system. Extensive phenotyping is being undertaken for a range of traits, both simple and complex, covering the whole spectrum of interest, from disease and pest resistance, abiotic stress tolerance, flowering time and adaptation, to physiological traits, to quality traits to yield and yield components. This increasing involves studying complex traits of low heritability, such as yield and yield components, and non-major gene components of quality (Snape et al. 2006). Because of these activities, a catalogue of mapped genes for both simple and complex traits is starting to be accumulated, and detailed genetical understanding in terms of trait architecture and genetic diversity is emerging. However, in such studies, it is often the cost and expertise for phenotyping rather than the molecular tools that is becoming the limiting factor for enlarging our understanding, including, for example, a paucity of physiologists able to dissect complex traits into individual components.

Another limiting factor is the need to bring together data from different sources covering phenotypic, genetic and molecular data into a common database so that researchers can get a common reference point for their own mapping studies. Many researchers have used the freely available recombinant inbred line International

Triticeae Mapping Initiative (ITMI) population, Opata x Synthetic, for their studies to enable a common reference point, and there is a good consensus SSR map based on this which is hugely valuable in developing maps and linking different maps (Somers et al 2004). However, at present, too many researchers and countries have their own local databases of maps and phenotypic data with limited access to others. The GrainGenes database (http://www.graingenes.org) does an admirable job in bringing together available mapping, genomics and phenotypic data. However, far too little data is being submitted in wheat by the major groups outside of the USA in comparison to the amount that is potentially available to the World community. There is a huge need to better coordinate and integrate data from different labs to produce the added value from all groups from combining the many different studies ongoing in the major wheat research labs around the World. We are all at fault in this!

THE PROMISE AND REALITIES OF MARKER-ASSISTED SELECTION IN WHEAT

The extent of gene discovery to date for wheat breeder's priority traits has now led to plant breeding applications through marker-assisted selection (MAS). The advantages have been extensively discussed (eg Koebner and Summers 2003), where plant selection is done on the basis of DNA profile associated with a useful trait in the laboratory, rather than phenotype in the glasshouse or field. In most wheat breeding programmes in Europe, Australia and at CIMMYT, MAS is routine. In Australia, for example, up to thirty alleles for biotic and abiotic stress resistance, and for quality, are being selected, and ideal marker ideotypes have been formulated. Indeed, crossing programme and selection programmes are being planned around MAS cycles to maximise genetic gain per generation. Concurrent with such studies are simulation and experimental studies to optimise these processes. The next step is to add MAS for QTL detected and validated through mapping, to this process.

However, marker implementation and validation are still major issues in wheat. Discovering a major gene or QTL and an associated marker by QTL analysis of a mapping population is often still a long way away from its efficient selection. It is not an easy task to find closely linked and diagnostic markers for traits of interest, particularly in areas where recombination is generally low, for example, in centromeric regions. There is a need for MAS to move to the use of 'functional' markers developed inside genes of interest, where allelic variation for the marker is directly associated with phenotype. This can come from using the extensive EST information to associate candidate genes with function, and then allelic variation at these candidate genes with function. The most suitable marker system to enable such associations has to be single nucleotide polymorphisms, SNPs, to allow haplotypes to be assembled which can become diagnostic for trait variation. Once we have widespread SNPs in wheat, it will also enable the more efficient use of the World's extensive germplasm collections through 'allele mining'.

In most current situations aimed at gene discovery of targets for MAS, functional markers are not available, and closely linked 'anonymous' markers have to be 'converted' in some way, usually to a PCR format, to be diagnostic. This is usually achieved by designing specific PCR primers from sequence information of the closest convenient marker to create, for example, a sequence tagged site. This is usually effective when the gene-marker combination is introduced from an exotic source into an adapted gene pool, creating quasi- linkage disequilibrium. However, it is often quite difficult to extend the diagnostic marker to other allelic variants or to other gene pools. One new method for doing this is to utilize the rice sequence to create markers targeted at a particular region of the wheat genome. First, a full-length gene sequence in rice is identified which is orthologous to a wheat gene in the target region by using a wheat EST identified by deletion or linkage mapping. Then, by identifying the exon-intron boundaries in the rice sequence, the conserved orthologous wheat sequence can be identified and used to create a diagnostic marker by designing primers that prime across exon-intron boundaries, utilizing sequence variation in the intron sequence for polymorphisms. Polymorphisms are recognized either by size or sequence differences in the intron using single strand conformational polymorphism (SSCP) analysis.

Finally, the cost of genotyping has to be competitive with the phenotyping costs or alternative selection systems. Although there are high-through-put platforms using fluorescent primers, these are limited by cost for the numbers that plant breeders would like to put through. So we have the problem of reducing the cost per data point, relative to the genetics gains that can be made by conventional selection. We are not yet at the stage of having high-through-put analytical platforms at a price, and throughput, worth the investment in large scale MAS other than for high value targets, mainly restricted to abiotic and biotic stresses which cause large yield or quality reductions, rather than desirable, more marginal traits in terms of the economic returns.

STRATEGIES FOR GENE CLONING IN WHEAT

Our knowledge of mechanisms by which mapped genes work is rare in wheat since cloning genes in the complex wheat genome is still a major undertaking both in terms of time and resources. Gene isolation by chromosome walking is the most direct, but also the most difficult route. The feasibility of this has now been demonstrated for key genes, such as *Vrn*, vernalization, genes (Yan et al. 2003; Yan et al. 2004), and the *Ph1* locus controlling homoeologous chromosome pairing (Griffiths et al. 2006). However, the resource implications for this in the past and in the present has meant that only a few key genes are important enough for the investment, and short cuts through other approaches are generally necessary. Most commonly this involves isolating orthologues from model species, usually *Arabidopsis* and rice. *Arabidopsis* is still the primary model system for fundamental plant science research, for the well documented reasons of small genome size, ease of growth, and short generation time, but rice is catching up, particularly through investment in China and Japan.

The production of the complete genome sequences of both of these species were historic landmarks in plant biology. However, it is still a point of debate amongst plant geneticists as to how far *Arabidopsis* will contribute to gene discovery and the facile cloning of important agronomic genes in major crops, particularly the cereals. An example of how*Arabidopsis* can be used to establish the function of important genes in cereals was illustrated by the cloning of the *Rht* dwarfing genes in wheat from the cloning of the *Arabidopsis* orthologue, *GAI* (Peng et al. 1999). However, there are still very few such examples, and although *Arabidopsis* is excellent at unravelling the 'gene circuitry' of a trait, experience has shown that although orthologues are often present in wheat, they don't necessarily have the predicted function, especially for genes showing the key allelic variants. Rice, is of course, much closer to wheat than *Arabidopsis*, and is a hugely valuable tool for isolating wheat genes of known function in rice. However, for instance the *Ppd1* gene controlling photoperiod response in wheat and barley (Turner et al. 2005) was cloned by chromosome walking because no photoperiod response phenotype had been mapped in the region of rice genome equivalent to the *Ppd1* region in wheat and barley. Moreover, experience is now showing that not all wheat genes are represented in rice, and vice versa, and the life style differences between a tropical and a temperate cereal may indicate many gene differences. Because of this, there is increasing interest in sequencing the whole genome. However, before this is available, sequencing other model species, such as the small grass, *Brachypodium*, can provide a useful substitute for wheat gene content, as well as providing closely linked markers for chromosome walking. A comparison of the corresponding regions between rice and *Brachypodium* will enable wheat genes to be clearly identified and annotated. Thus, an international consortium has been started to develop the full range of possible genomic resources in *Brachypodium*, (http://www.brachypodium.org/) including the complete genome sequence of the diploid species, *Brachypodium distychion*, which has all the advantages of a model species including a genome size of only 300 Mb. This should provide a good representation of a temperate cereal genome and the combination of rice and *Brachypodium* can provide an excellent representation of a region of the wheat genome (Griffiths et al. 2006).

TOWARDS A FULL WHEAT GENOME SEQUENCE

In the long term, the greatest resource available to the wheat genetics community will be the whole genome sequence. This objective must be supported by all wheat geneticists as a long term aim. However, this is a daunting undertaking in terms of cost and effort. The wheat genome is 40 times the size of the rice genome. At current costs of sequencing, this will require funding of tens of millions of dollars. An international consortium IWGSC has been set up (http://www.wheatgenome.org/) to facilitate the process. However, there is still some debate on how the aim is best achieved. There are two schools of thought. The first is to use the existing wheat BAC library of over 1.25 million clones developed by the French (Evry)

and UK (John Innes Centre) groups to develop a detailed physical map of the whole genome as a precursor to systematic sequencing. The second school of thought is to do the sequence chromosome by chromosome via the development of chromosome specific BAC libraries. The feasibility of this approach has already been demonstrated by the development of a 3B specific library, and an extensive sequence of one arm is already being compiled by INRA in France. However, chromosome 3B is the largest of the wheat chromosomes and the only one that is easily isolated. The others, can however, be isolated by using di-telosomics, isochromosomes, or as groups. However, the technical effort of isolating sufficient quantities of individual chromosomes and of making high quality BAC libraries from the small quantities of DNA available are not to be underestimated. Also, a chromosome by chromosome approach could take many years to assemble each and all of the necessary libraries, and in the end, a 'big bang' approach of whole genome sequencing may be preferable.

CONCLUSIONS

Since the last Wheat Congress in 2000, huge progress and successes have been achieved in wheat genetics, genomics and genetic analysis. Wheat now stands amongst all the other crops as a well resourced species for gene discovery. Important genes are starting to be cloned and their mechanisms of action understood. The one 'holy grail' still remaining to be developed is the whole genome sequence. Despite the advances, however, we have to admit that wheat breeding has benefited little other than through MAS at the present time. There is still a major challenge in translating gene discovery and understanding into facile tools for plant breeders. This still remains an objective and a duty for wheat researchers.

REFERENCES

Allouis S, Moore G, Bellec A, Sharp R, Faivre Rampant P, Mortimer K, Pateyron S, Foote TN, Griffiths S, Caboche M, Chalhoub B (2003) Construction and characterisation of a hexaploid wheat (Triticum aestivum L.) BAC library from the reference germplasm "Chinese Spring". Cereals Res Commun 31:331–337

Griffiths S, Sharp R, Foote TN, Bertin I, Wanous M, Reader S, Colas I, Moore G (2006) Molecular characterisation of Ph1 as a major chromosome pairing locus in polyploid wheat. Nature 439:749–752

Hayden MJ, Sharp PJ (2001) Targeted development of informative microsatellite (SSR) markers. Nucleic Acids Res 29:e44

Hayden MJ, Stephenson P, Logojan AM, Khatkar D, Rogers C, Elsden J, Koebner RMD, Snape JW, Sharp PJ (2006) Development and genetic mapping of sequence tagged microsatellites (STMs) in bread wheat (Triticum aestivum L.). Theor Appl Genet (in press)

Jaccoud D, Peng J, Feinstein K, Kilian A (2001) Diversity Arrays: a solid state technology for sequence information independent genotyping. Nucleic Acids Res 29:e25

Koebner RMD, Summers R (2003). 21st century wheat breeding: selection in plots or detection in plates. Trends Biotechnol 21:59–63

Peng J, Richards DE, Hartley NM, Murphy GP, Devos KM, Flintham JE, Beales J, Fish LJ, Worland AJ, Pelica F, Sudhakar D, Christou P, Snape JW, Gale MD, Harberd NP (1999) 'Green revolution' genes encode mutant gibberellin response modulators. Nature 400:256–261

Safar J, Bartos J, Janda J et al (2004) Dissecting large and complex genomes: flow sorting and BAC cloning of individual chromosome types from bread wheat. Plant J 39:960–968

Snape JW, Foulkes MJ, Simmonds J, Leverington M, Fish LJ, Wang Y, Ciavarrella M (2006) Dissecting environmental effects on wheat yields via QTL and physiological analysis. Euphytica (in press)

Somers DJ, Isaac P, Edwards K (2004) A high-density microsatellite consensus map for bread wheat (Triticum aestivum L.). Theor Appl Genet 109:1105–1114

Sorrells ME, La Rota CM et al (2003) Comparative DNA sequence analysis of wheat and rice genomes. Genome Res 13:1818–1827

Turner A, Beales J, Faure S, Dunford RP, Laurie DA (2005) The pseudo-response regulator Ppd-H1 provides adaptation to photoperiod in barley. Science 310:1031–1034

Yan L, Loukoianov A, Tranquilli G, Helguera M, Fahima T, Dubcovsky J (2003) Positional cloning of wheat vernalization gene VRN1. Proc Natl Acad Sci USA 100:6263–6268

Yan L, Loukoianov A, Blechl A, Tranquilli G, Ramakrishna W, SanMiguel P, Bennetzen JL, Echenique V, Dubcovsky J (2004) The wheat VRN2 gene is a flowering repressor down-regulated by vernalization. Science 303:1640–1644

IN VITRO STARCH BINDING EXPERIMENTS: STUDY OF THE PROTEINS RELATED TO GRAIN HARDNESS OF WHEAT

A. BAKÓ, M. GÁRDONYI AND L. TAMÁS*

Eötvös Loránd University, Department of Plant Physiology and Molecular Plant Biology Pázmány Péter sétány 1/C, Budapest, Hungary, H-1117
**E-mail: tamasl@ludens.elte.hu*

Abstract: Two friabilin components, puroindoline a and GSP-1 were expressed in *Escherichia coli*. Starch binding properties of the recombinant polypeptides and of friabilin extracted from wheat flour were compared *in vitro*. The produced proteins as well as native wheat friabilin bound to starch granules prepared from different (soft, hard and durum) wheat cultivars. Starch granules also bound specifically several wheat endosperm proteins other than friabilin

Keywords: puroindoline a, puroindoline b, grain hardness, friabilines

INTRODUCTION

Grain hardness (endosperm texture) forms the fundamental basis of commercial differentiation of wheat cultivars. It is possible to differentiate 'soft' and 'hard' hexaploid wheats and 'very hard' durum wheat as three distinct qualitative classes. Texture determines flour particle size, starch damage, water absorption and milling yield. Therefore, grain hardness is an indicator of the suitability of a particular flour for a particular product. To the grower, texture is important as generally higher premiums are paid for the harder wheats (Morris 2002).

The hardness of the grain is largely determined by the properties of the endosperm. The surface of the starch granules is covered by lipids and special proteins. Hard wheat starches prepared by water sedimentation have more material adhering to their surface than soft wheat starches prepared by the same method when examined by scanning electron microscopy (Barlow et al. 1973). It is generally

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 685–691

accepted that the adhesion between the granules and the protein matrix is stronger in hard wheats than in soft wheats. During the milling of hard wheat, starch granules fragmentize. This fragmentation is called starch damage. Starch damage is the most important factor in determining water absorption of flour. It also affects the amount of carbohydrates available to yeasts, the fermentative activity, gas production, loaf volume and, as a result, baking quality. So far the most well characterized source of variation in grain hardness is the *Ha* (Hardness) locus located on the short arm of chromosome 5D of hexaploid wheat. The genes for puroindoline a, puroindoline b and GSP-1 (Grain Softness Protein) (the three major components of the friabilin protein fraction) are tightly linked to the *Ha* locus. There is an unbroken linkage between mutations in any of the puroindoline genes and grain hardness. Friabilin is abundant on the surface of water-washed soft wheat starch granules, scarce on hard wheat starch and absent on durum wheat starch.

The biochemical mechanism governing endosperm texture is poorly understood. The cause of the strong adhesion between starch granules and protein matrix in hard and durum wheat cultivars is unknown. How friabilin located on the surface of the granules impairs the adhesion in soft wheats is also unclear. Detailed knowledge on the molecular background of grain hardness could help in producing cultivars specially fit for any one particular purpose.

Isolation of the components located at the starch-gluten interface and investigation of their interactions in *in vitro* experiments might help to elucidate the mechanism of adhesion between starch and matrix and the impairment of this adhesion by friabilin. There is no biochemical data available on interaction between matrix proteins and starch granules. Friabilin was proven to bind to the surface of starch granules in *in vitro* experiments (Bloch et al. 2001). Individual puroindoline proteins isolated by three consecutive chromatographic steps were shown to bind polar lipids, such as those present on the surface of starch granules (Dubriel et al. 1997). Extensive purification procedures might be avoided by heterologously expressing the individual components. Puroindoline a was produced earlier in *Pichia pastoris* (Issaly et al. 2001).

We aim to find the factors and mechanisms responsible for grain hardness/softness by investigation of the starch-lipid-friabilin-matrix protein complex. Here we present *in vitro* results on the interaction of native wheat friabilin and of *E. coli* expressed friabilin polypeptides with different types of starch granules. Starch granule binding by gluten components is also demonstrated.

MATERIALS AND METHODS

Plant Material

Flour and starch samples used in this study were prepared from bread wheat cultivars Riband (soft) and Mercia (hard) and from durum wheat cultivars Martondur and Svevo.

Friabilin Extraction

Wheat friabilin proteins were extracted using the Triton X-114 (TX-114) phase partitioning method of Blochet et al. (1993). The extracts were stored at 4 °C.

Heterologous Expression of Friabilin Proteins

Puroindoline a gene was amplified from a cDNA library. Oligonucleotide 1 contained a sequence, which replaced the signal peptide sequence by a codon for Met, and an *Nco*I restriction site was also added to the gene for cloning. Oligonucleotide 2 incorporated a *BamH*I restriction site next to stop codon of the gene. PCR fragment was cloned into pCR 2.1 TOPO vector and checked by DNA sequencing and was subcloned into pET11d expression vector. The clone was sequenced again and called PApET11d.

Part of a cDNA clone for *GSP-1* gene was amplified for cloning in pET17b vector and protein expression. Oligonucleotide 3 was designed to replace the sequence coding the N-terminal 40 amino acid residues (including the signal peptide) by a codon for Met and an *Nde*I restriction site was also added to the gene for cloning. Oligonucleotide 4 incorporated a *BamH*I restriction site next to stop codon of the gene. PCR fragment was cloned into pCR 2.1 TOPO vector and sequenced. After subcloning into pET17b expression vector, the construct was checked again by sequencing. This construct was called GSPUPpET17b.

Proteins were expressed in Origami B (DE3) as host cell. For SDS-PAGE and Western blot analysis, bacteria were directly suspended in protein loading dye. For starch binding experiments, bacterial pellet was resuspended in 8M urea, 50mM Tris-HCl (pH 6.8) and incubated for 4 h at room temperature with constant agitation. Sample was centrifuged and the supernatant was dialyzed overnight against 4M urea, 50mM Tris-HCl (pH 6.8) at 16 °C. The resulting solution was dialyzed for 8 h against 50mM Tris-HCl (pH 6.8) at 4 °C, changing the solution once. The final solution was used promptly for starch binding experiments, or mixed with protein loading dye.

Starch Binding Experiments

Starch binding experiments were performed using a method adapted from Bloch et al. (2001).

Separation and Identification of Proteins

Protein loading dye was added to the samples. After heating at 100 °C for 2 min, samples were loaded on 15% Tris-Tricine (Schägger and von Jagow 1987) minigels and proteins were separated under reducing conditions. Gels were then either Coomassie stained or used for Western blotting. Fermentas Prestained Protein Ladder was used as molecular weight standard.

Cation Exchange FPLC

The pH of the TX-114 extract was set to 4.9 by addition of sodium acetate. The sample was then applied to a Mono-S cation exchange column (Pharmacia). Proteins were eluted with a 50 mM to 1 M sodium acetate (pH 4.9) concentration gradient, which contained 20% (vv^{-1}) acetonitrile, and protein in the column effluent was monitored by absorbance at 280nm.

RESULTS AND DISCUSSION

Grain hardness is caused by strong adhesion between gluten and starch granules. Proteins present at the matrix-starch granule interface play a crucial role in this adhesion. Surface protein composition of starch granules prepared from wheat cultivars Riband (soft), Mercia (hard) and Svevo (durum) was checked after water washing and after washing five times with 50 mM sodium acetate (pH 4.0) buffer. Proteins present on sodium acetate-washed starch and on water washed starch showed similar patterns when analyzed by SDS-PAGE, although the amount of protein was significantly lower after sodium acetate treatment. There were several bands visible in the 33 to 54 kDa molecular weight region. The corresponding proteins in similar extracts are considered to be storage proteins remaining adsorbed to the surface of starch granules after starch extraction. Their presence is generally believed to be artifactual, caused by binding to the granules during the isolation/extraction process (Baldwin 2001). To our knowledge, there is no experimental evidence underlying the latter statement. It is worth to note, that the amount of protein extractable from the surface of starch granules was smallest in case of soft wheat, intermediate in case of hard wheat and most in case of durum wheat. These findings are in accordance with earlier scanning electron microscopic results (Barlow et al. 1973). Only the amount of friabilin followed an opposite pattern.

To test the protein binding properties of the different types of starch granules, TX-114 extract of Riband flour was incubated with different starch granules. SDS-PAGE analysis of the eluates showed (Fig. 1) that all three types of starch granules used in this study bound friabilin selectively, but also had affinity for other proteins present in the preparation. The additional bands visible in the eluates indicate that there are several other TX-114 soluble proteins in Riband flour that bound to starch granules in our assay. Not all the proteins in the TX-114 extract bound to starch granules and the relative amount of the bound components does not correspond to the amounts present in the original TX-114 extract. These results suggest that binding was specific. The band observed at approx. 60 kDa probably corresponds to GBSS I, a crucial enzyme of starch synthesis, located on the surface of and inside the starch granules in wheat endosperm. The bands in the 33–54 kDa region might correspond to storage proteins that are identical to the ones present on the untreated surface of starch granules isolated by water washing.

Nature of the observed protein bands was investigated by Western blot analysis. Using an anti-friabilin serum it was confirmed that Riband friabilin bound to all

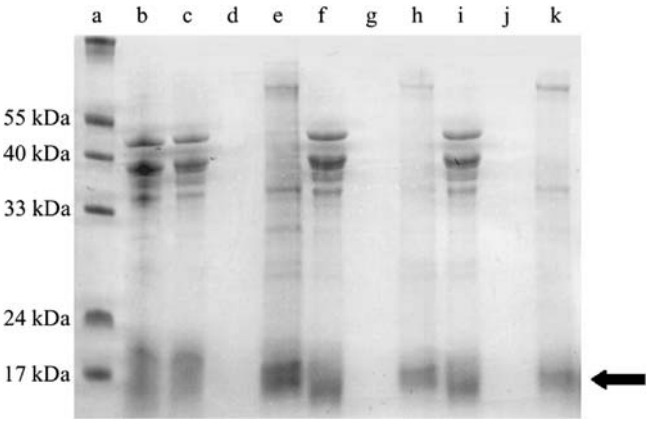


Figure 1. Binding of TX-114 extract of cultivar Riband to different starch granules. SDS-PAGE separations are shown of (a) molecular weight standard; (b) TX-114 extract; (c, f and i) flow through; (d, g and j) washing; (e, h and k) eluate. Starch columns were prepared from cv. Riband (c-e), cv. Mercia (f-h), cv. Martondur (i-k). The arrow indicates GSPP

three types of starch granules. Binding of friabilin to soft and hard wheat starch granules *in vitro* was demonstrated earlier by Bloch et al. (2001). Binding of friabilin to durum wheat starch granules was not reported before. Analysis of the eluates with anti-storage protein serum showed more bands in the eluates than Coomassie staining of SDS-PAGE gels. The results suggest that several storage protein components bind the granules. To test the starch binding ability of storage proteins, 70% ethanol extract (containing mainly gliadins) of Riband flour was used for binding to starch prepared from cv. Riband. SDS-PAGE analysis showed faint bands in the eluate at the 33–54 kDa region. This confirms that several wheat storage proteins are capable of starch binding *in vitro*.

It is possible that lipids bound to starch granules affect the binding of proteins. According to Greenblatt et al. (1995), propan-2-ol and water (90:10) is effective in removing bound polar lipids from starch. Following their procedure the three different types of starch granules were treated with the solvent and then used for starch binding experiments with Riband TX-114 extract. SDS-PAGE patterns of the eluates were similar to patterns without propan-2-ol treatment, but the amount of bound protein was significantly lower. Therefore, it is possible that starch bound polar lipids have a role in the granule-protein interaction, but further investigation is necessary to confirm these results and elucidate the role of lipids.

To be able to characterize the individual components eluted in the starch granule binding assay, we attempted to isolate them from the TX-114 extract by cation exchange FPLC. We were unable to completely purify the individual components or to identify the fraction containing the $M_r \approx 25000–54000$ proteins observed in the eluates after starch binding. Friabilin purified by FPLC bound to the granules. This suggests that the chromatographic procedure used in this study does not affect

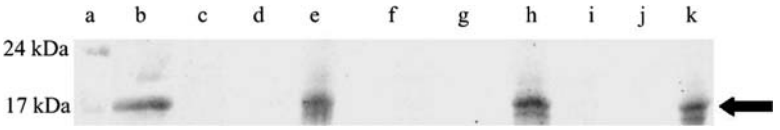


Figure 2. Binding of expressed GSPP to different starch granules. SDS-PAGE separations are shown of (a) molecular weight standard; (b) urea extract; (c, f and i) flow through; (d, g and j) washing; (e, h and k) eluate. Starch columns were prepared from cv. Riband (c-e), cv. Mercia (f-h), cv. Martondur (i-k). The arrow indicates GSPP

the functionality of the proteins. Further investigation is needed to characterize the additional bound proteins and their interactions with starch granules and friabilin.

To avoid the difficulties of separation of different proteins extracted from wheat samples, two friabilin polypeptides were expressed in an *E. coli* system. Friabilins are synthesized as preproteins in wheat endosperm. After the removal of the signal peptide, the maturing proteins are cleaved again at both the N- and C-terminals *in vivo*. Therefore, sequence of the cDNA clones was modified as follows: puroindoline a construct (PA) codes for a protein lacking the signal peptide only. GSP-1 construct (GSPP) codes for a protein completely processed at the N-terminal (designed according to Douliez et al. 2000). SDS-PAGE analysis of proteins produced heterologously by *E. coli* showed an additional band at approx. 15 kDa, when compared to the negative control. Western blot analysis using an antiserum rose against wheat friabilin also showed an extra band at approx. 15 kDa. Identity of the produced GSPP polypeptide was also confirmed by MALDI-TOF MS. Urea extracts of the bacterial cultures producing the heterologous proteins were assayed for starch binding. SDS-PAGE (Fig. 2) and Western blot analyses showed that both PA and GSPP are capable to bind to wheat starch granules (soft, hard and durum) *in vitro*.

We found that the expressed proteins show a functional trait similar to native wheat friabilin. The system is, therefore, suitable for exploring the interactions of the starch granule surface with recombinant polypeptides. The analysis of mutant and modified proteins produced using protein engineering may assist to clarify the molecular basis for friabilin binding and grain texture.

ACKNOWLEDGEMENTS

We thank Katalin Kremmerné Palczer for her excellent technical assistance. M. Gárdonyi is financially supported by a postdoctoral fellowship from OTKA (D 048401). A. Bakó is financially supported by KPI.

REFERENCES

Baldwin PM (2001) Starch granule-associated proteins and polypeptides: a review. Starch/Stärke 53: 475–503

Barlow KK, Butrose MS, Simmonds DH, Vesk M (1973) The nature of the starch-protein interface in wheat endosperm. Cereal Chem 50:443–454

Bloch HA, Darlington HF, Shewry PR (2001) In vitro binding of puroindolines to wheat starch granules. Cereal Chem 78:74–78

Blochet JE, Chevalier C, Forest E, Pebay-Peyroula E, Gautier MF, Joudrier P, Pezolot M, Marion D (1993) Complete amino acid sequence of puroindoline, a new basic and cystine-rich protein with a unique tryptophan-rich domain, isolated from wheat endosperm by Triton X-114 phase partitioning. FEBS Lett 329:336–340

Douliez JP, Michon T, Elmorjani K, Marion D (2000) Structure, biological and technological functions of lipid transfer proteins and indolines, the major lipid binding proteins from cereal kernels. J Cereal Sci 32:1–20

Dubriel L, Compoint JP, Marion D (1997) The interaction of puroindolines with wheat flour polar lipids determines their foaming properties. J Agr Food Chem 45:108–116

Greenblatt GA, Bettge AD, Morris CF (1995) Relationship between endosperm texture and the occurrence of friabilin and bound polar lipids on wheat starch. Cereal Chem 72:172–176

Issaly N, Solsona O, Joudrier P, Gautier MF, Moulin G, Boze H (2001). Optimization of the wheat puroindoline production in *Pichia pastoris*. J Appl Microbiol 90:397–406

Morris CF (2002) Puroindolines: the molecular genetic basis of wheat grain hardness. Plant Mol Biol 48:633–647

Schägger H, von Jagow G (1987) Tricine-sodium dodecyl sulphate poly-acrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. Anal Biochem 166: 368–379

HIGH THROUGHPUT *AGROBACTERIUM* TRANSFORMATION OF WHEAT: A TOOL FOR FUNCTIONAL GENOMICS

H.D. JONES*, M. WILKINSON, A. DOHERTY AND H. WU

Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK

**E-mail: huw.jones@bbsrc.ac.uk*

Abstract: At Rothamsted Research UK, we have developed a robust protocol for the *Agrobacterium*-mediated transformation of elite wheat and during the last year, made over one hundred independent transgenic lines in one durum and three bread wheat varieties. We are now able to provide training or generate transgenic wheat lines as a not-for-profit service (see www.bract.org). Approximately 40% of our lines contain a single, functional T-DNA insertion. This paper describes the current status of *Agrobacterium* transformation of wheat and some initial molecular analysis of our lines. It also describes our plan to further increase throughput using mature seed-derived callus and other approaches

Keywords: *Agrobacterium* transformation, wheat, mature seed derived callus, transgene insertions

INTRODUCTION

Genetic transformation is a powerful research tool that can be used to identify genes and study their function *in planta* and had significant potential for direct varietal improvement. It provides key underpinning knowledge about the genetic basis of key traits to inform and short-cut conventional breeding strategies. For specific crops (but not yet wheat), it has enabled the commercial planting of new genetically modified varieties by the introduction of novel genes directly into locally-adapted germplasm.

Wheat was among the last of the major crops to be transformed with the first fertile, transgenic plants being reported using particle bombardment little over a decade ago. Advances in the design of micro-projectile devices, choice of explant, media composition and selection systems has enabled the application of wheat transformation to study the role of specific genes in a wide range of agronomically

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 693–699.

important traits (reviewed by Barcelo et al. 2001, Jones 2005, Sahrawat et al. 2003). Particle bombardment remains a robust, relatively efficient method for the genetic manipulation of wheat (Altpeter et al. 2005), however at the molecular level, the DNA integration sites are often unnecessarily complex. There are several significant advantages to transferring DNA via *Agrobacterium*, including a reduction in transgene copy number, the stable integration with fewer rearrangements of long molecules of DNA with defined ends and the ability to generate lines free from selectable marker genes (Cheng et al. 2004, Dai et al. 2001, Jones 2005, Smith and Hood 1995, Travella et al. 2005). This has been a driving force in the development of methods using *Agrobacterium tumefaciens* to deliver DNA.

The soil pathogen *Agrobacterium tumefaciens* is the causative agent for crown gall disease and is well adapted for transferring a small segment of its DNA (T-DNA) to its host plant cell. Cereals are not natural hosts for *Agrobacterium* species and research was needed to match host strains, plasmids, selection systems, wheat genotypes and media compositions (for reviews see Cheng et al. 2004, Jones et al. 2005). To date there are relatively few reports describing successful regeneration of adult wheat plants containing T-DNA insertions and these are summarised in Table 1.

Transformation efficiencies (number of independently transformed plants per 100 explants infected) reported for *Agrobacterium* transformation are generally higher for the model, highly regenerable wheat genotype Bobwhite than for commercial wheat germplasm, but currently lower than the best published values for biolistics. Examples of reported average transformation efficiencies are included in Table 1 however, with the exception of Hu et al. (2003), these transformation rates were calculated from a relatively small number of experiments and, as with the early reports of biolistic transformation, they are expected to increase as protocols are further developed and refined. *Agrobacterium*-mediated DNA-delivery is already the method of choice in many major commercial laboratories and, except for some specialised applications, is set to displace biolistics for routine wheat transformation over the next five years.

ANALYSIS OF TRANSGENE INSERTIONS

In an ideal model, a single, intact T-DNA with no backbone sequence would integrate into the plant genomic DNA with no rearrangements. However, studies in various species have demonstrated that this is not always the case. To assess the situation in our transgenic wheat plants we took 34 randomly selected, independent lines transformed using a binary vector containing a single T-DNA comprising two expression cassettes (UidA (GUS) and bar (PAT)) and subjected them to PCR, Southern, segregation and expression analysis. In 13 of the 34 lines (38.2%), the UidA (GUS) and bar (PAT) genes segregated together as a single functional locus at the expected Mendelian ratio of 3:1. Southern analysis of a selection of these lines found single hybridising bands when probed with bar or UidA confirming

Table 1. Summary of main parameters reported for *Agrobacterium*-mediated transformation of wheat. IE – freshly isolated immature embryos; PCIE – pre-cultured immature embryos; EC – embryogenic callus; NS not specified. (Redrawn from Jones et al. 2005)

Wheat variety (S – spring) (W – winter)	Explant type	Axis removed	Agrobacterium strain (binary vector)	Transformation Freq. (%)	No of plants reported	Refs
Bobwhite (S)	IE (age *NS); 1-6d PCIE; 10-25d EC	NS	C58-ABI (pMON18365)	1.4–4.3	> 100	Cheng et al. 1997
Bobwhite (S)	4d PCIE	NS	C58-ABI (pMON18365)	4.4	3354	Hu et al. 2003
Bobwhite (S)	1-6d PCIE; 8-30d EC	NS	C58-ABI (pMON18365)	4.8–19	154	Cheng et al. 2003
Bobwhite (S)	3-6 PCIE	NS	C58C1 (pPTN155)	0.5–1.5	13	Halilloglu and Baenziger 2003
Cadenza (S) Florida (W)	0-72h IE	Yes	AGL1 (pAL154/156)	0.3–3.3	44	Wu et al. 2003
Fielder (S)	6-9d PCIE	Yes	AGL0 (pBGX1)	1.8	4	Weir et al. 2001
Veery-5 (S)	14d EC	Yes	LBA4404 (pHK21)	1.2–3.9	17	Khanna and Daggard 2003)
Vesna (S)	IE (age *NS)	NS	LBA4404 (pTOK233) AGL1 (pDM805)	0.13–0.41	6	Mitic et al. 2004
Various Chinese varieties (NS*)	EC (age *NS)	NS	AGL1 (p^{UNN-2})	3.7–5.9	44	Xia et al. 1999

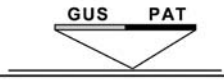
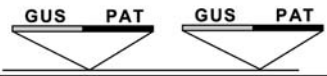
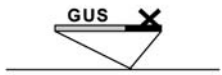
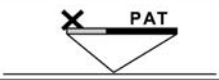
Type	No of independent events	Description
I	13	UidA and bar present as single copies that co-segregate 
II	6	UidA and Bar present as multiple copies that co-segragate 
III	11	More UidA copies than bar. Some plants contain UidA only 
IV	4	More bar copies than UidA. Some plants contain bar only 

Figure 1. Description of the four types of transgenic events found in 34 transgenic wheat lines

the presence of a single functional locus and indicating a single transgene copy. A further 6 of the 34 lines (17.6%) also showed complete UidA and bar linkage but possessed two or more functional loci as indicated by segregation ratios of 15:1 or greater, and the presence of multiple hybridising bands on Southerns. These were designated as type I and type II events respectively (Fig. 1).

A third group of lines (11 out of 34; 32.4%) possessed more functional UidA loci than bar. In most (8 lines) there was a single locus containing both UidA and bar which segregated 3:1 in the T1 and a second locus that contained only a functional UidA gene. The bar gene was undetectable in plants that had inherited only this locus. A fourth group of lines (4 out of 34; 11.8%) possessed more functional bar loci than UidA. These were designated as type III and type IV events respectively (Fig. 1). We were surprised to find so many of these events where part of the T-DNA had failed to copy or integrate and we intend to investigate this further.

TRANSFORMATION OF MATURE SEED CALLUS

One of the major bottlenecks to wheat transformation is the continual growth of juvenile plants and the isolation of immature embryos to serve as regenerable explants. It is costly to provide a year-round supply of donor wheat plants in glass house or contained environment growth rooms. It is also technically-challenging and laborious to dissect immature embryos from seeds at 10–14 days post anthesis.

We have been investigating the potential of mature seeds to provide embryogenic callus for transformation (Fig. 2). By optimising various tissue culture parameters we have shown that is it possible to recover fertile, phenotypically-normal, adult plants from mature seed-derived callus (further details in Wilkinson et al. 2005).

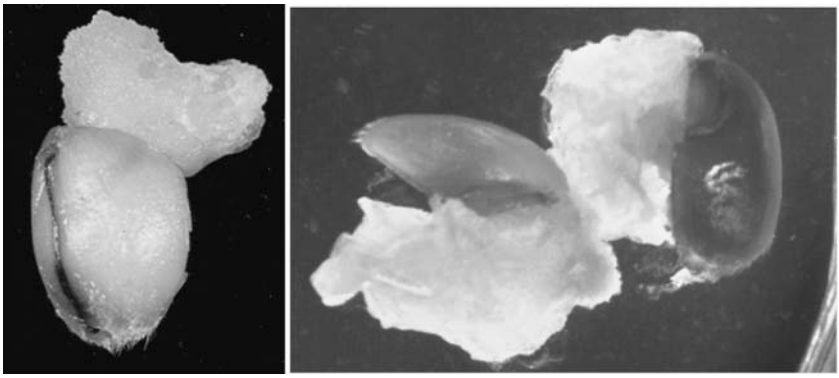


Figure 2. Embryogenic callus capable of regeneration initiated from the axis/scutellum of mature wheat seeds

This procedure can produce an almost limitless supply of embryogenic callus and may also eliminate the significant seasonal variation, which can affect the tissue culture response of immature embryos.

OTHER STRATEGIES FOR HIGH-THROUGHPUT TRANSFORMATION

It has been postulated that 'transformability' can be increased in recalcitrant genotypes by overexpression of genes involved in, for example, somatic embryogenesis, hormone perception/signalling, cell-cycle regulation etc. We intend to investigate the effects of such genes in wheat.

Protocols using Agrobacterium to deliver DNA to plant cells rather than physical methods such as the gene gun are more amenable to automation using robotics/liquid-handling machinery. Combined with an almost limitless supply of embryogenic callus from mature seeds, automation, even at significantly lower efficiencies, may provide a route to high-throughput transformation.

CONCLUSIONS

Genetic transformation is a powerful research tool for gene identification and functional analysis. In *Arabidopsis* and rice, populations tagged with T-DNAs or heterologous transposons are proving uniquely useful for gene identification. In addition, the increasing availability of strongly constitutive, tissue-specific or inducible promoters and siRNA technology is facilitating highly targeted overexpression and precise down-regulation of candidate genes. Wheat has many unique biologically and commercially important traits, including aspects of development, end-use quality and disease resistance that cannot easily be accessed via model species. There is already an increasing demand from the wheat research community

for access to these transformation-based tools and technologies and to provide this we are striving for high-throughput *Agrobacterium*-mediated methods.

ACKNOWLEDGEMENTS

Rothamsted Research receives grant aided support from the Biotechnology and Biological Sciences Research Council UK. HW and AD were supported by the Defra, UK, BRACT project.

REFERENCES

Altpeter F, Baisakh N, Beachy R, Bock R, Capell T, Christou P, Daniell H, Datta K, Datta S, Dix PJ et al (2005) Particle bombardment and the genetic enhancement of crops: myths and realities. Mol Breed 15:305–327

Barcelo P, Rasco-Gaunt S, Thorpe C, Lazzeri PA (2001) Transformation and gene expression. In: Shewry PR, Lazzeri PA, Edwards KJ (eds) Advances in botanical research incorporating advances in plant pathology, vol 34. Academic press, London, UK, pp 59–126

Cheng M, Fry JE, Pang SZ, Zhou HP, Hironaka CM, Duncan DR, Conner TW, Wan YC (1997) Genetic transformation of wheat mediated by *Agrobacterium tumefaciens*. Plant Physiol 115:971–980

Cheng M, Hu TC, Layton J, Liu CN, Fry JE (2003) Desiccation of plant tissues post-*Agrobacterium* infection enhances T-DNA delivery and increases stable transformation efficiency in wheat. Vitro Cell Dev Biol Plant 39:595–604

Cheng M, Lowe BA, Spencer TM, Ye XD, Armstrong CL (2004) Factors influencing *Agrobacterium*-mediated transformation of monocotyledonous species. Vitro Cell Dev Biol Plant 40:31–45

Dai SH, Zheng P, Marmey P, Zhang SP, Tian WZ, Chen SY, Beachy RN, Fauquet C (2001) Comparative analysis of transgenic rice plants obtained by *Agrobacterium*-mediated transformation and particle bombardment. Mol Breed 7:25–33

Haliloglu K, Baenziger PS (2003) *Agrobacterium tumefaciens*-mediated wheat transformation. Cereal Res Commun 31:9–16

Hu T, Metz S, Chay C, Zhou HP, Biest N, Chen G, Cheng M, Feng X, Radionenko M, Lu F et al (2003) *Agrobacterium*-mediated large-scale transformation of wheat (*Triticum aestivum* L.) using glyphosate selection. Plant Cell Rep 21:1010–1019

Jones HD (2005) Wheat transformation: current technology and applications to grain development and composition. J Cereal Sci 41:137–147

Jones HD, Doherty A, Wu H (2005) Review of methodologies and a protocol for the *Agrobacterium*-mediated transformation of wheat. Plant Meth 1:5.

Khanna HK, Daggard GE (2003) *Agrobacterium tumefaciens*-mediated transformation of wheat using a superbinary vector and a polyamine-supplemented regeneration medium. Plant Cell Rep 21:429–436

Mitic N, Nikolic R, Ninkovic S, Miljus-Djukic J, Neskovic M (2004) *Agrobacterium*-mediated transformation and plant regeneration of *T. aestivum* L. Biol Plantarum 48:179–184

Sahrawat AK, Becker D, Lutticke S, Lorz H (2003) Genetic improvement of wheat via alien gene transfer, an assessment. Plant Sci 165:1147–1168

Smith RH, Hood EE (1995) *Agrobacterium-tumefaciens* transformation of monocotyledons. Crop Sci 35:301–309

Travella S, Ross SM, Harden J, Everett C, Snape JW, Harwood WA (2005) A comparison of transgenic barley lines produced by bombardment and *Agrobacterium*-mediated techniques. Plant Cell Rep 23:780–789

Weir B, Gu X, Wang MB, Upadhyaya N, Elliott AR, Brettell RIS (2001) *Agrobacterium tumefaciens*-mediated transformation of wheat using suspension cells as a model system and green fluorescent protein as a visual marker. Aust J Plant Physiol 28:807–818

Wilkinson MD, Biradar D, Jones HD (2005) Mature seeds explants of wheat: a cheaper and easier alternative for wheat transformation? In: Abstracts of 7th international wheat conference, 27 Nov–2 Dec. Mar del Plata, Argentina, poster presentation

Wu H, Sparks C, Amoah B, Jones HD (2003) Factors influencing successful *Agrobacterium*-mediated genetic transformation of wheat. Plant Cell Rep 21:659–668

Xia GM, Li ZY, He CX, Chen HM, Brettell R (1999) Transgenic plant regeneration from wheat (*Triticum aestivum* L.) mediated by *Agrobacterium tumefaciens*. Acta Phytophysiologica Sinica 25:22–28

ACCELERATING THE TRANSFER OF RESISTANCE TO FUSARIUM HEAD BLIGHT IN WHEAT (*TRITICUM AESTIVUM* L.)

J. THOMAS*, C. HIEBERT, D. SOMERS, R. DEPAUW, S. FOX AND C. McCARTNEY

Research Branch, Agriculture and AgriFood Canada, 195 Dafoe Road Winnipeg, MB. R3T 2M9, Canada

**E-mail: jthomas@agr.gc.ca*

Abstract: Release of cultivars resistant to Fusarium Head Blight (FHB) from our wheat breeding program is hindered more by incidental flaws of resistant lines than by a lack of improved resistance. To increase the number of resistant lines to select among, we are experimenting with enhancing the fixation of three quantitative trait loci (QTL) for FHB resistance. This is achieved by hemizygosity of the chromosome segments that carry the QTL loci (3BS, 6BS and 5AS) in F1, followed by their reversion to euploidy upon inbreeding. Elite hemizygous parents were developed by combining a non-reciprocal translocation between 3BL and 6BL and a telocentric for 5AL in crosses with elite hard red spring wheat lines. Hemizygotes were tracked with DNA markers and confirmed by their chromosome pairing (iii + it + 18ii). Based on meiotic and test cross data, 3BS, 6BS and 5AS all showed rapid euploid reversion. Reversion was driven by cosegregation of 3BS and 6BS due to preferential "V" alignment of trivalents at meiotic metaphase and by natural selection in favour of euploid plants and gametes. Populations to fix FHB resistance are to be recovered from crosses of improved hemizygotes with triple QTL derivatives from the breeding program. Since hemizygous hybrids only will be advanced, their segregating progeny will go on to fix the targeted FHB QTL. With QTL recovery guaranteed, resources and crossing plans can focus on improving overall merit

Keywords: Fusarium Head Blight resistance, euploid reversion, hemizyosity

INTRODUCTION

One goal of the genetic analysis of *Fusarium* Head Blight(FHB) resistance in wheat (*Triticum aestivum* L.) is targeted breeding based on cross-applicable quantitative trait loci (QTL) (Somers et al. 2005). While backcrossing and intercrossing resistant

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 701–706.

lines will enhance recovery of QTL, it also confines the working gene pool. In wider crosses, recovery of FHB resistant lines with broad merit is made less probable by the enlarged number of loci that require selection (Thomas and DePauw 2003). Calculations show that with many loci segregating, a plant that retains all desirable alleles will occur only rarely in an inbreeding population whose size we can afford (Sneep 1977). Under the usual rules of inheritance, little can be done to evade this difficulty since selection for complex traits is ineffective in F_2 while combinatorial possibilities decline as heterozygotes disappear in F_3 or F_4. If we grant priority to a new trait without expanding the population, the most likely outcome will be lines that are flawed in some other aspect. This epitomises our experience with FHB. We propose instead to suspend the usual rules of inheritance for FHB resistance QTL by cytogenetic fixation (Thomas and DePauw 2003). For example, inter-varietal chromosome substitutions are fixed by reversion of a monosome from hemizygosity (one dose) to euploidy (two doses). However, as a non-Mendelian breeding technique, monosomic reversion is not efficient. Typically, unpaired monosomes misdivide at meiotic anaphase (A1), are excluded at second anaphase (A2) and revert slowly due to a low frequency of euploid ovules. Other hemizygous variants include telosomes and non-reciprocal translocations. Compared to monosomes, these types pair at metaphase (M1), separate undivided at A1, assort normally at A2 and revert quickly (Thomas et al. 2003, 2004). This report describes the assembly of a triple hemizygous stock from a translocation and a telocentric and demonstrates its ability to fix FHB resistance QTL on 3BS, 5AS and 6BS (Liu and Anderson 2003, Buerstmayr et al. 2003, Yang et al. 2005).

MATERIALS AND METHODS

An existing 3BL.6BL Robertsonian translocation and its 3BS.6BS reciprocal (Friebe and Gill 1994) were rendered hemizygous by pollinating a 3B-6B double monosomic with Alcedo. (The double mono was isolated from a cross of mono 3B with a wheat-alien substitution of 6B in Rescue). Progeny that were doubly monosomic for the translocated chromosomes were then pollinated with monotelodisomic 5AL. Four triply hemizygous plants (lacking the 3BS.6BS translocation but retaining the 3BL.6BL translocation, the 5AL telo and standard versions of 3B, 6B and 5A) were identified from the absence of appropriate polymorphic DNA marker alleles (Somers et al. 2004) and were confirmed by M1 pairing (telo bivalent plus trivalent). Gametic transmission of the three hemizygous arms was determined from the segregation of polymorphic markers in male and female test crosses using a well-characterised FHB resistant line (DH181) as a tester (Yang et al. 2005). The degree of fixation of FHB QTL to be expected among F_2 seed of triple hemizygous hybrids was estimated from cross products of the gametic frequencies. Prior to making crosses intended for the breeding program, hemizygous parents were improved by crossing with elite varieties to diversify their genetic background and increase the coefficient of hard red spring parentage in line with our other breeding priorities. Triple QTL parents were identified from their markers, first in a survey of lines in the breeding program and later among doubled haploids from a marker-assisted

backcross project to accelerate the pyramiding of FHB QTL (Somers et al. 2005). These were then used to pollinate improved triple hemizygotes.

RESULTS

Preferential "V" disjunction of the Robertsonian trivalent at first meiotic metaphase (M1), (previously noted for other non-reciprocal translocations; Thomas et al. 2004) was confirmed for 3B-3BL.6BL-6B trivalents (Table 1) and for 3B-3BS.6BS-6B trivalents (also recovered, data not shown). Overall, 3B-3BL.6BL-6B trivalents were found in 117/125 cells (0.94) with 104/117 (0.89) aligned as "V"s (i.e. with end chromosomes of the linear trivalent oriented in the same direction). Other observed alignments included a low frequency of "L"s (one end chromosome not oriented) and "I"s (end chromosomes opposed). Since the average pairing rate of the chromosome background was ca. 0.95 (Table 1), the expected incidence of intact trivalents is 0.95^2 (ca. 0.90). Thus the observed pairing efficiency of the trivalent (0.94) was above average. Incidence (88/92 = 0.96) and proper alignment (87/88) of heteromorphic 5A-5AL bivalents were also high (Table. 1).

A testcross of 3BS, 6BS and 5AS triple hemizygotes with the triple QTL line DH181 (Table 2) detected an enormous excess of gametes that were plus-plus and null-null for 3BS and 6BS over gametes that were null-plus and plus-null among pollen (100% vs. 0%) and among ovules (98% vs. 2%). This quasi-linkage between the hemizygous ends of the translocation trivalent (Table 2) arises from its preferential alignment and disjunction as a "V" (Table 1) probably due to a one-way conversion of "I" trivalents into "L"s and then into "V"s (Thomas et al. 2004). Telocentric (5A-5AL) bivalents aligned independently of "V" and "L" trivalents ($\sim 1/2$ were co-aligned; Table 1) and no strong association of 5AS with 3BS or 6BS was recorded among ovules (Table 2). Thus quasi-linkage did not involve 5AS. Ovules that were plus-plus for 3BS and 6BS were about as frequent as null-nulls (52% vs. 46%) while 61% of ovules carried 5AS compared to 39% nulls. Strong certation occurred against aneuploid pollen (69% of recovered male gametes were euploid compared to 31% in the three aneuploid classes not affected by quasi-linkage). Ratios of the genotypes expected in F_2 were calculated from cross products of the observed gametic frequencies (Table 2). The incidence of triple

Table 1. Pairing and alignment of trivalents and telocentrics at meiotic metaphase. Picture shows a "V" trivalent (V) and a telocentric bivalent (t) co-aligned (i.e. 3BS, 6BS and 5AS will cosegregate)

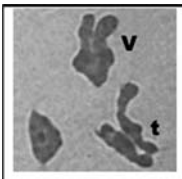
	Number of plants (cells)	2n	Trivalent types (see above)					Telo types		Translocation and telo co-aligned?	Pairing rate of the background
			"V"	"L"	"I"	"V"s/total	ii+i	it	i+t		
3BL.6BL + 5AL	4 (92)	40+t	0.83	0.05	0.04	0.89	0.08	0.96	0.04	37/75	0.94
3BL.6BL	1 (33)	41	0.85	0.12	0.00	0.88	0.03	-	-	-	0.96

Table 2. Observed gametic percentages (in italics) from male and female test crosses of the triple hemizygote and estimated F_2 percentages (Punnett square of cross products inside the heavy line). Cells between the dotted lines contain gamete and zygote classes affected by quasi-linkage of 3BS and 6BS. The underlined cell (21.1%) is the triple resistant class; progeny of the 26 shaded cells (total 65.1%) will continue to revert toward full resistance in F_3; the 37 remaining cells of the Punnett square (total 13.8%) have all lost one, two or three resistance QTL through deficiency. In a conventional tri-hybrid F^2, corresponding numbers are 1.6% (1/64) fixed for resistance (vs. 21.1%), 40.6% (26/64) capable of triple fixation (vs. 65.1% reverting) and 57.8% (37/64) fixed for one or more susceptible alleles (vs. 13.8% deficient).

Ovules (n = 95)	Pollen (n = 95)	3BS 6BS 5AS	3BS 6BS null	3BS null 5AS	3BS null null	null 6BS 5AS	null 6BS null	null null 5AS	null null null
		69.2	***23.1***	***0.0***	***0.0***	***0.0***	***0.0***	***2.6***	***5.1***
3BS,6BS,5AS	***30.5***	<u>21.1</u>	7.0	0.0	0.0	0.0	0.0	0.8	1.6
3BS,6BS,null	***21.1***	14.6	4.9	0.0	0.0	0.0	0.0	0.5	1.1
3BS,null,5AS	***0.0***	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3BS,null,null	***0.0***	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
null,6BS,5AS	***1.1***	0.7	0.2	0.0	0.0	0.0	0.0	trace	0.1
null,6BS,null	***1.1***	0.7	0.2	0.0	0.0	0.0	0.0	trace	0.1
null,null,5AS	***29.5***	20.4	6.8	0.0	0.0	0.0	0.0	0.6	1.4
null,null,null	***16.8***	11.7	3.9	0.0	0.0	0.0	0.0	0.3	0.7

QTL homozygotes in F_2 seed of triple hemizygotes, (21.1%) is predicted from the cross product of euploid gamete frequencies (30.5% ♀ × 69.2% ♂). This compares with 1.6%($= (1/4)^3 = 1/64$) expected in the F_2 of a conventional tri-hybrid. If we turn to the frequency of F_2 segregates with at least one dose of 3BS, 6BS and 5AS (i.e. fixed or with the capacity to fix all three QTL segments); this was estimated at 86% compared to 43% ($= (3/4)^3 = 27/64$) predicted for a tri-hybrid F_2.

Given space and good conditions, triple hemizygotes were only slightly subnormal and supported the development of crossed and selfed seed. A study of fertility in a population segregating for all three hemizygous segments is in progress. Early results (in preparation) show that seed production of revertants > hemizygotes > deficients; this differential fertility means the endpoint of QTL fixation will approach an asymptote of 100%. F_2s from the test cross with the triple QTL line DH181 were compared in a corn-inoculated irrigated FHB nursery in 2005. In the field, populations derived from triple hemizygotes were plainly less vigorous than those from comparable euploids. Transitory weakness and reduced fertility are the hallmarks of euploid reversion. Weak aneuploids in a triple hemizygous population correspond to unfixed or susceptible segregates in a tri-hybrid F_2. Neither category is wanted but only the former is obvious and self-eliminating.

Measurement of enhanced FHB resistance arising from QTL fixation is needed in order to validate euploid reversion. Unfortunately, maximum FHB incidence in 2005 was low (<1%). Although, as expected, fewer infected spikelets were found in

triple hemizygous plots than in comparable trihybrid populations (9.8 vs. 13.3), the difference was insignificant. Low disease pressure thus precluded a demonstration of reduced FHB. For the present, we predict a response similar to other methods that target the transfer of particular QTL (for example marker assisted selection).

Figure 1 illustrates crosses made to introduce euploid reversion into the breeding program. Two resistance genes (*Sm1* for midge resistance; *Lr21* for leaf rust) and a dwarfing gene (*RhtB1b*) were installed in the background of triple hemizygotes from an initial cross with PT432 and were maintained in further crosses using leaf rust, GA_3 and allele-specific or nearby markers. After further crossing with elite lines, hard red spring parentage of hemizygotes reached 93% and further upgrading is planned. Some parents contributed additional resistance to FHB not arising from the targeted QTL as judged by parentage or marker status. Two cycles of crosses were prepared between upgraded triple hemizygotes and lines with the targeted QTL (identified from relevant markers). Previously, entire QTL regions were surveyed; this guarantees the QTL but also risks the freezing of adverse linkages. More recently, QTL were identified based on single nearby markers: Qfhs.ndsu-3BS by STS3B-80 (Liu and Anderson 2003), the 6BS QTL by *xgwm644* (Yang et al. 2005) and Qfhs.ifa-5A by *xgwm304* (Buerstmayr et al. 2003). Per cycle, 15 to 20 hemizygous plants were crossed with 10–20 triple QTL lines to generate >4000 hybrid seeds and advance ~500 F_2s reverting for FHB resistance and fixed or segregating for *Lr21, Sm1* and *RhtB1b*.

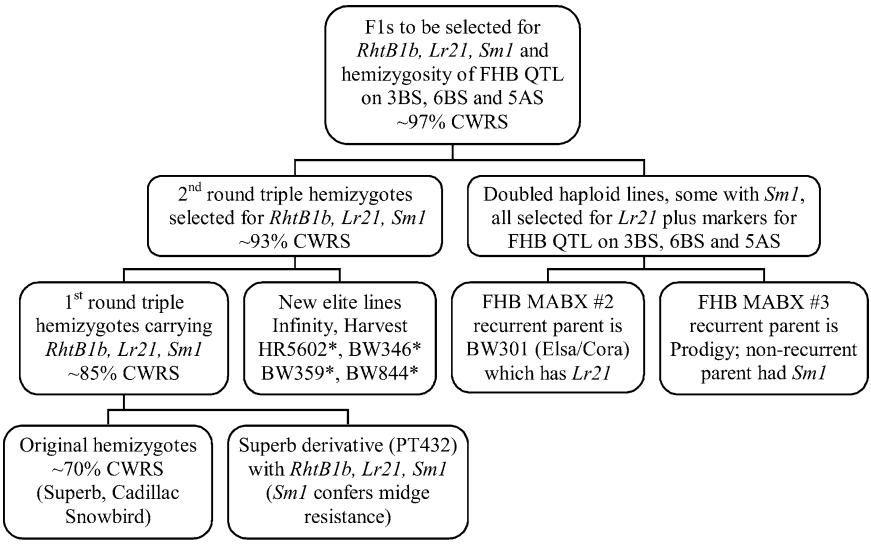


Figure 1. Convergence of hemizygosity, Canada Western Red Spring parentage (CWRS), additional important genes (*Lr21, Sm1* and *RhtB1b*) and targeted FHB resistance QTL in recent crosses. Parental lines marked * displayed additional FHB resistance (not attributable to the target QTL based on markers and/or parentage). MABX = marker-assisted backcrosses of FHB QTL

DISCUSSION

Undoubtedly, targeted genes could be fixed in purpose-made F_1s purely through the use of markers (*cf.* Somers et al. 2005) and so avoid the use of cytogenetics. We have used this approach to develop F_1s fixed for wheat midge resistance by marker-assisted screening for *Sm1* in four-way crosses (R/S//R/S) and top-crosses (R/S//R). The result was a technical success since 93% of F_2 populations bred true for midge resistance (unpublished). However, with this approach, a trait donor must appear on both sides of the final cross to the exclusion of some non-donor that could otherwise have been chosen based on any criterion. In retrospect, merit of these "fixed midge" populations was reduced by susceptibility to lodging, leaf rust and FHB, largely inherited from their midge-resistant parents. In any comparable crossing plan, euploid reversion requires the trait donor only as male, making enhanced recovery of trait-positive lines compatible with a wide range of elite, trait-negative parentage that is readily diversified and upgraded. Thus euploid reversion is a flexible adjunct to other breeding methods since revertants will recast the background of FHB-resistant selections over and over again, while inheriting a targeted set of resistance QTL intact.

ACKNOWLEDGEMENTS

Erica Riedel, Jadwiga Budzinski, Sasanda Nilmalgoda, Debbie Jones, Elaine Martineau, and Dennis Green gave technical assistance. Financial support was provided by Western Grains Research Foundation, Agripro, Agricore United and Agriculture and AgriFood Canada (MII).

REFERENCES

Buerstmayr H, Steiner B, Hartl L, Griesser M, Angerer N, Lengauer D, Miedaner T, Schneider B, Lemmens M (2003) Molecular mapping of QTLs for *Fusarium* head blight resistance in spring wheat. II. Resistance to fungal penetration and spread. Theor Appl Genet 107:503–508

Friebe B, Gill BS (1994) C-band polymorphism and structural rearrangements detected in common wheat (*Triticum aestivum*). Euphytica 78:1–5

Liu S, Anderson JA (2003) Targeted molecular mapping of a major wheat QTL for *Fusarium* head blight resistance using wheat ESTs and synteny with rice. Genome 46:817–823

Sneep J (1977) Selection for yield in early generations of self-fertilizing crops. Euphytica 26:27–30

Somers DJ, Isaac P, Edwards K (2004) A high density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theor Appl Genet 109:1105–1114

Somers DJ, Thomas J, DePauw R, Fox S, Humphreys G, Fedak G (2005) Assembling complex genotypes to resist *Fusarium* in wheat (*Triticum aestivum* L.). Theor Appl Genet 111:1623–1631

Thomas J, DePauw R (2003) Dodging the exponential challenge of breeding *Fusarium* head blight resistant cultivars. In: Proceedings of the 3rd Canadian workshop on fusarium head blight, Winnipeg, Canada, p 136

Thomas J, Riedel E, Fox S (2003) An efficient method for breeding monogenic traits in wheat. In: Pogna N (ed) Proceedings of the 10th international wheat genetics symposium, Paestum, Italy, pp 171–174

Thomas J, Riedel E, Benabdelmouna A, Armstrong K (2004) A cytogenetic method for stacking gene pairs in common wheat. Theor Appl Genet 109:1115–1124

Yang ZP, Gilbert J, Fedak G, Somers DJ (2005) Genetic characterization of QTL associated with resistance to *Fusarium* head blight in a doubled haploid spring wheat population. Genome 48:187–196

PRODUCTION AND MOLECULAR CYTOGENETIC IDENTIFICATION OF NEW WINTER WHEAT/WINTER BARLEY DISOMIC ADDITION LINES

M. MOLNÁR-LÁNG*, É. SZAKÁCS, G. LINC AND E.D. NAGY

Agricultural Research Institute of the Hungarian Academy of Sciences H-2462, Martonvásár, P.O. Box 19, Hungary

**E-mail: molnarm@mail.mgki.hu*

Abstract: Disomic wheat/barley addition lines were developed from hybrids produced with the German two-rowed winter barley cultivar 'Igri' and the Ukrainan six-rowed winter barley cultivar 'Manas'. The 2H, 3H and 4H disomic addition lines of winter wheat 'Martonvásári 9 kr1'/winter barley 'Igri' produced in Martonvásár were identified using GISH, FISH and SSR markers. A disomic addition of the 1HS isochromosome was also identified. The 2H, 3H and 4H addition lines have been multiplied in the nursery and characterized morphologically. Backcross progenies were produced and identified with molecular genetic and cytogenetic methods from the Japanese wheat 'Asakaze komugi' × Ukrainan six-rowed winter barley 'Manas' hybrids. A deletion and a translocated chromosome facilitated the physical localization of microsatellite markers on chromosome 5H. So far the 4H disomic addition line has been identified from the fertile BC_2 plants in this combination, but the development of other disomic additions is in progress

Keywords: disomic addition lines, wheat-barley addition lines, molecular cytogenetic identification

INTRODUCTION

The hybridization of wheat and barley makes it possible to transfer useful characters such as earliness, tolerance to drought and soil salinity, and various nutrition quality parameters from barley into wheat. The first wheat-barley hybrid was produced by Kruse (1973) and not much later a set of wheat/barley addition lines was developed by Islam et al. (1978). Since the production of the Chinese Spring/Betzes spring wheat/spring barley addition lines there has been no report on the development of a new set of wheat/barley addition lines except the 5H and 6H addition lines produced

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 707–713.

from a hybrid involving the wheat cultivar 'Shinchunaga' and the barley cultivar 'Nyugoruden' by Koba and co-workers in 1997. However, wheat/rye addition lines have been developed in many cultivar combinations (Shepherd and Islam, 1988). Wheat-alien addition lines form the starting point for producing translocations from selected chromosomes and are also suitable genetic materials for genome mapping. As there is great genetic variability between various barley cultivars for important agronomic traits (two or six-row, winter or spring habit, biotic and abiotic resistance, etc.) it would be advisable to develop addition lines using different barley genotypes in order to map and transfer favourable agronomical characters from barley. Many cultivated barley varieties were used for the pollination of wheat, and the development of addition lines was started from hybrids produced with the German two-rowed winter barley cultivar 'Igri' and the Ukrainan six-rowed winter barley cultivar 'Manas'.

MATERIAL AND METHODS

Plant Materials

Wheat × barley hybrids were produced using two wheat (*Triticum aestivum* L.) genotypes as the maternal plants: the Hungarian winter wheat line Martonvásári 9 kr1 (Mv9 kr1), (Molnár-Láng et al. 1996), and the Japanese wheat variety Asakazekomugi. The winter barley (*Hordeum vulgare* L.) cultivars used as the male parent were the following: Igri, (two-rowed, German) and Manas (six-rowed, Ukrainian).

The wheat × barley hybrids ('Mv9 kr1' × 'Igri', 'Asakaze komugi' × 'Manas') were multiplied in tissue culture as described earlier (Molnár-Láng et al. 2000). The backcross pollination was carried out using the wheat genotypes 'Mv9 kr1', 'Asakaze komugi' and 'Chinese Spring'. One day after pollination 100 ppm 2,4-D was injected into the stem, and embryo culture was initiated three weeks after pollination.

Cytology, sequential genomic and fluorescence in situ hybridization: Mitotic and meiotic chromosome counts were made using the Feulgen squash technique. Fluorescence in situ hybridization (FISH) was carried out with the GAA satellite sequences amplified from barley (Pedersen et al. 1996), pAs1 isolated from Ae. tauschii (Rayburn and Gill, 1986), HvT01 subtelomeric tandem repeat amplified from barley (Schubert et al. 1998) and pTa71 isolated from wheat (Gerlach and Bedbrook 1979). The DNA probes were labelled by PCR (Vrána et al. 2000) and by nick translation using either Fluorogreen (fluorescein-12-dUTP, Roche) or Fluorored (rhodamine-5-dUTP, Roche). FISH was carried out according to Linc et al. (1999). After rinsing the preparations, genomic in situ hybridization (GISH) was carried out on the same slides as described earlier (Molnár-Láng et al. 2000) following the method of Reader et al. (1994). Total barley genomic DNA was labelled with Fluorored by nick translation and used as a probe. Unlabelled wheat genomic DNA was sheared by autoclaving and used as blocking DNA at 40

times the quantity of the probe. The slides were counterstained with DAPI (4′,6-diamidino-2-phenylindole, Amersham). The chromosomes were examined under a Zeiss Axioskop 2 epifluorescence microscope equipped with Filter 10 for FITC, Filter 15 for Texas Red and Filter 01 for DAPI. The images were captured by means of a Spot CCD camera (Diagnostic Instruments, USA). The images obtained during FISH with different probes were merged using the computer program Image-Pro Plus 4.0 (Media Cybernetics, USA).

SSR marker analysis: Genomic DNA was isolated from the 'Mv9 kr1'/'Igri' disomic addition lines and the BC2 progenies of the 'Asakaze komugi' × 'Manas' hybrids and their progenitors. Altogether twenty-three SSR markers were selected from a highly saturated genetic map of barley (Ramsay et al. 2000). The markers were: Bmac0213 (1HS), Bmac0032 (1HS), EBmac0783 (1HL), HVHVA1 (1HL), HVM36 (2HS), Bmac0093 (2HS), EBmac0415 (2HL), HVM60 (3HL), HVM62 (3HL), HvLTPPB (3HS), Bmag0013 (3HL), HVM40 (4HS), HVM67 (4HL), Bmac0306 (5HS), Bmag0337 (5HL), Bmag0394 (5HL), Bmag0323 (5HL), Bmac0096 (5HL), EBmac0824 (5HL), Bmac0316 (6HS), Bmac0040 (6HL), EBmac0806 (6HL) and Bmag0120 (7HL). The PCRs were performed as described earlier (Molnár-Láng et al. 2005) following the method of Ramsay et al. (2000).

RESULTS AND DISCUSSION

Production of New Winter Wheat 'Martonvásári 9 kr1'/Winter Barley 'Igri' Disomic Addition Lines

The 'Martonvásári 9 kr1' × 'Igri' hybrid was vigorous and had good tillering ability, but showed complete male and female sterility, so it was multiplied in tissue culture from young inflorescences (Molnár-Láng et al. 2000). 101 plants were regenerated from one initial hybrid plant, of which 92 were raised to maturity. Six BC_1 plants were grown from the nine embryos developed after pollinating 4606 flowers on the regenerated 'Mv9 kr1' × 'Igri' hybrids with the wheat line 'Mv9 kr1'. Plants with 44 chromosomes were selected from the selfed progenies of the BC_2 plants. Chromosome pairing was analysed in metaphase I of meiosis to identify plants with 22 bivalents. As several different lines with 44 chromosomes and 22 bivalents in meiosis were selected, the next step was to identify the barley chromosomes added to the wheat background. At first the parental genotypes had to be identified under our laboratory conditions using FISH with the help of different DNA probes.

Identification of the Barley Chromosomes in the 'Mv9 kr1'/'Igri' Disomic Addition Lines Using FISH and SSR Markers

Wheat chromosomes can be identified using FISH with the GAA sequence combined with the pAs1 clone (Pedersen and Langridge 1997). Barley chromosomes can also be identified using FISH with the help of the GAA sequences

(Pedersen et al. 1996). The FISH hybridization pattern of barley chromosomes with the HvT01 tandem telomeric repeat and the pTa71 probe is described by Schubert et al. (1998) and by Leitch and Heslop-Harrison (1992). All the chromosomes of the parental genotypes, the wheat line 'Mv9 kr1' and the barley cultivar 'Igri' were identified with the help of these sequences in the molecular cytogenetic laboratory in Martonvásár.

The first addition line was identified using FISH with the help of the GAA sequence and the pAs1 probe. All the wheat chromosomes could be identified with the help of these sequences and a pair of 4H chromosomes was present. The 4H chromosome could be identified by its strong GAA FISH hybridization signals. The presence of the additional barley chromosome pair was confirmed with GISH. The GISH experiment was carried out on the same slides after washing off the FISH signals. Chromosome 4H was also identified with the help of probe HvT01, which gave telomeric signals on both chromosome arms, while the wheat chromosomes gave no signals. The presence of chromosome 4H in this addition line was also confirmed with SSR markers. Markers HvM 40 and HvM 67, previously mapped on chromosome 4H, gave the expected bands on this line. The 4H Mv9 kr1/Igri addition line has a compact spike with small awn stubs at the top. The spikes have good fertility. The 4H addition line has already been multiplied in our nursery, so a great number of seeds are available for further studies.

The next addition line contains a pair of 2H chromosomes, identified with a combination of the probes GAA and pAs1 using FISH. The GAA FISH hybridization signals on chromosome 2H are very similar to the C-banding pattern of this chromosome, having symmetrical interstitial hybridization sites on both arms (Jensen and Linde-Laursen 1992, Linc and Molnár-Láng 2003). The presence of 2H was also confirmed with sequential GISH analysis after FISH. When applying HvT01 as a probe, a FISH hybridization signal was observed only on the short arm of the barley chromosomes, which is typical of 2H. The other barley chromosome which has a signal only on the short arm is 5H, but that is a satellited chromosome which could be easily recognized. The SSR marker HvM36 also gave the expected PCR product size to confirm the presence of 2H in this line. This line has a long, loose spike, having fewer seeds/spike than the 4H addition line. The plants are taller than the 4H addition line. This line has also been multiplied in the nursery so a large number of seeds are available for further studies.

The third addition line contains the chromosome 3H. It was first identified using FISH with a combination of the DNA probes GAA and pAs1. The GAA hybridization sites on this chromosome did not allow the unequivocal identification of 3H, as it was mostly hybridization sites close to the centromere that could be recognized, which are very similar to the hybridization signals on 7H. Thus, the probe HvT01 was also used for FISH identification. This probe made it clear that this chromosome was 3H, as it is the only barley chromosome which has a strong subtelomeric signal and a weak interstitial signal besides the telomeric

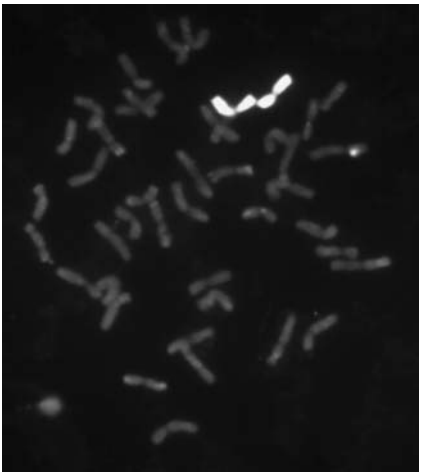


Figure 1. Genomic *in situ* hybridization on the metaphase chromosome complement of a 3H 'Mv9 kr1'/'Igri' disomic addition line. Total barley genomic DNA was labelled with Fluorored (two barley chromosomes are bright)

signal on its long arm (Schubert et al. 1998) (Fig. 1). The molecular cytogenetic identification was also confirmed with the help of molecular markers. The SSR markers HvM60 and HvM62 gave the expected bands, confirming the presence of the 3H chromosome in this line. The 3H addition line has a very compact ear with a great number of spikelets per ear. Small awnstubs can be seen at the top of the spikes. The plants are short but have good fertility. This addition line has also been multiplied in the nursery.

The fourth line was the most difficult to identify as it has a disomic addition of the isochromosome 1HS. In this addition line strong GAA FISH hybridization signals were observed around the centromere on the barley chromosome after the identification of all the chromosomes with the GAA sequence and the HvT01 repeat. It was thought to be 7H, as two telomeric bands were observed with the HvT01 probes at the ends of the chromosome pair. 5H and 6H could be excluded, as there was no satellite, and 2H and 4H on the basis of the GAA pattern, while 3H could be excluded as it has a very characteristic hybridization pattern with HvT01. However, molecular markers tested to confirm the presence of 7H did not give the expected PCR products. FISH hybridization with the probe pTa71 identified this chromosome pair as 1HS isochromosomes. Two minor, perfectly symmetrical, interstitial pTa71 signals were observed on both arms of this chromosome. The strength of this signal coincided with the strength of the signal on the 1H short arm. It thus became clear that this line carries a pair of 1HS isochromosomes. It has a long, loose spike with small awnstubs. The plant was grown in a controlled environment, producing enough seeds for further multiplication.

Production and Molecular Cytogenetic Identification of Backcross Progenies from the Wheat 'Asakaze Komugi' × Winter Barley 'Manas' Hybrids

The aim of this study was to produce backcross progenies on a new wheat ('Asakaze komugi') × Ukrainan six-rowed winter barley ('Manas') hybrid produced in Martonvásár. As no backcross seeds were obtained on the initial hybrids, young inflorescences of the hybrids were used for in vitro multiplication in three consecutive cycles until a backcross progeny was developed. The chromosome constitution of the regenerated hybrids was analysed using GISH after each in vitro multiplication cycle in metaphase I of meiosis (Molnár-Láng et al. 2005). The seven barley chromosomes were present even after the third in vitro multiplication cycle but abnormalities were observed. Sixteen BC2 plants shown by GISH analysis to contain one to three complete barley chromosomes, two deletion barley chromosomes and a dicentric wheat-barley translocation were grown to maturity from the single backcross progeny. The barley chromatin was identified using 20 chromosome-specific barley SSR markers. All seven barley chromosomes were represented in the BC2 plants. A deletion breakpoint at FL ±0.3 on the 5HL chromosome arm facilitated the physical localization of microsatellite markers. The chromosome constitution of each BC2 plant was determined with a combination of GISH and SSR markers, giving an exact demonstration of the number and origin of the barley chromosomes present in these plants. The presence of two barley chromosomes in a wheat background was detected by GISH from selfed BC2 seeds originating from a 4H monosomic addition, so three plants with 4H disomic additions were selected from the selfed seeds of the BC2 progenies. The development of the other disomic addition lines is in progress.

ACKNOWLEDGEMENTS

The technical assistance of Mrs I. Bucsi and Mrs. J. Havasi is gratefully acknowledged. This work was financed by the Hungarian National Scientific Research Fund (No. T 043502).

REFERENCES

Gerlach WL, Bedbrook JR (1979) Cloning and characterization of ribosomal RNA genes from wheat and barley. Nucleic Acids Res 7:1869–1885

Islam AKMR, Shepherd KW, Sparrow DHB (1978) Production and characterization of wheat-barley addition lines. In: Ramunujam, S (ed) Proc. 5th Int. Wheat Genetics Symp, New Delhi, India, pp 356–371

Jensen J, Linde-Laursen I (1992) Statistical evaluation of length measurements on barley chromosomes with a proposal for new nomenclature for symbols and positions of cytological markers. Hereditas 117:51–59

Koba T, Takumi S, Shimada T (1997) Isolation, identification and characterization of disomic and translocated barley chromosome addition lines of common wheat. Euphytica 96:289–296

Kruse A (1973) Hordeum × Triticum hybrids. Hereditas 73:157–161

Leitch IJ, Heslop-Harrison JS (1992) Physical mapping of the 18S-5.8S-26S rRNA genes in barley by in situ hybridization. Genome 35:1013–1018

Linc G, Friebe BR, Kynast RG, Molnár-Láng M, Kőszegi B, Sutka J, Gill BS (1999) Molecular cytogenetic analysis of Aegilops cylindrica Host. Genome 42:497–503

Linc G, Molnár-Láng M (2003) Two methods for the development of wheat/barley disomic additions in winter wheat varieties, and identification using molecular cytogenetic techniques (C-banding, GISH). Növénytermelés 52:3–13

Molnár-Láng M, Linc G, Sutka J (1996) Transfer of the recessive crossability allele *kr1* from 'Chinese Spring' into the winter wheat variety Martonvásári 9. Euphytica 90:301–305

Molnár-Láng M, Linc G, Logojan A, Sutka J (2000) Production and meiotic pairing behaviour of new hybrids of winter wheat (*Triticum aestivum*) x winter barley (*Hordeum vulgare*). Genome 43:1045–1054

Molnár-Láng M, Novotny C, Linc G,Nagy ED (2005) Changes in the meiotic pairing behaviour of a winter wheat-winter barley hybrid maintained for a long term in tissue culture, and tracing the barley chromatin in the progenies using GISH and SSR markers. Plant Breed 124:247–252

Pedersen C, Langridge P (1997) Identification of the entire chromosome complement of bread wheat by two- colour FISH. Genome 40:589–593

Pedersen C, Rasmussen SK, Linde-Laursen I (1996) Genome and chromosome identification in cultivated barley and related species of the Triticeae (Poaceae) by in situ hybridization with the GAA-satellite sequence. Genome 39:93–104

Ramsay L, Macaulay M, degli Ivanissevich S, MacLean K, Cardle L, Fuller J, Edwards KJ, Tuvesson S, Morgante M, Massari A, Maestri E, Marmiroli N, Sjakste T, Ganal M, Powell W, Waugh R (2000) A simple sequence repeat-based linkage map of barley. Genetics 156:1997–2005

Rayburn AL, Gill BS (1986) Isolation of a D genome specific repeated DNA sequence from *Aegilops squarrosa*. Plant Mol Biol Rep 4:102–109

Reader SM, Abbo S, Purdie KA, King IP, Miller TE (1994) Direct labelling of plant chromosomes by rapid *in situ* hybridization. Trends Genet 10:265–266

Schubert I, Shi F, Fuchs J, Endo TR (1998) An efficient screening for terminal deletions and translocations of barley chromosomes added to common wheat. Plant J 14:489–495

Shepherd KW, Islam AKMR (1988) Fourth compendium of wheat-alien chromosome lines. In: Miller TE, Koebner RMD (eds) Proc 7th Int. Wheat Genet Symp, Cambridge, England, pp 1373–1398

Vrána J, Kubaláková M, Simková H, Číhalíková J, Lysák MA, Dolezel J (2000) Flow sorting of mitotic chromosomes in common wheat (*Triticum aestivum* L.). Genetics 156:2033–2041

GENETIC ENGINEERING OF RUSSIAN WHEAT GENOTYPES FOR ABIOTIC STRESS RESISTANCE

D. MIROSHNICHENKO[1,2,*], M. FILIPPOV[1], A. BABAKOV[2] AND S. DOLGOV[1,2]

[1] *'Biotron', Branch of Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Pushchino, Moscow Region, Russia 142290*

[2] *All Russian Institute of Agricultural Biotechnology RAAS, Timiryazevskaja 42, Moscow, Russia 127550*

[*] *E-mail: miroshnichenko@fibkh.serpukhov.su*

Abstract: The purpose of our investigations was the introduction of desirable genes into Russian wheat cultivars by biolistic transformation system. The ultimate yield of 47 independent transgenic lines of Andros variety, Russian spring wheat, with the herbicide resistance gene *bar* was produced. In 2004 field tests for herbicide resistance (1.0% Basta) of T3 homozygous offspring obtained from seven transgenic lines demonstrated that agronomic performance of treated transgenic and non-treated control plants were similar. Na^+/H^+ vacuolar antiporter *hvnhx2* gene from barley genome was introduced into wheat under *Ubi1* promoter so as to produce the salt- resistant plants. The total number of 14 transgenic T0 wheat plants was generated, and the RT-PCR confirmed the presence of high m-RNA concentration in extracts of 9 independent lines. Significant differences in plant growth were observed between the *hvnhx2* transgenic and non-transgenic T1 progeny. The growth of the T1 transgenic plants in saline conditions was about 80% of the same in non-saline conditions, while the non-transgenic plants showed 50% delay in the growth

Keywords: biolistic transformation, abiotic stress resistance, transgenic wheat

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the major cereal crop in Russia. Though the demand for Russian wheat varieties with resistance to diseases and other environmental stress is great, still there is a limitation for transferring resistance to crop plants because of the complexity of stress tolerance traits, as most of them are quantitatively linked ones. Nevertheless, the direct introduction of a small number of genes by genetic

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 715–721

engineering offers convenient alternative and a rapid approach for the improvement of stress tolerance. Weeds compete with crop plants for available nutrients and light energy, and thus reduce crop yields on the average from 10 to 15%. Biotechnology makes possible to provide crop plants with resistance to a certain herbicide, which allows selective elimination of weeds. Another problem of cereal crops productivity, including wheat, is soil salinity. Today, about 20% of the world's cultivated land and almost half of irrigated lands are affected by salinity. Thus, if salt tolerance would be conferred upon wheat plants, the production of wheat could be increased.

In the present study the herbicide resistance was investigated under field conditions in populations of transgenic wheat transformed with *bar* gene. The enhancement of salt tolerance in wheat plants transformed with a barley vacuolar Na^+/H^+ antiporter gene *hvnhx2* is demonstrated .

MATERIALS AND METHODS

The monocot transformation vector psGFP-BAR (Richards et al. 2001) was used to generate herbicide-resistant wheat plants. It contains *gfp* gene driven by the rice *Act1* promoter (McElroy et al. 1990) and *bar* gene, which confers resistance to the herbicide Basta, driven by the maize ubiquitin *Ubi1* promoter (Christensen et al. 1992). A particle inflow gun was used to deliver DNA-coated tungsten particles into the immature embryos. A dual screening/selection approach based on the combination of *gfp* gene as vital reporter gene and *bar* gene as transgene selection gene was chosen for generation of transgenic wheat. T1, T2 and T3 seeds were obtained by self-pollination of T0 (primary transformants), T1 and T2 wheat plants, respectively. Segregation tests were performed at the expression level using GFP fluorescence, and at the structural level using PCR to confirm the presence of bar gene. Following the segregation analysis fifteen T1 homozygous progenies from seven T0 transgenic lines were selected for evaluation of herbicide resistance in field tests.

Field trials were grown without irrigation, using a randomized complete block design with two replicates. Each transgenic line was sown in four plots. Two plots were tested for herbicide resistance; another two plots were left untreated. Plant height and weight of seeds from one spike were determined using 25 individual plants collected from the central rows of each plot. Mean values of yield were calculated by bulking the plants from each plot.

Barley *hvnhx2* gene driven by *Ubi1* promoter was introduced into psGFP-BAR vector in order to enhance salt tolerance. All plants selected within transformation were screened for the presence of transgene sequences by PCR analysis. Reverse transcription (RT)-PCR was performed with total RNA extracted from flowering T0 plants. T1 transgenic plants with expression of *hvnhx2* gene were grown in pots containing a mix of peat moss, perlite, and vermiculite to analyze the effects of salt. Plants were divided into two groups: one group was watered with a solution of high salt concentration (200 mM NaCl), the other group of plants was watered with distilled water.

RESULTS AND DISCUSSION

Wheat Transformation

Currently the biolistic transformation approach is the best choice for generation of useful transgenic wheat germplasm in a genotype-independent manner. Earlier we have improved the reproducibility of biolistic transformation efficiency using the combination of *gfp* as vital reporter gene and *bar* gene for transgene selection. This combination allowed establishing efficient escape-free protocols for elite Russian wheat cultivars. We have generated 47 independent transgenic lines of the Russian spring wheat variety Andros with both *gfp* and *bar* genes using immature zygotic embryos as the target tissue for bombardment. T1 and T2 hetero- and homozygous progenies from most of the transgenic lines showed resistance to high herbicide concentration (up to 2.5% Basta) while spraying.

Herbicide Resistance

In 2004 we have performed field trials to establish the real effect of transformation on the agronomic performance of transgenic plants under field conditions. T3 homozygous plants resulted from the T1 progeny of seven self-pollinated T0 plants of Andros were sprayed with 1% Basta. Non-transgenic wheat plants showed no signs of resistance in a week after the treatment, while most of transgenic plants exposed high level of herbicide resistance (Fig. 1). Only a slight sensitivity to the herbicide was observed in transgenic line A-46/1, expressed as the yellowing of leaves. At the end of wheat cultivation several traits such as plant height, weight of seeds at one spike and total yield were evaluated to determine the effect of herbicide treatment. Clear variations in agronomic traits were found; first of all between non-treated transgenic lines and non-transgenic control, and then between transgenic lines treated with herbicide and control (Table 1). Two of 15 tested transgenic T1 lines had a genotypic difference when compared to the non-transgenic control. Line A-46/1 had a statistically valid lower plant height as

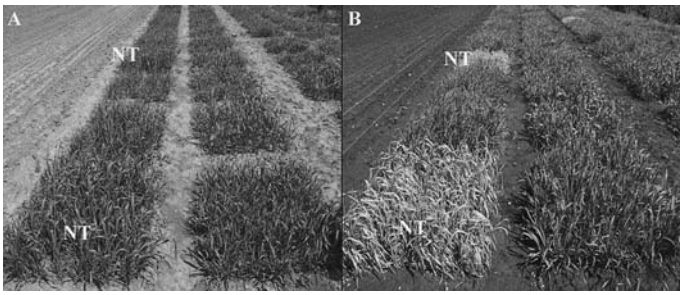


Figure 1. Fields trials for herbicide resistance of transgenic wheat lines (T3 progeny). A - Plots before spraying with herbicide (1% Basta); B – Same plots one week after spraying. NT- plots with non-transgenic plants

Table 1. Evaluation of the field performance of transgenic wheat lines (T3 homozygous plants) transformed with *bar* gene after spraying with herbicide (Basta 1.0%). Means followed by the same letter are not significantly different at the 0.05 probability level

Transgenic line (T0 → /T1)	Plant height (cm)		Weight of seeds from one spike (g)		Yield (kg m^{-2})	
	− Basta	+ Basta	− Basta	+ Basta	− Basta	+ Basta
Non transgenic	1.13a	0.81c	1.32ab	0.83c	0.51ab	0.12c
A-03/4	1.07a	1.09a	1.56ab	1.48ab	0.48ab	0.63a
A-03/34	1.10a	1.12a	1.50ab	1.56ab	0.60ab	0.53ab
A-06/12	1.14a	1.15a	1.70a	1.40ab	0.56ab	0.44bc
A-18/47	1.04ab	1.12a	1.26ab	1.40ab	0.34bc	0.37bc
A-20/26	1.14a	1.14a	1.65ab	1.78a	0.47ab	0.50ab
A-20/28	1.10a	1.12a	1.63ab	1.46ab	0.65a	0.59ab
A-20/36	1.13a	1.07a	1.62ab	1.80a	0.59ab	0.59ab
A-20/38	1.14a	1.15a	1.78a	1.38ab	0.64ab	0.55ab
A-20/40	1.13a	1.12a	1.77a	1.60ab	0.47ab	0.55ab
A-34/9	1.09a	1.10a	1.68ab	1.66ab	0.61a	0.58ab
A-37/28	1.10a	1.08a	1.60ab	1.25ab	0.54ab	0.50ab
A-37/30	1.13a	1.15a	1.78a	1.71a	0.57ab	0.60ab
A-37/39	1.13a	1.12a	1.39b	1.47ab	0.51àb	0.52ab
A-37/45	1.14a	1.14a	1.55ab	1.37ab	0.61a	0.50ab
A-46/1	0.81c	0.77c	1.50ab	1.50ab	0.55ab	0.40bc

compared to non-transgenic plants, whereas differences in the other traits were statistically no significant. Transgenic line A-18/47 showed decrease of yield up to 30% as compared with non-transgenic plants. It should be noted that both plots of these two lines (treated and non-treated with herbicide) resulted in similar decreasing of plants height (line A-46/1) or the loss of yield (line A-18/47); thus indicating the somaclonal nature of such deviations. Despite some variations observed for the other lines between non-treated transgenic plants and non-transgenic plants, the analysis of variance showed that those differences were not significant and could be attributed to the environmental effect.

Somaclonal variations are well documented for many plant species, including cereals regenerated through the somatic embryogenesis. However, the transformation method and selection of transgenic plants may represent an additional stressful factor that may induce somaclonal variation after plant regeneration. Earlier Bregitzer et al. (1998) showed the most important agronomic distinctions between transgenic lines of barley owing to somaclonal variations, and the transformation procedure appears to induce greater somaclonal variation than tissue culture itself. Furthermore, Arencibia et al. (1999) and Svitashev et al. (2000) showed that particle bombardment is a method of transformation more stressful than others, such as agrobacterial or cell electroporation, increasing the frequency of rearrangements and chromosome breakage.

In most cases the difference between transgenic lines treated with herbicide and non-treated transgenic/non-transgenic plants was not statistically proved, though

certain losses and gains in yield were observed (Table 1). The tendency to decrease yield compared to non transgenic plants was observed for lines A-6/12, A-18/47 and A-46/1. The fact that non-treated plants of A-18/47 line showed the same loss of yield indicated that the difference of this trait could be more related with somaclonal variation, rather than with herbicide treatment. For lines A-6/12 and A-46/1 the loss of yield after herbicide treatment is associated with insufficient expression of the *bar* genes, as the non-treated transgenic plants did not expose such decrease. That suggestion was confirmed by the damage of leaves observed for line A-46/1 a week after spraying with herbicide. Line A-6/12 did not show any visible damages of plants after herbicide application; however we noticed the decrease in number of spikes per m^2 for this line after treatment (data not shown).

The result of this study showed that agronomic traits and yields of transgenic and non-transgenic plants were comparable or even higher for transgenic lines. Since the observed differences were not proved statistically, we associate those variations with an environmental influence. Although the results of this study do not address to the underlying molecular phenomena, the observed expression patterns provide important empirical information that would be useful in designing breeding approaches for stress resistance. Furthermore, it was the first attempt of field evaluation of transgenic wheat in Russia.

Salt Resistance

Soil salinity affects the cereal productivity severely in Russia. The identification of genes imparting tolerance and production of salt-tolerant genotypes is one of the objectives of wheat breeders. Recently, genes encoding vacuole-type Na^+/H^+ antiporters have been shown to increase resistance to salinity in several transgenic species including rice (Ohta et al. 2002) and wheat (Xue et al. 2004). Na^+/H^+ antiporters exclude Na + from the cytosol and are localized in both plasma and vacuolar membranes. Many naturally salt-tolerant plants (halophytes) rely on this strategy, whereas salt-sensitive plants including wheat displayed little Na^+/H^+ antiporter activity in tonoplast vesicles.

In this study we report that one of the vacuolar Na^+/H^+ antiporters isolated from the salt-resistant barley genotype can alter the salt sensitivity of transgenic wheat plants. The barley *hvnhx2* antiporter gene was introduced into spring wheat genotype Andros under control of the maize *Ubi1* promoter. 14 independent transgenic plants were generated after biolistic transformation. The expression of the *hvnhx2* gene in the transgenic plants was confirmed by mRNA detection (Fig. 2). Over expression was found in three of nine analyzed transgenic lines; endogenous mRNA for the *hvnhx2* gene was also presented in non-transgenic plants though. It might be due to a high homology (94.9%) of cDNA sequence of barley *hvnhx2* gene with the vacuolar Na^+/H^+ antiporter of wheat.

Some of T1 offspring from self pollinated T0 transgenic wheat plants were grown in different saline conditions in order to examine whether the over expression of the *hvnhx2* gene conferred resistance to salt stress. The growth performance of

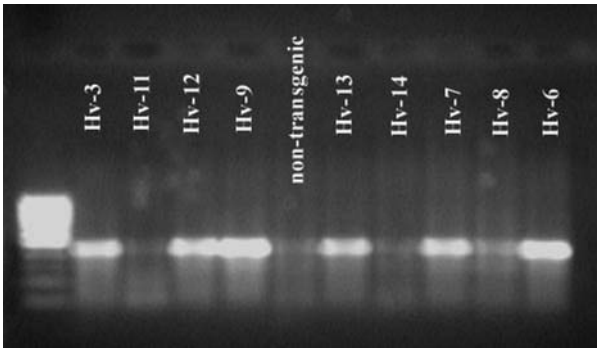


Figure 2. Expression analysis of *hvnhx2* transgenic wheat lines. DNase-treated RNA prepared from T0 transgenic plants was used for RT-PCR

hvnhx2-negative progeny was examined along with *hvnhx2*-positive progeny so as to confirm that the salt tolerance of the *hvnhx2* transgenic lines is not acquired through tissue culture process. Two-weeks-old plants, which had developed first true leaves, were treated with 200 mM NaCl solution for 15 weeks. The appreciable differences in plant growth were observed between the *hvnhx2* transgenic and non-transgenic progeny of Hv-09 line (Fig. 3). The growth of the non-transgenic plants under the influence of NaCl was near 50% of the non-saline conditions. Transgenic progeny showed the delay of the growth as well; but it was about 20% only. Increased growth of the *hvnhx2* transgenic plants could be explained by the compartmentalization of Na^+ in vacuoles. It is quite possible because the

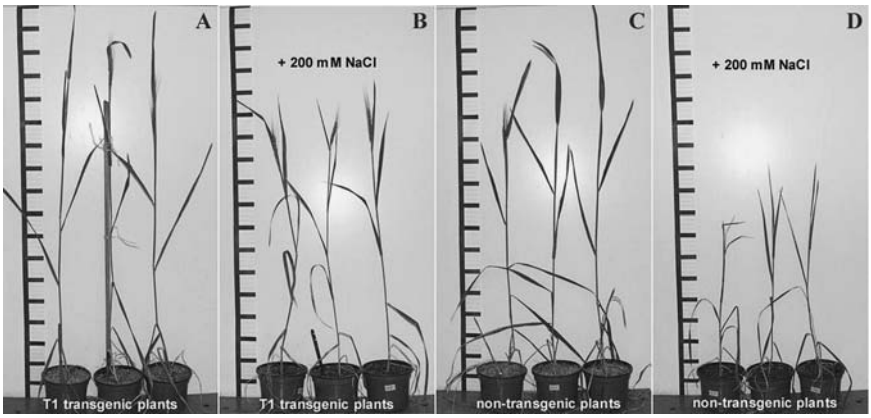


Figure 3. Effect of salt on the growth of the T1 progeny obtained by self-pollination of the primary T0 transgenic plant Hv-9. Plants were watered with a distilled water (A and C) or 200 mM NaCl (B and D). T1 transgenic plants (A and B) and non-transgenic plants (C and D) are shown after 15 weeks of salt treatment

over-production of a vacuolar Na^+/H^+ antiporter confers the transgenic wheat a higher capacity of sequestering Na^+ into vacuoles. These results demonstrate that transgenic wheat plants containing the *hvnhx2* gene are suitable for practical applications and are capable to produce crops even if they are exposed for a long period to a high saline concentration.

REFERENCES

Arencibia AD, Carmona ER, Cornide MT, Castiglione S, Chinea A, Oramas P, Sala F (1999) Somaclonal variation in in-sect- resistant transgenic sugarcane (*Saccharum* hybrid) plants produced by cell electroporation. Transgenic Res. 8:349–360

Bregitzer P, Halbert SE, Lemaux PG (1998) Somaclonal variation in the progeny of transgenic barley. Theor Appl Genet. 96:421–425

Christensen AH, Sharrock RA, Quail PH (1992) Maize poly-ubiquitin genes: genes, structure, thermal perturbation of expression and transcript splicing, and promoter activity following transfer to protoplast by electroporation. Plant Mol Biol 18:675–689

McElroy D, Zhang W, Cao J, Wu R (1990) Isolation of an efficient actin promoter for use in rice transformation. Plant Cell 2:163–171

Ohta M, Hayashi Y, Nakashima A, Hamada A, Tanaka A, Nakamura T, Hayakawa T (2002) Introduction of a Na + /H+ antiporter gene from *Atriplex gmelini* confers salt tolerance to rice. FEBS Lett 532:279–282

Richards HA, Rudas VA, McDaniel JK, Tomaszewski Z, Conger BV (2001) Construction of a GFP-BAR plasmid and its use for switchgrass transformation. Plant Cell Rep. 20:48–54

Svitashev S, Ananiev E, Pawlowski WP, Somers DA (2000) Association of transgene integration sites with chromosome rear-rangements in hexaploid oat. Theor Appl Genet 100:872–880

Xue ZY, Zhi DY, Xue GP, Zhang H, Zhao YX, Xia GM (2004) Enhanced salt tolerance of transgenic wheat (*Triticum aestivum* L.) expressing a vacuolar Na + /H+ antiporter gene with improved grain yield in saline soils in the field and a reduced level of leaf Na+. Plant Sci 167:849–859

MOLECULAR MAPPING OF DURABLE RUST RESISTANCE IN WHEAT AND ITS IMPLICATION IN BREEDING

H.S. BARIANA[1,*], H. MIAH, G.N. BROWN, N. WILLEY[1] AND A. LEHMENSIEK[2]

[1] *University of Sydney Plant Breeding Institute-Cobbitty, Faculty of Agriculture, Food and Natural Resources, PMB11, Camden, NSW2570, Australia*

[2] *University of Southern Queensland, Centre for Systems Biology, Toowoomba, QLD4350*

[*] *E-mail: harbansb@camden.usyd.edu.au*

Abstract: Genetic characterization of sources of durable resistance enables their strategic deployment in breeding programs. Genomic locations of uncharacterized adult plant resistance (APR) sources to leaf rust and stripe rust diseases of wheat were determined. Two genomic regions, 3DS (Halberd) and 5DS (Cranbrook) controlled APR to both leaf rust and stripe rust. Chromosomes 6B (Cranbrook) and 7B (Halberd) reduced leaf rust severity. Chromosomes 2DS, 3BS and 7A also reduced stripe rust severities in at least one crop season. Stem rust resistance genes *Sr2* (3BS) and *Sr30* (5DL) from Cranbrook explained stem rust response variation. Regression analysis also indicated strong positive interaction of these two loci in controlling stem rust. Expression of *Sr2*-linked psuedo black chaff (Pbc) was controlled by a major gene on chromosome 3BS and three modifiers located on chromosomes 6A, 3D and 7A. The chromosome 7A located region was not consistent across all seasons and sites. QTLs detected consistently in different experiments were temporarily designated as *QYr/Lr3D, QYr/Lr5D, QLr6B* and *QLr7B*

Keywords: durable resistance, adult plant resistance, stem rust, stripe rust

INTRODUCTION

Breeding for rust resistance is a continuing process and demands sustained effort on identification and characterization of diverse sources of resistance. Australian wheat cultivars Halberd and Cranbrook, released in 1969 and 1984, respectively, displayed low to moderate levels of APR against stripe rust and leaf rust pathogens of wheat. Cultivar Cranbrook also carries moderate levels of resistance to stem

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 723–728.

rust. The aim of this study was to identify genomic regions that control durable resistance to leaf rust and stripe rust and to relate adult plant stem rust response variation to genetically characterized genes *Sr2* and *Sr30*.

MATERIAL AND METHODS

A doubled haploid population derived from a cross involving cultivars Cranbrook and Halberd was screened against three rust diseases under field conditions for two or more seasons. Molecular mapping of this population was performed in a nationally coordinated program (Chalmers et al. 2001, Lehmensiek et al. 2005)

Pathotypes used included:

Puccinia striiformis f.sp. *tritici* (Pst): 110 E143A+ and 134 E16A+.

Puccinia triticina (Pt): 104-1,2,3, (6), (7), 11 and 104-1,2,3, (6), (7), 11, 13 *(Lr24)*.

Puccinia graminis f.sp. *tritici* (Pgt): 98-1,2,3,5,6.

Both cultivars did not carry any effective resistance genes against Pst and Pt pathotypes used in this study. Details of seedling screening against stem rust were described in Bariana and McIntosh (1993). Field assessments were performed on a 1–9 scale (Table 1). Quantitative trait loci (QTL) for adult plant rust resistance were characterized by single marker regression and interval mapping using Mapmanager QTXb20 (Manly et al. 2001). The likelihood ratio statistic (LRS) threshold for declaring statistical significance of an association was calculated empirically for each set of data using the permutation test set at 1000 iterations.

Pbc expression was scored on a 1-5 scale as described in Brown (1997). *Sr2*-linked seedling chlorosis was also scored according to Brown (1997). Stripe rust responses were scored for four years. Leaf rust and stem rust responses and Pbc expression were observed for two years.

RESULTS AND DISCUSSION

Both Cranbrook and Halberd exhibited low to moderate levels of resistance against predominant Australian Pst and Pt pathotypes. Cultivar Cranbrook carries stem rust resistance genes *Sr2* and *Sr30* and displayed moderate level of stem rust resistance.

QTL mapping indicated the involvement of five genomic regions in controlling resistance to stripe rust (Table 2). Of these, two genomic regions each, contributed by Cranbrook (5DS) and Halberd (3DS) also reduced leaf rust severity. Genomic regions on chromosomes 2D, 3B and 7A decreased stripe rust severity at least in one experiment. The genomic region located on the chromosome 3BS also reduced leaf rust severity. *Yr30* was located close to the stem rust resistance gene *Sr2* in the chromosome arm 3BS (RP Singh pers. comm.) and *Lr27* was reported to be very closely linked with *Sr2* (McIntosh et al. 1998). These genes may have been responsible for reducing stripe rust and leaf rust severities. The involvement of genomic regions located on chromosomes 3D and 5D in reducing stripe rust and leaf rust severities has not been reported in the literature and therefore are unique. Genomic regions from the chromosome arms 6BS and 7BS exhibited

Table 1. Description of rust response assessment scale (1–9) used to assess rust responses in the field

Scale	Response	Stripe rust	Leaf rust	Stem rust
1	Very resistant (VR)	No disease	No disease	No disease
2	Resistant (R)	<5% necrotic dots	<5% necrotic dots with/without occasional medium sized pustules	<5% small sized unruptured uredinia on various plant parts
3	Resistant to Moderately resistant (R-MR)	10–15% necrotic stripes, scattered and rarely sporulating, top leaves often free from infection	10–15% infected area, pustule size and response may vary between genotypes	10–15% small to medium pustules. Rupturing of some pustules may occur
4	Moderately resistant (MR)	20–30% necrotic to lightly sporulating stripes	20–30% infected area, pustule size and response may vary between genotypes	20–30% medium size susceptible pustules, often ruptured. Rupturing more pronounced near the node
5	Moderately resistant-Moderately susceptible (MR-MS)	35–45% blotchy islands with light sporulation, can have moderate to high chlorosis and necrosis, sporulating islands often appear all over the leaf but in some cases restricted towards the tip	35–45% infected area, pustule size and response may vary between genotypes with light to moderate sporulation	35–45% medium to large sized moderately sporulating pustules. Again more pronounced near the node
6	Moderately susceptible (MS)	50–60% large stripes often sporulating, stripes can turn necrotic under windy and warmer conditions. Stripes in this category remain distinct and largely uncoalesced	50–60% susceptible pustules, pustule size may vary	50–60% susceptible pustules with larger pustules near the node and relatively smaller pustules on the rachis
7	Moderately susceptible to susceptible (MS-S)	65–75% sporulating stripes start to coalesce, chlorosis present. Sporulating stripes can turn necrotic under windy and warmer conditions	65–75% susceptible pustules with moderate sporulation	65–75% susceptible pustules and coalescing of pustules occurs. Small holes on stem (near nodes) may be visible
8	Susceptible (S)	80–90% moderate to heavy sporulation. There is always a small green island left near the base of the leaf	80–90% susceptible pustules with heavy sporulation	80–90% moderate to heavy sporulation. Medium to large holes on stem visible near nodes and can cause breakage of stems
9	Very susceptible (VS)	95–100% infection and heavy sporulation leading to defoliation	95–100% Heavy sporulation leading to defoliation	95–100% Heavy sporulation and breakage of stem due to large holes

Table 2. List of QTLs contributing towards reduction of rust severities in Cranbrook/Halberd-derived doubled haploid population

Disease	Chromosome involved	Closest marker(s)/ gene(s)	LRS	% Variation explained	Contributed by
Stripe rust	3DS	gwm456	11.2–20.2	7–14	Halberd
	5DS	psr326b	13.3–14.5	8–9	Cranbrook
	2DS	gwm261/wmc190	21.8	15	Halberd
	3BS	gwm533	12.1	8	Cranbrook
	7AL	wmc283	12.1	8	Cranbrook
Leaf rust	3DS	gwm456	13.8–20.2	11–13	Halberd
	5DS	psr326b	12.6–14.5	8	Cranbrook
	6BS	gwm88	16–16.5	9–10	Cranbrook
	7BS	gwm537	17.2–17.7	11	Halberd
	3BS	gwm533	19.4	14	Cranbrook
Stem rust	3BS	gwm533	37.6	25	Cranbrook
	5DL	*Sr30*	28.0	40	Cranbrook
Pbc*	3BS	gwm533	53.1–157.6	35–73	Cranbrook
	6AS	abg466	11.8–16.6	8–12	Cranbrook
	3DS	cfd70	13.9–20.3	13–17	Halberd
	7AL	P37/M48-3	14.6	10	Halberd

**Sr2*-linked psuedo black chaff

involvement in the reduction of leaf rust severities (Table 2). Cultivars Cranbrook and Halberd have been crossed with completely susceptible parents to develop single gene stocks for genes involved in controlling resistance to both stripe rust and leaf rust diseases of wheat. Populations derived from these crosses will enable identification of markers closely linked with the target loci. The genomic regions identified in this study to control APR to stripe rust and leaf rust pathogens of wheat will be formally designated once the single gene stocks are ready. In the meantime these QTLs were temporarily designated as *QYr/Lr3D, QYr/Lr5D, QLr6B* and *QLr7B*.

Detection of genotypes carrying better levels of resistance than parents Cranbrook and Halberd, indicated that these QTLs could be pyramided in a single genotype to achieve high levels of resistance. The durability for leaf rust and stripe rust resistance displayed by cultivars Cranbroook and Halberd would ensure durable performance of cultivars derived from these sources of resistance. Since these genes are present in commercial cultivars, the single backcross method described by Bariana et al. (2004) and Singh et al. (2004) would be suitable for transfer of resistance. This approach proved successful in recovering total genetic variation in backcross derivatives in the Australian Cereal Rust Control Program. Additional backcrosses can be made on individuals showing high levels of resistance at the BC1F2 stage to enrich recurrent parent genetic background.

Two genomic regions controlled stem rust resistance in cultivar Cranbrook. Comparison of *Sr30* and *Sr2*-linked seedling chlorosis scores from the greenhouse with the stem rust responses from field screening clearly demonstrated the involvement of *Sr30* and *Sr2* in reducing stem rust severity under field conditions. Cultivars carrying *Sr2* alone are known to support higher levels of stem rust development (60–80%) compared to *Sr30* (40–60%) carrying cultivars. Different stem rusting behaviour of *Sr30* carrying cultivars (based on seedling assays) was reported by Bariana et al. (2001). Some cultivars although postulated to carry *Sr30* based on comparative tests with avirulent and virulent Pgt pathotypes showed susceptible stem rust responses against avirulent pathotypes under field conditions, whereas other cultivars produced moderate responses against both avirulent and virulent pathotypes. It appeared that expression of adult plant resistance attributed to *Sr30* was suppressed in some genetic backgrounds. For example, Australian cultivar Frame displayed intermediate levels of resistance against even *Sr30* virulent Pgt pathotypes, whereas Yitpi, a Frame-derivative, postulated to carry *Sr30* based on seedling tests exhibited highly susceptible response under field condition against both *Sr30* avirulent and virulent Pgt pathotypes. Similarly involvement of *Sr12* in decreasing stem rust severity against *Sr12*-virulent Pgt pathotype was observed in two mapping populations. Expression of *Sr2*-linked pseudo black chaff (Pbc), was controlled by a major gene located on the chromosome arm 3BS and was enhanced by genes on chromosomes 3D, 6A and 7A. Involvement of chromosome 7A in enhancing Pbc expression needs further investigation.

Only two sources (*Yr18/Lr34* and *Yr29/Lr46*) of minor gene based APR against both leaf rust and stripe rust of wheat have been reported (Singh 1992; Singh et al. 1998). Two such loci detected in this study would be useful in achieving durability of rust resistance in breeding programs. Two soft wheat cultivars, Datatine and Bullaring, released by the Department of Agriculture Western Australia, exhibited slow rusting for leaf rust and stripe rust under field conditions (Bariana HS unpublished). Cultivar Datatine was derived from a cross involving a *Sr24/Lr24* derivative of Halberd and the susceptible club wheat Tincurrin. Datatine showed much slower leaf rust and stripe rust development in comparison to Tincurrin. It appeared that slow rusting of Datatine was derived from Halberd. Cultivar Bullaring in turn may have derived its leaf rust and stripe rust resistance from Datatine. Molecular marker comparison with Halberd will be made to confirm this hypothesis. The previously reported locations of stem rust resistance genes *Sr2* and *Sr30* based on cytogenetic studies (McIntosh et al. 1998) served as an internal check to detect any error during data processing.

ACKNOWLEDGEMENTS

We thank GRDC Australia for financial support through the Australian Cereal Rust Control Program and the Australian Winter Cereal Molecular Marker Program and Prof. R. Appels for continuous encouragement.

REFERENCES

Bariana HS, Bell JA, Standen GE, Kaur K (2001) Comparative evaluation of wheat cultivars carrying *Sr30* against avirulent and virulent *Puccinia graminis* f.sp. *tritici* pathotypes. In: Proceedings of 10th wheat breeders assembly, 16–21 Septemeber, Mildura, Victoria, Australia, pp 230–232

Bariana HS, McIntosh RA (1993) Cytogenetic studies in wheat XV. Location of rust resistance genes in VPM1 and their genetic linkage with other resistance genes in chromosome 2A. Genome 36:476–482

Bariana HS, Willey N, Venkata BP, Lehmensiek A, Standen GE, Lu M (2004) Breeding methodology to achieve durability for rust resistance in wheat. In: Proceedings of the 54th Australian cereal chemistry conference and 11th wheat breeders assembly, 21–24 September, Canberra, ACT, Australia, pp 8–12

Brown GN (1997) Inheritance and expression of leaf chlorosis associated with gene *Sr2* for adult plant resistance to wheat stem rust. Euphytica 95:67–71

Chalmers KJ, Cambell AW, Kretschmer J et al (2001) Construction of three linkage maps in bread wheat, *Triticum aestivum*. Aust J Agr Res 52:1089–1119

Lehmensiek A, Eckermann PJ, Verbyla AP, Sutherland MW, Appels R, Daggard GE (2005) Curation of wheat maps to improve map accuracy and QTL detection. Aust J Agr Res 56 (in press).

Manly KF, Cudmore RH Jr, Meer JM (2001) Map manager QTX, cross-platform software for genetic mapping. Mamm Genome 12: 930–932

McIntosh RA, Hart GE, Devos KM, Gale MD, Rogers WJ (1998) Catalogue of gene symbols for wheat. In: Slinkard AE (ed) Proceedings of the 9th international wheat genetics symposium, Saskatoon, Canada, vol 5. University Extension Press, University of Saskatchewan, pp 1–229

Singh RP (1992) Genetic association of leaf rust resistance gene *Lr34* with adult plant resistance to stripe rust in bread wheat. Phytopathology 82:835–838

Singh RP, Huerta-Espino J (2004) The use of single backcross, selected-bulk breeding approach for transferring minor genes based rust resistance into adapted cultivars. In: Proceedings of the 54th Australian cereal chemistry conference and 11th wheat breeders assembly, 21–24 September, Canberra, ACT, Australia, pp 48–51

Singh RP, Mujeeb-Kazi A, Huerta-Espino J (1998) *Lr46*: a gene conferring slow-rusting resistance to leaf rust in wheat. Phytopathology 88:890–894

POTENTIAL USES OF MICROSATELLITES IN MARKER-ASSISTED SELECTION FOR IMPROVED GRAIN YIELD IN WHEAT

B. KOBILJSKI[1,*], S. DENČIĆ[1], N. HRISTOV[1], N. MLADENOV[1], S. QUARRIE[2,3], P. STEPHENSON[2] AND J. KIRBY[2]

[1] *Institute of Field and Vegetable Crops, M. Gorkog 30, 21000 Novi Sad, Serbia and Montenegro*
[2] *John Innes Centre, Norwich Research Park, Colney, Norwich, NR4 7UH, UK*
[3] *Present address: Kraljice Natalije 39, 11000 Belgrade, Serbia and Montenegro*
[*] *E-mail: kobboris@ifvcns.ns.ac.yu*

Abstract: Seven hundred and ten wheat genotypes from the Novi Sad Core Collection, originating from 38 countries, have been evaluated for 54 traits under both field and lab conditions during seven growth seasons. In each year, the field experiment comprised 3–7 replications, each with basic plot size of 1.2 m^2. Based on the obtained data, a subset of 96 genotypes with the highest phenotypic variability for 26 of the most important breeding traits was identified for screening with microsatellites. A set of 36 SSRs was used for molecular screening, covering the 42 wheat chromosomes. To test the effectiveness of the concept of association between marker alleles and yield, the 96 accessions were grouped according to the presence of particular microsatellite fragments in at least three genotypes and average group yields were compared using one-way ANOVA. Associations were determined for 13 microsatellites (gwm46, gwm155, gwm190, gwm192, gwm295, gwm337, gwm484, gwm539, gwm540, psp3088, psp3103, psp3153 and psp3200). In a subsequent analysis of 20 genotypes with either the highest or lowest yields it was possible to determine the presence of high and low-yielding alleles. Two varieties with the lowest yields in this research carried no high-yielding alleles, while a further four genotypes from the same group carried only two high-yielding alleles. Conversely, cv. Pobeda, which is presently a standard variety in the Commission for Variety Approval of Serbia, possesses all 14 high-yielding alleles, followed by Renesansa, NS 55-25, NS 66/92 and NS 79/90 with 10 high-yielding alleles. In the 20 highest-yielding genotypes, 61.8% of the total alleles were high-yielding alleles, while in the 20 lowest-yielding genotypes the frequency of high-yielding alleles was almost three times lower (22.1%). The difference in informativeness of the 13 microsatellites were determined in terms of their informative value (IV), calculated as the ratio between the number of high-yielding alleles determined in the low and high-yielding genotypes. The results are discussed with emphasis placed on the problems and prospect of such studies in the molecular and breeding context

Keywords: yield alleles, marker assisted selection, microsatellites

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 729–736.

INTRODUCTION

We are, undoubtedly, only at a start of MAS in most plant breeding programmes, and its current applications are limited in extent. This statement of Snape (2004) perfectly demonstrates the present status of MAS in plant breeding in general and wheat breeding in particular. Oddly enough, although hopefully all of us are aware of the truthfulness of this statement, we keep introducing more and more complex biotechnologies thus further deepening the gap between research and application. For example, in last decade, the large majority of molecular marker papers dealt with the identification of genomic regions associated with traits based on the screening of populations derived from single crosses. This strategy is almost exclusively sub-optimal for direct application in breeding programs for numerous reasons (Eisemann et al. 2004). The QTL – trait association strategies with single-cross population screenings frequently use obscure or outdated parents and the QTL effects are very dependent on environment (playing a "hide and seek" game) so the results could be applied only to the actual breeding site or those environmentally similar to the one where the research was performed. Also, this strategy competes directly for significant resources for phenotypic evaluation and provides no information about the frequency or value of the QTLs in breeding populations, thus further steps are required to validate the usefulness of markers for direct application in MAS. Consequently, despite the widespread use of such a QTL analysis approach, marker application is being impeded through the lack of information on the polymorphism and marker-trait associations in genetic material relevant to most breeding programs worldwide (Eisemann et al. 2004). Thus it seems that Snape (2004) was right in saying that we need to modify the present strategy in order to diminish the gap between research and application. Many examples from both ancient and more recent human history have shown that the majority of the greatest achievements are postulated and performed on a very simple basis. In regard to this, Snape (2004) also stated: *Plant breeding is, in its essence, a simple process that can be described euphemistically as – cross the best with the best, select the best, and hope for the best*. Simple as that?! But, to discover ways to achieving the "best", especially in four crucial wheat breeding targets – grain yield, quality, biotic and abiotic stress tolerance, we have to use modern biotechnology in a way that should be as simple, informative and useful as possible, thus in this paper we are proposing a strategy which we believe could significantly narrow the gap between research and application.

MATERIALS AND METHODS

Seven hundred and ten wheat genotypes from the Novi Sad Core Collection, originating from 38 countries, have been evaluated for 54 traits under both field and lab conditions during seven growth seasons. In each year, the field experiment comprised 3–7 replications, each with a basic plot size of 1.2 m^2. Based on the data obtained, a subset of 96 genotypes with the highest phenotypic variability for

26 of the most important breeding traits for Serbia and Montenegro and the UK were identified for screening with a set of 36 microsatellites covering the three wheat genomes and all 42 chromosomes (for details see Kobiljski et al. 2002). Microsatellites were selected at random from those optimized for use with an ABI 377 Sequencer at the John Innes Centre (JIC), Norwich, UK. The standard protocol of sample preparation for an ABI 377 Sequencer was applied, and the gels were run using a standard ABI 377 Sequencer procedure. The output from the sequencer was analyzed with Genescan™ software to measure the molecular size of each SSR allele. To test the effectiveness of the concept of association between marker alleles and yield, the 96 accessions were grouped according to the presence of particular microsatellite fragments in at least three genotypes and average group yields were compared using one-way ANOVA.

RESULTS AND DISCUSSION

From the breeder's point of view it seems logical that for making a "QTL puzzle", especially for extremely complex traits such as grain yield, it should be more simple and useful to start from the top – grain yield itself. The majority of recent genetic research on cereals deals with quantitative traits, many of which are directly or indirectly related to grain yield, with emphasis on determining a small fragment of the puzzle which in wheat is made up of approximately 30,000 pieces (the wheat genes). Theoretically, if we are able to find association between certain alleles and grain yield or reliable evidence that a particular molecular fragment is related to grain yield we could select it in progenies without any particular need, in the initial phase, to know exactly what sub-trait we are selecting for. Further analysis of either the main yield components or any other trait significantly linked to a particular molecular marker could indicate the direction in which we should go if we want to analyse further the nature of the grain yield benefit. By starting from grain yield itself we actually "see" the whole and final picture that comprises the "grain yield QTL puzzle" and we could then work with particular identifiable fragments (traits) of the whole picture of the "grain yield puzzle". To test the feasibility of such an approach, seven-year grain yield data for 96 genotypes were collected and analyzed with 36 randomly selected microsatellites for which significant allele – grain yield associations were identified for 13 SSRs: gwm46, gwm155, gwm190, gwm192, gwm295, gwm337, gwm484, gwm539, gwm540, psp3088, psp3103, psp3153 and psp3200 (Tables 1 and 2).To analyse the presence and frequency of the alleles in relation to grain yield performance, the initial 96 genotypes were arranged in three groups. The first group comprised 20 genotypes with the highest average yields, the second comprised 56 average-yielding genotypes and the third comprised 20 genotypes with the lowest average yields. Analysis of molecular data for each of the three groups revealed some particularly interesting findings. For the SSRs listed above it was possible to identify a pattern of differences between allele frequencies of highest yielding genotypes (HYGs) and lowest yielding genotypes (LYGs), with specific alleles being significantly associated for each SSR with high yield. Based

Table 1. Distribution of "high-yielding" alleles in the 20 highest-yielding wheat genotypes(Alleles presence or absence is indicated by "+" or "-"-respectively)

Primer Genotype	Xgwm46.2	Xgwm155	Xgwm190	Xgwm192.2	Xgwm295	Xgwm337	Xgwm484	Xgwm539	Xgwm540	Xpsp3088	Xpsp3103.1	Xpsp3103.2	Xpsp3153.2	Xpsp3200	Yield (t ha^{-1}) Mean = 7.84	*No. of alleles Mean = 8.7*	*Rht8 locus*
NS 66/92	+	+	+	+	+	+	−	+	+	+	−	−	−	+	8.91	*10*	175
NS 79/90	+	−	+	+	+	−	−	+	−	+	+	+	+	+	8.88	*10*	192
Mina	+	−	−	+	−	+	−	+	−	−	−	+	−	+	8.41	*6*	192
NS 559	+	−	+	+	+	+	+	−	−	+	−	−	−	+	8.06	*8*	192
Centurk	+	+	−	+	+	+	−	+	−	−	−	−	−	+	8.05	*6*	200
Pobeda	+	+	+	+	+	+	+	+	+	+	+	+	+	+	8.03	*14*	192
BCD 1302/83	−	+	−	+	+	+	+	−	−	−	−	−	−	+	8.01	*7*	192
NS 33/90	−	−	+	+	+	−	−	+	+	+	+	+	+	+	7.88	*9*	192
Ana	+	−	+	+	+	−	−	+	+	+	−	−	−	−	7.83	*7*	165
NS 55-25	−	−	+	+	−	+	−	+	+	+	+	+	+	+	7.77	*10*	192
NS 46/90	−	−	+	+	−	+	+	+	+	+	−	−	−	−	7.70	*7*	192
Sofija	+	−	+	+	+	−	−	+	−	+	−	−	+	+	7.66	*9*	175
UC 65680	+	+	+	+	+	−	+	+	−	+	−	−	−	−	7.66	*8*	192
Slavija	+	−	+	+	−	+	−	+	−	+	+	+	+	+	7.56	*9*	192
Sava	−	−	+	+	−	−	−	+	−	+	+	+	−	+	7.52	*7*	192
Nizija	−	−	+	+	+	−	+	+	+	+	−	−	+	+	7.44	*9*	192
Renesansa	+	−	+	+	−	+	+	+	+	−	+	+	−	+	7.38	*10*	192
Triple dirk B	−	−	+	+	+	+	−	+	+	+	−	−	−	+	7.38	*9*	null
Nova Banatka	+	−	+	+	+	+	−	+	+	+	−	−	−	+	7.32	*9*	nd
Rusalka	−	+	+	+	+	−	−	−	+	+	+	−	+	+	7.26	*9*	192
Total high alleles.	*12*	*7*	*17*	*20*	*14*	*13*	*7*	*16*	*11*	*16*	*8*	*8*	*8*	*17*			

Table 2. Distribution of "high-yielding" alleles in the 20 lowest-yielding wheat genotypes(Alleles presence or absence is indicated by "+" or "-"-" respectively)

Primer Genotype	Xgwm46.2	Xgwm155	Xgwm190	Xgwm192.2	Xgwm295	Xgwm337	Xgwm484	Xgwm539	Xgwm540	Xpsp3088	Xpsp3103.1	Xpsp3103.2	Xpsp3153.2	Xpsp3200	Yield (t/ha) Mean = 4.11	*No. of alleles Mean* = 3.1	*Rht8 locus*
L-1	–	–	+	+	–	–	–	–	–	–	–	–	–	+	4.96	3	192
Purdue/Loras	–	–	+	+	–	–	–	–	+	–	–	–	–	+	4.95	4	165
Helios	+	–	–	+	–	–	–	–	–	–	–	–	–	+	4.79	3	175
Purdue 39120	+	–	+	+	–	–	–	–	+	+	–	–	–	+	4.79	6	165
Al-kan-tzao	–	–	–	–	–	–	–	+	+	+	–	–	+	–	4.78	4	165
Saitama 27	–	–	–	+	–	–	–	–	–	+	–	–	+	–	4.65	3	165
Ai bian	–	–	+	+	–	–	–	–	–	+	–	–	–	–	4.58	4	165
ZG 987/3	–	–	+	–	+	–	–	–	–	–	–	–	–	–	4.53	2	null
Norin 10	–	–	+	+	–	–	–	–	–	–	–	+	–	+	4.48	4	null
Tr. compactum	–	–	–	–	–	–	–	+	–	–	–	–	–	+	4.27	2	175
Timson	–	–	+	+	–	–	–	–	–	–	–	–	–	+	4.26	2	165
INTRO 615	–	–	–	+	–	–	–	+	+	+	–	–	–	–	4.13	3	null
Magnif 41	–	+	+	+	–	–	–	–	–	+	–	–	–	–	4.11	6	165
Tibet Dwarf	–	–	+	+	–	–	–	–	–	+	–	–	–	–	4.10	4	165
ZG K 238/82	–	–	+	+	–	–	–	–	–	–	–	–	–	+	3.87	4	null
L 1A/91	+	–	–	+	–	–	–	–	–	+	–	–	–	–	3.42	3	192
L1/91	+	–	–	+	–	–	–	–	–	+	–	–	–	–	3.33	3	165
Tr. spherococc.	–	–	–	+	+	–	–	–	–	–	–	–	–	–	3.02	2	175
Min dwarf.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2.63	0	175
Tom Thumb	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2.62	0	175
Total high alleles	*4*	*1*	*10*	*14*	*2*	*4*	*0*	*3*	*4*	*9*	*0*	*1*	*2*	*8*			

on this, for each of these SSRs we were able to differentiate the alleles into high or low-yielding ones. Tables 1 and 2 summarise the results with the 13 SSRs. In the 20 HYGs, 61.8% of the total alleles were high-yielding ones (black boxes), while in the 20 LYGs the frequency of high-yielding alleles was almost three times lower (22.1%). Of the 20 HYGs, 14 were from the Novi Sad (NS) wheat breeding programme, while no NS germplasm was found in the group of 20 LYGs. This indicates that desirable alleles for those SSRs in respect to grain yield have been fixed in the germplasm from NS and also that high-yielding alleles were fixed at a higher frequency in the HYGs compared with LYGs. It is noteworthy that cv. Pobeda, which is presently a standard variety in the Commission for Variety Approval of Serbia, possesses all 14 high-yielding alleles. Also, the high frequency of high-yielding alleles (10) in cv. Renesansa and advanced lines NS 55-25, NS 66/92 and NS 79/90 explains at least to some extent why these genotypes are frequently and successfully used for hybridization in the NS wheat breeding programme (Table 1).

In contrast, in LYGs, two varieties with the lowest yields carried no high-yielding alleles (Minister Dwarf and Tom Thumb), while additional four genotypes carried only two high-yielding alleles (Table 2).

From the data in Tables 1 and 2 it is clear that there exists a difference in informativeness of the microsatellites. Thus, we calculated an informative value (IV) for all 14 microsatellite loci as the ratio between the numbers of high-yielding alleles determined in the low and high yielding genotypes. There were four main groups based on their frequencies and IV of the microsatellites used in this research: 1) High-yielding alleles that were frequently present in both HYGs and LYGs *(Xgwm190, Xgwm192, Xpsp3088, Xpsp3200)*. From the breeder's point of view, such alleles may not be very useful (IV = 47–70%), but as they occurred very frequently in the HYGs their presence should be desirable in the genetic background of high yielding ideotypes. 2) High-yielding alleles that were present in a majority of the HYGs and rarely present in the LYGs *(Xgwm46, Xgwm295, Xgwm337, Xgwm539)*. This case is very interesting for breeders since such alleles could be good indicators of increased yield potential. The IV values for this group ranged from 14.3 to 33.3%. 3) High-yielding alleles that were often present in the HYGs and rarely present in the LYGs *(Xgwm155, Xgwm540, Xpsp3103, Xpsp3153)*. The usefulness of such alleles is likely to be lower than that mentioned for group 2 but still could be worthwhile for practical application in breeding. The IV values for these microsatellites ranged from 12.5 to 36.4%. 4) High yielding alleles that were partially present in the HYGs but entirely absent in the LYGs *(Xgwm484, Xpsp3103)*. This would be a perfect solution for MAS if the frequency of high-yielding alleles was much higher than that described as "partially present". Nevertheless, from the point of view of helping to resolve the QTL puzzle, the value of these alleles could be helpful for use as criteria for MAS targeting increased grain yield.

Our results suggest the possibility of accumulating high yielding alleles in a single genotype (at least for 13 microsatellites from this research – as shown by cv. Pobeda). Also, this approach allows us to choose parents for hybridization

on the basis of their number of high-yielding alleles, presuming that, through recombination, we will be able to recover progenies with higher proportions of high-yielding alleles than those present in either parent. With respect to the relevance and applicability of such data, the most useful option would be to measure grain yield at the breeding site and to do molecular screening with as many markers as possible.

By doing this, the data obtained would be much more useful for a certain breeding programme than is the case nowadays. Of course, there are limitations in applying such a strategy. Grain yield, as the most complex trait, usually has a low heritability and it would be very optimistic to believe that we could find a strict linkage disequilibrium between marker polymorphism and high-yielding alleles controlling grain yield. Despite these limitations, we believe that, from the application point of view, the approach of "going directly for grain yield" could at least direct us towards "good indicators" of improved yield.

Thus, to test this concept, we analyzed the seven-year data for main yield components and several other important traits for both HYGs and LYGs (Table 3).

The HYGs were on average 5.5 days earlier, 6 cm taller and with much higher kernel weight per spike and 1000 kernel weight compared with the LYGs, indicating that a major factor contributing to the better yield performance of the high yielding group was better adaptation to the environment. Based on the differences in grain weight, we may conclude further that the critical period for formation of high grain yield at the NS breeding site was grain filling. Earlier flowering enables either a longer grain filling duration or the avoidance of terminal drought and heat stress at the end of the grain filling period, which are very frequent stresses at the Novi Sad breeding site. As the major genes (Rht, Ppd and Vrn) play the most important role in wheat adaptability, we also analysed the data previously obtained by molecular screening of the Rht8 locus (Tables 1 and 2). In the Novi Sad wheat breeding programme, the vast majority of varieties and lines carry a 192bp fragment with SSR gwm261 diagnostic for the Rht8 gene (and due to close linkage probably Ppd-D1 as well) indicating a certain phenotypic advantage in relation to other Rht gene locus alleles (Kobiljski et al. unpublished data). Nevertheless, the genotype with the highest grain yield in this research (NS 66/92) does not have Rht8 (Ppd-D1), and neither do Centurk, Ana, Sofija and Triple Dirk B. Contrary to this, in the LYGs,

Table 3. Average values of main yield components and few other important traits in high and low yielding genotypes

	Grain yield (t ha^{-1})	Earliness (days from 1st. Jan to flowering)	Plant height (cm)	No. of kernels per spike	Kernel weight per spike (g)	No. of spikes per unit area (spikes m^{-2})	1000 kernel weight (g)
High yielding genotypes	7.84	132.5	78.3	40.1	1.62	652.7	41.4
Low yielding genotypes	4.11	138.0	72.7	42.3	1.15	626.9	28.2

the 192bp fragment is very rare though still present in two genotypes, L-1 and L1A/91. From this it is logical to conclude that some other major genes or QTLs are also playing important roles in determining grain yield in these varieties. Using this approach we are dissecting grain yield into its main components, and then into subcomponents and subunits. Here is where modern biotechnology can make its greatest contribution in determining the molecular basis of improved grain yield (or any other complex trait). By using this approach we are going from the general to the particular in solving the "QTL puzzle". There is a risk of some results being "false positives", but in the "QTL darkness" it is much simpler, easier and wiser to reach a destination following a main road rather than using shortcuts.

ACKNOWLEDGEMENTS

Dr. B. Kobiljski is grateful to the Royal Society for a fellowship to visit the John Innes Centre.

REFERENCES

Eisemann B, Banks P, Butler D, Christopher M, DeLacy I, Jordan D, Mace E, McGowan P, McIntyre PD, Rodgers D, Sheppard J (2004) Pedigree-based genome mapping for marker-assisted selection and recurrent parent recovery in wheat and barley. 4th International Crop Sci. Congress, Brisbane, Australia. http://www.cropscience.org.au/icsc2004/poster/3/4/1/1422_eisemannb.htm

Snape J (2004) Challenges of integrating conventional breeding and biotechnology: a personal view. 4th Inter. Crop Sci. Congres, Brisbane, Australia. http://www.regional.org.au/au/cs/2004/plenary/3/1394_snapejw.htm

Kobiljski B, Quarrie S, Dencic S, Kirby J, Iveges M (2002) Genetic diversity of the Novi Sad wheat core collection revealed by microsatellites. Cel Mol Biol Lett 7:685–694

MARKER IMPLEMENTATION IN THE DEPARTMENT OF AGRICULTURE, WESTERN AUSTRALIA WHEAT BREEDING PROGRAM

R. MCLEAN[1,*], I. BARCLAY[1], R. WILSON[1], R. APPELS[2], M. CAKIR[2], G. DEVLIN[2] AND D. LI[2]

[1] *Department of Agriculture, South Perth, WA 6151, Australia*
[2] *WA State Agricultural Biotechnology Center, Murdoch University, Murdoch, WA 6150, Australia*
[*] *E-mail: rmclean@agric.wa.gov.au*

Abstract: Marker Assisted Selection (MAS) is being applied to a large breeding program that makes approximately 3,000 crosses and has a field program of 180,000 plots across 14 sites each year. In the breeding program MAS is seen as complementing the existing screening for yield, quality and disease resistance. This paper describes the experiences of applying MAS, role of MAS in the breeding program, its effect on breeding methodology, and future directions for MAS in the program

Keywords: marker assisted selection, marker discovery, marker-trait validation, marker implementation

INTRODUCTION

The Department of Agriculture, Western Australia (DAWA) wheat breeding program has three wheat breeders with complementary targets in quality grades and different environments. There is a high degree of co-operation, collaboration and exchange of germplasm between the plant breeders, but each breeder controls a separate germplasm pool. The program aims to produce commercially competitive cultivars in five major quality grades for a target environment in Western Australia which in the 2005/06 season is expected to produce 10.2 m. tonnes on 4.4 m. ha., 48% of the estimated Australian crop (Department of Agriculture 2005). Currently cultivars from the program are sown on 81% of the W.A. crop area, and in 2004/05 made up 42% of Australian exported wheat (AWB Ltd.).

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 737–746

The breeding program primarily uses the F_2 bulk progeny method, but uses backcrossing, doubled haploid (DH), modified single seed descent (SSD) and bulk methods as appropriate. We are currently making approximately 3,000 crosses each year and manage a field program of 180,000 plots across 14 sites. In the breeding program MAS is seen as complementing the existing screening for yield, quality and disease resistance.

Molecular marker technologies offer a range of novel approaches to improve the efficiency of a breeding program. The DAWA breeding program uses molecular markers to accelerate wheat breeding and hence to increase genetic gain per year by way of optimising parental selection in crossing program and enriching the populations for the desired traits (Cakir et al. 2003). The success of these strategies is often dependent on the availability of polymorphic and also closely linked markers to the trait of interest. During the past ten years marker discovery and linkage studies have identified a large number of QTL regions that could be used for the breeding of wheat. However, these QTL studies are not easily applicable in breeding programs and therefore further validation experiments for the markers within those QTL regions need to be carried out using breeding populations.

There are three steps in developing MAS; discovery, validation and refinement, and implementation.

MARKER DISCOVERY

The majority of wheat marker development in Australia occurs through the Australian Winter Cereal Molecular Marker Program (AWCMMP). The AWCMMP is a national research and development effort funded by the Grains Research and Development Corporation (GRDC) that has projects across 14 research organisations. The aim of the AWCMMP is to provide overall coordination of wheat and barley molecular marker development and implementation in Australia, specifically to:

- Identify markers linked to traits of importance to wheat/barley improvement
- Apply markers in all Australian wheat/barley breeding programs
- Provide molecular marker service laboratories associated with each of the major publicly funded wheat/barley improvement programs
- Provide the most effective research effort and most efficient utilisation of resources through a nationally coordinated effort (http://www.grdc.com.au/AWCMMP/index.html).

The annual budget for the AWCMMP is A$3.2 m., with A$400,000 specifically directed to marker discovery. Within the AWCMMP breeders are involved in the prioritization of target traits of most significance to breeding programs and in the development and phenotyping of populations for marker development and validation. Access to information is available to all participants via regular meetings and an electronic site. Current target traits include disease resistances, agronomic and physiologic traits, and end-use quality. Australian breeders support the AWCMMP and would hope to see continuing investment by GRDC in this important project.

The Department of Agriculture is also a core participant in the Molecular Plant Breeding Cooperative Research Centre. The prime objective of the MPBCRC is to ensure the competitiveness of Australian crop and pasture industries by developing the latest molecular technologies and delivering them to the grain and pasture industries through breeding programs. The research program provides enabling intellectual property, new molecular technologies, tools and software to increase the efficiency and speed of plant breeding (http://www.molecularplantbreeding.com). Participation of the wheat breeding program in MPBCRC research gives the program access to both markers and new molecular technologies.

MARKER/TRAIT VALIDATION

Marker validation is a key step to marker implementation, which has been largely neglected when funding and resource allocations are made. To take a newly discovered marker from another laboratory to routine implementation within a breeding program involves considerable effort from both molecular biologists and breeders. Experience has shown that there are considerable phenotyping and laboratory costs in this process and sufficient time also needs to be allocated to the process. Because of these costs it is important to have close collaboration between breeders and molecular biologists to prioritise marker validation for the program. Close involvement of breeders in validation has also proved valuable in explaining some of the early results with a new marker because of their experience and understanding of the germplasm and populations being used.

The first step in validation is to understand the genetic distance between a linked marker and the gene of interest to determine the likely recombination rate and to evaluate the usefulness of the marker to a breeding program. Some reported marker/trait linkages are so far apart that their value in a breeding program is low as high recombination rates make the marker an unreliable screening tool. If the marker is close enough to the gene of interest the next step is to transfer the specific conditions for the assay developed in one laboratory to another. When this has been achieved the marker has to be validated in relevant germplasm for that breeding program. In Australia the major breeding programs have quite different germplasm bases, and for linked markers a marker that may work in the germplasm of one program may not be applicable in another breeding program due to lack of polymorphism at the marker site. Another consideration at this stage is that it may be necessary to convert a marker to a more appropriate marker type for the large-scale implementation used in that laboratory, or to facilitate the opportunities for multiplexing.

Often there is an unnecessary delay between marker discovery and the validation process. Researchers often wish to continue work on marker development until a publishable stage, and not make information more broadly available until then. This needlessly delays the validation process in breeding programs, and delays implementation. In Australia this is an area where the process of implementing

markers could be made more efficient, and could also help develop more robust markers.

The validation step can identify some unexpected results. For example the *Lr24* and *Sr24*-linked marker, developed by Schachermayr et al. (1995) on red-grained wheat, was not diagnostic in white grained Australian cultivars carrying these genes. In Australia breeders have utilized a shortened segment version of the original *Thinopyrum ponticum* (Podp.) Z.-W. Liu & R.-C. Wang (*Agropyron elongatum* (Host.) Beauv.) derived translocation where the genes for red grain are not present, and the linked marker is not able to be used. New markers have now been developed that work in white grained wheat populations (Mago et al. 2005).

MARKER IMPLEMENTATION – TARGET TRAITS

The experience in Australia has been that marker implementation has been evolutionary rather than revolutionary. Breeding programs have been using markers in situations where they are not necessarily the most efficient selection tool, but are considered more as an exercise in 'building for the future' to develop marker technology and strategies, though this is now changing as breeders are becoming more selective in their application. More attention is now being paid to genetic and economic effectiveness (Kuchel et al. 2005) since different tools may be appropriate for different traits, stages of the breeding program, breeding strategies or specific crosses and breeders will select the most appropriate and cost effective tool for any particular situation.

Breeders see an important role for MAS in targeting traits that are difficult or expensive to phenotype, or where the breeding program is not well equipped to carry out the testing. The reality is that these are also the most difficult markers to develop. Consequently most of the markers available are for simply inherited and phenotyped traits, though progress in developing markers for pre-harvest sprouting on chromosome 4AL (Mares and Mrva 2001), cereal cyst nematode (*Heterodera avenae* Woll.) (Ogbonnaya et al. 2001), root lesion nematode (*Pratylenchus neglectus* (Rensch) Filipjev and Schuurmans Stekhoven) (Williams et al. 2002), and Barley Yellow Dwarf Virus (BYDV) (Stoutjestijk et al. 2001) are examples of more difficult/expensive to measure traits, or traits our breeding program does not have good screening facilities for. Particular challenges for the future would include markers for some of the expensive to phenotype quality and physiologic traits such as water absorption, extensibility, stem carbohydrate storage and waterlogging that are important for our environment. Phenotyping quality traits also often requires large amounts of seed for destructive testing and this as well as costs contribute to delaying direct selection for these traits.

There are still efficiencies for a breeding program in using markers for simpler traits where markers can be multiplexed, or where phenotyping can cause plant setback (e.g. Al and B tolerance screening), or where phenotyping can not be done until late in the plants development. For example polyphenol oxidase, while simple to score, can only be scored on the grain produced so could not be phenotyped

before crossing. Similarly adult plant resistances for rust diseases fall into this category.

Other target traits for markers are those controlled by recessive genes, where a co-dominant marker can enable tracking of genes without the need for progeny testing.

Pre-emptive breeding for diseases that may be expected to arrive in the future ("Quarantine traits"), which in Australia include diseases and insects such as Karnal bunt (*Tilletia indica* Mitra), Russian Wheat Aphid (*Diuraphis noxia* Mordvilko) and Hessian Fly (*Mayetiola destructor* Say), can be handled efficiently with the use of markers. With these traits international collaboration for phenotyping is essential. Unfortunately the area of pre-emptive breeding is not currently receiving the attention it requires in Australia.

In 2004 20,096 assays were conducted, and this needs to be significantly increased to meet the breeding program requirements, particularly as more markers are implemented. Markers currently implemented in the DAWA wheat breeding program include:

- Disease resistance – many genes for leaf, stem and stripe rusts, cereal cyst nematode (*Cre1* and *Cre3*), yellow spot (or tan spot *Pyrenophora tritici-repentis* (Died.) Drechs.), root lesion nematode, BYDV
- Grain quality - grain texture/hardness (pinA, pinB), flour colour on chromosome 7AL, GBSS Null 4A, and pre-harvest sprouting on 4AL, PPO, late maturity alpha-amylase (LMA), presence of rye segments
- Mineral toxicities (Boron toxicity)
- Phenology (*Rht* genes)
- Herbicide tolerance - imidazolinone.

The program currently has a considerable list of markers at various stages in the validation process, including numerous genes for rust resistance and quality traits, including validating the use of the MALDITOF for identifying glutenin subunits.

MARKER IMPLEMENTATION – PLACE IN THE BREEDING PROGRAM

As more markers have become available MAS has resulted in changes in breeding strategies used by the breeding program. It is now possible to develop more complex crossing and selection strategies involving intercrosses between various restricted backcrosses and/or topcrosses to pyramid specific gene combinations based on MAS of individual parent plants, and MAS of resulting progeny to more quickly assemble desirable gene combinations for specific selected traits. As an example we have a project to eliminate some defects and enhance with other traits the variety Wyalkatchem, currently the leading variety in WA. We aim to enhance Wyalkatchem through improved rust resistance, pre-harvest sprouting tolerance and resistance to cereal cyst nematode whilst maintaining its excellent yield, plant morphology and resistance to *Pratylenchus*. We will be bringing together the traits through MAS of parental individuals from relevant crosses for

rust resistance, pre-harvest sprouting, cereal cyst nematode, root lesion nematode and *Rht* genes and also look at selecting for background genotype closest to Wyalkatchem.

Our selection strategies have also changed. It is now possible to bring forward testing for traits in earlier generations to improve the targeting of material entering yield trials, and also to work with traits that the program has previously not been able to target. Specific examples include pre-harvest sprouting and resistance to cereal cyst nematode and *Pratylenchus*, traits which our program does not have the resources to evaluate through phenotyping.

Marker assisted selection (MAS) will be increasingly applied to three major areas within the DAWA breeding program.

CHOICE OF PARENTS

First is the use of MAS in choice of parents in crossing programs, particularly screening of F1s in backcross and topcross situations. The efficiency over conventional crossing strategies is greatest where crossing targets multiple traits, and particularly in tracking recessively inherited traits where the need for progeny testing is removed. MAS has proved very effective in increasing the presence of *Sr2*, a gene for adult plant resistance to stem rust resistance that shows recessive inheritance (Knott 1968), in our breeding program.

Breeders have considered the use of MAS in accelerated backcrossing by selection for the recurrent parent background genotype. At this stage the high cost of whole genome screening and the need to handle larger numbers of plants within a substantial backcrossing program has resulted in the decision to not use this strategy generally within the breeding program. We are planning to use this approach for limited very complex crossing strategies currently being carried out. In other situations, such as the introduction of a new disease where new replacement cultivars were urgently required, whole genome screening would be a desirable strategy. At the end of a complex crossing process MAS is also used to select F_1 plants which carry targeted genes for production of F_2 seed or DHs. Experience in the breeding program has also shown the advantage of pre-screening of parents prior to crossing. In several instances new parents have unexpectedly been found to be mixed for the gene(s) of interest.

PYRAMIDING GENES

Second is the use of MAS for gene pyramiding. In particular the breeding program is using MAS to pyramid genes for rust resistance. The wheat breeding program currently has a major effort in upgrading resistance to the three rusts, currently actively working with approximately 40 resistance genes, and is using MAS where possible in the process of backcrossing genes into adapted germplasm for each of our quality gene pools. In future the program aims to move from single resistance gene cultivars to developing cultivars with multiple effective genes to each of the

three rusts to reduce the risk of development and multiplication of new rust races via simple step-wise mutations resulting in loss of effectiveness of resistance genes. A significant effort is being put into validating and developing markers for these genes to enable efficient gene pyramiding strategies.

POPULATION ENRICHMENT

Third is the enrichment of populations for target traits earlier in the breeding process. Greatest efficiency is achieved by selection as early as possible in the breeding process, but this is also where the number of individual lines is greatest. As the affordability, throughput and range of assays improves the amount of screening the breeding program can do in F_2 and F_3 generations, and in the F_5 and F_6 generations after re-selection will increase significantly. Effective selection at early generations increases the overall efficiency of the breeding process as lines entering expensive replicated, multi-site yield trials are more targeted and have a greater probably of success. The DAWA breeding program has integrated MAS in the F_2 bulk progeny, DH and SSD breeding systems.

Currently we estimate that 65% of assays relate to parent screening, 20% to gene/trait pyramiding in early generations, and 15% to fixed line screening.

MAS and more traditional screening methods are often complementary, and both utilised depending on the generation. As an example MAS may be utilised to select for rust resistance genes at an early generation to truncate a population. At later stages in the breeding program, where the number of lines is significantly reduced, screening nurseries may be used to evaluate the impact of background genotype on gene expression and to evaluate the resistance against multiple rust races. Similarly for a more complex trait controlled by multiple QTLs one or a few components may be selected for at early generations through MAS. At later generations more expensive testing may be utilised to evaluate the trait overall. This strategy is particularly relevant for complex traits such as yield and end product quality, however a better understanding of epistatic interactions will be needed to best use markers for these traits.

MARKER IMPLEMENTATION – IN PRACTICE

The program has standardized on the collection of leaf samples into deep welled 96 well plates. The single step high throughput DNA extraction method is based on Xin et al. (2003). To each plate well 200 μl of extraction buffer and a steel ball-bearing are added. After shaking for 2.5 minutes using a genogrinder, the green solution is transferred to a fresh 96 well plate and 400 ul of ethanol is added. A BioMek 2000 (Beckman) robot is used for setting up PCRs in a 384 well format, and this has greatly increased throughput and reduced costs of the marker implementation. Fragment analyses of PCR products are conducted running either agarose or acrylamide gels. While simple presence/absence type of markers are analysed with

agarose gels fragment analysis of SSR markers is carried out using acrylamide gels where there is a need of resolving a few base pair differences among test lines.

Taking markers from use in experimental populations to implementation in a large breeding program is proving to be a considerable challenge to both breeders and molecular biologists. This can be attributed to the significantly greater number of assays required by a breeding program, and the more complex crossing structure used in a breeding program compared with the simple crosses between two fixed line parents of most experimental populations. Timeliness of results is also a significant issue to the breeding program. Late arrival of marker information in a large crossing program creates immense problems and additional work for the breeding program.

Most experimental crosses are made between relatively genetically divergent parents, whilst in a breeding program crosses may be made between more closely related individuals which may result in difficulties in finding polymorphic markers. There is therefore an advantage in testing parents as soon as possible to find appropriate polymorphic markers to use before time critical results are required from segregating populations. From this year breeders will be attempting to sample all crossing parents for storage of DNA samples. As early as possible the breeders will document critical crosses where MAS is planned to enable the marker laboratory to determine appropriate markers in preparation for testing the cross populations. Earlier work on parents will also help to identify problems where some parents are unexpectedly mixed for a target trait.

The most obvious need for efficiency is in the laboratory to improve assay throughput, but producing and sampling leaf tissue on a large scale has proved challenging. The program has used both glasshouse and field grown leaf material and cutting leaf material to insert into 96 well plates for large numbers of individuals has proved very demanding of resources. Some progress has been made towards developing a sampling tool that automatically punches a leaf disc directly into a cell of a 96 well plate to improve sampling efficiency and accuracy. More recently preliminary studies have shown the potential of extracting DNA directly from seed in the 96 well plates in cases where it is not necessary to keep the tested plants. Successful development of this method will remove the need to sample leaf material, but will also have advantages of evening work flows across the year. Currently marker samples are being taken at a few major times during the year that fit in with plant breeding workloads. This results in significant workload peaks and troughs for the marker laboratory. With DNA extraction from seed plant breeding staff can load seed into 96 well plates at the same time as they are handling seed for trial preparation. Plates can then be stored with dry seed for processing throughout the year, with plates being brought out of storage as required. We aim to convert the majority of our marker screening program to seed DNA extraction from next year.

Good data handling and laboratory management systems become essential to handle the larger numbers of lines and assays required by a breeding program. A new plant breeding database is being developed to help handle the additional data

being generated, and to track samples and results between plant breeders and the marker laboratory. It has been necessary to develop more standardised procedures and processes with better documentation and labelling of samples and data between plant breeding and laboratory. In particular systems of standardised plate numbering and bar coded labelling and electronic transfer files accompanying each plate have been developed. The transfer file contains all information required by the laboratory, such as plate layout of lines, controls, pedigrees, generation, target traits, date results are required by and other relevant information. Results are then returned to breeders via the transfer file. With three wheat breeders all submitting samples for assay it has been important to develop a common prioritisation classification system so laboratory staff can schedule work according to the urgency with which results are required. For example samples relating to a crossing program are given a high rating to ensure results are available in time for crossing.

There is a need for good communications and a close working relationship between plant breeders and molecular biologists, but this relationship also extends to the technicians in the respective groups. To develop this relationship the technical groups have arranged tours of both workplaces to gain some insight of work carried out. Close location of plant breeding facilities and the molecular marker laboratory has been an advantage in developing communications. Particularly at this early stage of integrating molecular markers into breeding programs having molecular marker service laboratories associated with each of the major publicly funded breeding programs has been a much better model than a single large national marker laboratory.

MARKER IMPLEMENTATION – A WISH LIST

A plant breeder's wish list would include:

- The ability to target more traits through development of more marker/trait associations requiring continued investment by research funding bodies
- Better markers, ideally this would be perfect markers, but also covers more co-dominant markers to distinguish homozygotes from heterozygotes and more tightly linked markers
- Better distinguishing of alleles at a locus
- Greater laboratory throughput and cheaper costs
- Improved and more efficient sampling methods
- Cheaper whole genome systems
- Better data handling.

ACKNOWLEDGEMENTS

GRDC for research investment in the wheat breeding program and marker development and implementation projects. AWCMMP and MPBCRC for investment in marker development and molecular technologies. GRDC and MPBRC for travel grants.

REFERENCES

Cakir M, Appels R, Carter M, Loughman R, Francki M, Li C, Johnson J, Bhave M, Wilson R, McLean R, Barclay I (2003) Accelerated wheat breeding using molecular markers. In: Pogna N (ed) X Int wheat genet symp, Paestum, Italy, Istituto Sperimentale per la Cerealicoltura, Roma, Italy, 1: 117–120

Department of Agriculture (2005) Western Australian agri-food and fibre industry outlook bulletin 4664 ISSN 1448-0352

Knott DR (1968) The inheritance of resistance to stem rust races 56 and 15B-1L (Can.) in the wheat varieties Hope and H-44. Can J Genet Cytol 10:311–320

Kuchel H, Ye G, Fox R, Jefferies S (2005) Genetic and economic analysis of a targeted marker-assisted wheat breeding strategy. Mol Breed 16: 67–78

Mago R, Bariana HS, Dundas IS, Spielmeyer W, Lawrence GJ, Pryor AJ, Ellis JG (2005) Development of PCR-markers for the selection of wheat stem rust resistance genes Sr24 and Sr26 in diverse wheat germplasm. Theor Appl Genet 102: 942–949

Mares DJ, Mrva K (2001) Mapping quantitative trait loci associated with variation in grain dormancy in Australian wheat. Aust J Agr Res 52: 1257–1265

Ogbonnaya FC, Subrahmanyam NC, Moullet O, de Majnik J, Eagles HA, Brown JS, Eastwood RF, Kollmorgen J, Appels R, Lagudah ES (2001) Diagnostic DNA markers for cereal cyst nematode resistance in bread wheat. Aust J Agr Res 52:1367–1374

Schachermayr GM, Messmer MM, Feuillet C, Winzeler H, Winzeler M, Keller B (1995) Identification of molecular markers linked to the Agropyron elongatum-derived leaf rust resistance gene Lr24 in wheat. Theor Appl Gen 90: 982–990

Stoutjestijk P, Kammholz SJ, Kleven S, Matsay S, Banks PM, Larkin PJ (2001) PCR-based molecular marker for the BYDV source of barley yellow dwarf virus resistance in wheat. Aust J Agr Res 52:1383–1388

Williams KJ, Taylor SP, Bogacki P, Pallotta M, Bariana HS, Wallwork H (2002) Mapping of the root lesion nematode (Pratylenchus neglectus) resistance gene Rlnn1 in wheat. Theor Appl Genet 104: 874–879

Xin Z, Velten JP, Oliver MJ, Burke J (2003) High throughput DNA extraction method suitable for PCR. Biotechniques 34: 820–826

EFFICIENT INTEGRATION OF MOLECULAR AND CONVENTIONAL BREEDING METHODOLOGIES

D. BONNETT*, J. HYLES AND G. REBETZKE

CSIRO Plant Industry, GPO Box 1600, Canberra ACT 2601, Australia
**E-mail: David.Bonnett@csiro.au*

Abstract: Molecular markers make it possible for breeders to combine desirable alleles at a greater number of loci and at earlier generations than is possible with conventional breeding methodologies. With an increasing number of markers for important traits, marker assisted selection (MAS) strategies that minimize population sizes and assay numbers while ensuring important alleles are not lost become increasingly important. In practical breeding, strategies will often have to balance MAS with the need to retain variability for important traits that are selected partly or wholly by conventional means

This paper will discuss strategies to maximize the number of traits that can be combined in a single breeding cycle taking account of practical limits such as reasonable population sizes that can be managed in a breeding program. Examples from our breeding program and mapping populations will be used to illustrate the effects of different MAS strategies on population size and levels of residual genetic variation at unselected loci. It is these unselected loci that become important where conventional selection follows MAS

Keywords: marker assisted selection, mapping populations, population size

INTRODUCTION

With the development and implementation of an increasing number of markers for important traits, marker assisted selection (MAS) strategies that minimize population sizes and assay numbers while maximizing the probability that important alleles are not lost become increasingly important. In practical breeding, strategies will often have to balance MAS with the need to retain variability for important traits selected partly or wholly by conventional means. This selection must often be delayed until later generations to increase heritability or greater seed quantities

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 747–752.

are available. In such cases, strategies to avoid loss of desirable alleles prior to selection are needed.

It is clear that even with large numbers of useful markers and the most efficient methodologies, there are limits to the number of traits that can be combined in a single breeding cycle.

Based on theory, inbreeding, backcrossing and enrichment of allelic frequencies in BCF1 and the F2 of biparental and backcross populations should be useful in minimizing populations sizes needed to obtain a target genotype (Bonnett et al. 2005). Data from breeding and mapping populations support these predictions and are being used to develop and test theories on efficient strategies combining MAS and phenotypic selection.

Strategies discussed in this paper are being implemented in our germplasm development program.

RESULTS

The Cost of Certainty

If the number and frequency of important unlinked alleles segregating in a cross is known, the population size needed to recover a target allelic combination with a probability of failure (P) can be calculated according to the formula (Hanson 1959):

$$N = \frac{\log_n P}{\log_n (1 - G)}$$

where G = genotypic frequency (see Bonnett et al. 2005 for formulae to calculate G).

Reducing P can come at a substantial cost (Fig. 1). Numbers needed to lower the chance that a population does not contain at least one individual with a target

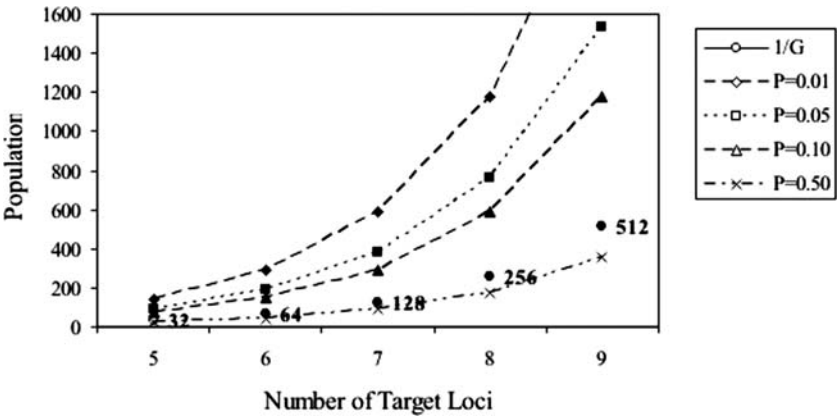


Figure 1. Population sizes to reduce probability of failing to recover a target genotype to different levels (P) in unselected biparental-derived DHs polymorphic at multiple target loci

genotype to 5% ($P = 0.05$) are around three times greater than the inverse of G. For example, if the frequency of the target genotype is 1/128, as expected in a doubled haploid (DH) population polymorphic at 7 target loci, a population of 382 is required. With only 128 individuals there is a 63% chance of obtaining at least one with the target genotype ($P = 0.37$). To achieve $P = 0.01$ in the same cross, 587 individuals are needed. This relationship holds for all genotype frequencies.

On a positive note, populations sufficient to recover a target combination of known alleles at low P-values will usually contain multiple individuals with this genotype providing a reasonable probability of capturing a small number of additional unknown alleles and the opportunity for further genetic gain.

Inbreeding and Enrichment Reduce Population Size

Both inbreeding and enrichment should substantially reduce population sizes. For example, in a biparental cross segregating at 6 unlinked loci, inbreeding from F_2 to $F_{2:3}$ should reduce the population size needed to recover a target homozygous genotype from more than 12,000 to just over 1,000; a reduction of 90% ($P = 0.05$). In a highly inbred or DH population, only 190 individuals would be needed to recover the target genotype.

The method of F_2 enrichment consists of selecting in F_2 all individuals carrying a target allele, whether homozygous or heterozygous, and removal of homozygous nulls. In a biparental cross the expected frequency of carriers is 0.75 at each polymorphic locus; much higher than the allelic frequency of 0.5 or the homozygote frequency of 0.25. Because of the high frequency of carriers, only a small F_2 population is required even with relatively large numbers of polymorphic loci. For example, an F_2 of only 19 individuals is needed in a cross segregating at 6 loci to recover an individual carrying all target alleles in at least heterozygous condition ($P = 0.05$). Following enrichment the frequency of selected alleles is increased to 0.67. The population size that must be produced for selection of homozygotes in a subsequent inbred or DH generation can then be calculated based on allelic frequencies of 0.67 (Bonnett et al. 2005). These are much smaller than needed for non-enriched populations. For example, in a biparental cross segregating at 6 loci, F_2 enrichment is predicted to reduce the population size and number of individuals that need to be tested by over 90% in $F_{2:3}$ (Fig. 2). Similarly, in a BCF_1 enrichment involves removal of homozygous nulls and retention of carriers to increase allelic frequencies and reduce population size.

Partial F_2 enrichment can be applied in populations where markers are not available for one or more important polymorphic loci. A larger F_2 is required to ensure non-enriched alleles are not lost through genetic drift, but useful efficiencies can still be achieved. Partial enrichment forms the basis of strategies combining marker-assisted and phenotypic selection and should allow reduced population sizes even with relatively high numbers of non-unenriched loci (Fig. 2, Table 1).

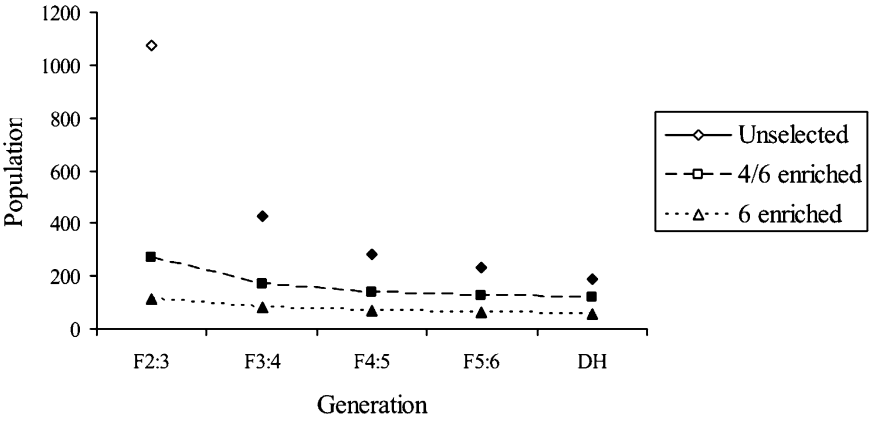


Figure 2. Line numbers needed for recovery of a target genotype in DH populations polymorphic at 6 loci following F_2 enrichment for target alleles at 0 (Unselected), 4 and 6 loci ($P = 0.05$)

Use of Cranbrook/Halberd Mapping Data to Test Theoretical Predictions on Combining Marker-assisted and Phenotypic Selection

The Cranbrook/Halberd population consists of 161 DH lines developed for mapping studies in the Australian Winter Cereals Molecular Marker Program (AWCMMP). With around 900 markers distributed over all chromosomes and extensive sets of phenotypic data on a range of traits, it represents a useful genetic framework to study the effects of selection at single and multiple loci and to test alternative breeding methodologies.

Table 1. Population sizes required to recover a target genotype ($P = 0.05$) in DHs derived from biparental F_2 populations with different numbers of enriched (Enrich) and unenriched (Rand) loci. Combined F_2 and DH population size indicated with F_2 needed for the enrichment step shown in parentheses

Loci	Genot. freq.	All loci rand	All loci enrich	1 locus rand	2 loci rand	3 loci rand	4 loci rand	5 loci rand
1	1/2	4	(3) 6	–	–	–	–	–
2	1/4	10	(4) 11	(8) 17	–	–	–	–
3	1/8	22	(7) 17	(11) 26	(18) 38	–	–	–
4	1/16	46	(10) 26	(16) 38	(24) 55	(37) 80	–	–
5	1/32	94	(14) 40	(21) 57	(33) 81	(50) 115	(77) 163	–
6	1/64	190	(19) 59	(29) 83	(45) 117	(68) 165	(103) 233	(155) 330
7	1/128	382	(26) 87	(39) 121	(60) 170	(91) 238	(138) 334	(207) 470
8	1/256	765	(33) 127	(53) 177	(81) 246	(122) 344	(184) 480	(277) 672
9	1/512	1532	(47) 187	(72) 258	(108) 358	(163) 497	(246) 691	(370) 964
10	1/1024	3066	(63) 274	(96) 377	(145) 520	(218) 719	(329) 997	(494) 1385
11	1/2048	6134	(85) 329	(129) 551	(194) 757	(292) 1044	(439) 1442	(659) 1997
12	1/4096	12269	(114) 401	(172) 806	(259) 1105	(390) 1519	(586) 2091	(879) 2888

To compare 2 alternative strategies, 9 independently inherited alleles controlling rust resistance, glutenins and coleoptile length were targeted. The target genotype was homozygous for rust resistance genes *Sr2, Sr30* and *Yr7/Sr9g*, high molecular weight glutenins *GluB1i* and *GluD1d* and low molecular weight glutenin *GluA3b*. For simplicity, a single marker located close to each allele was used and assumed perfect (i.e. no recombination between gene and marker). The 3 alleles controlling coleoptile length were assumed to be selected only by phenotypic means in DH material (i.e. no F_2 enrichment applied at these loci) and to account for 95% of the phenotypic variance. Although MAS was not applied for coleoptile alleles, for the purpose of testing the alternative selection methodologies, their presence and frequencies were tracked using markers psr931 (3BS), P32/M61–2 (7BL) and csME1 (4BS).

The alternative strategies and outcomes of their application to the Cranbrook/Halberd data set were as follows:

1. Unselected biparental DH population

In an unenriched biparental-derived DH population, the expected frequency of a genotype combining the target alleles at all 9 loci is 1/512. The probability of such an individual not being present among the 161 Cranbrook/Halberd DH lines is 73% so observed the absence of the target genotype was not unexpected. To increase the population size and better test the theoretical prediction, DH lines from Sunco/Tasman and CD87/Katepwa were used to supplement Cranbrook/Halberd lines with selection applied for the same regions. With a combined population size of 531, $P = 0.35$ a single individual of target genotype was identified (Table 2).

2. Partial F_2 enrichment

To examine partial F_2 enrichment, a hypothetical F_2 population of 160 individuals was 'synthesized' from the genotypes of 160 Cranbrook/Halberd DH lines by twice combining pairs of lines at random. Population size targets in F_2 and DH were set to

Table 2. Comparison of unselected DH and a DH derived from a partially enriched F_2 in a Cranbrook/Halberd cross segregating for 9 target loci

Strategy	Frequency of target genotype	Number of individuals to recover target genotype ($P = 0.05$)	Number of individuals screened and P-value	Number of individuals recovered with target genotype	Total number of individuals screened[ab]
1. Selection of target genotype in unenriched DH					
DH	0.002 (1/512)	1532	531 (P = 0.35)	1	531
2. F_2 enrichment of 6/9 target alleles followed by selection of homozygotes in the DH Population required for F_2 enrichment = 163 (160 screened)					
Part A- F_2 enrichment	0.022	163	160 (P = 0.03)	10	
Part B- DH	0.011	333	333 (P = 0.03)	3.6	493

[a] rounded to nearest integer value

[b] includes plants screened in F_2

achieve a cumulative 5% probability of failing to recover the target genotype across both selection stages. Genotypes of hypothetical DHs derived from the enriched F_2 population were predicted based on genotypic frequencies in the enriched F_2.

While both strategies were successful in recovering at least one individual with the target genotype, partial enrichment produced a significantly greater number of target individuals with a smaller population size (Table 2). While the differences may not seem great in this instance, the enrichment strategy provides a 95% confidence of recovering the target genotype while the unenriched DH will fail to recover a target genotype in over 1/3 cases. For the same probability of recovering a target genotype in the non-enriched DH, a population of 1532 is needed; three times the number required in the enriched population.

DISCUSSION

With increasing knowledge of the number and location of genomic regions controlling important traits, breeders are able to design targeted strategies to combine multiple alleles controlling one or several traits. The availability of markers not only allows reductions in population size through enrichment strategies, but earlier application of selection and selection for more traits than is possible with phenotypic selection. In comparing MAS and phenotypic selection schemes it has been reported that this difference in the timing of selection is one of the key benefits of MAS strategies (Kuchel et al. 2005). In many cases, MAS makes possible the stacking of traits that could not be combined within the limits of reasonable population sizes with phenotypic selection.

In order to maximize the advantages of MAS, it is important to develop and test theory on the most efficient strategies. Based on our theoretical studies and growing body of data from populations in our germplasm development program, strategies combining F_2 enrichment and inbreeding (with or without DH production) form useful components of MAS strategies in many crosses and still offer advantages when combined with phenotypic selection.

In addition to the strategies discussed in this paper, we are developing and implementing strategies to deal with repulsion phase linkage between target alleles, recombination between target alleles and linked markers and beginning to use more sophisticated simulation modeling to assist in this process. Computer modeling is also providing a more effective means of identifying key points where loss of important alleles through genetic drift is most likely and what strategies can be applied to efficiently minimize this risk.

REFERENCES

Bonnett DG, Rebetzke GJ, Spielmeyer W (2005) Strategies for efficient implementation of molecular markers in wheat breeding. Mol Breed 15:75–85

Hanson WD (1959) Minimum family sizes for the planning of genetic experiments. Agron J 51:711–715

Kuchel H, Ye G, Fox R, Jefferies S (2005) Genetic and economic analysis of a targeted marker-assisted wheat breeding strategy. Mol Breed 16:67–78

GENETIC DIVERSITY IN TURKISH DURUM WHEAT LANDRACES

T. AKAR* AND M. OZGEN
Central Research Institute for Field Crops
POBox: 226 06042 Ulus-Ankara Turkey
Fax: (+90) *312 287 89 58*
**E-mail: yezakar66@yahoo.com*

Abstract: The aim of this study was to determine genetic diversity of durum wheat (*T.durum* L.,) landraces collected from various regions of Turkey by means of RAPD markers. In addition to this, some morphological, pathological and technological traits were also investigated in this study. In this research, 100 durum wheat lines selected from 10 land races and 3 cultivars were screened by using ten decamer of 15 more polymorphic and reputable RAPD markers selected from 300 RAPD primers. Totally, 92 loci were determined based on RAPD data. 80 of these loci were polymorphic while the others were monomorphic. Estimated genetic diversity parameters investigated in this research such as mean effective allele numbers, observed heterozygote, proportion of polymorphic loci and gene diversity were generally higher in land races than those of the cultivars. Effective allele numbers, observed heterozygoty, proportion of polymorphic loci and gene diversity were 1.40, 0.24, 89.96 and 0.25, respectively. Genetic distance of the material which was calculated based on RAPD data changed between 0.74 and 0.99. Large variation observed in morphological (plant height, spike length, grain number per spike, biological yield, and resistance to lodging, etc.), pathological (stripe and leaf rusts), technologic traits (1000 kernel weight, hectoliter weight, protein ratio, SDS sedimentation etc.) and RAPD markers within and among land races population suggests that these germplasm can be used in improving new durum cultivars with higher yield, resistance to rusts and desirable quality traits

Keywords: durum wheat, landraces, genetic diversity, molecular characterisation, RAPD

INTRODUCTION

Durum wheat is mainly cultivated under semi-dry areas of the world along 9 million ha acreage. Middle east, Northern Africa and America and Mediterranean countries are known main production areas of this species (Sirivasta 1984). Turkey is one

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 753–760.

of the most important durum wheat producer and exporter countries in the world after Canada and USA (Anonymous 2000). This species has been cultivated along three different regions of Turkey and mainly used for making bulgur, macaroni and flat bread (Eser 1995). As for other plant species, Turkey is also one of the gene centres of durum wheat (Gökgöl 1939). Determination of the genetic diversity among and within landraces is a crucial factor for introduction and use of any species. Characterization of plant cultivars and landraces has mainly been made on morphological traits which are affected by environmental factors. In order to overcome the problem, isoenzymes, RFLP and RAPD markers have been developed since 1960 (Mori et al. 1993). As other PCR based molecular techniques, RAPD markers are preferred for mapping and characterization studies due to time and cost effectiveness. Since for RAPD system a smaller DNA fragment is required, many promising results have been gathered for cereal and also other plant species (Williams et al. 1990, Devos and Gale 1992) compared with RFLP markers. RAPD markers are commonly used for genetic diversity studies in landraces and inter specific crosses due to higher polymorphism ratio (Karp and Edwards 1995). In addition to morphologic characterization, we aimed to determinate genetic diversity within and among some Turkish durum wheat landraces by RAPD markers in this study.

MATERIAL AND METHODS

Plant Material

Three durum wheat cultivars (Kunduru-1149, C-1252 and Altıntas) developed by Central Research Institute for Field Crops and Anatolia Research Institute, and ten durum wheat landraces collected from different provinces of Southeast Anatolia, Middle and Western Blacksea and Central Anatolian regions were used. In order to determine genetic diversity, ten single spikes were selected from each population including cultivars.

Genomic DNA Isolation and RAPD Primers

Genomic DNA of each plant was extracted from a whole seed using the protocol modified by Göçmen (2001). Totally, 300 ten-base oligonucleotide primers of Operon Technologies (California, USA) including two kits (A (OPA) to O (OPO)) were used to screen genetic diversity. Each kit has 20 primers and the sequences of them were arbitrary. They were generated on a random basis with a requirement that their **G** + C content are 60%–70% and that ends were not complementary.

DNA Amplification Conditions

In order to generate RAPD markers by PCR, conditions reported by Williams et al. (1990) were optimised for durum wheat landraces populations DNA.

PCR Mixture and Cycling Condition

Optimum PCR mixture for 25 µl reaction volume consisted of 8 ng template DNA, 3 mM $MgCl_2$, 0.2 µM primer, 200 µM deoxynucleotidetriphosphates, 1 unit Taq DNA polymerase, 2.5 µl PCR buffer from 10x, and 0.13 µl Tween-20. Cycling condition used in this experiments was 45 cycles of 1 minute at 94 °C (denaturing) 1 minute at 37 °C (annealing) and 2 min at 72 °C (extension) with post-holding at 72 °C for 10 minutes in a thermal cycle developed by Appligene Oncor (Crocodile III).

Strategy for Identification and Evaluation of RAPD Data

In the first step of the study, 300 RAPD primers were screened against a selected landrace population. If a band was found in only one of the lines selected from the population, it was assumed as polymorphic. Primers with high number of RAPD loci were used to screen genomic DNA's of 96 lines. These single locus segregation data were used to determine genetic distance, similarity and related molecular parameters; then, a cluster tree based on these data was constructed by using POP GENE 32 package program (Yeh et al. 1997).

Evaluation of Quantitative Data

MINITAB 12.1 programs were used to analyse all morphological, pathological and quality data collected under field, greenhouse and laboratory conditions during the study.

RESULTS AND DISCUSSION

Among the 300 RAPD primers which were screened against a selected landrace population DNA's, more than 85 of them revealed at least one polymorphic locus. Considering cost of the experiments, the most polymorphic 15 RAPD primers were selected; they were reproducible and clear alike. When these 15 polymorphic primers were screened against DNA's of 96 landrace lines, totally 92 RAPD loci were determined (Table 1). Of these 92 RAPD loci, 80 loci were polymorphic while the others were monomorphic, so 7.66 segregating loci per screened primer were determined (Table 1). Polymorphic loci number and percentage of polymorphic loci for landraces changed between 30 to 61 and 32.6% to 66.30%, respectively (Table 2). This indicates that long-term breeding studies have resulted in lowering polymorphic loci number and percentage which were the lowest value at two registered durum wheat cultivars (C-1252 and Altıntas) (Table 2). In addition to these, rate of mean effective allele and gene diversity were generally higher in landraces than those of durum wheat cultivars (Table 2). When all landraces populations were generally evaluated, effective allele numbers, observed heterozygocity, proportion

Table 1. Total and monomorphic loci number over all primers

Primers	Sequence of the primers	Total loci number	Monomorphic loci number	Segregating loci detected (bp)	Populations screened
OPA-11	CAATCGCCGT	4	1	500	All pop.
OPA-16	AGCCAGCGAA	10	–	–	“
OPB-11	GTAGACCCGT	6	–	–	“
OPE-09	CTTCACCCGA	15	1	1400	“
OPE-13	CCCGATTCGG	4	1	700	“
OPJ-09	TGAGCCTCAC	7	1	800	“
OPK-09	CCCTACCGAC	11	–	–	“
OPL-03	CCAGCAGCTT	8	4	900; 800; 700; 600	“
OPL-13	ACCGCCTGCT	8	1	900	“
OPM-15	GACCTACCAC	6	2	1000; 900	“
OPM-16	GTAACCAGCC	6	1	900	“
OPM-18	CACCATCCGT	7	–	–	“
TOTAL	–	92	12	–	–

Table 2. Single locus-population description statistics

Populations	Sampling number/ locus	Normal allele number	Effective allele number	Shannon information index	Polymorphic locus number	Polymorphic locus (%)	Gene diversity
Kunduru-1149	5	1.500*	1.244*	0.235*	46	50.0	0.151
		0.052**	0.033**	0.027**			0.018
C-1252	4	1.413	1.282	0.237	38	41.31	0.161
		0.050	0.038	0.030			0.204
Altıntas	5	1.365	1.207	0.185	30	32.62	0.122
		0.049	0.034	0.027			0.019
Sanlıurfa	7	1.554	1.305	0.275	51	55.43	0.181
		0.050	0.037	0.028			0.020
Adıyaman	6	1.543	1.366	0.293	50	54.35	0.196
		0.051	0.038	0.029			0.021
Tokat-1	5	1.597	1.327	0.299	49	53.26	0.196
		0.050	0.035	0.027			0.020
Tokat-2	6	1.532	1.240	0.242	49	53.26	0.154
		0.051	0.030	0.026			0.018
Amasya	8	1.608	1.322	0.294	56	60.87	0.193
		0.050	0.037	0.028			0.020
Cankırı-1	8	1.543	1.264	0.250	50	54.35	0.161
		0.051	0.035	0.027			0.019
Cankırı-2	7	1.630	1.357	0.321	58	63.04	0.212
		0.049	0.035	0.028			0.020
Corum	7	1.641	1.396	0.343	59	64.13	0.230
		0.049	0.039	0.029			0.021
Sinop	7	1.576	1.291	0.274	53	57.61	0.178
		0.050	0.035	0.027			0.019
Kastamonu	7	1.663	1.402	0.351	61	66.30	0.235
		0.048	0.038	0.029			0.020

of polymorphic loci and gene diversity were 1.40, 0.24, 89.96 and 0.25, respectively. Mean genetic distance of the landraces calculated on the basis of RAPD data was 0.1050 and changed between 0.74 to 0.99. The most similar populations (0.9968) were Cankiri-2 and Corum which were from two provinces geographically very close while the most distance (0.7473) germplasm were C-1252 and Altintas cultivars which were long and short plant height cultivars (Fig. 1). In addition to these, genetic distance data were also used to construct dendograms based on the UPGMA method (Nei 1978). Populations have been grouped into seven different clusters consisted of seven different groups by using the mean molecular data (Fig. 1). There is a tendency that geographically close populations were generally gathered into the same or near groups. These results show that RAPD data can be used for classification of material stored at gene banks and determination of core collections. Variation in morphological traits is highly convincing especially in biological yield (Table 3). Biological yield level of Cankırı-1, and 2 and Corum populations has out yielded between all cultivars (Table 3). These results indicate that landraces can be used for development of new high yielding winter germplasm suitable for dry lands. The same situation is also true for grain quality traits.

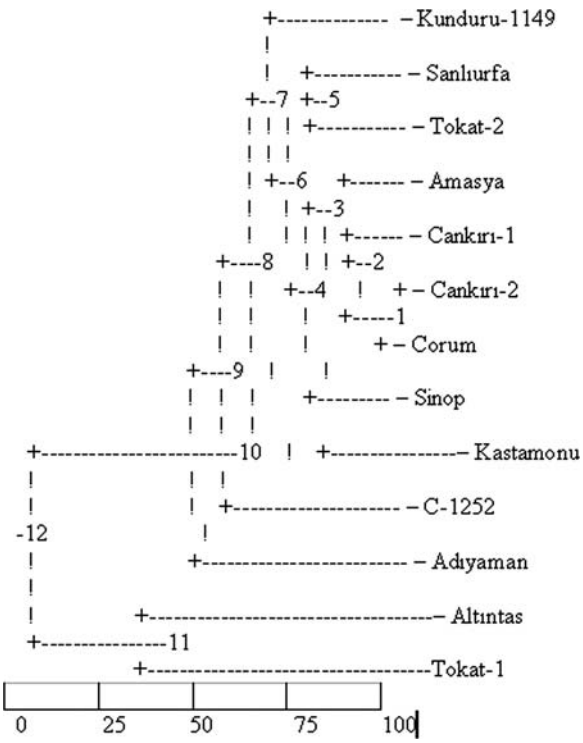


Figure 1. Multi-population Description Statistics (Dendogram of genetic distance)

Table 3. Morphological Variation within Populations

	Plant height (cm)			Spike height (cm)			Kernel number per spike			Spike weight (g)			Lodging (0–9)			Biological Yield (g)		
Pop/Cult	Min.	Max.	Mean. St.dev	Min	Max.	Mean St.dev	Min.	Max.	Mean St.dev	Min.	Max.	Mean St.dev	Min.	Max.	Mean St.dev	Min.	Max.	Mean St.dev
Kunduru-1149	117,3	129, 0	123,4 3,8	6, 3	8, 5	7, 3 0, 4	55,0	66,0	60, 1 3, 4	3,7	5,0	4,2 0,5	3,0	4,0	3,4 0,2	573,3	1507,3	1137, 4 279, 7
C-1252	85,3	92, 3	88,5 2,1	7, 1	7, 7	7, 3 0, 2	48,3	60,3	55, 4 4, 0	3,5	5,3	4,3 0,6	1,0	1,0	1,0 0,0	713,0	1891,7	1360, 0 353, 3
Altıntas	103,7	118, 7	112,6 4,9	6, 3	9, 1	7, 5 1, 0	53,7	60,7	57, 3 2, 9	3,2	5,3	4,0 0,7	1,7	2,0	1,8 0,2	745,3	1823,3	1143, 1 311, 6
Sanlıurfa	85,0	115, 0	93,5 8,7	3, 8	4, 9	4, 2 0, 3	36,0	55,7	46, 3 7, 5	1,9	3,5	2,7 0,6	1,3	3,7	2,2 1,0	531,7	1956,7	995, 9 510, 9
Adıyaman	115,3	141, 3	124,7 8,8	6, 2	8, 3	7, 1 0, 6	49,0	61,0	55, 1 3, 8	2,5	4,2	3,4 0,6	1,3	3,7	2,9 0,8	428,3	1861,7	1212, 2 487, 5
Tokat 1	115,7	130, 3	121,0 33,8	6, 3	8, 5	7, 0 2, 0	42,3	71,7	58, 7 17, 5	3,1	6,2	4,5 1,5	1,0	4,0	2,0 1,2	759,3	1984,0	1396, 2 541, 8
Tokat 2	121,0	138, 0	129,1 5,2	6, 3	9, 3	8, 2 1, 2	42,3	71,7	58, 7 17, 5	2,3	5,4	4,0 0,9	1,3	3,3	2,4 0,7	716,7	1963,7	1319, 3 470, 6
Amasya	123,3	134, 0	128,3 3,4	5, 2	8, 9	7, 3 1, 1	44,7	66,0	55, 7 6, 2	3,2	4,4	4,0 0,4	1,0	1,3	1,2 0,2	624,0	2031,0	1308, 9 430, 6
Cankırı 1	120,7	144, 7	133,6 7,4	4, 7	7, 4	5, 9 0, 9	36,0	60,3	51, 3 7, 7	2,6	5,3	3,8 0,7	2,0	4,7	3,9 1,0	1172,7	2027,0	1673, 9 324, 9
Cankırı 2	85,0	103, 3	93,3 6,2	5, 2	7, 3	6, 3 0, 8	49,7	60,3	54, 8 3, 9	2,6	4,6	3,7 0,6	1,0	1,3	1,2 0,2	716,7	1725,3	1406, 7 317, 8
Corum	118,0	140, 0	128,7 7,8	5, 2	9, 2	7, 0 1, 2	42,3	65,0	53, 5 6, 1	2,7	5,3	3,9 0,9	1,0	2,3	1,7 0,6	725,0	1962,7	1435, 2 466, 4
Sinop	81,7	105, 0	94,3 30,9	3, 2	6, 2	5, 0 1, 9	39,3	50,0	43, 3 13, 3	1,7	2,7	2,2 1,1	1,0	4,7	2,1 1,1	591,3	1539,7	881, 5 436, 8
Kastamonu	113,3	133, 0	124,6 6,4	5, 2	8, 1	6, 3 1, 1	44,3	62,7	56, 5 6, 5	2,5	4,3	3,7 0,6	2,0	4,3	3,0 1,0	560,0	1684,7	1051, 2 422, 2

Table 4. Leaf Disease Variation within Populations

	Yellow rust			Brown rust		
Pop/Cultivars	Min.	Max.	Mean St.dev	Min	Max.	Mean St.dev
Kunduru-1149	40	80	66,0 10,75	0	4	3,10 1,19
C-1252	24	70	44,4 17,98	3	4	3,30 0,48
Altıntas	8	70	39,4 22,16	2	4	2,80 0,63
Sanlıurfa	5	60	31,5 16,33	0	4	2,10 1,59
Adıyaman	4	30	16,0 9,93	0	0	0
Tokat 1	50	70	63,3 7,07	2	3	2,88 0,33
Tokat 2	30	70	54,0 15,76	0	4	2,40 1,71
Amasya	20	50	37,0 12,51	0	4	2,90 1,59
Cankırı 1	50	70	59,9 6,91	0	4	3,20 1,28
Cankırı 2	16	40	30,2 9,95	0	4	3,40 1,26
Corum	4, 5	40	24,4 11,67	0	4	2,90 1,59
Sinop	10	50	32,2 13,0	3	4	3,33 0,50
Kastamonu	9	50	32,4 13,75	3	4	3,70 0,48

Combination of higher sedimentation values and lower yellow berry rate within Corum, Tokat and Amasya populations clearly shows importance of landraces for germplasm improvement. Landraces are also crucial sources for disease resistance. Response of these germplasm was tested against yellow and brown rusts, both under greenhouse and field conditions (Table 4). Some populations have demonstrated good yellow and brown rusts resistance together with desirable grain quality even within the same population (Table 4). Therefore, breeders can select disease resistance germplasm combined with higher grain quality, using this material rather than registered cultivars for highland conditions.

CONCLUSION

Molecular, morphological, pathological and grain quality analyses of some Turkish durum wheat populations show that variation in these populations is crucial to develop new winter type germplasm suitable for dry land conditions of Turkey and the highlands of the region alike.

REFERENCES

Anonymous (2000) International grains council grain market report. Canada Square, Camary Whorf London, €14SAE, England

Devos KM, Gale MD (1992) The use of random amplified polymorphic DNA markers in wheat. Theor Appl Genet 84 (5/6):567–570

Eser V (1995) Durum breeding in turkey. Proceedings of the SEWANA. Durum network workshop, 20–23 March 1995, ICARDA, Aleppo, Syria. pp 43–54

Göçmen B (2001) Genetic Characterization of 150 F6 inbred durum wheat lines derived from Kunduru-1149 x Cham-1 cross using molecular markers and economically important traits, (Ph. D thesis), METU, Ankara

Gökgöl M (1939) Türkiye buğdayları cilt II. 955 sayfa. Yeşilköy Tohum Islah Enstitüsü Yayın No:14. Tan Matbaası, İstanbul

Karp A, Edwards KJ (1995) Techniques for the analysis, characterization and conservation of plant genetic resources. Reports of an IPGRI work shop, Rome, Italy, 9–11 Oct 1995

Mori M, Hosaka K, Umenura Y, Kaneda C (1993) Rapid identification of Japanese potato cultivars by RAPDs. Jpn J Genet 68:167–174

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583–590

Sirivasta JP (1984) Durum wheat. Its world status and patential in the Middle East and Nort Africa. Rachii 3:1-B

Williams JGK, Kubelic AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphism amplified by arbitrary primers one useful as genetic markers. Nucleic Acids Res 18:6531–6535

Yeh CF, Yang R, Boyle T (1997) POPGENE 32 version 1.31, Windows-based software for population genetic analysis

HISTORICAL CROSS-SITE ASSOCIATION BASED ON CULTIVAR PERFORMANCE IN THE SOUTHERN CONE

M.M. KOHLI[1,*], J. CROSSA[2] AND J. FRANCO[3]

[1]*Consultoria Internacional de Tecnología Agrícola, México*
[2]*Centro Internacional de Mejoramiento de Maíz y Trigo, México*
[3]*Facultad de Agronomía, Universidad Nacional, Uruguay*
[*]*E-mail: mmkohli@gmail.com*

Abstract: Southern Cone wheat growing region is considered one large epidemiological zone separated by the Andes Mountain Range. Although local breeding programs have exchanged germplasm successfully and results have been published regularly, very little effort has been made to determine site similarities based on historical data. The present study uses 12 years of agronomic data on days to heading (DH), leaf rust reaction (LR) and septoria leaf blight infection (SB) from Southern Cone Wheat Advanced Lines Nursery (LACOS) to define and characterize cross-site associations. A modified pattern analysis using classification (cluster analysis) and ordination (Factor analysis) based on distances and similarities (average correlations between sites) were utilized for sites representing at least three years of data for each trait. Two factors accounted for very large proportion (73 percent) of the variation for DH. However, in the cases of LR and SB slightly lower variation, 45.6 and 53.5 percent respectively, was explained by two factors. Using three factors, 68.4 percent of the variation in SB and 53.5 percent of the variation in LR could be explained. Fourteen common sites were used classify the region in two distinct groups with regards to DH. The key factor seems to be the temperature regime during the vegetative phase of the crop in the two regions separated by the parallel 27° South. The higher temperature regime of the north (Bolivia, Brazil and Paraguay) can be further subdivided to include very high temperature sites of Sao Paulo, Brazil, and drought prone site, San Benito, Bolivia, in one subgroup separating it from other sites in this group. Lower temperature locations grouped together represent sites in Argentina, Chile and Uruguay. LR represented a large variation in grouping the sites together. While all sites in Brazil grouped together as a unit, explaining the virulence similarity in this country, two Chilean sites, La Platina and Chillan grouped independently. Surprisingly, Chillan grouped with locations in Argentina. The fourth group includes sites in Paraguay, Uruguay and Mexico; all of them with heavy infection ratings over the years. Similar variation was observed in the groupings of seven SB sites. While Toluca, Mexico, and Marcos Júarez, Argentina, represented two independent groups, highland sites of Patzcuaro, Mexico, and Escalante, Bolivia, made up the third group. The southern locations of Chillan, Chile, and La Estanzuela, Uruguay, represented the high infection sites as an independent group. The site similarities

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 761–769.

demonstrated in this study should increase our understanding of the variation present in the Southern Cone Wheat Region and enhance cultivar adaptation across programs

Keywords: cultivar adaptation, septoria leaf blight, rust infection

INTRODUCTION

Southern Cone Region, comprising Argentina, Bolivia, Brazil, Chile, Paraguay and Uruguay is the major wheat producing area in Latin America. The crop seeded mostly under rainfed conditions and in rotation with soybeans and maize is spread over approximately eight million hectares and produces approximately 20 million tons of grain annually. Wheat is grown in diverse agro-climatic regions ranging from tropical Lowland in Bolivia to vast dry/humid Pampas in Argentina, acid soils with aluminum toxicity in Brazil and temperate Pre-Cordillera region in the southern Chile. In spite of the vast geographic expansion it covers, the whole region is considered to be one large epidemiological zone for the diseases and insect pests; only separated by the Andes Mountain Range.

In order to take advantage of this situation and generate early agronomic information on genetic materials in the region, the national wheat breeding programs have been very conscious of the usefulness of the germplasm exchange (Kohli et al. 2003). Such exchanges have been done regularly since 1950s when the first regional and international nurseries were started. The International Rust Nursery (USDA), the Latin American Wheat Yield Trial organized by Dr. Norman Borlaug of the Office of Special Studies, Mexico, the Southern Cone Wheat Yield Nursery (ERCOS) organized by EMBRAPA, Brazil, and Latin American Disease and Observation Nursery (VEOLA) organized by CIMMYT have been some of the formal vehicles for regional evaluation of germplasm.

The regional screening of advanced lines under diverse conditions has not only helped develop critical disease and agronomic information but also introduced increased genetic variability in each program on a regular basis. While this informal or formal exchange of germplasm has taken place for almost 50 years, very little effort has been made to determine site similarities based on historical data. Such information can be used to build complementarities among different wheat improvement programs of the region and understand cultivar adaptation (Peterson and Pfeiffer 1989).

MATERIALS AND METHODS

The present study uses 12 years of key agronomic data from Southern Cone Wheat Advanced Lines Nursery (LACOS), based on the evaluation of a total of 3560 advanced lines during 1991 and 2002. Three agronomic characters viz. days to heading (DH), leaf rust reaction (LR) and septoria leaf blight infection (SB) were

selected to define and characterize cross-site association. While LACOS is evaluated at more than 30 sites annually, the data on these agronomic parameters is not always reported. As a result, only those sites that reported information on a given character for at least three years were taken into consideration. The sites used in the analysis, their geographic location and overall averages for each of the three characters are presented in Table 1.

Factor analysis was based on standardized correlation coefficients as utilized by Peterson and Pfeiffer (1989). It used the correlation between sites representing at least three years of data for each trait. Using this criterion on the total 37 sites that provided data over the years, the number of sites represented for DH, LR and SB were 14, 11 and 7 respectively. The factor analysis computes factor loadings that are correlations between the variables and the unobserved factors so that it simplifies correlation matrices. In spite of apparent bias introduced by the analysis of agronomic characters from different number of years and lines tested, they do provide an estimate of general environmental conditions at each location that affect germplasm performance and adaptation.

RESULTS AND DISCUSSION

The sites presented in the Table 1 are not only different in terms of their geographic location but also have strong differences among their climatic conditions, especially with regards to the precipitation regime, which affects the performance of germplasm each year. Such variation added to the testing of different set of advanced lines each year should generally make it difficult to characterize a site and use it to predict the performance of a line under study. However, long term averages of the evaluated parameters presented in the Table 1 seem to characterize the locations quite well and assign the importance to each location based on these traits. As a result and so far as the crop cycle till heading is considered, the locations in Argentina, Southern Brazil, Chile and Uruguay, as a group, seem to be very different than the sites under study in Bolivia, Northern Brazil and Paraguay. The site associations as measured by correlation coefficients for crop cycle (days to heading) are presented in the Table 2. Similar correlation coefficients for leaf rust and septoria leaf blight infection are presented in Tables 3 and 4 respectively.

Two independent factors accounted for very large proportion (73 percent) of the variation for DH. As mentioned earlier, this character seems to be playing a very important role in deciding the adaptation of a germplasm in the region. Given the lesser number of locations studied for LR and SB, two independent factors explained slightly lower variation, 45.6 and 53.5 percent respectively, for them. While the utilization of three factors improved the level of confidence to explain 68.4 percent of the variation in SB and 53.5 percent of the variation in LR, this increase was not considered significant. As a result, two factors characterization was considered as a base to study the site associations in detail.

The data from 14 sites used to classify the DH, mostly falling in the same quadrant, divides the region in two distinct groups (Fig.1a). Only one location, Santa

Table 1. Geographic location and overall average for days to heading and disease index for leaf rust and septoria leaf blight for the sites under study

Site	Country	Location	Latitude	Longitude	Elev. (mts)	Heading (days)	Leaf rust	Sept leaf
2	Mexico	Toluca	19°16′N	99°51′W	2640	81	–	34
3	Mexico	Patzcuaro	19°27′N	101°44′W	2350	74	–	51
4	Mexico	El Batan	19°31′N	98°50′W	2249	67	–	14
6	Paraguay	Cap. Miranda	27°17′S	55°49′W	200	82	16	–
8	Brazil	Passo Fundo	28°15′S	52°24′W	684	93	9	–
9	Brazil	Cruz Alta	28°38′S	53°36′W	473	–	9	–
11	Brazil	Ponta Grossa	25°13′S	50°01′W	880	77	7	–
12	Brazil	Londrina	23°22′S	51°10′W	540	73	14	–
13	Brazil	Palotina	24°17′S	53°50′W	330	70	3	–
14	Brazil	Campinas	22°54′S	47°05′W	669	76	6	–
15	Argentina	Pergamino	33°56′S	60°33′W	66	91	29	40
16	Argentina	Marcos Juarez	32°42′S	62°07′W	110	100	16	38
19	Argentina	Tres Arroyos	38°20′S	60°13′W	120	94	5	42
23	Uruguay	La Estanzuela	34°20′S	57°41′W	81	98	11	51
24	Uruguay	La Estanzuela	34°20′S	57°41′W	81	101	10	52
26	Bolivia	Saavedra	17°14′S	63°10′W	320	69	17	–
27	Bolivia	Cochabamba	17°30′S	66°06′W	2730	64	1	–
29	Chile	Chillan	36°31′S	71°55′W	217	93	9	–
30	Chile	Hidango	34°06′S	71°47′W	296	123	–	39
31	Chile	Santiago	33°34′S	70°38′W	625	113	4	–
36	Bolivia	Escalante	17°46′S	65°24′W	32	90	–	53

Table 2. Correlation coefficients among sites for days to heading

NAME	S1	S2	S3	S8	S12	S13	S15	S16	S23	S26	S27	S29	S30	S31
S1	-	0, 88	0, 65	0, 40	0, 74	0, 67	0, 60	0, 56	0, 53	0, 60	0, 63	0, 58	0, 56	0, 54
S2		–	0, 24	0, 42	0, 74	0, 40	0, 49	0, 39	0, 41	0, 37	0, 64	0, 50	0, 54	0, 33
S3			–	0, 03	0, 70	0, 62	0, 46	0, 04	0, 40	0, 51	0, 66	0, 50	0, 43	0, 31
S8				–	0, 68	0, 75	0, 63	0, 67	0, 62	0, 35	0, 57	0, 57	0, 69	0, 51
S12					–	0, 87	0, 43	0, 47	0, 54	0, 63	0, 70	0, 49	0, 53	0, 58
S13						–	0, 55	0, 53	0, 64	0, 59	0, 70	0, 52	0, 58	0, 56
S15							–	0, 67	0, 59	0, 34	0, 52	0, 70	0, 69	0, 72
S16								–	0, 59	0, 13	0, 51	0, 58	0, 73	0, 54
S23									–	0, 36	0, 55	0, 61	0, 70	0, 63
S26										–	0, 48	0, 29	0, 24	0, 13
S27											–	0, 54	0, 53	0, 46
S29												–	0, 79	0, 70
S30													–	0, 78
S31														–

Table 3. Correlation coefficients among sites for leaf rust infection

NAME	S4	S6	S8	S9	S11	S12	S14	S16	S23	S29	S31
S4	–	0, 25	0, 17	0, 07	0, 31	0, 10	0, 20	−0, 01	0, 23	0, 11	0, 06
S6		–	0, 17	0, 14	0, 33	−0, 05	0, 27	0, 25	0, 31	0, 31	−0, 06
S8			–	0, 63	0, 53	0, 58	0, 42	0, 22	0, 33	0, 13	0, 18
S9				–	0, 42	0, 49	0, 55	0, 18	0, 37	0, 12	0, 00
S11					–	0, 50	0, 41	−0, 01	0, 39	0, 06	0, 04
S12						–	0, 44	0, 33	0, 33	0, 28	0, 10
S14							–	0, 12	0, 20	0, 11	−0, 05
S16								–	0, 16	0, 36	−0, 08
S23									–	0, 26	0, 08
S29										–	0, 20
S31											–

Cruz, Bolivia, though closely associated, falls in a different quadrant. The temperature regime during the vegetative phase of the crop, separated around parallel 27° South, seems to be the key dividing factor.

The higher temperature regime of the north (Bolivia, Brazil and Paraguay) can be further subdivided to specify very high temperature sites of Campinas, Brazil and Santa Cruz, Bolivia, as separate entities in one or two independent subgroups separating them from other sites in this group. It is notable to mention that drought prone site of Cochabamba, Bolivia, because of is affect on crop cycle, also falls in this group. Lower temperature locations in Argentina, Chile and Uruguay, all grouped together, show little variation for heading days except for the location of Tres Arroyos, Argentina, which has the longest period to heading.

Although the wheat region east of the Andes is considered to be a single epidemiological zone, a large variation was observed while grouping sites for LR (Fig.1b). All sites in Brazil grouped together together as a unit, explaining similarity in virulence pattern. The closest location to the Brazilian sites for this character is La Estanzuela, Uruguay, which seems to have similar virulences for the disease. Two Chilean sites, Santigo and Chillan are grouped independently. Surprisingly Santiago shows a closer association with locations in Argentina than with Chillan, a location less than 500 km south. It seems that early stripe rust infection at Chillan is

Table 4. Correlation coefficients among sites for infection to septoria leaf blight

NAME	S2	S3	S16	S23	S24	S29	S36
S2	–	0, 16	−0, 06	0, 18	0, 25	0, 13	0, 02
S3		–	0, 17	0, 29	0, 37	0, 53	0, 39
S16			–	0, 20	0, 22	0, 13	−0, 16
S23				–	0, 41	0, 54	0, 19
S24					–	0, 29	0, 02
S29						–	0, 26
S36							–

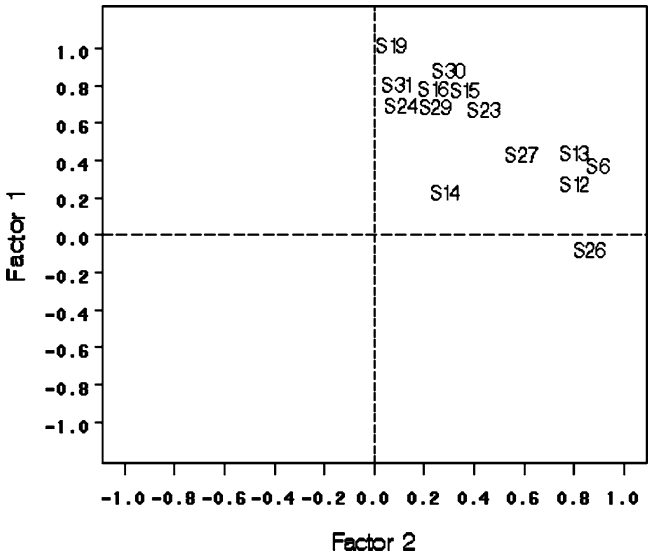


Figure 1a. Biplot, Factor Analysis Models

either causing a conflict with leaf rust evaluations or allows selection of a different virulence pattern. All heavy leaf rust infection sites in Paraguay, Uruguay and Mexico though dissimilar in their virulence pattern, group closer to each other. It seems that CRIA, Capitan Miranda, Paraguay, site is completely different from others and may be a key site to evaluate germplasm in the future.

Seven sites analyzed for septoria leaf blight infection, although much lower in number, show similar variation their groupings (Fig. 1c). Toluca, Mexico; Marcos Júarez, Argentina and Escalante, Bolivia; were each observed as independent sites unrelated with each other or with the remaining four that showed some closeness as a group. Except for Toluca, Mexico, which shows high SB infection every year, the other two sites depend on the precipitation regime of the year. Patzcuaro, Mexico is grouped together with southern locations of Chillan, Chile and La Estanzuela, Uruguay, all representing high infection sites every year. It is interesting to see that early seeding of wheat at La Estanzuela, Uruguay, to observe higher SB infection does separate out from the normal seeding date in a significant manner.

The present study was done on a limited number of characters to define the site similarities. However, factor analysis provides an effective measure to determine relationships among test locations and an indication of the germplasm adaptation. While the interpretation of the resulting groupings can be subjective, biological significance of site associations can be better assessed through correlation coefficients and the means of the agronomic parameters studied at each location. It is very clear that environmental stresses strongly influence the character evaluations in the region each year. However, the similarity among sites demonstrated in this study should serve to improve the efficiency of screening as well targeting of

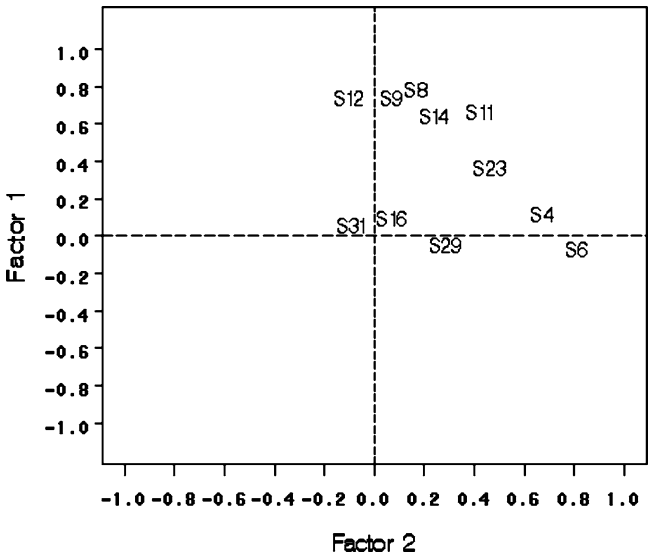


Figure 1b. Heading

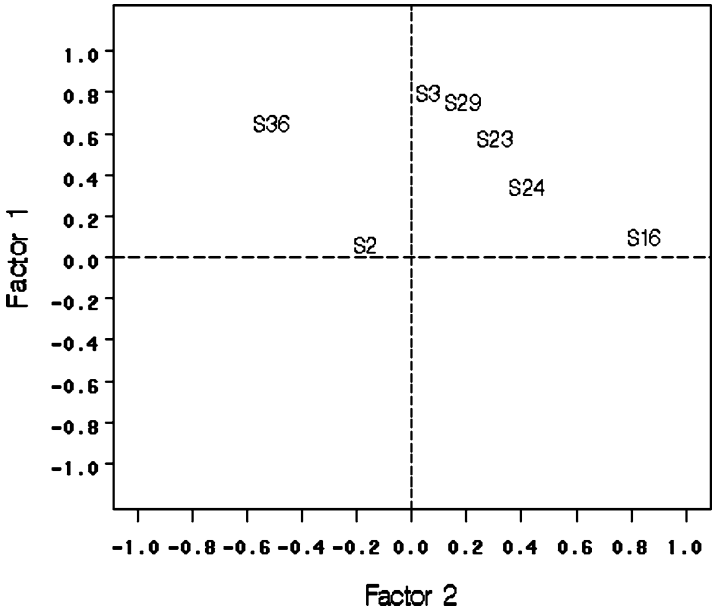


Figure 1c. Leaf rust Septoria leaf blight

future exchange of germplasm in the region. Besides modifications to the existing germplasm exchange research projects, such targeting will help enhance cultivar performance and adaptation across programs in the Southern Cone Region.

REFERENCES

Kohli MM, Ulery A, Quincke M (2003) Intercambio de germoplasma regional de trigo en el Cono Sur. En: Kohli MM, Díaz de Ackermann M y Castro M (eds) Estrategias y metodologías utilizadas en el mejoramiento de trigo: un enfoque multidisciplinario. Seminario Internacional. CIMMYT-INIA, La Estanzuela, Uruguay.

Peterson CJ, Pfeiffer WH (1989) International winter wheat evaluation: relationship among test sites based on cultivar performance. Crop Sci 29:276–282

MICROSATELLITES AS A TOOL TO EVALUATE AND CHARACTERISE BREAD WHEAT CORE COLLECTION

L. LEIŠOVÁ*, L. KUČERA AND L. DOTLAČIL

Research institute of Crop Production, Drnovska 507, 161 06 Prague, Czech Republic
**E-mail: leisova@vurv.cz*

Abstract: Hexaploid bread wheat (*Triticum aestivum*) is one of the most important crop species. 5778 accessions of winter wheat and 3929 accessions of spring wheat are hold in RICP Gene bank. Core collection is used to be established as tool for germplasm study, conservation of genetic variability and for the identification of useful genes. This information is valuable for breeders when a source of new alleles is needed

This study examines the use of neutral genetic markers to guide sampling from a large germplasm collection with the objective of establishing from it a smaller, but genetically representative sample. Genetic variation of 430 bread wheat accession was analysed using 40 microsatellite loci, covering all three wheat genomes and all 42 chromosomes (Röder et al. 1998). The cultivars originated from 32 countries mainly of the Europe. For 40 microsatellite loci, 542 alleles were detected. The average number of alleles per locus was 13.6. Cluster analysis based on microsatellites data showed that diversity in the studied file of bread wheat accessions is not randomly distributed. It can be explained mainly by geographical and by temporal impact to the breeding processes in different countries during last century

Accessing diversity at the molecular level using microsatellite analysis gave us valuable information on the genetic structure of bread wheat core collection and provided new insights on diversity of the set of winter bread wheat

Keywords: microsatellite, allelic diversity

INTRODUCTION

Loss of genetic diversity has become a problem not only of the natural plant and animal population but also agriculturally important species. Ancient cultivars or landraces and wild relatives of domesticated species are being lost as modern varieties become adopted by farmers. This had led to calls for genetic conservation

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 771–778.

of crop germplasm (Frankel and Bennett, 1970). Currently, a large number of materials from major crop species and their wild relatives are stored in gene banks, in in situ conservation sites and on farm programs of conservation. Because the large size of some of these collections, together with limited funding, combine to restrict the characterisation of the material available and hinder their use breeding purposes (Brown, 1995), an increasingly popular proposal for germplasm management is to construct smaller core collections from these larger collections. Ideally, core collections should be chosen to represent the maximum of the genetic diversity contained in the larger collection. Construction of such collection usually starts by stratifying the larger collection into a series of groups according to their genetic distances (Bataillon et al. 1996).

To obtain genetic distances, molecular markers that are highly polymorphic and easy to use are beneficial. The degree of genetic variation in wheat (*Triticum aestivum* L.) has been accessed using different types of DNA molecular markers (Koebner et al. 2001). Microsatellites are commonly used to study genetic relationships among genotypes within species because of their high level of polymorphism (Devos et al. 1995, Plaschke et al. 1995, Röder et al. 1995, Korzun et al. 1997). In addition, microsatellites exhibit a codominant inheritance (Hernandez et al. 2002), which is essential for effective discrimination between closely related lines (Akkaya et al. 1992). Microsatellite markers are currently used to identify genotypes, quantitative trait loci (QTLs) and genetic diversity (Medini et al. 2005).

Bread wheat (*Triticum aestivum* L.) is one of the most important crops in the world. It is an allohexaploid ($2n = 42$) containing three distinct but related genomes A, B and D, each with seven chromosomes. It has a large genome of 16×10^9 bp, of which more than 80% is repetitive DNA. Knowledge of genetic diversity of wheat varieties is a prerequisite for the successful management of conservation programs. The estimation of genetic variability is more efficient if highly polymorphic molecular markers as for example microsatellites are used. The first microsatellite map in wheat possessed 279 microsatellites (Röder et al. 1998), by now a total of 1235 microsatellite loci were developed and mapped (Somers et al. 2004). Recently 101 microsatellite markers derived from expressed sequence tags (EST-SSRs) were added into a set of microsatellites (Gao et al. 2004). In wheat, microsatellites have been used in genome-mapping experiments (Röder et al. 1995, Röder et al. 1998), as markers for phenotyphically desirable traits (Schmidt et al. 2004) and to study the genetic relationship of different varieties (Plaschke et al. 1995).

The aim of our study was to characterise the allelic diversity of the collection of bread wheat accessions originating from different countries in the world using a set of 40 microsatellite markers.

MATERIALS AND METHODS

Four hundred and thirty bread wheat accessions as an empiric core collection were chosen from 32 different countries (Table 1). All accessions were obtained from Gene Bank Prague-Ruzyne. About 30 wheat plants per accession were

Table 1. List of wheat accessions

Country of origin	Number of genotypes	Country of origin	Number of genotypes
Armenia	1	China	3
Australia	6	Italy	7
Austria	15	Japan	1
Belgium	4	Kazakhstan	1
Bulgaria	8	Netherlands	18
Belarus	1	Poland	3
Canada	2	Romania	5
Czech Republic	124	Russia	20
Germany	56	Slovenia	1
Denmark	3	Slovakia	1
Estonia	1	Sweden	15
Finland	3	Turkey	1
France	16	Ukraine	22
United Kingdom	28	United States of America	27
Hungary	12	Uzbekistan	1
Switzerland	5	Yugoslavia	9
Total			430

pooled and genomic DNA was isolated using a CTAB method according to Saghai-Maroof (1984).

To the study of the collection of bread wheat accessions a set of 40 microsatellite markers developed by Röder et al. (1998) were chosen. PCRs with fluorescently labelled primers (fam, hex a tet) were performed in reaction volume of 15μl according to the optimised protocol. The annealing temperature and the concentration of Mg^{2+} ions are listed in Table 2. Reactions were run in the cycler UNO II (Biometra). The analysis of PCR products was performed using the method of capillary electrophoresis on the sequencer ABI PRISM 310 (Perkin-Elmer, Foster City, USA). A multiplexed configuration of three or four reactions was used in one analysis. As to interne size standard TAMRA500 (Applied Biosystems, Foster City, USA) was used. Electrophoretograms were processed by GeneScan and Genotyper software.

For each locus the presence or absence of bands in each size category through all genotypes was scored. Data were set in a binary matrix. Genetic similarities were calculated using the Dice coefficient and dendrogram obtained by clustering according to the UPGMA method using the program Statistica. For the detection of alleles' frequency Microsoft Excel software was used.

RESULTS AND DISCUSSION

Cultivars were sampled from 32 countries all over the world. They were chosen as an expert core collection from Prague Gene Bank accessions so all distinct genotypes of

Table 2. List of microsatellite markers used in this study

SSR primer	Repetition motif	Chromosome location	Annealing temper. (°C)	Mg2+ conc.	Range of product size (bp)	Number of alleles
Xgwm 2	(CA)18	3AS	60	1.5 mM	112–128	6
Xgwm 11	(TA)6CATA(CA) 19(TA)6	1BS	60	2.0 mM	191–221	13
Xgwm 44	(GA)28	7DS	60	1.5 mM	164–190	13
Xgwm 46	(GA)2GC(GA)33	7BS	60	2.0 mM	146–188	16
Xgwm 99	(CA)21	1AL	60	2.0 mM	98–142	20
Xgwm 120	(CT)11 (CA)18	2BL	60	1.5 mM	120–159	14
Xgwm 135	(GA)20	1AL	60	2.0 mM	137–199	18
Xgwm 149	(GA)23imp	4BL	55	2.0 mM	135–177	8
Xgwm 169	(GA)23	6AS	53	2.0 mM	176–238	22
Xgwm 186	(GA)26	5AL	53	2.0 mM	101–151	16
Xgwm 190	(CT)22	5DS	60	2.0 mM	202–236	8
Xgwm 194	(CT)32imp	4DL	60	2.0 mM	113–141	15
Xgwm 219	(GA)35imp	6BS	60	2.0 mM	149–223	20
Xgwm 233	(CT)24	7AL	45	1.5 mM	238–276	10
Xgwm 234	(CT)16(CA)20	5BS	50	1.5 mM	225–251	11
Xgwm 251	(CA)28	4BL	55	2.0 mM	81–123	17
Xgwm 257	(GT)30	2BS	60	1.5 mM	193–199	4
Xgwm 260	(GA)20	7AS	60	2.0 mM	146–176	17
Xgwm 261	(CT)21	2DL	60	1.5 mM	165–215	13
Xgwm 272	(CA)17	5DL	50	2.0 mM	127–145	7
Xgwm 285	(GA)27	3BS	50	1.5 mM	204–258	14
Xgwm 312	(GA)37	2AS	55	2.0 mM	192–268	22
Xgwm 325	(CT)16	6DL	60	2.0 mM	133–151	9
Xgwm 337	(CT)5(CACT) 6(CA)43	1DS	55	2.0 mM	171–207	16
Xgwm 341	(CT)26	3DL	55	1.5 mM	124–154	16
Xgwm 372	(GA)> 51	2AL	60	1.5 mM	285–345	23
Xgwm 400	(CA)21	7BS	60	1.5 mM	130–160	15
Xgwm 408	(CA)> 22(TA)(CA)7(TA)9	5BL	55	1.5 mM	147–205	17
Xgwm 413	(GA)18	1BS	60	2.0 mM	88–118	15
Xgwm 415	(GA)25imp	5AS	55	2.0 mM	127–133	6
Xgwm 427	(CA)31 (CA)22	6AS	50	2.0 mM	192–234	12
Xgwm 437	(CT)24	7DL	60	2.0 mM	91–127	16
Xgwm 469	(CT)19(CA)10	6DL	60	2.0 mM	152–184	14
Xgwm 480	(CT)16 (CA)13	3AL	57	1.5 mM	171–197	9
Xgwm 539	(GA)27	2DS	60	1.5 mM	123–201	31
Xgwm 566	(CA)21(GA)2(TA)8	3BL	60	2.0 mM	118–136	9
Xgwm 610	(GA)17imp	4AL	60	2.0 mM	152–196	14
Xgwm 626	(CT)5(GT)13	6BS	50	2.0 mM	104–135	5
Xgwm 642	(GT)14	1DL	60	2.0 mM	177–209	8
Xgwm 664	(GA)22	3DL	55	2.0 mM	151–155	3
					Total:	**542**

bread wheat were represented. Leaves from about 30 plants in stage of 2 leaves were used for DNA extraction and microsatellite analysis. Most amplification products revealed only one allele per genotype. When two or three alleles were present at a single locus and genotype, the all were taken into account because especially landraces and the old varieties had a character of populations with several different lines and some of them could carry an agronomically interesting allele. In total 542 alleles were detected from the 40 amplified loci. The total number of alleles per locus ranged from 3 for locus *Xgwm664* to 31 for locus *Xgwm539*, with an average of 13.6 alleles per locus. 4.1% of the data were considered to be null alleles. Null microsatellite alleles are relatively common in wheat (Plaschke et al. 1995) and can arise from point mutation(s) in one or both of the priming sites or by deletions.

The UPGMA cluster analysis established from the similarity data reflects geographical origins of the accessions. The Czech lines/varieties are mainly included in three clusters according to their genetic kinships with foreigner lines/varieties. The first cluster is mainly formed by landraces and old varieties from the Czech Republic. The second cluster is formed by accessions from Germany and from countries of the north of Europe. In the third cluster accessions from countries of central Europe are included. The fourth and the sixth cluster are represented by English and Dutch and French varieties. In the fifth cluster there is a lot of Czech varieties whose breeding was influenced by varieties form the south-east of Europe. Accessions from the USA and Canada form the sixth cluster. In the seventh cluster there are Czech varieties influenced with Russian and Ukrainian varieties. There are also a lot of Bulgarian and Hungarian varieties. This clustering is not surprising, it generally agrees with previous study accomplished by Roussel et al. (2005). The fact that Czech varieties are placed in several clusters could be explained by the localisation of the Czech Republic in the centre of the Europe and by four different trends in bread wheat breeding during last century.

In total, 542 alleles were found. The analysis of alleles' requency showed that some of them are widely spread through all studied genotypes, for example allele 238 of the locus *Xgwm642* or allele 133 of the locus *Xgwm415* (Fig. 1). Other alleles are specific to some region; for example allele 177 of the locus *Xgwm408* and allele 152 of the locus *Xgwm260* are specific to Czech varieties. Allele 113 of the locus *Xgwm186* occurs in Russian variety Mironovska808 and also in many varieties in whose pedigree Mironovska 808 is included. Allele 193 of the locus *Xgwm480* is specific for mainly Czech varieties with an alternative form of sowing. 16% of detected alleles are found to be unique within the studied set of bread wheat varieties. Locus *Xgwm610* can be an example with several unique alleles (Fig. 2).

The distribution of alleles differs from a normal distribution. The creation of a core collection on the base of a random sampling may lead to the considerable change of a character of alleles' frequency distribution. A lot of unique and rare alleles may be lost. That is why it is necessary to take into account this fact and choose the strategy that conserve the most unique or rare alleles as possible. Two classes of alleles: specific to some region with high frequency and widespread with low frequency are of more interest, especially as the former may be a source of

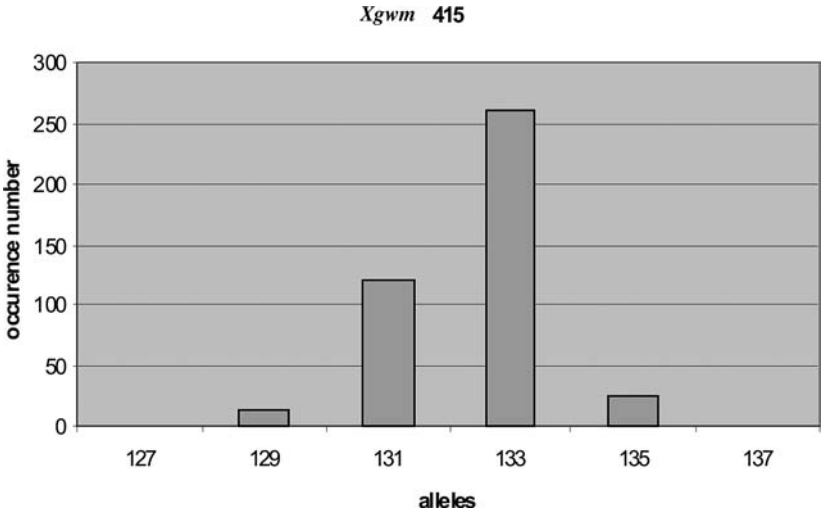


Figure 1. The alleles' frequency of microsatellite locus *Xgwn 415*

local adaptation (Bataillon et al. 1996). Not only core collection creation but also breeding processes can lead to the loss of alleles as showed Roussel et al. 2005.

Alleles appear or disappear as they fall prey to a mutation processes, in case of microsatellites to deletions or insertions of the repeat motifs. That is why it is interesting to follow if bread wheat breeding results in higher frequency of shorter or

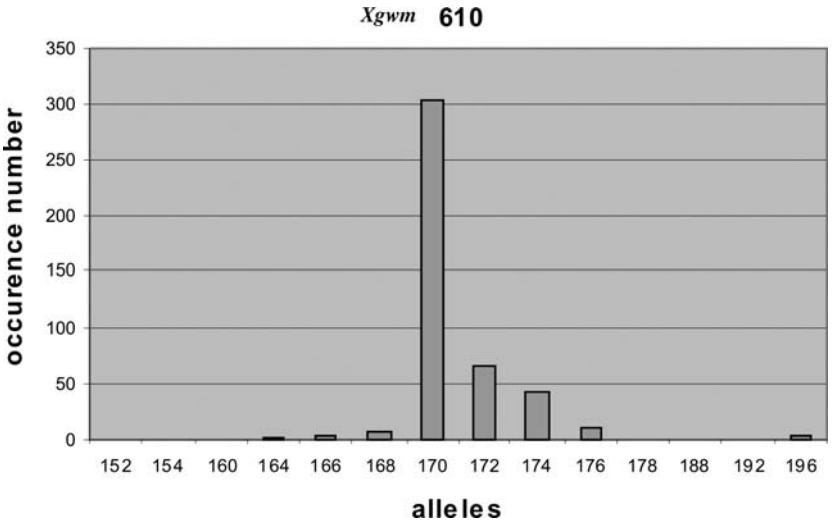


Figure 2. The alleles' frequency of microsatellite locus *Xgwm 610*

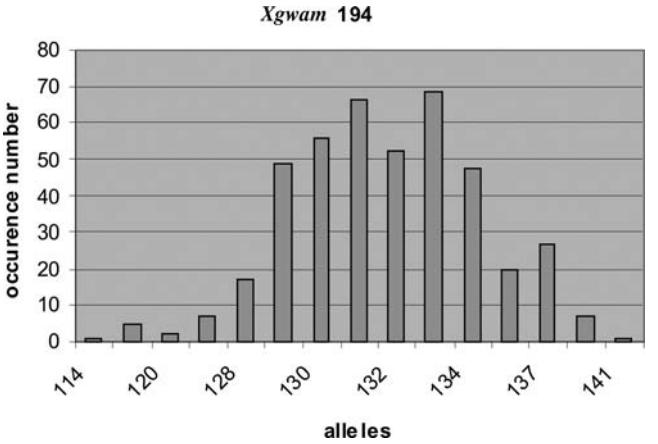


Figure 3. The alleles' frequency of microsatellite locus *Xgwm 194*

longer alleles. As for locus *Xgwm 194* older varieties and landraces carry shorter and low frequency alleles and newer varieties contain rather shorter alleles (Fig. 3). But there are other microsatellite locuses where shorter alleles appear during breeding processes or rise up their frequency.

For the development of core collections, it is necessary to take into account all these facts. As genebank collections are increasingly searched for specific genotypes or even structural features of a specific gene the information about alleles' structure and frequency is very important. That is why molecular markers including microsatellites are powerful tools to help in the development of the really contributing bread wheat core collection.

ACKNOWLEDGEMENTS

This work was supported by the Czech Ministry of Agriculture, project n°: 0002700602.

The authors thank Hana Udavská for an excellent technical assistance.

REFERENCES

Akkaya MS, Bhagwat AA, Cregan PB (1992) Length polymorphism of simple sequence repeat DNA in soybean. Genetics 132:131–139

Batallion TM, David JL, Schoen DJ (1996) Neutral genetic markers and conservation genetics: simulated germplasm collections. Genetics 144:409–417

Brown AHD (1995) The core collection at the crossroads, In: Hodgkin T, Brown AHD, van Hintum TJL, Morales AEV (eds) Core collections of plant genetic resources, John Wiley, Sons Ltd., Chichester, UK. pp 3–19

Devos KM, Bryan GJ, Collins AJ, Stephenson P, Gale MD (1995) Application of two microsatellite sequences in wheat storage proteins as molecular markers. Theor Appl Genet 90:241–252

Frankel OH, Bennett E (1970) Genetic resources in plants – their exploration and conservation. Davis FA. Philadelphia, USA

Gao LF, Jing RL, Huo NX, Li Y, Li XP, Zhou RH, Chang XP, Tang JF, Ma ZY, Jia JZ (2004) One hundred and one new microsatellite loci derived from ESTs (EST-SSRs) in bread wheat. Theor Appl Genet 108:1392–1400

Hernandez P, Laurie DA, Martin A, Snape JW (2002) Utility of wheat simple sequence repeat (SSR) markers for genetic analysis of *Hordeum chilense* and *H. tritordeum*. Theor Appl Genet 104:535–739

Koebner RMD, Powell W, Donini P (2001) The contribution of current and forthcoming DNA molecular marker technologies to wheat and barley genetics and breeding. Plant Breed Rev 21:181–220

Korzun V, Röder MS, Worland AJ, Börner A (1997) Intrachromosomal mapping of the genes for dwarfing (*Rht12*) and vernalisation response (Vrn1) in wheat by using RFLP and microsatellite markers. Plant Breed 116:227–232

Medini M, Hamza S, Rebai A, Baum M (2005) Analysis of genetic diversity in Tunisian durum wheat cultivars and related wild species by SSR and AFLP markers. Genet Resour Crop Evol 52:21–31

Plaschke J, Ganal MW, Röder MS (1995) Detection of genetic diversity in closely related bread wheat using microsatellite markers. Theor Appl Genet 91:1001–1007

Röder MS, Plaschke J, König SU, Börner A, Sorrels ME, Tanksley SD, Ganal MW (1995) Abundance, variability and chromosomal location of microsatellites in wheat. Mol Genet Genom 246:327–333

Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier MH, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007–2023

Roussel V, Leisova L, Exbrayat F, Stehno Z, Balfourier F (2005) SSR allelic diversity chynges in 480 European bread wheat varieties released from 1840 to 2000. Theor Appl Genet 111:162–170

Saghai Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal locations, and population dynamics. Proc Natl Acad Sci Unit States Am 81:8014–8018

Schmidt AL, Gale KR, Ellis MH, Giffard PM (2004) Sequence variation at a microsatellite locus (Xgwm261) in hexaploid wheat (*Triticum aestivum*) varieties. Euphytica 135:239–246

Somers DJ, Isaac P, Edwards K (2004) A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theor Appl Genet 109:1105–1114

MOLECULAR MAPPING OF LEAF AND STRIPE RUST RESISTANCE GENES IN *T. MONOCOCCUM* AND THEIR TRANSFER TO HEXAPLOID WHEAT

K. SINGH[1,*], P. CHHUNEJA[1], M. GHAI[1], S. KAUR[1], R.K. GOEL[1], N.S. BAINS[1], B. KELLER[2] AND H.S. DHALIWAL[3]

[1]*Dept. Plant Breeding, Genetics and Biotechnology, Punjab Agricultural University, Ludhiana 141 004, India*

[2]*Institute of Plant Biology, University of Zurich, Switzerland*

[3]*Indian Institute of Technology, Roorkee, India*

[*]*E-mail: kuldeep35@yahoo.com*

Abstract: Wheat as a crop has benefited immensely from genetic improvement programmes but wheat production is still being challenged constantly by several diseases; among them, rusts are the most prominent. Leaf rust is the most widely distributed desease of wheat despite the fact that major emphasis has been made to develop rust resistant varieties. Deployment of major genes has often turned out to be non-durable and in India most of the genes identified from cultivated germplasm are not effective against the prevalent pathotypes of leaf rust. A spring type *Triticum monococcum* (acc. 14087) has maintained a high level of resistance to Indian isolates of leaf and stripe rust. Genetic studies using a set of 125 recombinant inbred lines (RILs), developed from a cross *T. monococcum* (acc. 14087)/*T. boeoticum* (acc. 5088) revealed that both *T. monococcum* and *T. boeoticum* have one APR gene that confers resistance to stripe rust and one seedling and one adult plant resistance gene for leaf rust. A genome linkage map with more than 150 markers, including RFLPs, SSRs and bin mapped ESTs, has been generated using the RIL population. QTL analysis revealed the presence of leaf and stripe rust resistance genes on chromosome 2A. Attempts were made to transfer both leaf and stripe rust resistance genes from *T. monococcum* to hexaploid wheat using *T. durum* cv N59 as a bridging species. Screening of F_1 and backcross generations revealed that B genome of *T. durum* suppresses resistance of *T. monococcum*. With subsequent backcrosses one seedling and one APR gene for leaf rust and one APR gene for stripe rust resistance have been transferred from *T. monococcum* to bread wheat cv. WL711 and one APR gene for leaf rust has been transferred in PBW343 background. Chromosome number of the resistant plants varied from 39–41. The leaf and stripe rust resistant plants are being analyzed with SSR markers that were found to be associated with leaf and stripe rust resistance genes based on QTL mapping in the RIL population

Keywords: QTL, rust resistance

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 779–786.

INTRODUCTION

Wheat as a crop has benefited immensely from genetic improvement programmes but wheat production is still being challenged constantly by several diseases; among them, rust diseases are the most prominent. Leaf rust is the most widely distributed disease of wheat, causing an average annual yield loss of 3 per cent worldwide (http://www.usda.gov/nass) despite the fact that major emphasis has been made to develop rust resistant varieties. However, after a period of time, resistance gene deployments have often turned out to be non-durable. Most of the genes identified from cultivated germplasm are not effective against the prevalent isolates of *P. triticina* f. spp. *tritici* I India (Tomar and Menon 2001). Due to a narrow genetic base and continuously evolving pathogen races, resistant varieties become susceptible to leaf rust when grown in vast areas. Some of the genes transferred from distantly species related have been exploited to some extent but others seem to be associated with yield penalty due to linkage drag that is difficult to eliminate even after repeated backcrosses, once it is selected in the first segregating generation (Young et al. 1988). The diploid 'A' genome progenitor gene pool of wheat contains many economically important alleles for resistance to diseases which might be transferred to cultivated wheat and utilized for its improvement (Feldman and Sears 1981). *Triticum monococcum* (A^mA^m) and *T. boeoticum* (A^bA^b) that harbour several desirable genes (Dhaliwal et al. 1993, Hussien et al. 1998) shares considerable homology with the A genomes of cultivated tetraploid ($2n = 2x = 28$; genome AABB) and hexaploid wheat ($2n = 2x = 42$; genome AABBDD) (Dvorak et al. 1993). Thus, direct hybridization may enable efficient transfer of desirable alleles from the 'wild' chromosome into its 'cultivated' homologues without any linkage drag. These wheats have a distinct mechanism of rust resistance, which acts at the pre-haustorial stage (Anker and Niks 2001). The same mechanism of resistance is indicated in the two best-known durable resistance genes in wheat: *Lr34* and *Lr46* (Rubales and Niks 1995, Singh and Rajaram 2002) and is the most likely cause of that durability (Niks and Rubiales 2002). While such genes are rare in cultivated germplasm, diploid A genome wheats can serve as valuable donors. A spring type *T. monococcum* (Acc. 14087) has maintained a high level of resistance for years to most virulent pathotypes of leaf and stripe rusts over years (Dhaliwal et al. 1993). A recombinant inbred line population (RIL) has been developed by crossing *T. monococcum* (Acc. 14087) and *T. boeoticum* (Acc. 5088), using single seed descent method. The RIL population segregated for resistance to leaf and stripe rust, and several other diseases and domestication traits (Dhaliwal et al. 2003). A set of 125 F_8 RILs has been screened for studying genetics of leaf and stripe rust resistance and mapping of genes governing the resistance to these diseases using a frame work map consisting of more than 150 markers. Also these genes have been introgressed into hexaploid wheat through direct crosses using *T. durum* as a bridging species.

MATERIAL AND METHODS

Mapping of Leaf and Stripe Rust Resistance Genes in *T. monococcum*

A set of 125 RILs generated from a cross involving *T. monococcum* acc. 14087 and *T. boeoticum* acc.5088 was used to study inheritance of leaf and stripe rust resistance in *T. monococcum* as detailed in Dhaliwal et al. (2003). Briefly, the RIL population was screened at the seedling stage against three leaf rust pathotypes 109R 31-1 (77-2), 121R 63-1 (77-5) and 21R5 (104-2) and three stripe rust pathotypes 46S103, 46S102, 46S119 following Nayer et al. (1997). Infection types 0, 1, 2, and X were considered resistant and 3 and 4, susceptible. The RIL population was also screened at adult plant stage against stripe rust pathotype 46S119, that is virulent on *Yr*9. A framework linkage map having both SSR and RFLP markers, including bin mapped ESTs, was generated using the *T. monococcum*/*T. boeoticum* RIL population. Both parents were resistant to various pathotypes of leaf and stripe rust but the population showed segregation thereby indicating that the resistance genes in these lines were different, since the population was segregating for more than one gene for both the pathotypes. A linkage map was generated using a series of SSR markers including *gwm*, *gdm*, *wmc*, *cfa*, and *cfd* and RFLP markers including bin mapped ESTs. The PCR amplified products were first resolved in 2.5% agarose gel and the ones which were monomorphic in agarose gels were resolved in 8.0% polyacrylamide gels using LICOR sequencer. QTL analysis was performed using the software package PLAB QTL (Utz and Melchinger, 1996, http://www.uni-hohenheim.de)

Transfer of Leaf and Stripe Rust Resistance to Hexaploid Wheat

T. monococcum acc. 14087 and one RIL (designated as RIL 101) were crossed to a *T. durum* line N59 that is susceptible to both leaf and stripe rusts. PBW343 and WL711 were used as the hexaploid recipient parent. WL711 was a land mark variety that has now become susceptible to both the leaf and stripe rusts and PBW 343 is the currently most widely grown variety in India occupying around 7.0 million hectares. This variety is resistant to stripe rust but has now become susceptible to leaf rust pathotype 77-5, both at seedling and adult plant stages. The triploid F_1 plants of the cross N59/*T. monococcum* and N59/RIL101 were backcrossed to N59 and both hexaploid wheat varieties WL711 and PBW 343. The resulting tetraploid and pentaploid plants, respectively, were screened against both rusts at adult plant stage as described above and the F_1 pentaploid plants of the crosses N59/*T. monococcum*//WL 711 or PBW 343 were backcrossed to the recurrent hexaploid parents to generate BC_1F_1 plants. Crosses with RIL 101 were also followed the same way. The backcross progenies were screened at the seedling stage with leaf rust pathotype 77-5 and at adult plant stage with a mixture of above mentioned pathotypes of leaf rust and stripe rusts. The plants, after screening at seedling stage, were transplanted in the field and evaluated for terminal disease severity for leaf rust and stripe rust at adult plant stage. Chromosome number of

the plants in various generations was analyzed from pollen mother cells (PMCs) using standard acetocarmine squashing technique.

RESULTS AND DISCUSSIONS

Generation of A genome linkage map: A total of 117 *gwm* primer pairs were tested for polymorphism among which 92 gave amplified products thereby showing a very high level of transferability of the markers generated from cultivated hexaploid wheat. Similarly other SSR markers were also analyzed and polymorphic ones mapped onto the linkage map. Majority (78%) of the A genome specific markers of cultivated hexaploid wheat are translated in *T. monococcum* and *T. boeoticum*. Similarly 60% of B genome specific markers and 42% of D genome specific markers were amplified indicating that the A and B genomes are more closely related than the D genome. A framework linkage map with more than 150 markers has been generated, and includes both SSR and RFLP markers.

Mapping of leaf and stripe rust resistance genes in *T. monococcum* and *T. boeoticum*: The RIL population was screened extensively against three leaf rust and three stripe rust pathotypes both at seedling and at adult plant stages and the number of genes conferring resistance in *T. monococcum* and *T. boeoticum* were identified (Dhaliwal et al. 2003). Table 1 shows disease reaction of parents and segregation pattern of RILs against leaf rust pathotype 77-2. Both parents were resistant but the population segregated for two genes ($\chi^2 = 1.03$), thereby indicating presence of one gene in *T. monococcum* and one gene in *T. boeoticum*. Phenotypic data of the RILs was regressed on the genotypic data using PLABQTL software package. Two QTL for leaf rust resistance genes and one for stripe rust resistance gene mapped on chromosome 2. Closely linked markers were fba374 and *Psr*540 for leaf rust and Cfd267 for stripe rust. Thus both leaf rust and stripe rust resistance gene seem to be located in chromosome 2. No leaf or stripe rust resistance genes have been reported in this region of the chromosome before. These thus may be new genes. The map is being enriched and we are in the process of identifying markers closely linked to the leaf and stripe rust resistance genes in *T. monococcum*.

Table 1. Seedling reaction of parents and RIL population against races 77–2 of *Puccinia triticina*

Parent/generation	No. of plants/RILs with infection type								
	0;	;	;1$^-$ to ;1	1– 1$^+$	2	X	3	3$^+$	Total plant/RILs
T. monococcum Acc (14087)	–	4	18	–	–		–	–	22
T. boeoticum Acc (5088)	19	–	–	–	–		–	–	19
F_6RILs	18	9	37	12	10	4	13	13	116

Diploid wheat *Triticum monococcum* L. (2n = 2x = 14, AA) has also been reported as highly resistant to wheat leaf rust (Dyck and Bartos 1994, Niks and Dekens 1991). Some accessions of *T. monococcum* show a high degree of pre-haustorial resistance (Anker and Niks 2001). This resistance, common in non-host interactions, is not based on hypersensivity and is presumed to be more durable. The non-hyperensitive resistance is more useful because it does not force selection pressure on the pathogen and reduces the chances of evolution of new races. The A-genome of *T. monococcum*, closely related to A- genome of polyploid wheat, can be used to transfer useful variability by direct hybridization, homologous chromosome recombination, backcrossing and selection with minimum linkage drag.

Gene Transfer in Tetraploid and Hexaploid Wheat

T. durum suppresses the resistance genes of T. monococcum: Gene transfer from *T. monococcum* to *T. durum* and *T. aestivum* was carried out by direct crosses and no embryo rescue was required. These F_1 plants were vigorous, triploid with 2n = 21 but were completely male sterile, although the chromosome pairing between the A genome of tetraploid wheat and the A genome of *T. monococcum* was high. Up to six bivalents were observed in the F_1 (Fig. 1a). Similarly the BC_1F_1 plants showed chromosome number ranging from 2n = 28 to 29 (Fig. 1b & c), attaining stable chromosomal constitution in one backcross. The F_1 hybrids between common or durum wheat and related species generally are male sterile but usually set seed when backcrossed, although seed set was very low (Table 2).

T. durum cv N59 is highly susceptible to both rusts at seedling as well as adult plant stage. The F_1 was susceptible at seedling as well as adult plant stage thereby indicating that the resistance in *T. monococcum* is either recessive or is being suppressed by *T. durum* genome. Further, the F_1 plants were backcrossed with recurrent parent N59 to generate BC_1F_1 and with two hexaploid wheat cultivars WL711 and PBW343. The BC_1F_1 plants of the cross N59/*T. monococcum*//N59 and F_1 plants of the cross N59/*T. monococcum*//WL711 or PBW343 were all susceptible

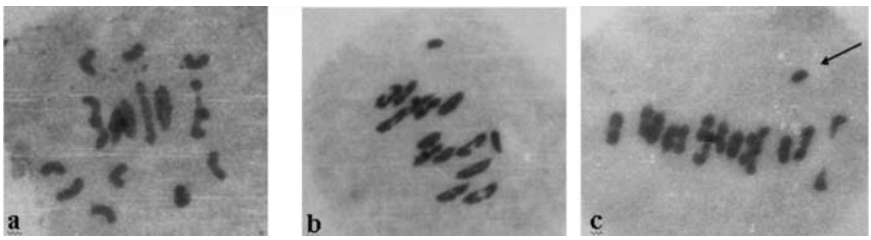


Figure 1. Pollen mother cells of the F_1 (a) and BC_1F_1 (b & c) plant of the cross *T. durum* cv N59/*T. monococcum* and *T. durum* cv N59/*T. monococcum* with $2n = 21(6'' + 9')$, $2n = 28\ (13'' + 2')$ and $2n = 29\ (14'' + 1')$ respectively

Table 2. Seed set in triploid F_1 hybrids when pollinated with tetraploid and hexaploid wheat

Cross	Florets pollinated	Seeds obtained	Per cent seed set
N59/*T. monococcum*//N59	772	35	4.53
N59/RIL86//N59	2234	47	2.10
N59/RIL101//N59	905	18	1.99
N59/RIL 130//N59	1484	39	2.63
N59/*T. monococcum*//PBW343	1230	37	3.0
N59/RIL86//PBW343	2475	21	0.84
N59/RIL101//PBW343	1152	35	3.04
N59/RIL 130//PBW343	719	10	1.039
Total	10971	242	2.2

at seedling stage as well as adult plant stages (Table 3). Similarly BC_1F_2 plants of N59/*T. monococcum*//N59 were also susceptible when tested at adult plant stage under filed conditions. This clearly shows that the B-genome of *T. durum* suppresses the leaf as well as stripe rust resistance of A genome of *T. monococcum* when transferred in tetraploid background. To further clarify whether only N59 has this mechanism or it is present in other durum varieties as well, the *T. monococcum* was crossed with two other susceptible *T. durum* lines MACS1967 and Malvi local. The F_1s of both crosses were susceptible to both leaf and stripe rusts, thereby indicating that the suppression mechanism in *T. durum* may be widespread. *T. durum* genome suppressing the stem rust resistance genes in durum itself has been shown elegantly by Knott (2000) and *T. durum* suppressing the disease resistance of D genome in the amphiploids has been shown in a series of publications (Ma et al. 1997, Qiu et al. 2005).

Generation of Backcross Progenies and Transfer of Leaf and Stripe Rust Resistance into Hexaploid Wheat

Based on the disease reaction of the F_1 of the cross N59/*T. monococcum*, its BC_1F_1 and BC_1F_2 and the F_1 of the crosses N59/*T. monococcum*//*T. aestivum* cv WL711 or PBW 343, it became clear that the B genome of *T. durum* suppresses

Table 3. Leaf rust reaction of BC_1F_1 progeny under field conditions

Cross	No. of plants	Disease reaction
BC_1F_1 [N59/*T. monococcum*//N59]	33	60–80S
BC_1F_1 [N59/R86//N59]	30	60–80S
BC_1F_1 [N59/R101//N59]	17	60–80S
BC_1F_1 [N59/RIL 130//N59]	20	60–80S
Total	100	

Table 4. Frequency of leaf and stripe rust resistant plants in the backcross progenies of various crosses

Cross	No. of plants resistant to			
	Total plants screened	Leaf rust only	Stripe rust only	Leaf & Stripe rust
N59/*T. monococcum*//2*PBW343	161	8	–[a]	–
N59/RIL101//2*PBW343	170	14	–	–
N59/RIL130//2*PBW343	65		–	–
N59/*T. monococcum*//2*WL711	25	14	0	1
N59/RIL86//2*WL711	27	3	0	1
N59/RIL101//2*WL711	111	40	4	10
Total	559	83	4	12

Note: RILs are from the cross *T. monococcum*/*T. boeoticum*

[a] Since PBW343 is resistant to stripe rust at adult plant stage it was not possible to identify the plant having stripe rust resistance transferred from *T. monococcum*

the leaf and stripe rust resistance of *T. monococcum*. Therefore it should be possible to obtain some resistant plants in the BC_1F_1 generation of the cross N59/*T. monococcum*//2*WL711 or PBW343. All the F_1 plants of the crosses N59/*T. monococcum*//WL711 or PBW343 were backcrossed with the recurrent parent. The B genome of *T. durum* would segregate in this generation and plants having resistance genes from *T. monococcum* but no suppressor gene should be resistant. All the seedlings were screened with leaf rust pathotype 77-5 and transplanted in the field after screening. These were screened with a mixture of leaf and stripe rust races under field conditions as well. Disease reaction of the BC_1F_1 plants at seedling and adult plant stage is presented in Table 4. A good number of plants having resistance to leaf rust or stripe rust or both have been obtained. Chromosome number of these plants ranged from 38–42. These plants have been selfed to identify the plants homozygous for leaf and stripe rust resistance genes. Molecular analysis of the resistant plants for chromosome 2 is in progress.

ACKNOWLEDGEMENTS

Financial support for this work by DBT, Govt. of India and SDC, Switzerland under Indo–Swiss collaboration in biotechnology is gratefully acknowledged.

REFERENCES

Anker CC, Niks RE (2001) Prehaustorial resistance to the wheat leaf rust fungus, Puccinia triticina, in Triticum monococcum (ss). Euphytica 117:209–215

Dhaliwal HS, Chhuneja P, Singh I, Ghai M, Goel RK, Garg M, Keller B, Röder M, Singh K (2003) *Triticum monococcum* - a novel source for transfer and exploitation of disease resistance in wheat. In 10th International Wheat Genetics Symposium, Paestum Italy, Sept. 1–6, 2003. pp 346–349

Dhaliwal HS, Singh H, Singh KS, Randhawa HS (1993) Evaluation and cataloguing of wheat germplasm for disease resistance and quality. In: Damania AB (ed) Biodiversity and wheat improvement. John Wiley, Sons Pub. pp 123–140

Dvorak J, Terlizzi P, Zhang HB, Resta P (1993) The evolution of polyploidy wheat: identification of the A genome donor species. Genome 36:21–31

Dyck PL, Bartos P (1994) Attempted transfer of leaf rust resistance from *Triticum monococcum* and durum wheat to hexaploid wheat. Can J Plant Sci 74:733–736

Feldman M, Sears ER (1981) The wild gene resources of wheat. Sci Am 244:102–112

Hussien T, Bowden RL, Gill BS, Cox TS, Marshall DS (1997) Performance of four new leaf rust resistance genes transferred to common wheat from *Aegilops tauschii* and *Triticum monococcum*. Plant Dis 81:582–586

Knott DR (2000) Inheritance of resistance to stem rust in medea durum wheat and the role of suppressors. Crop Sci 40:98–102

Ma H, Singh RP, Mujeeb Kazi A (1997) Resistance to stripe rust in durum wheats, A-genome diploids, and their amphiploids. Euphytica 94:279–286

Nayar SK, Prashar M, Bhardwaj SC (1997) Manual of current techniques in wheat rusts. Regional Station, Flowerdale, Shimla 171002, India. Res Bull No.2 pp 32

Niks RE, Rubiales D (2002) Potentially durable resistance mechanisms in plants to specialized fungal pathogens. Euphytica 124:201–216

Niks RE, Dekens RG (1991) Prehaustorial and posthaustorial resistance to wheat leaf rust in diploid wheat seedlings. Phytopathology 81:847–851

Qiu YC, Zhou RH, Kong XY, Zhang SS, Jia JZ (2005) Microsatellite mapping of a *Triticum urartu* derived powdery mildew resistance gene transferred to common wheat (*Triticum aestivum* L.). Theor Appl Genet DOI 10.1007/s00122-005-0081-5

Rubiales D, Niks RE (1995) Characterization of Lr34 a major gene conferring nonhypersensitive resistance to wheat leaf rust. Plant Disease 79:1208–1212

Singh RP, Rajaram S (2002) Breeding for disease resistance in wheat. In: Curtis BC, Rajaram S, Gómez Macpherson H (eds) Bread Wheat – Improvement and Production Edited. FAO Plant Production and Protection Series

Tomar SMS, Menon MK (2001) Genes for resistance to rusts and powdery mildew in wheat. IARI publication, New Delhi, India pp 1–52

Utz HF, Melchinger AE (1996) PLABQTL: A program for composite interval mapping of QTL. J Quantitative Trait Loci Band 2, Heft 1, 1996 (www.uni-hohenheim.de)

Young ND, Tabksley SD (1989) RFLP analysis of size of chromosomal segment retained around TM-2 locus of tomato during backcross breeding. Theor Appl Gent 77:353–357

BORLAUG, STRAMPELLI AND THE WORLDWIDE DISTRIBUTION OF *RHT8*

M.H. ELLIS[1,2,*], D.G. BONNETT[1,2] AND G.J. REBETZKE[1]

[1]*CSIRO Plant Industry, GPO Box 1600, Canberra ACT2601, Australia*
[2]*Graingene, 65 Canberra Avenue, Griffith ACT2603, Australia*
[*]*E-mail: marc.ellis@csiro.au*

Abstract: The height-reducing gene *Rht8* was introduced by breeder Nazareno Strampelli from the Japanese landrace 'Akakomugi' and has been widely used in wheats adapted to southern and eastern Europe

Definitive identification of *Rht8* is difficult because it does not make seedlings insensitive to gibberellin application and its height effect may be confounded with other genes affecting plant height or flowering time. Following identification of a close linkage between microsatellite *WMS261* and *Rht8*, the marker has been used extensively to screen large numbers of diverse international germplasm. A 192bp allele at the *WMS261* locus was assumed to be 'diagnostic' for *Rht8* and its international distribution was inferred from the marker data

We report several instances of cultivars and mapping populations which vary for the presence of the *Xgwm261*-192bp allele, but with no associated reduction in height, suggesting a lack of linkage with *Rht8*

Our studies have identified that Norin10, used by Norman Borlaug to introduce *Rht-B1b* and *Rht-D1b* into Mexican wheats, also carries *WMS261*-192bp, and this allele was retained in some of his varieties. The widespread use of Norin10-derived germplasm during and after the green revolution represents a second source of *WMS261*-192bp microsatellite allele in international germplasm, with no linkage to *Rht8*. Therefore, the presence of this allele can only be indicative of *Rht8* in wheat varieties with a clear pedigree relationship to Akakomugi or Strampelli wheats

Keywords: wheat, gene distribution, height-reducing gene

INTRODUCTION

Height reduction is one of the single most important adaptations introduced in cereals by breeders in the last century. Shorter plants are less susceptible to lodging and partition more of the available assimilates to the grain. In wheat, the large

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 787–791

increases in yield achieved during the green revolution have been associated with the introduction of the reduced height (Rht) genes *Rht-B1b* and *Rht-D1b* in the CIMMYT breeding program by Norman Borlaug. Originally from the Japanese variety 'Norin10', these genes became prevalent in CIMMYT wheats, and are now found in the majority of modern wheat cultivars. Pre-dating these plant breeding breakthroughs, the Italian wheat breeder N. Strampelli used height reducing genes from another Japanese wheat, 'Akagomugi', to improve the lodging resistance and yield potential of the landrace 'Rieti originale'. Akagomugi is known to contain the height-reducing genes *Rht8* and *Rht9*, as well as a photoperiod-insensitivity gene linked to *Rht8*.

Using a chromosome substitution line between 'Capelle Desprez' and the Strampelli wheat variety Mara, Korzun et al. (1998) reported a tight linkage (0.6 cM) between *Rht8* and a 192bp allele at the WMS261 microsatellite locus (*Xgwm261)*, located on chromosome 2DS. The paper was followed by a second part (Worland et al. 1998), in which the prevalence of the *WMS261-192bp* allele, and by interpolation *Rht8*, was determined in a wide range of germplasm. Since then, a number of studies have surveyed current wheat varieties for the presence of *Rht8* using the *WMS261-192bp* allele as a diagnostic marker for *Rht8*, either to determine its prevalence in worldwide wheat cultivars (Chebotar et al. 2001, Worland et al. 2001) or to ascertain the effect of *Rht8* on other agronomical traits (Bai et al. 2004).

Rht8 has been shown to have potential for Australian wheat breeding, as it offers the opportunity to reduce adult plant height without reducing coleoptile length or early vigour (Rebetzke et al. 1999). This renewed interest in *Rht8* led us to use the marker WMS261 in our studies. In this paper, we present evidence that the *WMS261-192bp* is often not associated with any height-reducing gene, and discuss the implications on our understanding of the prevalence of *Rht8* in worldwide wheat cultivars.

RESULTS AND DISCUSSION

Evidence for a Lack of Linkage Between *Xgwm261* and *Rht8* in Modern Australian Cultivars

In a previous study (Bonnett et al. 2001), we reported that several Australian wheat cultivars contained the *WMS261-192bp* allele. Some varieties were mixed for *WMS261-192bp* and other alleles at this locus. For example, cultivars Mitre and Perenjori contained both *WMS261-192bp* and *WMS261-165bp* variants. Similar observations were reported by Shmidt et al. (2004). Microsatellite marker variation within a cultivar is not uncommon, but if a marker is linked to a height-reducing gene, one would expect it to be fixed since varieties are selected for uniformity in height. This observation, and the lack of pedigree relationship of Australian cultivars with Akakomugi or Strampelli wheats, suggested that the presence of *WMS261-192bp* in several Australian wheats is not associated with *Rht8*. Indeed, we

have found several lines of evidence to support this lack of association. We selected a number of individual plants from the mixed cultivar Mitre, genotyped the individuals for the *Xgwm261* locus and progeny-tested them for height in field rows. As shown in Table 1, the selections with the *WMS261-192bp* allele were not significantly shorter than the ones carrying the 165bp allele at this locus.

To pursue this matter further we investigated a doubled haploid population issued from a cross between Sunco (*WMS261-165bp*) and Tasman (*WMS261-192bp*). As shown in Table 1, the progeny genotypes at *Xgwm261* showed no significant difference in height. In fact virtually all the variance in height (>95%) in this population could be accounted for by the genotypes at the *Rht-B1* and *Rht-D1* loci. A similar situation was observed in another DH population developed from Trident (*WMS261-208bp*) and Molineux (*WMS261-192bp*). Progeny lines containing the *WMS261-192bp* allele were not different in height to those containing the alternative *WMS261-208bp* allele (Table 1). However, this is not the case for *WMS261-192bp* allele wheats ancestrally derived from Akagomugi. For example, *WMS261-192bp* derivatives from a cross between the Strampelli wheat Mara (*WMS261-192bp*) and Halberd (*WMS261-176bp*) were significantly shorter than their sister *WMS261-176bp* counterparts (Table 1). Similarly, for a RIL population derived from Trident (*WMS261-208bp*) and Chuan-Mai18 (*WMS261-192bp*), progenies containing the *WMS261-192bp* allele were significantly shorter than those containing the alternative allele (Table 1). Chuan-Mai 18 has a direct pedigree relationship with Mara (Chuan-Mai18 = 51Mai/Mara//Datouhuang/3/Dardo/4/69-1776, Y. Zhou, pers. comm.). Our interpretation of these results is that the

Table 1. Plant height for wheats varying for alleles at the *Rht-B1*, *Rht-D1* and *Xgwm261* loci.

Variety/Population	Locus	Allele	Mean Height (cm)	t-test
Mixed Cultivar				
Mitre	*Xgwm261*	192bp	68.4	ns
	Xgwm261	165bp	68.8	
Segregating populations				
Sunco × Tasman	*Rht-B1*	Rht-B1a	89	**
	Rht-B1	Rht-B1b	71	
	Rht-D1	Rht-D1a	92	**
	Rht-D1	Rht-D1b	68	
	Xgwm261	192bp	82	ns
	Xgwm261	165bp	79	
Trident × Molineux	*Xgwm261*	192bp	79	ns
	Xgwm261	208bp	81	
Halberd × Mara	*Xgwm261*	192bp	96	**
	Xgwm261	176bp	116	
Chuan-Mai 18 × Trident	*Xgwm261*	192bp	83	**
	Xgwm261	208bp	92	

WMS261-192bp allele is linked to *Rht8* in Mara and Chuan-Mai18, whereas the same sized PCR product from Molineux and Tasman is height-neutral and not linked to any reduced height gene.

A Height-Neutral *WMS261-192bp* Allele is found in Norin10 and Early CIMMYT Wheats

The Japanese variety Norin10 also contains a 192bp allele at *Xgwm 261* (Fig. 1). This was confirmed in two separate accessions of Norin10 (Australian Winter Cereal Collection codes AUS12053 and AUS22845, Tamworth, Australia), as well as an ancestor of Norin10, Daruma (AUS21849). Cloning and sequencing showed that the *WMS261* PCR product in Akakomugi and Norin10 are 100% identical. Norin10 does not have *Rht8*, and therefore its chromosome 2D region containing *WMS261-192b*p should have no effect on height. This is an important observation since Norin10 is almost ubiquitous in wheat pedigrees (cultivars containing either *Rht-B1b* or *Rht-D1b* have Norin10 as an ancestor). It is therefore likely that the height-neutral *WMS261-192bp* has been drifting into pedigrees and becoming fixed in some current cultivars. For instance, the early Mexican cultivar Pitic62 (Norin10/Brevor/Yaktana54) has a *WMS261-192bp* genotype. Pitic62 is a progenitor of Molineux ((Pitic62/Festiguay//2*Warigal) 21/7/16), and is therefore the likely source of the height-neutral allele found in this variety. Likewise, the *WMS261-192bp* allele found in Tasman (Torres/3/Gaboto/SieteCerros T66//Bluebird/CianoF67) can be traced to the early Norin10-derived CIMMYT wheat Siete Cerros.

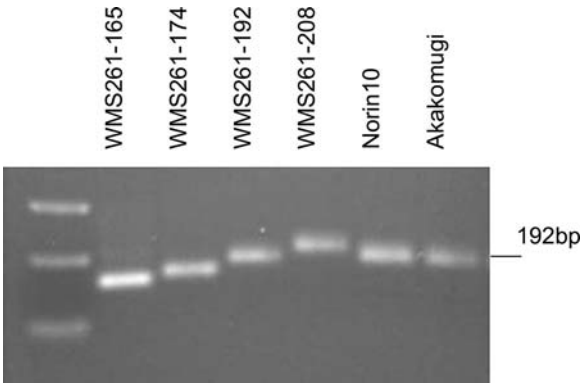


Figure 1. WMS261 genotypes of Norin10 and Akakomugi. A selection of varieties with a range of WMS261 genotypes is included for comparison

CONCLUSION

There is clear evidence that *WMS261-192bp* is not always diagnostic for *Rht8*. Caution should be exerted when interpolating from linkage within a mapping population, to a wider unrelated group of cultivars. For instance, even when tight linkage can be demonstrated, the large number of generations involved in plant breeding provides many opportunities for recombination to occur.

In this paper, we show that two key landraces in the history of wheat breeding, Akakomugi and Norin10, happened to have identical alleles at the *Xgwm261* locus. In the case of Akakomugi, used by N. Strampelli in his breeding program, *Xgwm261* was linked to *Rht8*. In Norin10, used by N. Borlaug as a source of *Rht-B1b* and *Rht-D1b*, *Xgwm261* was not linked to any height-reducing gene. Therefore the 192bp allele of *Xgwm261* can only be taken as diagnostic of *Rht8* when it can be shown to be derived from Akagomugi.

REFERENCES

Bai GH, Das MK, Carver BF, Xiangyang Xu, Krenzer EG (2004) Covariation for microsatellite marker alleles associated with *Rht8* and coleoptile length in winter wheat. Crop Sci 44(4):1187–1194

Bonnett DG, Ellis MH, Rebetzke GJ, Condon AG, Spielmeyer W, Richards RA (2001) Dwarfing genes in Australian wheat - present and future. Proc. 10th Australian Wheat Breeders Assembly. Mildura, 16–21 September, 2001. pp 154–157

Chebotar SS, Korzun VN, Sivolap YM (2001) Allele distribution at the locus WMS261 marking the dwarfing gene *Rht8* in common wheat cultivars of Southern Ukraine. Russ J Genet 37:1075–1080

Korzun V, Roder M, Ganal M, Worland AJ (1998) Genetic analysis of the dwarfing gene *Rht8* in wheat 1. Molecular mapping of *Rht8* on the short arm of chromosome 2D of bread wheat. (*Triticum aestivum*). Theor Appl Genet 96:1104–1109

Rebetzke GJ, Richards RA, Fischer VM, Mickelson BJ (1999) Breeding long coleoptile, reduced height wheats. Euphytica 106:159–168

Schmidt AL, Gale KR, Ellis MH, Giffard PM (2004) The significance of sequence variation at a microsatellite locus (XGWM261) in hexaploid wheat (Triticum aestivum) varieties. Euphytica 135:239–246

Worland AJ, Korzun V, Ganal MW, Roder M, Law CN (1998) Genetic analysis of dwarfing gene *Rht8* in wheat. Part II. The distribution and adaptive significance of allelic variants at the *Rht8* locus of wheat as revealed by microsatellite screening. Theor Appl Genet 96:1110–1120

Worland AJ, Sayers EJ, Korzun V (2001) Allelic variation at the dwarfing gene *Rht8* locus and its significance in international breeding programmes. Euphytica 119:155–159

WHEAT BREEDING IN GLOBAL CONTEXT

S. RAJARAM
Director, Integrated Gene Management (MP2) ICARDA, Aleppo, Syria
E-mail: s.rajaram@cgiar.org

We are honored to have Dr Norman E. Borlaug amongst us. He laid the foundation for International Wheat Breeding almost 50 years ago, which resulted into Green Revolution in Asia in late 1960's and early 1970's. South America and rest of the world greatly benefited due to Dr Borlaug's generous decision to share germplasm first through USDA Rust Nursery and later through CIMMYT's International Yield Trials (ISWYN) and Screening Nursery (IBWSN).

Argentina and Brazil contributed a lot to Dr Borlaug's international efforts by providing special genetic resources such as those varieties from Buck and Klein Companies and INTA and varieties such as Frontana from Brazil. These genetic stocks contributed in improving leaf rust, stem rust resistance and industrial quality. The variety Marcos Juarez INTA was the result of an international undertaking between INTA of Argentina and CIMMYT, Mexico. This variety should be considered a hallmark germplasm as it fitted well into soybean-wheat cropping system due to its earliness and wide adaptation across Argentina.

International Wheat Breeding efforts were boosted due to contribution of varieties Kaukaz and Aurora from USSR. These gene pools contributed to the production of varieties Veerys and Bobwhites, respectively through 1B/1R translocation. The Veerys varieties as a group represented 15–20% yield potential increase over previously grown varieties in all major mega environments especially in semi arid (ME4) and favorable irrigated (ME1). Bobwhites proved to be the best wheat for transformation technology.

The international breeding further benefited when Oregon State University contributed NdD/P101 germplasm as a source of yellow rust resistance and consequently we distributed many lines including Atila. The Atila group of lines has assumed prominence from Indian Subcontinent to Turkey in the North and Morocco

H.T. Buck et al. (eds.), Wheat Production in Stressed Environments, 793–794

in the West. The lines have unusual breadth of adaptation and higher yield than Veerys. These have demonstrated good adaptation to drought, higher temperature tolerance, efficient in N&P extraction and higher root biomass. Atila lines are grown on more than 10 million hectares in 2005 and have been proven excellent breeding germplasm.

In late 1980's, fusarium head-blight (FHB) became a world wide problem. Earlier, it was sporadic and localized to China, South America and a few European countries. We were able to expand the genetic base of wheats for additional variability through International Shuttle Breeding program between CIMMYT and China (Jiangsu Academy, Sichuan Academy and Heliongjiang Academy). Varieties resistant to FHB such as Wuhan 1, 2 and 3 and Shanghai 7 and 8 and Suzhoe wheats were the product of generous collaboration between various Chinese institutions. These are now internationally used by the breeders.

The International Wheat Breeding would not be possible without free exchange of germplasm and trust built amongst the breeders. We have come long way towards achieving this goal.

Lately, I have been engaged in creating another international wheat center based at ICARDA in Syria. This Center is situated within wheat diversity and hopefully would furnish necessary germpalsm needs of NARS in CWANA and beyond.

The Wheat Center at ICARDA is already engaged in the following disciplines with substantial scientific and human resources committed to it. These are:

- Plant Breeding (Spring Bread Wheat, Winter Bread Wheat and Spring Durum Wheat)
- Prebreeding based on wild species
- Biotechnology (Transformation, Molecular markers and DH Productions)
- Integrated Pest Management (Diseases and Insects)
- Human Resources Development (Training)
- Industrial Quality
- Sustainable management of wheat based dry land farming system
- Conservation of wheat and wheat related genetic resources

I am very hopeful that ICARDA's wheat program would be a credible scientific and information Center for CWANA and whole world.

I want to thank GOA for hosting the 7th IWC and particularly Drs Hilda Buck and Jorge Nisi for their continuous hard work towards realization of this Conference in this wonderful city of Mar del Plata. It is great success for all of us. I want to thank my fellow member of IOC for their advice and guidance.

I wish the best to all of you for your future endeavor in wheat research and safe return to your home and family.