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Scale and Complexity in Plant Systems Research

Gene-Plant-Crop Relations

Edited by
J.H.J. Spiertz, P.C. Struik and H.H. van Laar

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Edited by

J.H.J. SPIERTZ
*Crop and Weed Ecology Group,
Wageningen University and Research Centre, The Netherlands*

P.C. STRUIK
*Crop and Weed Ecology Group,
Wageningen University and Research Centre, The Netherlands*

and

H.H. VAN LAAR
*Crop and Weed Ecology Group,
Wageningen University and Research Centre, The Netherlands*

Springer

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Physiology and modelling of crop adaptation

Diversity, resource use and crop performance

Outlook and dialogue

PREFACE

The growing demand for food and increasing scarcity of fertile land and other resources (water, energy, etc.) present multiple challenges to plant and crop scientists to meet the demands of future generations while protecting the environment and conserve biological diversity. Novel directions in linking basic plant sciences to crop and systems research are needed to meet the growing demand for food in a sustainable way. Crop performance can be changed by modifying genetic traits of the plant through plant breeding or changing the crop environment through agronomic management practices. To achieve that, systems analysis and modelling play an important role by integrating and evaluating new findings at the gene and plant level at higher scales of aggregation. Robust crop-physiological modelling can become an essential tool to use insights from functional genomics in explaining crop behaviour. Current crop models can predict crop performance over a range of environmental conditions. Recently QTL information has been incorporated into crop models, and this has proved the potential of narrowing genotype–phenotype gaps and of applying QTL-based models for the analysis of genotype-by-environment interactions. To make further progress, model structure must be upgraded to allow for more physiological feedback features. Model input parameters should be designed to be potentially grounded in gene-level understanding. Integration of crop modelling into genetic and genomic researches can enhance the future position of crop physiology in 'plant breeding by design' (Yin, X., Struik, P.C. and Kropff, M.J., 2004. Role of crop physiology in predicting gene-to-phenotype relationships. *Trends in Plant Science*, 9 (9), 426-432).

New tools derived from advances in molecular biology, genomics and plant physiology have yet not been widely adopted in plant breeding and integrated crop management because of inability to connect information at the gene level to the phenotype, crop and agro-ecosystem level. The complexity and need for integration can be illustrated by quotes of:

a. *Lloyd Evans*: "Crop yields are the integrated end-product of many processes being researched by reductionist scientists at various levels. For such research to be effectively used in agriculture there must be continuing and effective interactions between researchers at the various levels of complexity" (*The Journal of Agricultural Science*, 2005, 143 (1), 7-10).
b. *Matthew Reynolds and Norman Borlaug*: "In addition to genetic challenges of crop improvement, agriculturalists must also embrace the problems associated with a highly heterogeneous and unpredictable environment. Not only are new genetic tools becoming more accessible, but a new generation of quantitative tools is available to enable better definition of agro-ecosystems, of cultivar by environment interactions, and of socio-economic issues." (*The Journal of Agricultural Science*, 2006, 144 (2), 95-110).

The international Frontis Workshop "Gene–Plant–Crop Relations: Scale and

Complexity in Plant Systems Research", held at Wageningen, The Netherlands, 23-26 April 2006, aimed at presenting and discussing new directions to bridge knowledge from the gene to plant, crop and agro-ecosystem level and at solving problems in production ecology and resource use by identifying and applying new research tools. This workshop was attended by about 80 participants from 20 countries and comprised 9 keynote presentations, 12 invited oral presentations and four poster sessions. For each of the poster sessions the posters were evaluated by two senior scientists; based on the scores four young scientists received an award. Presentations and discussions during the workshop sought to identify the most promising opportunities in this emerging field, and also the more recalcitrant challenges.

Keynote and selected contributions to the workshop were the building material for the contents of this book. All papers have been reviewed by two international experts and the editors. The sections of the book cover the following themes:

- Genetics of plant performance; from molecular analysis to modelling
- Modelling genotype × environment interactions
- Genetics and physiology of crop adaptation
- Physiology and modelling of plant functioning and crop performance
- Diversity, resource use and crop performance
- Outlook and dialogue on future research.

The contents of most contributions combine the presentation of the state of the art in specific fields, based on a concise review of progress made and illustrated by results of ongoing research, and an outlook on future research.

The support of the Royal Netherlands Academy of Sciences (KNAW), Frontis – Wageningen International Nucleus for Strategic Expertise, the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) and the European Cereal Atlas Foundation (ECAF) is greatly acknowledged.

People make things work; the cooperation of participants and authors and the enthusiasm of the staff of the Group Crop and Weed Ecology (CWE) and of Frontis were vital for the success of a challenging scientific event and a high scientific quality. We hope that this book reflects the inspiring scientific atmosphere of the workshop and will give food for thoughts on new research directions in plant sciences.

Huub Spiertz, *Wageningen University, The Netherlands*
Convenor, International Frontis Workshop GPC2006

Ken Giller, *Wageningen University, The Netherlands*
Professor of Plant Production Systems Group, Wageningen University, The Netherlands

Ken Cassman, *University of Nebraska, USA*
Steering Committee, International Frontis Workshop GPC2006

Maarten Koornneef, *Director, Max Planck Institute for Plant Breeding Research (MPIZ), Cologne, Germany, and Professor of Plant Genetics, Wageningen University, The Netherlands*

GENETICS OF PLANT PERFORMANCE: FROM MOLECULAR ANALYSIS TO MODELLING

CHAPTER 1

GENETIC AND MOLECULAR ANALYSIS OF GROWTH RESPONSES TO ENVIRONMENTAL FACTORS USING *ARABIDOPSIS THALIANA* NATURAL VARIATION

M. REYMOND[#], B. PIEPER[#], H. BARBIER[#], A. IHNATOWICZ[#],
M. EL-LITHY[##], D. VREUGDENHIL[##] AND M. KOORNNEEF[#,##]

[#] Max Planck Institute for Plant Breeding Research, Carl von Linné Weg 10,
50829 Cologne, Germany.
[##] Departments of Genetics and Plant Physiology, Wageningen University,
Wageningen, The Netherlands.
E-mail: reymond@mpiz-koeln.mpg.de; pieper@mpiz-koeln.mpg.de;
barbier@mpiz-koeln.mpg.de; ihnat@mpiz-koeln.mpg.de; m_ellithy@yahoo.com;
dick.vreugdenhil@wur.nl; koornnee@mpiz-koeln.mpg.de

Abstract. *Arabidopsis thaliana* natural accessions have been collected in the Northern hemisphere in a wide range of habitats, with a specific environment in each habitat, suggesting that selection for adaptation to these local environments occurred and provided genetic variation of responses to environmental factors.

Plant performance traits are complex traits and are fluctuating under contrasted environmental conditions (e.g., temperature, day length, nutrient nutrition, drought). The genetic architecture of such traits and of their responses to environmental conditions could be analysed by detecting QTL (Quantitative Traits Loci). Accessions from contrasted geographical regions have been used to detect such QTL. QTL analysis and subsequent QTL cloning of genetic variation for growth and plant performance traits in *Arabidopsis* using *Arabidopsis thaliana* natural accessions are powerful tools to understand the genetic and the molecular basis of plant performance in contrasted environments. QTL analysis of growth-related traits and their response to temperature, light and nutrient starvation in hydroponic system are in progress in different populations (recombinant inbred lines, backcross inbred-line populations) Near-isogenic lines or heterogeneous inbred lines are also selected and used to confirm the effect of QTL and to isolate recombinant events in the QTL region in order to fine-map and clone the QTL.

The challenge of these studies is to understand this genetic variation, which is also very relevant for plant breeding, since it involves the traits determining yield and yield stability. It is difficult to predict which processes underlie this genetic variation, but candidate processes are primary and secondary metabolism, nutrient uptake, transport processes and aspects of development etc. This implies that a

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thorough and broad (whole plant) approach needs to be applied to identify the nature of the observed variation. Apart from being relevant for breeding it is assumed that genetic variation for plant performances contributes to the adaptation of specific genotypes to a specific ecological system and therefore has ecological and evolutionary relevance.

ARABIDOPSIS AS MODEL PLANT FOR MOLECULAR STUDIES

Arabidopsis thaliana is a plant species of no economic importance and is not considered to be a problematic weed. However, the progress made in dissecting the genetic and molecular basis of many plant processes by using *Arabidopsis* and its resources, is impressive. The main reasons why so much progress has been made in the past 25 years are the large number of scientists working with this species and the resources that have been made available (Somerville and Koornneef 2002). The choice of *Arabidopsis* was based on some biological characteristics such as the short generation time, it being a self-fertilizer and its small plant size, which all make it very suitable for genetic studies. However, nowadays the large resources which are publicly available for this species might even be more important. Especially the complete genome sequence published in 2000 (Kaul et al. 2000) and the availability of mutations in almost every gene and for which insertion-mutant seeds can be easily ordered through Internet (http://www.arabidopsis.org) are important resources that are still unique to *Arabidopsis*. Nowadays, the amount of genomic resources available is also increasing rapidly for crop plants. For instance, for rice a complete genome sequence is available and accessible through the Internet (see: http://www.cbi.pku.edu.cn/mirror/GenomeWeb/plant-gen-db.html) and other data bases such as Solgenes, Gramene etc. However, completeness of the knock-out mutant collection (> 300.000 mutants, Sessions 2005) in *Arabidopsis* will not easily be met in other plants because their generation in *Arabidopsis* depends on the extremely efficient ‘floral dip’ transformation procedure.

Based on the technology and resources available in *Arabidopsis*, one can establish a functional analysis of every gene by the analysis of the phenotype of mutants in which a gene of interest is disrupted. This procedure is called reverse genetics (from gene to phenotype). The functional analysis of one gene can be complicated by the fact that many genes are duplicated (redundant). Therefore knock-outs disturbing all the genes of the same family need to be generated to see phenotypes clearly distinct from the wild type. It may also be difficult to detect a mutant phenotype because the effect of the studied gene is not detectable on the phenotypic level. For example, growing plants on soil will not reveal a seed-dormancy phenotype because this requires a specific germination test performed on Petri dishes.

The opposite approach to identify gene function starts with mutant phenotypes and tries to find the corresponding gene(s) by accurate mapping, sequencing and complementation of mutant phenotypes by wild-type DNA; it is called forward genetics (from phenotype to genotype). In addition to its use for reverse genetics the complete DNA sequence also allows the construction of whole-genome micro-arrays (called DNA chip), which allows the study of the expression of all genes at once.

NATURAL VARIATION IN *ARABIDOPSIS*

The above-mentioned approaches rely on mutants often induced in a single genetic background, which are pure lines with favourable properties in the laboratory, usually including earliness and low seed dormancy. However, natural variation is an increasingly important resource for genetic variation in addition to induced mutants. *Arabidopsis* accessions have been collected in a wide range of sites in the Northern hemisphere. Because these accessions can come from different habitats, it is assumed that selection for adaptation to these local environments has occurred and has provided genetic variation in responses to these environmental factors. The traits and genes for which natural variation is present differ from induced mutants by the fact that they survived in nature, implying that natural selection allowed the survival of the various alleles. Furthermore, many of the traits showing natural variation are related to properties that are important for crop plants, including (biotic and abiotic) stress resistance, developmental traits, phenology, seed dormancy and aspects of growth.

The genetic analysis of natural variation

In segregating progenies derived from crosses between diverse accessions such traits can be analysed genetically. Because the genetic differences between two accessions are often determined by more than one gene and because of large environmental effects on the phenotype, methods of quantitative genetics need to be applied. Especially the association of trait phenotypes with the genotype, assayed by molecular markers, is very effective for the analysis of quantitative-trait loci (QTL). QTL analysis reveals the regions on the genetic map where a gene or several closely linked genes are located and their contribution to the total variance of the trait in that experiment. For QTL analysis the use of so-called immortal mapping populations, consisting of homozygous inbred lines is effective because trait values can be obtained from replications and from experiments performed in different environments. In such populations, genotyping (analysis of the marker phenotypes in all lines) needs to be done only once.

Recombinant inbred lines (RILs) are the most frequently used immortal mapping populations in *Arabidopsis*. These are obtained by single-seed descent from F2 plants until the F9 or further generation. The use of RILs is relatively easy in *Arabidopsis*, as it is a self-pollinating plant with a short generation time. A number of introgression lines (ILs) will also become available soon. Another advantage, especially of RIL populations that are used by many researchers, is that an increasing number of traits are mapped in the same populations. Combining the results of multiple studies can lead to the discovery that some loci control more than one trait, sometimes in an unexpected way (Koornneef et al. 2004). Co-location of QTLs can also provide a clue of what pathways might be involved in complex traits. In a recent study this concept was applied and even expanded by combining the results of gene expression analysis with QTL mapping of metabolite levels and enzyme activities (J.J.B. Keurentjes and colleagues, personal communication). Common genetic-map positions of differentially expressed genes and QTLs allow the construction of genetic networks. A combined analysis of this information within the context of a systems framework is very useful for the future identification of the gene(s) underlying the QTLs because differences in expression that co-locate with the QTLs provide candidates for the QTGs (Quantitative Trait Genes).

The cloning of the genes underlying QTLs

The cloning of genes based on the map position of mutants (positional cloning) is very effective in *Arabidopsis* because of the efficiency with which a segregating population can be analysed and because of the abundance of markers available (Lukowitz et al. 2000). Nowadays the mapping of a mutant down to less than 50 kbp (on average 0.2 centi-Morgan) is sufficient to search for candidate genes. Subsequent DNA sequencing of these genes will rapidly reveal the precise location of the mutations. Complementation of the mutant phenotype after transformation with the wild-type allele provides proof that the causative gene has been positively identified. Similar approaches have been used to clone QTLs. However, because positional cloning is done gene-by-gene, care has to be taken that only one segregating locus is studied at a time. This can be achieved by using so-called near-isogenic lines (NILs) that contain an introgression of one parent's alleles at a QTL position into the genetic background of the other (recurrent) parent. Subsequently, a population of lines with different recombinant events is selected by genotyping the offspring of a cross between the NIL and the recurrent parent with polymorphic markers surrounding the QTL. The QTL is eventually fine-mapped by repeating this process with progeny lines that still show the effect of the QTL on the phenotype. The procedure becomes similar to that of mutant approaches once the QTL has been fine-mapped to a sufficiently small region. Another approach is to make use of residual heterozygosity present at a QTL in RILs after several generations of selfing. The genetic background of the progeny of such lines is a mixture of both parental accessions. This so-called HIF (heterogeneous inbred families) concept (Tuinstra et al. 1997) is effective because one does not first have to create the NILs, which requires several generations of backcrossing and marker-assisted selection (MAS). Using either NILs and/or HIF will allow the validation/confirmation of the presence and the effect of a QTL.

Accurate phenotyping of QTLs with small effects and with relatively large environmental influence is difficult. Therefore, it is often more effective to select homozygous recombinants in a genomic region surrounding the QTL and then analyse their phenotype in replicates. Such procedures are also described by Peleman et al. (2005) and are commonly referred to as QTL Isogenic Recombinant Analysis. A complication of QTL cloning compared to positional cloning of mutants is finding the gene(s) responsible for the phenotype among the candidate genes in the region where the QTL was fine-mapped. In case of mutants in a self-pollinating plant such as *Arabidopsis*, any DNA sequence difference that is detected indicates that the gene has been identified. However, among *Arabidopsis* accessions polymorphisms of sequences occur at an abundance of about one in every 350 bp (Schmid et al. 2003), even in coding regions. This implies that several differences will be found in each gene. As a consequence, sequence information is in most cases not informative for the detection of the QTN (Quantitative Trait Nucleotide). However, sometimes the presence of deletions (Alonso-Blanco et al. 2005a) or mutations in known important parts of the gene (Teng et al. 2005) is informative. Therefore, transformation experiments are crucial to prove which gene(s) is (are) responsible for the phenotypic differences observed between NILs or HIFs. That this concept works in *Arabidopsis* has been shown by the cloning of several QTLs affecting traits such as flowering time, frost tolerance and seed dormancy (for review: Alonso-Blanco et al. 2005b). Mutants are complementary tools for the identification of QTGs.

Fine-mapping and cloning QTLs is also a powerful way to distinguish pleiotropic effects from the effect of linked genes when QTLs of several traits are co-localized. It is also helpful in confirming or disproving unexpected co-location of QTL for diverse traits. Examples are the effect of *FLC* on flowering time and length of circadian periods (Swarup et al. 1999), the effect of an invertase gene on invertase activity and root growth (Sergeeva et al. 2006), the effect of the *ERECTA* locus on plant length and water use efficiency by affecting stomatal densities (Masle et al. 2005) and the resistance to *Plectospaerella cucumerina* (Llorente et al. 2005). The fine-mapping of QTL in combination with candidate genes, expression analysis and the search for mutants with related phenotypes in the QTL region are the tools available at the moment to identify genetic variation at the molecular level. Because some tools are still unique to *Arabidopsis*, QTL cloning is most efficient in this species.

A summary of the various approaches used to identify the molecular basis of genetic variation is shown in Figure 1.

THE GENETICS OF PLANT PERFORMANCE

Plant performance traits (photosynthetic performance, growth performance, yield performance, etc.) are complex traits and their values fluctuate with the variability in environmental conditions encountered in the field. Finding the pertinent environmental variables to which these traits are responding is an important aspect of the genetics of plant performance. It is a challenge to find the genes that underlie variation in plant performance in contrasting environments.

Genes with relatively large effects on phenotype (or trait value) have been identified in *Arabidopsis* using mutant approaches, often accompanied with over-expression of the genes of interest. However, this is often a brute-force approach which requires high-throughput technologies for making transformation constructs, the transformation and, very important, trait analysis. Examples where transgenic approaches resulted in yield enhancement have been reviewed by Van Camp (2005), who also described the high-throughput platforms developed mainly by the industry. Finding the genes controlling the variation present in nature and identifying these genes within the germplasm pool of crop plants will require the application of the technology described above in section *The cloning of the genes underlying QTLs*.

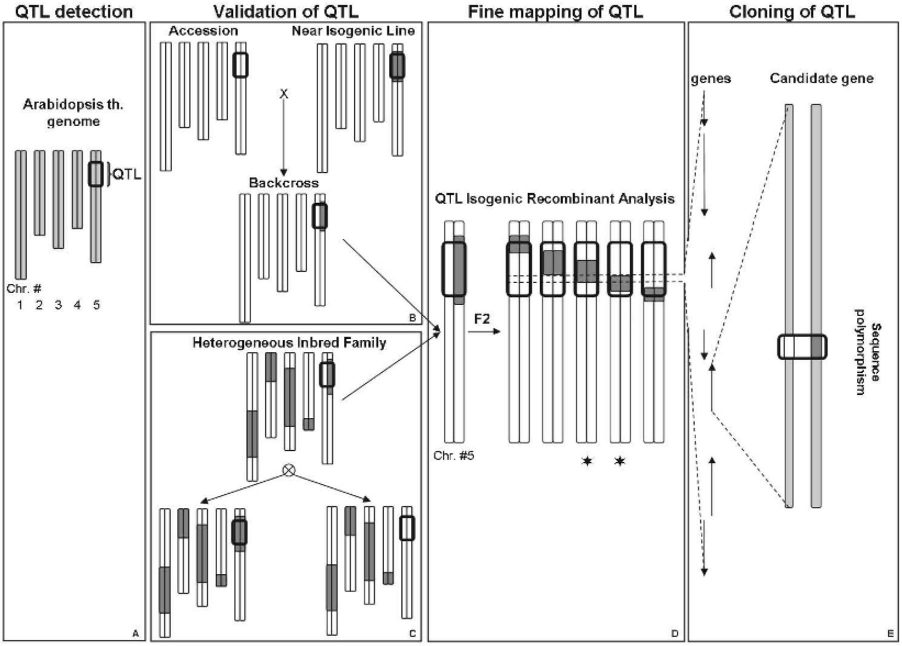

Figure 1. *Various approaches used to identify the molecular basis of a QTL.* **(A)** *QTL detection. QTLs could be detected using several types of populations (Recombinant Inbred Lines, F2, Backcrossed lines,...). For this example on* Arabidopsis thaliana*, a QTL on chromosome 5 (Chr.#5) has been detected.* **(B and C)** *QTL validation. Validation of a QTL could be done by comparing either* **(B)** *a Near Isogenic Line (NIL) at the position of the detected QTL and the corresponding accession (having the same genetic background) or* **(C)** *lines from a Heterogeneous Inbred Line (HIL at the position of the detected QTL).* **(D)** *Fine mapping of a QTL. Fine mapping could be performed by making use of recombinant events from selfed heterozygote lines at the position of the detected QTL. These recombinant lines are then phenotyped. In this example, asterisks indicate lines showing a phenotype significantly different from the accession having the same genetic background. The dotted traits indicate the fine position of the QTL.* **(E)** *Cloning a QTL. Recombinant events occurring during the fine-mapping of the QTL are usually not enough to get a recombinant event between each gene within the QTL. Hence, once the QTL is fine-mapped, several genes are included in the highlighted regions (arrows represent predicted Open Reading Frames within the region of the fine-mapped QTL). Candidate-gene approach could then be performed. In the case of obvious candidate gene(s), this approach could already be performed just after the QTL detection*

QTL analysis for yield in crop plants has been performed in many studies. In *Arabidopsis*, natural variation for growth-related traits has been detected in various studies but the number of studies where QTL analyses have been done is limited. El-Lithy et al. (2004) identified QTLs for growth-related traits under normal (laboratory) conditions. Many of these QTLs co-localized with QTLs of flowering time, although growth was analysed during the vegetative phase. Loudet et al. (Loudet et al. 2003b) showed that growth QTLs (biomass accumulation) differ depending on the growth conditions. Another example, obtained when analysing the same population, is root growth under phosphate starvation (Reymond et al. 2006). In experiments in which the supply of nutrients is varied, their concentration in plant tissue can also be analysed (Loudet et al. 2003a). QTLs for the accumulation of nitrogen in several cases co-located with QTLs for growth. El-Lithy (2005) also found co-locations of loci for starch accumulation in the leaves and for plant growth. These co-locations suggest a functional relationship between traits. However, due to the inaccuracy of QTL mapping, co-locations can be due to different but linked genes. An analysis of a number of trait co-localizations was described by El-Assal et al. (2004). The authors made use of a transgenic line different from the reference genotype in one of the confirmed alleles conferring a flowering-time QTL encoded by the Cryptochrome-2 (*CRY2*) gene. They revealed that some co-locating QTLs were pleiotropic effects of *CRY2* (flowering time, ovule number and fruit length) but others (seed dormancy and invertase activity) were due to allelic variation at linked genes.

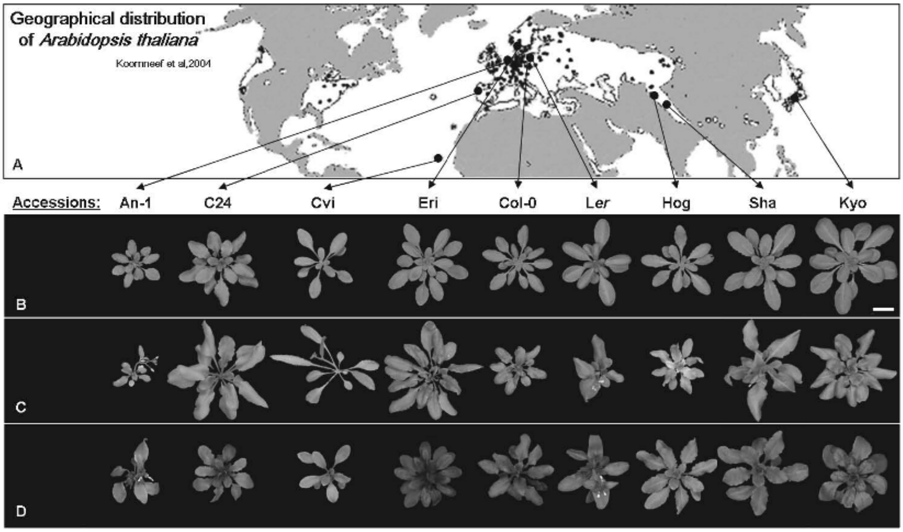

Figure 2. *Example of 'Genotype × Environment' interactions using accessions of* Arabidopsis thaliana **(A)** *Geographical distribution of* Arabidopsis thaliana *accessions (after Koornneef et al. 2004). Black points on the map indicate the location where accessions have been collected for this example.* **(B, C and D)** *Rosette shape and colour of the selected accessions growing in a range of temperature and light conditions (white bar represents 2 cm)*
(B) *Photoperiod: 12 h; temperature: 16/14 °C (day/night); light intensity:150 (±30) μmol* m^{-2} s^{-1}
(C) *Photoperiod: 16 h; temperature: 4/4 °C (day/night); light intensity: 15-20 μmol* m^{-2} s^{-1}
(D) *Photoperiod: 16 h; temperature: 13/4 °C (day/night); light intensity: 200-400 μmol* $m^{-2}s^{-1}$

For the analysis of the genetics of plant performance RIL populations derived from crosses between *Arabidopsis* accessions from diverse origins are used. These include Ler (from Poland), Kas-2 (from Kashmir), Sha (from Tajikistan) and Cvi (from the Cape Verde Islands). QTL analysis of growth-related traits and their response to light, temperature and nutrient starvation (using hydroponics) are performed in different populations. An example of the large genotype × environment interaction in *Arabidopsis* is shown in Figure 2. NILs are also selected and used to confirm the effect of QTL and to isolate recombination events in the QTL region in order to fine-map and clone the QTL. In addition to QTLs for growth, QTLs can also be detected for metabolites, enzyme activities and gene expression. The combined QTL analysis of all such traits now provides the possibility to correlate these various traits on the basis of their common map position, thereby unravelling the processes and pathways underlying plant performance traits. Examples are the control of root length by a QTL encoding an invertase (Sergeeva et al. 2006). These combined studies will help to dissect variation for integrative traits (such as plant growth) into variation of various underlying processes and component traits. Candidate processes are primary and secondary metabolisms, nutrient uptake, transport processes, aspects of development, etc. It is of interest that in rice two major yield QTGs that have been cloned deal with hormone metabolism (Ashikari et al. 2005). A cytochrome oxidase was found to underlie a major QTL for grain number per panicle and the yield enhancing allele was conferred by a loss of function mutation. A major QTL for seed length called GS3 revealed a gene for which the function, and thereby the pathway involved, could not be predicted with any certainty from its sequence (Fan et al. 2006). Interestingly, also here the allele positive for yield is a loss-of-function allele. A thorough and broad (whole plant) physiological and biochemical approach is needed to identify the nature of the observed variation in addition to the molecular study of genes involved.

THE TRANSLATION FROM MODEL TO CROP PLANTS

Based on the common molecular basis of many processes in plants, it is now becoming more obvious that many genes, for which the function is discovered in *Arabidopsis*, also underlie the genetic variation that is exploited by plant breeders in crop plants. However, in more distantly related species similar processes may just as well be regulated by unrelated genes. The best known example is flowering time, where QTL cloning in rice revealed a number of genes that, based on mutant analysis, previously had been shown to control flowering time in *Arabidopsis* (Hayama and Coupland 2004). However, the two major genes controlling flowering-time variation in nature in *Arabidopsis* (*FRI* and *FLC)* are not found in monocots. In wheat, in which genes based on phenotype and physiology were predicted to be similar to *FLC,* another regulatory gene was found (Yan et al. 2004). For the major determinant of the day-length response in barley a gene was found that was not identified as a major player in *Arabidopsis* and rice (Turner et al. 2005), although the process in which the gene was involved (circadian rhythm) was known from

Arabidopsis research. However, especially when dicot species are compared many examples of similar genes controlling similar processes have been described.

Another possibility for exploitation of the vast amount of research done on *Arabidopsis* and the availability of its genome sequence is to make use of synteny between genomes. With the complete genome sequence of rice now available it has become clear that there are reasonably sized blocks of highly conserved synteny between this Graminea and *Arabidopsis*, even though both species are estimated to have diverged around 200 million years ago. The largest of these regions spans no less than 119 *Arabidopsis* genes (Goff et al. 2002). The level of synteny can be much higher between more related species. For instance, highly conserved genetic synteny has been reported up to the multi-megabase scale between *Arabidopsis* and other members of the Brassicaceae (Lukens et al. 2003; Parkin et al. 2005). In an older study, comparative mapping had already demonstrated genetic co-linearity of vernalization-responsive flowering-time loci in *Brassica napus* with the top of chromosome IV and V in *Arabidopsis*, the location of *FRI* and *FLC* (Osborn et al. 1997). Evidence that FLC is indeed central to the vernalization response in *B. napus* was provided some years later (Tadege et al. 2001), demonstrating the feasibility of comparative genetics.

In cereals, much has been expected from the synteny among Gramineae. However, not many cases in which similar map positions of QTLs are due to variation at the same genes have been published. Especially, for complex traits controlled by many genes one cannot expect that variation in the same gene set will determine variation in different species. There are too many candidates in the genome.

CONCLUSIONS

From the research done in *Arabidopsis* and rice the procedures for the identification of genes underlying QTLs are well developed. The methodology has been shown to be successful also in plants with large genomes such as wheat and maize for some major-effect QTLs. The need for this knowledge is large because when transgenic approaches are used, these genes are needed. Also for marker-assisted breeding, markers within the gene to follow are preferred over linked markers, because the latter can be separated from the target locus by recombination and therefore do not predict the desired phenotype in 100% of the cases. Research on *Arabidopsis* will contribute to the finding of the genes underlying QTLs, also in crop plants because the candidate genes and pathways for crop-plant QTLs will be identified. The analysis of larger-effect genes has already started for flowering time but will also become available for more complex traits dealing with growth and genotype × environment interactions.

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CHAPTER 2

FROM QTLS TO GENES CONTROLLING ROOT TRAITS IN MAIZE

R. TUBEROSA AND S. SALVI

Department of Agro-environmental Sciences and Technology, Viale Fanin 44, 40127 Bologna, Italy.
E-mail: roberto.tuberosa@unibo.it

Abstract. In maize, two major quantitative-trait loci (QTLs) on chromosome bins 1.06 and 2.04 have been shown to affect root architecture and a number of agronomic traits, including grain yield. The QTL on bin 2.04 (*root-ABA1*) also affects root lodging and ABA concentration in the leaf. To evaluate the effects of *root-ABA1* better, near-isogenic lines (NILs) have been produced and evaluated *per se* and in testcross combinations under different water regimes. Additionally, the NILs have been crossed to obtain large mapping populations suitable for the fine-mapping of *root-ABA1* and, eventually, its map-based cloning. The identification of the sequence responsible for a target QTL can be facilitated by the candidate-gene approach coupled with a comparative *in silico* analysis based on sequence information of model species and other crops. Genomics, when appropriately integrated with other relevant disciplines, will positively impact our understanding of root growth and functions.

INTRODUCTION

The main challenge faced by plant scientists during the 21st century will be to increase crop productivity per unit area while enhancing the sustainability of agricultural practices and preserving the remaining biodiversity (European Plant Science Organization 2005). Of all the factors that presently limit crop yield, irrigation water and fertilizers play an increasingly important role, due to their escalating costs and diminished availability. Clearly, a better knowledge of the developmental processes that impart tolerance to drought and low nutrients will allow for a more effective identification of target traits for boosting yield potential while optimizing water and nutrient use efficiency.

Among the morphological factors that affect tolerance to drought and low nutrients, root traits play a major role. In a landmark review, Ludlow and Muchow (1990) listed a number of traits and their orders of priority for improving drought

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resistance in both intermittent and terminal stress environments and under conditions of either subsistence or modern agriculture. In three of these four situations, rooting depth and density were considered to be of primary importance. Roots show a high level of developmental plasticity in response to external cues, an essential feature for the adaptation of plants to different environmental conditions and for optimizing the utilization of water and nutrients. Among the multiple factors that regulate root plasticity in maize, abscisic acid (ABA) has shown a positive role for sustaining root elongation under conditions of water deficit that inhibit shoot elongation (Sharp et al. 2004).

From a breeding standpoint, although extensive genetic variation for root architecture has been reported (O'Toole and Bland 1987), root traits have seldom been considered as selection criteria to improve yield, mainly due to the difficulty in their measurement and their quantitative mode of inheritance. As compared to conventional breeding approaches, the identification of quantitative-trait loci (QTLs) that may concurrently affect root traits and grain yield under varying water and nutrient regimes would allow for more targeted selection approaches, such as marker-assisted selection (Ribaut and Hoisington 1998). Additionally, cloning the sequences underlying such QTLs (Salvi and Tuberosa 2005) and exploring allelic variability through EcoTILLING (Comai et al. 2004) or association mapping (Yu and Buckler 2006) pave the way for the identification of agronomically superior alleles at these loci. Therefore, the advent of genomics has ushered in novel opportunities to elucidate the genetic and functional basis of root growth and to manipulate allelic diversity more effectively at relevant loci affecting yield under low-input conditions (Tuberosa et al. 2002a; 2005; Sharp et al. 2004; Zhu et al. 2005a; 2005b). In this context, the objectives of this review are to (i) summarize briefly the information available on two major QTLs for root traits in maize and (ii) describe the procedures required for their cloning based also on the exploitation of model species.

QTLS FOR ROOT TRAITS IN MAIZE

When QTL data become available from two or more populations of the same species, it is possible to compare their position by using common anchor markers (e.g., RFLPs and SSRs) and/or through the use of a reference map (Tuberosa et al. 2002b; Tuberosa and Salvi 2004). In maize, the comparative analysis of QTLs from different populations is facilitated by the subdivision of the reference map into 100 sectors (bins) of comparable size (Davis et al. 1999). Based on the bin framework of the UMC reference map, a comprehensive survey on QTLs for root traits in maize was recently presented (Tuberosa et al. 2003). Here, we summarize the main results for two major QTLs (on bins 2.04 and 1.06) shown to affect root architecture and other traits, including grain yield.

In maize, QTLs for root architecture were first reported by Lebreton et al. (1995) using 81 F_2 plants derived from the cross between two lines (Polj17 and F-2) known to differ for a number of root traits, including root pulling force (RPF), and for ABA concentration in the leaf and xylem sap. For all QTLs but one, the signs of the additive effects for ABA concentration and RPF were similar. As to nodal root number (NRN), the comparative analysis of QTL effects revealed a striking correlation between the QTL effects of NRN and ABA concentration in the xylem sap (r = 0.84) and suggested that variation in xylem ABA content is largely determined by variation in NRN. The QTL region with the strongest concurrent effect on root traits and leaf ABA concentration (L-ABA) was mapped near *csu133* on bin 2.04. A strong effect of this region on L-ABA was also reported in the Os420 × IABO78 background (Tuberosa et al. 1998). It should be noted that none of the major mutants impaired in ABA biosynthesis mapped to this region, a result that led the authors to hypothesize that the effect of the QTL on L-ABA may actually be due to differences in the water status of the plant consequent to a primary effect of the QTL on root size/architecture. More recently, the meta-analysis conducted by Sawkins et al. (2004) has validated the key role of bin 2.04 on yield in water stressed maize.

QTLs for root traits were also investigated by Tuberosa et al. (2002c) using 171 F_3 families derived from the cross Lo964 × Lo1016. In an experiment conducted in hydroponics, 11, 7, 9 and 10 QTLs influenced primary-root length (R1L), primary-root diameter (R1D), primary-root weight (R1W) and the weight of the adventitious seminal roots (R2W), respectively. The high LOD values (> 5.0) of 10 QTLs and their sizeable R^2 values (from 14.7 to 32.6%) suggested the presence of highly significant QTLs. The most significant QTLs (LOD values of 14.7, 6.4 and 8.3 for R1D, R1L and R2W, respectively) were mapped on bin 1.06. In order to verify to what extent the QTLs influencing root growth in hydroponics may also modulate root growth in the field, a random sample of 118 (Lo964 × Lo1016) F_3 families was tested for RPF in replicated field trials (Landi et al. 2002). Among the 30 bins with QTLs for RPF and/or number of brace roots, 15 (including 1.06) also harboured QTLs for root traits in hydroponics. QTLs for root traits on bin 1.06 have also been reported in Polj17 × F-2 (Lebreton et al. 1995) and B73 × Mo17 (Kaeppler et al. 2000). Additionally, Hirel et al. (2001) reported a major QTL for nitrogen use efficiency and grain yield (GY) on bin 1.06, a finding that underlines the importance of this region for GY in conditions not only of limited water availability but also of limited N supply. Field trials conducted during two seasons to measure GY under well-watered (GY-WW) and water-stressed (GY-WS) conditions with the Lo964 × Lo1016 F_3 families revealed a number of QTLs whose peaks overlapped with those for root traits measured in hydroponics (Tuberosa et al. 2002c) and/or in the field (Landi et al. 2002). In particular, QTLs for R2W co-localized with QTLs for GY-WW and/or GY-WS in bins 1.03, 1.06, 1.08, 7.02, 10.04 and 10.07. In five of these six regions, an increase in root weight was associated with a higher GY. Of all regions that concomitantly influenced root traits and GY, the strongest and most consistent effects were revealed by a 10-cM interval on bin 1.06 that influenced root traits and GY in both years and under both water regimes.

In order to evaluate the effects of the QTLs on bins 2.04 and 1.06 on root traits and grain yield more accurately, near-isogenic lines (NILs) differing for the parental segment at these QTL regions have been developed (Landi et al. 2005, S. Salvi et al. unpublished).

Isogenization of QTLs for root traits

The isogenization of a QTL is an essential prerequisite for its fine-mapping and to investigate in greater detail its direct effects on the target trait as well as the associated effects on other traits (Shen et al. 2001). Near-isogenic lines (NILs) contrasted for the parental chromosome regions at the target QTL can be obtained through repeated selfings (at least 5–6) of one or more individuals heterozygous at the QTL region followed by the identification of the homozygotes for each one of the two parental segments. Alternatively, each parental line of the original mapping population evaluated for discovering the QTL can be used as recurrent parent in a backcross scheme in which a single plant heterozygous at the QTL in question is utilized as donor of the alternative QTL regions; in this case, the congenic lines are identified as backcrossed derived lines (BDLs). For a cross-pollinated species such as maize, the evaluation of the effects of a particular QTL on yield or other highly heterotic traits should preferably be carried out using near-isogenic hybrids (NIHs), which can be obtained by crossing BDLs at the same target region introgressed in different genetic backgrounds. Depending on the BDLs used as parents, NIHs are either homozygous or heterozygous at the target QTL region, while being heterozygous for most of the remaining portion of the genome.

The major drawback to the utilization of NILs is the long time required for their production. This problem can be partially overcome by producing a library of introgression lines (ILs), namely a collection of NILs obtained by backcross and differing only for a small portion (usually ca. 10–30 cM) of the donor genome. In maize, an adequate coverage of the genome requires ca. 80 lines. Once the ILs are made available, the fine-mapping of any major QTL segregating in the original cross can be readily pursued. We have developed a library of ILs derived from B73 (recurrent parent) × Gaspé Flint (donor parent) to identify major QTLs for root growth and architecture. The preliminary results are quite encouraging as to the possibility of using this approach to identify major QTLs for root traits.

Root-ABA1 *affects root traits and ABA concentration in maize*

The isogenization of the region near *csu133* on bin 2.04 in the Os420 × IABO78 background allowed Landi et al. (2005) to obtain pairs of BDLs contrasted for the parental chromosome segments at this region, herein identified as (+/+) and (–/–) for their effects on L-ABA. Field testing of the BDLs under both water-stressed (WS) and well-watered (WW) regimes confirmed the effect of the QTL on L-ABA. Subsequently, NIHs for the QTL near *csu133* were developed and field-tested for two years under WW and WS conditions. Differences among NIHs for L-ABA and other morpho-physiological traits were not affected by water regimes (Giuliani et al. 2005b). Interestingly, the (+) QTL allele for high L-ABA markedly reduced root lodging. To further elucidate the effects of the QTL on root architecture and L-ABA, root traits of two pairs of BDLs were measured in plants grown in soil columns at three water regimes. On average, the QTL confirmed its effect on L-ABA and showed a significant, concurrent effect on the angle, branching, number, diameter and dry weight of the roots. Based on these results, Giuliani et al. (2005b) suggested that the QTL affects root lodging through a constitutive effect on root architecture and size which, in turn, affects L-ABA. Consequently, the QTL has been identified as *root-ABA1.*

The effects of *root-ABA1* on grain yield were also evaluated in various genetic backgrounds. For this purpose, the (+/+) and (–/–) BDLs were factorially crossed with five and 13 inbred lines of different origin, thus producing two sets of test crosses (TCs) that were tested in Italy and China, respectively, under both WW and WS conditions (Landi et al. in press). In Italy, the TCs derived from (+/+) BDLs were less susceptible to root lodging across both water regimes than the TCs derived from (–/–) BDLs (28.0 vs. 52.5%), but were also less productive under WS conditions (4.88 vs. 6.27 Mg ha^{-1}). The TCs derived from (+/+) BDLs were also less productive in China (6.83 vs. 7.49 Mg ha^{-1}; average of WW and WS conditions). In both sites, the lower grain yield of the TCs derived from (+/+) BDLs was prevalently due to a lower number of both ears/plant and kernels/plant. These results indicate that the (+) *root-ABA1* allele confers a lower susceptibility to root lodging but also a lower grain yield, especially when root lodging does not occur. The yield loss associated with the (+) *root-ABA1* allele has tentatively been ascribed to a negative effect of an excessive accumulation of ABA on reproductive fertility (Landi et al. in press). An alternative explanation might be that *root-ABA1* affects biomass production in response to sensing of drought stress. The fine-mapping of *root-ABA1* has now been undertaken as a preliminary step to its positional cloning. In order to investigate the effects of *root-ABA1* on the transcriptome and identify functional markers tightly linked to the QTL, microarray analysis has been used to profile the transcripts of the contrasting BDLs (Giuliani et al. 2005a). This study has led to the identification of several genes preferentially expressed in only one of the two BDLs; among these genes, those that map within the supporting interval of *root-ABA1* may provide useful clues as to the functional polymorphisms associated with its effects.

CLONING QTLS FOR ROOT GROWTH

Different options are available to proceed from a supporting interval delimiting a target QTL to the actual gene(s) responsible for the QTL effect. According to the mapping accuracy and the magnitude of the QTL effect, the support interval of the QTL may span several hundreds of genes. Additionally, non-coding regions may also be responsible for QTLs through a cis-acting effect on the promoter region of nearby genes. Clearly, identifying the right ‘needle’ in the ‘genome haystack’ is a daunting undertaking, although one well-worth pursuing for the possible applications and for elucidating the genetic basis of quantitative traits.

The positional cloning of a major QTL (reviewed in Salvi and Tuberosa 2005) requires the availability of (i) a large mapping population (> 1,500 plants) derived from the cross of two NILs for the target QTL, (ii) a contiged genomic (BACs or YACs) library spanning the QTL region and (iii) a system for validating the identity and testing the effects of candidate genes. Only a few of the root QTLs so far described are amenable to a positional-cloning approach, particularly in view of the large amount of resources required to measure roots accurately in the hundreds of plants that are phenotyped in any QTL-cloning project. Additionally, positional cloning in maize is made more complex by its large genome size and redundancy.

The candidate-gene approach

At its simplest, the candidate-gene approach exploits information on the role and function of a particular coding sequence and verifies whether it may represent a feasible candidate for the QTL in question (Pflieger et al. 2001). Therefore, candidate genes can also be identified in species other than the one being directly investigated. If a plausible cause–effect relationship can be hypothesized between a QTL and a candidate gene mapping nearby, then validation of its role could be attempted through genetic engineering and/or the screening of knockout mutants, avoiding the tedious procedures of the positional-cloning approach. The identification of suitable candidate genes and the elucidation of their function can be facilitated by combining different approaches and high-throughput platforms (Schnable et al. 2004; Giuliani et al. 2005a; Guo et al. 2005; Salvi and Tuberosa 2005). The recent progress in the high-throughput profiling of the proteome (Hochholdinger et al. 2005; Wen et al. 2005; Sauer et al. 2006) and metabolome (Steuer et al. 2003) provides additional leads to analyse the changes in the concerted expression of the genes involved in root growth and their response to environmental cues. However, it should be appreciated that, at present, proteomics and metabolomics can indirectly report changes occurring in only a fraction of the genome; additionally, proteomics is often unable to detect the changes in gene products (e.g., transcription factors) that, despite their low level, are more likely to play a pivotal role in root growth. A detailed study on proteome profiling is in progress to ascertain the role of cell-wall proteins (CWPs) in the elongation of the primary root in maize (Zhu et al. 2006). Although many of the CWPs identified in this study have previously been shown to be involved in cell-wall metabolism and cell elongation, a number of CWPs (e.g., endo-1,3;1,4-β-D-glucanase and α-L-arabinofuranosidase) were not described in previous cell-wall proteomic studies.

From a technical standpoint, it should be noted that the combination of the ‘omics’ platforms with laser-capture microdissection allows for unprecedented levels of functional resolution at the anatomical level. In maize, a combination of laser-capture microdissection and subsequent microarray analyses applied to the root pericycle of wild-type and *rum1* mutant allowed Woll et al. (2005) to identify 19 genes involved in signal transduction, transcription and the cell cycle that are active before lateral-root initiation; these findings will contribute to the identification of the developmental checkpoints involved in lateral-root formation in maize downstream of *rum1*.

Arabidopsis *as a model*

Although root development in *Arabidopsis* and rice differs from maize in both overall architecture and the anatomy of individual roots, genes cloned in

Arabidopsis and rice may in some cases provide interesting leads for QTL cloning in maize, particularly for those functional/morphological features of root development which from a phylogenetic standpoint may have been conserved to a greater extent across species (e.g., signalling cascades, cell elongation, growth and density of root hairs, etc.). The power of combining QTL analysis for root morphology and target metabolites with fine-mapping and mutant analysis, in order to elucidate the genetic and functional basis of root growth, was recently shown in *Arabidopsis* (Sergeeva et al. 2006) where the possible role in root elongation of the sucrose-splitting enzymes sucrose synthase and invertase was tested. Several QTLs affected both invertase activity and root length. The fine-mapping of a major QTL for root length revealed consistent co-location with the locus for invertase activity containing a gene coding for a vacuolar invertase. The role of this invertase gene in root elongation was confirmed by the analysis of a functional knockout line.

An area worthy of exploration relates to the mechanisms regulating the level of gene expression. Also in this case, the model species *Arabidopsis* has provided useful insights. Although several plant microRNAs (miRNAs) have been shown to play a role in plant development, a study in *Arabidopsis* has shown for the first time an effect on the root phenotype due to a reduced expression of a miRNA (Guo et al. 2005). *Arabidopsis thaliana* miR164 was predicted to target five NAM/ATAF/CUC (NAC) domain-encoding mRNAs, including NAC1, which transduces auxin signals for lateral-root emergence. Cleavage of endogenous and transgenic NAC1 mRNA by miR164 was shown to be blocked by NAC1 mutations that disrupt base-pairing with miR164. Compared with wild-type plants, *Arabidopsis* miR164 mutants expressed less miR164 and more NAC1 mRNA and produced more lateral roots. The results of this landmark study indicate that auxin induction of miR164 provides a homeostatic mechanism to clear NAC1 mRNA to down-regulate auxin signals and clearly show the value of using *Arabidopsis* as a model for elucidating the complex molecular mechanisms regulating root growth. Further insights on the role of auxins on root growth were provided by the study of Okushima et al. (2005): their data suggest that the ARF7 (Auxin Response Factor 7) and ARF19 proteins play essential roles in auxin-mediated growth of lateral roots by regulating both unique and partially overlapping sets of target genes.

Recently, the screening of nine *Arabidopsis* accessions grown under rigorously controlled conditions revealed that one accession was unaffected by water deficit in terms of root growth (Granier et al. 2006). A mapping population including this accession as one of the parents might facilitate the identification of QTLs modulating the response of roots to decreasing soil moisture.

CONCLUSIONS AND PERSPECTIVES

The sequencing of the maize genome is now well underway (http://www.maizegdb.org/sequencing_project.php). Once the annotated sequence is released, additional opportunities will become available for identifying the genes controlling root traits. The genomics approach, when appropriately intersected and integrated with other relevant disciplines (e.g., soil science, agronomy, crop physiology, biochemistry, etc.) will positively impact our understanding of root growth and development. As shown in this article, QTL analysis and genomics are powerful tools to disentangle the genetic complexity governing root growth and its plasticity. In a limited number of cases, such genetic complexity has been ‘Mendelized’ as a prerequisite to QTL cloning, now a reality, albeit applicable only to a few major QTLs. QTL cloning will shed light on the mechanisms regulating the quantitative expression of root traits. In this respect, modulation of gene expression is likely to play a pivotal role, and new insights will derive from a better understanding of the role of miRNAs. On the molecular side, extensive EST databases and unigene sets derived from cDNA libraries of root tissues provide valuable markers to construct functional maps that will facilitate the identification of QTL candidates. High-throughput genomic profiling based on the detection of single-nucleotide polymorphisms (SNPs) and other enabling platforms (Kilian 2005; Woll et al. 2005) will vastly improve our capacity for QTL cloning and/or allele mining. From an applicative standpoint, the challenge faced by plant scientists is how to integrate best and most effectively into extant breeding programmes the deluge of information generated through the ‘omics’ platforms. Despite the spectacular progress on the molecular side, our capacity to phenotype roots accurately on the massive scale that is often required by genomics studies remains the major limiting factor. A partial solution to the shortcomings of phenotyping is provided by an appropriate use of modelling, an approach which expands our capacity to predict the effects that specific environmental (e.g., water availability) and genetic (e.g., QTL effects, Tardieu 2003) variables might have on plant growth and final yield. Clearly, integrative and interdisciplinary approaches will be instrumental to further our understanding of root growth and, eventually, enhance our ability in tailoring root architecture in order to improve water and nutrient use efficiency of crops.

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CHAPTER 3

MULTI-TRAIT MULTI-ENVIRONMENT QTL MODELLING FOR DROUGHT-STRESS ADAPTATION IN MAIZE

M. MALOSETTI[#,###], J.M. RIBAUT[##], M. VARGAS[##], J. CROSSA[##],
M.P. BOER[#,###] AND F.A. VAN EEUWIJK[#,###]

[#] *Laboratory of Plant Breeding, Department of Plant Sciences, Wageningen University, P.O. Box 386, 6700 AJ Wageningen, The Netherlands.*
[##] *International Maize and Wheat Improvement Center (CIMMYT), 6-641, 06600 Mexico D.F., Mexico.*
[###] *Biometris, Wageningen University, P.O. Box 100, 6700 AC Wageningen, The Netherlands.*
E-mail: marcos.malosetti@wur.nl

Abstract. Water shortage is a major cause of yield loss in maize. Thus, breeding for adaptation to water-stressed environments is an important task for breeders. The use of quantitative-trait loci (QTL) models in which the response of complex phenotypes under stressed environments is described in direct relation to molecular information can improve the understanding of the genetic causes underlying stress tolerance. Mixed QTL models are particularly useful for this type of modelling, especially when the data stem from multi-environment evaluations including stressed and non-stressed conditions. The study of complex phenotypic traits such as yield under water-limited conditions can benefit from the analysis of trait components (e.g., yield components) that can be exploited in indirect selection.

Multi-trait multi-environment QTL models help to identify the genome regions responsible for genetic correlations, whether caused by pleiotropy or genetic linkage, and can show how genetic correlations depend on the environmental conditions. With the objective of identifying QTLs for adaptation to drought stress, we present the results of a multi-trait multi-environment QTL-modelling approach using data from the CIMMYT maize-breeding programme.

INTRODUCTION

Water shortage is a major cause of yield loss in maize (*Zea mays* L.). The supply of water by irrigation can alleviate drought stress, but irrigation is costly and not realistic in most of the maize production areas. Yield loss due to water stress can be

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tackled by developing varieties better adapted to water-limited conditions. Thus, drought tolerance is a prime objective of many maize-breeding programmes.

Maize is particularly sensitive to drought stress occurring just before and during flowering when the crop's yield potential is defined. When drought stress occurs just before flowering, a delay of silk emergence in relation to male flowering is observed, and this increase of the anthesis-silking interval (ASI) is correlated with lower yield (Bolaños and Edmeades 1993). Therefore, the selection of genotypes that have a short ASI under water-limited conditions has been shown to be useful to improve drought tolerance in maize (Edmeades et al. 1999; 2000; Ribaut et al. 1996).

Quantitative-trait loci (QTLs) associated with drought tolerance can be used in breeding strategies for drought tolerance, especially for selection under stress conditions, where traits typically show low heritability (Ribaut et al. 1996). In QTL mapping, complex phenotypes are modelled in direct relation to molecular information contributing to the understanding of the genetic causes underlying stress tolerance. Mixed models offer a particularly useful statistical framework for QTL analysis (Malosetti et al. 2004), especially when the data stem from multi-environment evaluations including stressed and non-stressed conditions. The study of complex phenotypic responses, such as yield under water-limited conditions, can benefit from the study of their trait components, which can be exploited in indirect selection.

In contrast to single-trait single-environment QTL models, multi-trait multi-environment QTL models simultaneously fit QTLs as affecting several traits in several environments. The attractiveness of such models is that they can help to identify the genome regions responsible for genetic correlations between traits, say yield and its components, whether caused by pleiotropy or genetic linkage, and can show how these genetic correlations depend on the environmental conditions. With the objective of identifying QTLs for adaptation to drought stress, we present the results of a multi-trait multi-environment QTL-modelling approach using data on grain yield (GY) and ASI from the CIMMYT maize-breeding programme.

MATERIALS AND METHODS

Field data and molecular-marker data

The data used in this paper were generated at CIMMYT, Mexico, with the objective of detecting QTLs related to yield and other yield-related traits under stressed conditions. A detailed description of field experiments and production of molecular-marker information is given in Ribaut et al. (1996). Briefly, an F_2 population derived from the cross between a drought-resistant parent (Ac7643S_5) and a high-yielding but drought-susceptible parent (Ac7729/TZSRWS_5) was genotyped by RFLP markers. A population of 211 $F_{2:3}$ families derived from that F_2 was subsequently evaluated in three years under different water and nitrogen stress conditions in Mexico (Table 1). Several traits were registered, but in the present chapter we concentrate on GY and ASI.

Table 1. *Description of the environments in which maize genotypes were evaluated: environment codes, location, water regime, nitrogen availability, mean GY and ASI*

Environment	Location	Water regime [¶]	Nitrogen	Mean GY ($t\ ha^{-1}$)	Mean ASI (days)
NS92a	Tlaltizapán	WW	normal	10.5	–1.6
IS92a	Tlaltizapán	IS	normal	6.4	–1.0
SS92a	Tlaltizapán	SS [§]	normal	3.7	–0.9
IS94a	Tlaltizapán	IS	normal	4.2	1.8
SS94a	Tlaltizapán	SS	normal	4.1	1.9
LN96a	Poza Rica	WW	low	1.8	2.9
HN96b	Poza Rica	WW	high	4.9	–1.1
LN96b	Poza Rica	WW	low	1.0	3.3

[¶] WW: well watered; IS: intermediate stress; SS: severe stress

[§] rainfall around flowering caused only intermediate stress

Multi-trait multi-environment phenotypic model

We first conducted an analysis without introducing molecular-marker information in the model. The multivariate multi-environment mixed model used was (random terms underlined):

$$\underline{y}_{ijt} = \mu_t + E_{jt} + \underline{G}_{it} + \underline{GE}_{ijt} + \underline{\varepsilon}_{ijt} \tag{1}$$

with $\underline{y}_{ijt}$ a vector containing the observations of genotypes (i=1…211), in each of the eight environments (j=1…8), and for the two traits (t=1…2); μ_t an intercept for each trait (overall trait means across genotypes and environments), E_{jt} the environmental effect (fixed), $\underline{G}_{it}$ and $\underline{GE}_{ijt}$ the trait-specific genotypic main effects and genotype-by-environment interaction (GEI) effects, respectively (both random terms), and finally a residual term, which we considered heterogeneous among environments. An unstructured variance–covariance matrix was assumed for the $\underline{G}_{it}$ term thus introducing genetic correlations between traits due to genotypic main effects. For the $\underline{GE}_{ijt}$ term we imposed a factor-analytic model of order 1 (FA1). This model allows parsimonious modelling of genotypic correlations between environments and traits, since it requires fewer parameters than an unstructured model (Smith et al. 2001). In summary, the mixed model as defined above, with an unstructured variance–covariance model for the genotypic main effects of the traits and a FA1 model for the GEI part, allows to consider heterogeneity of genetic variance for the traits across environments, genetic correlations between environments for the same trait, and genetic correlations between traits within and across environments. Residuals were not estimated directly from the analysis since the data consisted of genotypic means per trial, but estimates were available from previous analysis.

Genotypic and GEI variance components per trait were estimated based on Model (1) and used to estimate heritability and genotypic and phenotypic correlations between GY and ASI. The association between GY and ASI was graphically represented by a number of scatter plots. Further investigation of the GEI patterns was performed by plotting the factor loadings for both axes of a factor-analytic variance–covariance model of order 2 (FA2), which can be regarded as an analogue of the AMMI analysis within a mixed-model framework (Smith et al. 2001).

The multi-trait multi-environment QTL analysis

The multivariate mixed model previously described was upgraded to include molecular information, leading to the following QTL model:

$$\underline{y}_{ijt} = \mu_t + E_{jt} + x_i^{add}\alpha_{jt} + x_i^{dom}\delta_{jt} + \underline{G}_{it} + \underline{GE}_{ijt} + \underline{\varepsilon}_{ijt} \qquad (2)$$

where the two extra terms in the model (both fixed effects) account for environment-specific additive QTL (α_{jt}) and dominance QTL (δ_{jt}) effects on GY and ASI. The covariables x_i^{add} and x_i^{dom} are called genetic predictors and are a function of the inferred genotypic constitution of the QTL at one particular point on the chromosome (Jiang and Zeng 1997). In short, for a given genotype, the additive genetic predictor (x_i^{add}) had a value –1 when homozygous of the maternal type, 0 when heterozygous, and +1 when homozygous of the paternal type. The dominance genetic predictor (x_i^{dom}) had a value +1 when the genotype was heterozygous at the locus, and 0 otherwise. With the genetic predictors estimated along the chromosomes we fitted the model at the different chromosome positions. The fixed QTL effects were tested by a Wald test (Verbeke and Molenberghs 2000) and the test statistic was plotted along the chromosomes to produce an analogue to the LOD score profile usually presented in QTL-mapping results. A QTL was revealed by a peak value exceeding a threshold value defined to control for multiple testing. Note that the described test is a global test for the presence of a QTL, with an effect on GY, ASI or both. Therefore, at positions where the global test indicated a QTL, we subsequently estimated and tested for the specific effects on GY and ASI being different from zero, the equivalent of a t-test using estimated standard errors. We restrict the analysis here to chromosomes 1 and 10.

When a QTL was found significant for both GY and ASI, a second scan was performed to investigate whether a single pleiotropic QTL or two closely linked QTLs were involved. In the initial scan a pleiotropic model was assumed as the genetic predictors represented the genotypic constitution at the same position for both traits. However, in this later stage, we allowed the genetic predictors to represent different positions on the chromosomes within a window of 20 cM around the initial pleiotropic position. The result of the two-dimensional scan was plotted in a contour plot to identify the region where the maximum for the test statistic was located, with close-linkage detected when that maximum resided far from the diagonal of the plot.

RESULTS AND DISCUSSION

Phenotypic analysis

The genotypic and GEI variance components for both traits are presented in Table 2. As expected, genotypic-variance components were larger for ASI than for GY. Conversely, GEI was more important for GY than for ASI, although the latter showed considerable GEI in some of the environments (particularly the water-stressed trials in 1994 and the low-nitrogen trials in 1996). Estimated heritability per environment was similar for GY and ASI, an observation that was already mentioned in previous studies of this population (Ribaut et al. 1996) and other similar populations used in CIMMYT trials (Bolaños and Edmeades 1996).

Table 2. *Estimates of genotypic variance across environments (V_G), environment-specific genotypic variances ($V_{G(E)}$), and environment-specific error variances (V_E) for grain yield (GY) and anthesis-silking interval (ASI), plus estimates of heritability (h^2), and genotypic (r_G) and phenotypic (r_P) correlations between traits*

	GY (t ha^{-1})				ASI (days)					
	V_G	$V_{G(E)}$	V_E	h^2	V_G	$V_{G(E)}$	V_E	h^2	r_G	r_P
NS92a	0.09	2.71	2.07	0.58	0.96	0.06	1.63	0.38	–0.11	–0.05
IS92a	0.09	1.04	2.03	0.36	0.96	0.21	0.98	0.54	–0.16	–0.07
SS92a	0.09	0.92	1.43	0.41	0.96	0.13	1.27	0.46	–0.12	–0.05
IS94a	0.09	1.33	1.53	0.48	0.96	1.13	2.45	0.46	–0.57	–0.27
SS94a	0.09	1.39	1.52	0.49	0.96	1.36	4.06	0.36	–0.48	–0.20
LN96a	0.09	0.17	0.39	0.41	0.96	1.07	2.04	0.50	–0.01	–0.01
HN96b	0.09	1.56	0.97	0.63	0.96	0.46	0.23	0.86	0.00	0.00
LN96b	0.09	0.20	0.21	0.59	0.96	2.33	2.64	0.56	0.05	0.03

Figure 1 shows the patterns of GEI in the experiments. For interpretation, the lengths of the vectors representing the environments correspond to the amount of GEI in that environment. The (cosine of the) angle between environmental vectors is proportional to the correlation between the two environments with respect to the GEI. Acute angles represent high positive correlations, obtuse angles indicate high negative correlations, and right angles point to low correlations. For GY, GEI was mainly caused by the contrast between environments in 1992 and 1994 versus those in 1996. This pattern reflects the contrast between two different locations, Tlaltizapán and Poza Rica, that represent rather different growing environments for maize, especially in terms of temperatures and water availability (Tlaltizapán is a drier and cooler location than Poza Rica) (Edmeades et al. 1999). GEI for ASI was mainly caused by the contrast of the trials in 1994 versus the ones in 1992 and 1996. In this case, the contrast seems to reflect the effect of a water-stressed environment (1994) versus those that did not have or had mild water restrictions (though some of the environments in Poza Rica were stressed due to low nitrogen). We emphasize that in those trials of 1992 that were managed in such a way that water stress was supposed to occur, rainfall occurred around flowering and, therefore, the plants did not experience water shortage. This lack of water stress is reflected by the values for average ASI observed in those trials: these values were similar to the ones observed in environments in which water stress was not imposed (Table 1).

Figure 1 also reflects the correlations between GY and ASI, and shows that in most of the environments the association between both traits was rather low (right angle between vectors). The only example of a negative association between GY and ASI was observed in the trials of 1994, where an obtuse angle between GY and ASI vectors indicates a negative correlation (Figure 1). This conclusion is in agreement with the estimated genetic correlations between both traits (Table 2). The lack of association between GY and ASI in most of the environments is also evident from Figure 2, where only in the water-stressed trials of 1994 a moderate association is observed (Figure 2). This observation is consistent with previous results in which the correlation between GY and ASI was mainly observed in water-stressed environments (Bolaños and Edmeades 1996; Chapman and Edmeades 1999).

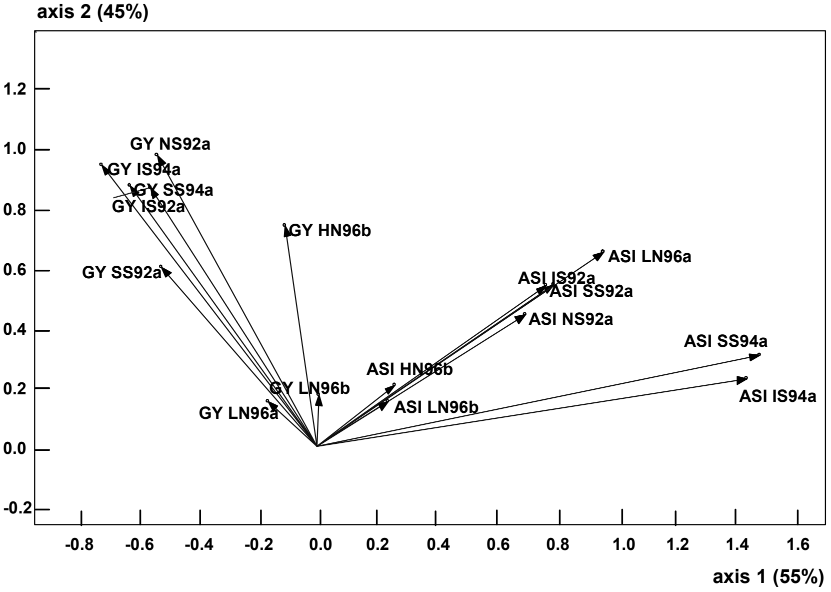

Figure 1. *Plot of loadings from a factor-analytic model of order 2 (FA2) fitted on maize trials carried out in eight environments in Mexico. The labels associated to each vector indicate the observed trait (GY or ASI) and environment*

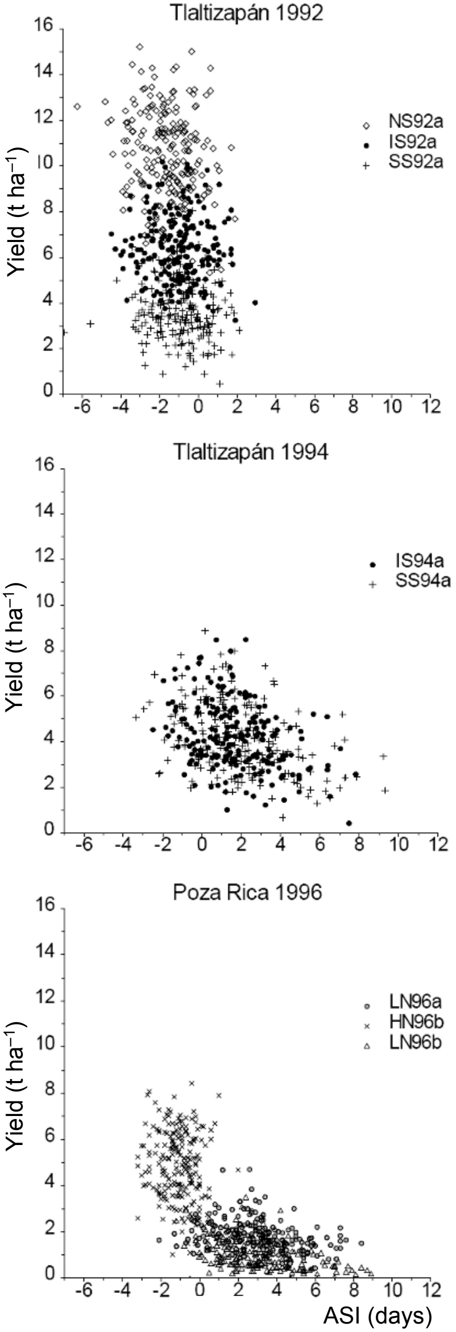

__Figure 2.__ Yield versus ASI in eight environments, with environments grouped by year

QTL analysis

The profiles resulting from the QTL scan of chromosomes 1 and 10 are presented in Figure 3. The profiles show three regions where significant additive QTL effects were found, two on chromosome 1 and one on chromosome 10. The two regions on chromosome 1 were at 137 cM and at 215 cM and the one on chromosome 10 at 62 cM, which agreed with previous studies in the same population, although using different QTL models (Ribaut et al. 1996; Vargas et al. 2006). No significant dominant effects were found on any of the chromosomes.

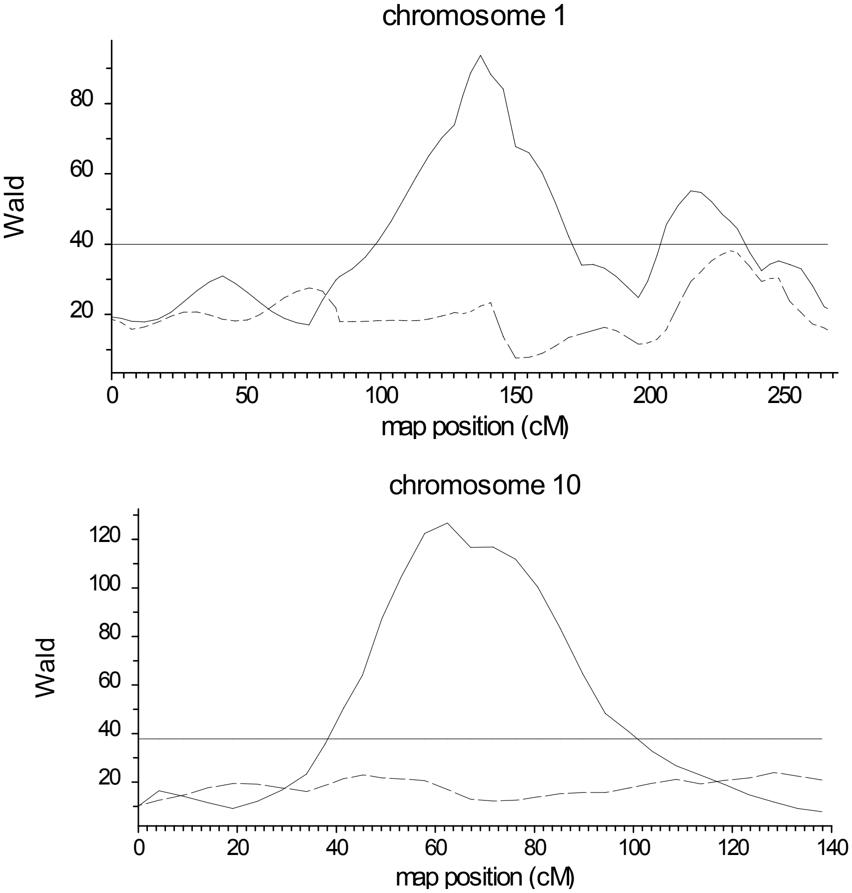

Figure 3. Result of a multi-trait multi-environment QTL-mapping scan (simple interval mapping) on chromosomes 1 and 10 of maize. The profile represents the test statistic under the null hypothesis of no additive (solid line) or dominance (broken line) QTL effect on GY or ASI in any environment. The horizontal line represents a threshold above which the null hypothesis is rejected

Two linked QTLs for GY and ASI on chromosome 1

The estimates of the QTL effects revealed that while the QTL at 137 cM on chromosome 1 had an effect only on GY, the one at 215 cM on the same chromosome had an effect exclusively on ASI (Table 3). The magnitudes and signs of the effects for GY reflect the higher GEI observed in this trait, as for example the allele coming from the high-yielding parent (the father) resulted in higher yields in four environments, but lower yield in the high-nitrogen environment of Poza Rica (Table 3), and no superiority in the rest of the environments. The effects for ASI were more consistent across the environments, with some variation in the magnitude, but not in sign (Table 3). Considering the signs of the effects of these two QTLs, the genetic correlation that those two QTLs induce is in the expected direction (except in HN96b), that is, a negative correlation between GY and ASI. However, the impact of this correlation might be low in view of the weak linkage between the QTLs (137 and 215 cM).

Table 3. *Environment-specific QTL effects for GY (t ha^{-1}) and ASI (days). A negative sign indicates that the high-value allele is coming from the maternal line (drought-resistant) and a positive sign indicates that the high-value allele is from the paternal line (high-yielding line)*

	chr 1, 137 cM		chr 1, 215 cM		chr 10, 62 cM	
Environment	GY	ASI	GY	ASI	GY	ASI
NS92a	0.65 *	–0.1	–0.26	–0.4 *	0.50 *	0.4 *
IS92a	0.63 *	–0.1	–0.12	–0.5 *	0.60 *	0.5 *
SS92a	0.82 *	–0.1	0.09	–0.6 *	0.19	0.5 *
IS94a	0.61 *	–0.3	–0.21	–0.7 *	0.47 *	0.5 *
SS94a	0.31	–0.3	0.00	–1.1 *	0.62 *	0.7 *
LN96a	0.01	0.3	0.01	–0.7 *	0.11	0.8 *
HN96b	–0.37 *	0.0	0.07	–0.4 *	0.97 *	0.2
LN96b	0.05	0.1	0.00	–0.5 *	0.10	0.5 *

* $P<0.05$

The QTL on chromosome 10

In contrast to chromosome 1, the QTL on chromosome 10 had a significant effect on both GY and ASI, in four of the eight environments (Table 3). Another remarkable difference was that the induced correlation was positive rather than negative. From a physiological point of view, a short ASI is an indicator of a better crop status (higher crop and ear growth rates), which relates to a higher yield (Edmeades et al. 2000; Westgate 2000). However, in our example, the allele coming from the high-yielding parent also caused a higher ASI value (Table 3), inducing a positive correlation between GY and ASI. On the one hand, and since GY is a complex trait determined by many processes during development, it is possible that the disadvantage of a longer ASI determined by this QTL is compensated by an advantage given by the same QTL at a later developmental stage, e.g., grain filling. On the other hand, this result may point to less-explored physiological mechanisms, which determine the increase of both GY and ASI. From a breeder's point of view, our result suggests that phenotypic selection for short ASI will not necessarily retain all positive alleles for GY, which highlights the potential of marker-assisted selection as a complement to conventional phenotypic selection.

A relevant question that follows from the results found for chromosome 10 is whether pleiotropy or genetic linkage is present. We addressed this question by refitting the model allowing for changing positions of the putative QTLs for GY and ASI. The results are presented in Figure 4 where the pleiotropic model (indicated in the figure by a dotted diagonal line) can be compared with alternative linkage models. Our result indicates that the area where the maximum of the test statistics was found (white area) included the pleiotropic model, though a close-linkage model cannot be excluded either (Figure 4). Whichever of the two models is the real underlying genetic model, the region would be considered as 'functionally pleiotropic' as breaking this association will always be difficult in practice.

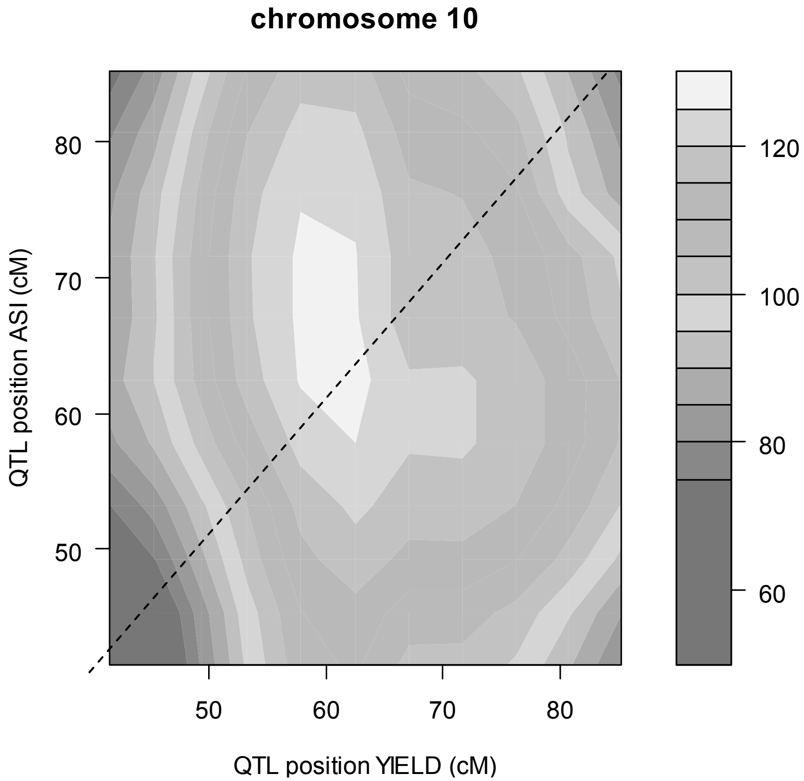

Figure 4. *Contour plot of the Wald statistic for QTL effects on chromosome 10 with varying positions for GY (horizontal axis) and for ASI (vertical axis). The results of pleiotropic models are represented on the diagonal (dotted line)*

CONCLUSIONS

With an example in maize we showed how information stemming from managed-stress trials can be exploited to investigate the genetic causes of drought-stress adaptation. Mixed models are particularly suitable to model complex phenotypic responses across environments (stressed and not stressed), including the commonly observed GEI. In addition, multivariate mixed model approaches allow to model the association between traits in their dependence on the environmental conditions. One step further, molecular marker information can be incorporated to identify the genome regions underlying variation and co-variation between traits, thereby providing relevant information for practical plant breeding. Questions on the relevant regions to select for and on pleiotropy versus genetic linkage determining correlations between traits can be addressed. This information can be advantageously integrated in breeding procedures for direct and indirect selection of better adapted genotypes.

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CHAPTER 4

ACCOUNTING FOR VARIABILITY IN THE DETECTION AND USE OF MARKERS FOR SIMPLE AND COMPLEX TRAITS

S.C. CHAPMAN[#], J. WANG[##], G.J. REBETZKE[###]
AND D.G. BONNETT[###]

[#] *CSIRO Plant Industry, Queensland Bioscience Precinct, St. Lucia, QLD 4067, Australia.*
[##] *Crop Research Informatics Laboratory, International Maize and Wheat Improvement Center (CIMMYT), Institute of Crop Science, and The National Key Facility for Crop Gene Resources and Genetic Improvement, Chinese Academy of Agricultural Sciences, Beijing 100081, China.*
[###] *CSIRO Plant Industry, P.O. Box 1600, Canberra, ACT 2601, Australia.*
E-mail: scott.chapman@csiro.au

Abstract. There are many sources of variability in gene–phenotype associations. During the measurement of genotype and phenotype and during selection, researchers must deal with experimental error in trials; gene-gene interaction (epistasis) for sub-traits and observed traits; trait-trait interaction (pleiotropy) and gene- or genotype-by-environment interaction. These effects can be structured in a framework that allows simulation of the entire gene-environment 'landscape'. Studies of these landscapes have been published by others. Here we aim to explain with simple examples some of the types of insights that can be made. A current challenge for breeders working with simple marker–phenotype associations is to design selection strategies that can rapidly create new combinations of multiple marker-based traits. For a real-world example in wheat, we have used simulation to show how gene enrichment during early generations (selection of homozygotes and heterozygotes with desirable alleles) can greatly reduce resource requirements when combining 9 genes into one genotype through marker-assisted selection. Another wheat example compares phenotypic and QTL-based selection for coleoptile length where the QTL also had a pleiotropic association with plant height. These simulations show the relative negative effects of either low heritability, or less than complete detection of QTL associated with traits. Finally, we revisit a marker-assisted selection (MAS) example whereby a QTL study is undertaken on a population for a complex trait, and then those QTL are used in selection. This process is subject to all sources of error described above. If the trait is complex, then interactions among sub-traits; between sub-traits and the environment; or between the chromosomal locations of controlling genes, create an extremely 'rugged' selection landscape that slows breeding progress. In this situation, a detailed understanding of some of these interactions is required if MAS is to be able to exceed the progress of conventional breeding.

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INTRODUCTION AND BACKGROUND

Many breeding programmes are now utilizing marker–trait associations as part of their selection process. Some of the typical applications include introgression of traits from donor (‘unadapted’ lines) into parental germplasm; broadening the genetic base of a crop (Xu et al. 2004); selection of parental combinations, based on marker profiles (Wang et al. 2005); selection of cross progeny during early and late generations of selfing and evaluation (Eagles et al. 2001); and recurrent selection based on marker–trait associations (Podlich et al. 2004). The value of markers is heightened when the target trait is difficult or expensive to screen, like resistance to cereal cyst nematodes (Ogbonnaya et al. 2001). With the continued expansion of information on quantitative trait loci (QTL) for more complex traits, there is an increasing desire to implement these efficiently in plant-breeding programmes, with new strategies being proposed for this (Podlich et al. 2004).

In considering how to utilize markers, several issues arise around the association of markers with genes that affect trait expression, and around the precision of the estimates of the relationship between alleles and trait expression. In perhaps the simplest relationship, the presence of a single allele at a single locus explains 100% of the observed phenotype in a particular environment, such as in the case of a gene that confers resistance to a single rust pathotype. This association is effectively the same as a qualitative gene effect like seed colour, apart from the need to have the ‘rust environment’ to see the effect. If the gene sequence is known, and/or the phenotype has been carefully mapped in crosses or screened across a large number of resistance and susceptible lines, then a ‘perfect’ allele marker may be available (Ogbonnaya et al. 2001). So, the gene-trait relationship is 100% explained; there is no genetic-background effect (the marker works in different pedigrees); and the relationship can be predicted without error by the presence/absence of a marker for the desired allele. The challenge for breeders then is to combine sets of essential alleles into single backgrounds.

At the other extreme is the case where many genes interact with each other (epistasis) in different environments (gene-by-environment interaction) and affect sub-traits that interact to determine the desirable trait (pleiotropy). For a particularly complex trait like yield, there are networks of interactions, including recursive effects, among these components of control of the desirable trait. A QTL study will never explain 100% of the genetic variation typically observed. During selection these effects will be apparent as low heritability for the trait and/or poor linkage between QTL and their markers. But it may not be clear how the main sources of error (epistasis, gene-by-environment or pleiotropy) result in the residual variance that is not explained by QTL.

Recently, Cooper et al. (2005) proposed a gene-to-phenotype modelling framework to utilize molecular breeding for complex traits. This illustrates, for a large number of genetic models, how the ‘context-dependent’ relationships between genes (epistasis, gene-by-environment interaction and pleiotropy) impact on genetic progress in both molecular and phenotypic breeding strategies. They propose, as an alternative to ‘traditional’ quantitative genetic models (say, comprised of genotype and genotype-by-environment interaction effects), to work with models where phenotype is described as a function of 'explained' and 'unexplained' sources of variation, and these sources are associated with vectors of 'known' and 'unknown' gene (or QTL) and gene-by-environment effects. For example, a simulation may use the predicted effects and the QTL/marker locations from a QTL study to simulate genetic progress in a breeding programme, assuming that 100% of the genetic variance is explained by the QTL. In an actual study, for all but the simplest gene–trait relationships, the 'unexplained' variance for a complex trait is typically 20 to 80%. The simulation can then be re-run multiple times, while adding in each an ensemble of different gene effects (representing epistasis and G×E etc.) to determine the potential effect of this 'unexplained variance' on expected genetic progress. Not so surprisingly, real-world QTL studies where the unexplained variance was high suffered more in terms of potential impact on selection, but now there is a method to quantify this effect in terms of expected context dependencies. These methods can help breeders to decide on the likely usefulness of the QTL in their selection scheme, given a better understanding of how robust the QTL are for expected (or unexpected) levels of complexity in the 'unexplained' variance.

While there has been some application of simulation approaches to examine the value of QTL for complex traits in Australian sorghum (Hammer et al. 2005), marker technology is still being developed for application in that breeding programme, but is focusing on utilization for QTL associated with complex traits such as midge resistance and stay-green (see Hammer et al., Chapter 5). Markers for single-gene/single-trait applications have been used in wheat-breeding programmes in Australia for over 10 years, e.g., Ogbonnaya et al. (2001) and Eagles et al. (2001). In general, their use has been in introgressing into breeding lines (in BCF_1) and in screening progeny in early (F_2) and later generations of evaluation. A pertinent task for these breeding programmes is devising strategies to combine these many 'simple' genes together into breeding lines.

Cooper et al. (2005) explored a large number of breeding scenarios, focusing on QTL for complex traits, and were able to summarize from these that gene-by-environment effects were still a substantial impediment to marker selection for complex traits. We aimed to present three practical scenarios of applying marker-assisted selection:

1. from a 3-way cross, recovering a target genotype comprising 9 desirable genes that have near-perfect markers;
2. from a 2-way cross, selecting for a quantitative trait (coleoptile length), given different levels of knowledge of the genetic variation explained by the QTL;
3. for a sorghum-breeding programme, selection for yield based on QTL detected in a single environment, compared to progress based on knowledge of underlying 'physiological pleiotropy' controlling yield.

Throughout the chapter, we aim to demonstrate how these approaches can account for sources of variability and assist breeders to deal with them.

MATERIALS AND METHODS

While some of the calculations presented here can be applied quite simply to sets of unlinked genes, the QU-GENE simulation platform was used for more complex scenarios (Podlich and Cooper 1998). The programme generates populations of genotypes and provides a library of subroutines to develop simulation modules of breeding programmes. For the wheat examples, we simulated selection using QuLine, a breeding module used to simulate wheat-breeding programmes (Wang et al. 2003), and to predict cross performance for quality traits (Wang et al. 2005). For sorghum, the original simulations were done using a proprietary breeding module (Hammer et al. 2005). More details of the examples are given in the Results section.

RESULTS

Example 1: Single-gene control of traits – Using F_2 enrichment to combine 'simple' genes in a complex cross

This example is the subject of a paper (Wang et al. in press) that explores additional details beyond those given here. Where 5 genes are unlinked and a simple cross is considered, the frequency (f) of the desired homozygote in the F_2 can be estimated as $0.25^5 = 0.00098$. To select one target genotype at an acceptance probability (α) of 0.01, this would require an F_2 population size of about 4,700 individuals, estimated from log (α) / log (1 – f). Delaying selection until lines are homozygous requires only 145 individuals as the frequency becomes 0.5^5. For 12 independent loci, > 77 million lines are needed to identify a single homozygote in the F_2, or > 18,000 in fixed lines. In this case, F_2 'gene enrichment' (selection of homozygotes and heterzygotes (Bonnett et al. 2005)) is a useful strategy as only 144 F_2s would need to be screened to retain the desired gene combination ($f = 0.75^{12} = 0.03168$), followed by screening of 596 fixed lines to recover then a homozygote individual ($f = (2/3)^{12} = 0.00771$).

Using simulation (QU-GENE/QuLine, Wang et al. 2003), we examined progeny from a 3-way cross ((Silverstar + *tin* × HM14BS) × Sunstate) segregating at 9 loci (7 independent). The aim was to recover a target genotype (at overall acceptance α = 0.01) that had the required alleles (bottom line of Table 1).

In the TCF_1, selection of *Rht-B1a* and *Glu-B1i* homozygotes could be fixed, and enrichment (selection for heterozygotes) done for *Rht8*, *Cre1*, and *tin*. If no selection was applied in the F_2, then a total of > 3500 lines (> 25,000 marker screens) were needed to recover the target genotype (Table 2). This was reduced to < 600 lines (< 3500 marker screens) if F_2 enrichment was used for the 7 loci that had not been fixed in the TCF_1. The effect of linkage between the *Glu-A3* and *tin* loci, and the non-perfect marker for *tin* (Table 1) resulted in a final frequency of the *tin* gene of 0.79, while other genes were all fixed at frequencies of 1.0 or > 0.98. Therefore, the presence of *tin* would still need to be confirmed by phenotyping after production of the fixed lines. So, in this example of multiple gene selection, the desired gene combinations can be achieved with a relatively small number of screens, even given slightly imperfect markers for three trait loci, and linkage-in-repulsion for two of the loci.

Example 2: Polygenic control of quantitative traits – Selection for increased coleoptile length and reduced height

The GA-insensitive, height-reducing gene, *Rht-D1b*, also reduces coleoptile length (cl) by about 20% in wheat seedlings, while the *Rht8* gene has virtually no effect. Long coleoptiles are desirable so that seeds can be planted deeper to access soil water better at sowing. The screening of the cl phenotype is taken after a set period growing in dark conditions in a controlled-temperature environment.

Based on QTL-mapping studies (Rebetzke et al. 2001; in press), 8 QTL were considered to affect height (ht) and coleoptile length in addition to the major height genes. Supposing the reduced height alleles at *Rht-D1* and *Rht8* reduce the plant height by 10 and 8 cm (explaining 48% and 31% of genetic variance, respectively; Ellis et al. 2002), then these additional QTL affecting plant height by 2 to 3 cm each explain between 2 and 5% of the genetic variance (data not shown). The QTL for coleoptile length explain similar proportions of genetic variance (equating to –3 to +4 mm), while the 18-mm reduction due to *Rht-D1b* explains about 80% of the

***Table 1**. Selected genes, their chromosomal location and the genotypes for the three parents*

Gene symbol	Rht-B1	Rht-D1	Rht8	Sr2	Cre1	VPM	Glu-B1	Glu-A3	tin
Chromosome	*4BS*	*4DS*	*2DL*	*3BS*	*2BL*	*7DL*	*1BL*	*1AS*	*1AS*
Marker type	*Cod.*	*Cod.*	*Cod.*	*Cod.*	*Dom.*	*Dom.*	*Cod.*	*Cod.*	*Cod.*
Marker–gene distance (cM)	*0*	*0*	*0.6*	*1.1*	*0*	*0*	*0*	*0*	*0.8*
Silverstar+ *tin*	***Rht-B1b***	*Rht-D1a*	*rht8*	*sr2*	***Cre1***	*vpm*	***Glu-B1i***	*Glu-A3c*	***tin***
HM14BS	*Rht-B1a*	*Rht-D1a*	***Rht8***	*sr2*	*cre1*	*vpm*	*Glu-B1a*	*Glu-A3e*	*Tin*
Sunstate[a]	*Rht-B1a*	***Rht-D1b***	*rht8*	***Sr2***	*cre1*	***VPM***	***Glu-B1i***	***Glu-A3b***	*Tin*
Target genotype	*Rht-B1a*	*Rht-D1a*	***Rht8***	***Sr2***	***Cre1***	***VPM***	***Glu-B1i***	***Glu-A3b***	***tin***

[a] The bold-printed alleles at *Rht-B1*, *Rht-D1* and *Rht8* reduce plant height; those at *Sr2*, *Cre1*, and *VPM* confer resistance to rusts or cereal-cyst nematode; those at *Glu-B1* and *Glu-A3* improve dough quality; and, the bold-printed allele at *tin* reduces the tiller number. The genes are all unlinked, except for *Glu-A3* and *tin*, which are linked in repulsion at 3.8 cM apart on chromosome 1AS.

***Table 2**. Selected proportion and number of individuals (or families) selected in each marker selection scheme*

Breeding population	*No enrichment selection in TCF_2*		*Enrichment selection for all target genes in TCF_2*	
	Selected proportion	*Minimum population size*	*Selected proportion*	*Minimum population size*
TCF_1	0.0313	145	0.0316	144
TCF_2			0.1190	37
DHs	0.0013	3440	0.0112	408

genetic variance. We undertook a series of simulations of a cross between HM14BS (ht 82 cm; cl 125 mm) and Sunstate (ht 78 cm; cl 75 mm) to attempt to recover a target genotype with increased coleoptile length and reduced height, but with a greater proportion of the desirable Sunstate genetic background.

In all cases, the process was to make the cross, produce ten F_1 plants, and then produce 1000 doubled haploid (fixed) lines prior to selection by either phenotype or by combinations of the markers for the two major and eight minor QTL. In an initial simulation (1), we made a single cross between HM14BS and Sunstate, assuming broad-sense heritabilities of 0.7 and 0.8 for height and coleoptile length, respectively, and undertook selection for coleoptile length in the 1000 DH lines, with no selection for height (Table 3). As might be expected, this led to a taller phenotype with a long coleoptile, i.e. a greater proportion of lines carrying both the *Rht-D1b* and *Rht8* alleles, and minor QTL for both coleoptile length and height. The next two simulations (2 and 3) show the effect of experimental precision in the measurement of coleoptile length. Compared to the initial simulation ($H_b = 0.8$), the final length of the selected lines decreased or increased by 5 mm or more as the phenotyping was made less precise ($H_b = 0.5$) or more precise ($H_b = 1.0$).

The remaining simulations (4 to 6) involve selection using the QTL information. For the major QTL, selection was against *Rht-D1b* and for *Rht8*, to increase the coleoptile length while trying to minimize the effect on height. When selection was applied only to these major QTL (simulation 4, Table 3), followed by selection on coleoptile phenotype, the plant height was close to that of HM4BS.

Table 3*. Breeding schemes and final height (ht) and coleoptile length (cl) of top 2% of lines*

Scheme	*Heritability*		*Selection for QTL*		*Selection for cl*	*Mean value*	
	Ht	*Cl*	*Major*	*Minor*		*ht (cm)*	*cl (mm)*
1	0.7	0.8	No	No	Yes	91.1	132.8
2	0.5	0.5	No	No	Yes	90.7	127.8
3	1.0	1.0	No	No	Yes	90.9	138.3
4	0.7	0.8	Yes	No	Yes	82.6	126.6
5	0.7	0.8	Yes	8	No	82.9	123.5
6	0.7	0.8	Yes	4	No	82.0	133.7

Example 3: Polygenic control of complex traits – Selection for 'yield' QTL in sorghum

At the other extreme of gene–trait relationships, is the example of selection of markers linked to QTL controlling complex traits. In this situation, many sources of error exist, which include: experimental error in measuring the phenotype during the QTL study (trait heritability); error in selection of the marker or markers for the QTL (poor linkage); lack of observation (or knowledge) about how 'sub-traits' combine physiologically to affect the trait of interest; and, most critically, lack of knowledge of the gene action of the 'unexplained' variance in the QTL study.

Using simulation, Chapman et al. (2003) and Hammer et al. (2005) illustrated that when simple additive gene action was defined for four sub-traits ('trait parameters') in a sorghum-cropping system, complex gene-by-gene-by-environment interactions could still be generated for expression of yield. Models of gene action (i.e. for phenotypes associated with QTL) were used to define trait parameters that are input values to a crop simulation model. For example, one trait parameter was the relationship between crop development rate (toward flowering) and temperature. For this trait, a simple additive three-gene model, based on existing knowledge of QTL, calculated the parameter for each of the simulated genotypes created in a population. The calculated parameters (for this and the other three traits) were input to a crop simulation model, and the model was then run for each genotype using soil data for six locations and weather data over 100 years. This generated a complex 'gene–environment landscape' from which environments could be sampled (e.g., several locations in a single year) and genotypes could be selected on the basis of the expression of the trait value as it affected yield. The best lines were crossed to create new generations in a manner similar to a conventional breeding programme. Chapman et al. (2003) quantified how bias in the sampling of environments by the breeding programme (because of variability in rainfall between successive seasons) reduced the efficiency of selection, through the generation of substantial genotype-by-environment interaction. Using the same dataset, Hammer et al. (2005) showed how even 'simple' combinations of traits across genotypes and environments could easily confound detection of QTL associated with yield.

DISCUSSION

In example 1, there was no attempt to select for 'background' alleles during the process of combining the essential genes. In practice, the breeding programme screens a large number of lines (ca. 10 to 20% more than indicated) using F_2 enrichment so that more than one target genotype is recovered. These target lines are then tested for field performance and may then be used as cultivars and/or parents in crossing and selection. In Australian wheat breeding greater disease resistance and grain quality are deemed 'essential' and have often taken priority over selection for yield *per se*, with integration of new sources of yield adaptation taking quite some time. This contrasts with the situation illustrated in sorghum, and in US corn breeding, where a major objective is to maintain and build upon elite combinations of genes for complex traits like yield (Duvick et al. 2004; Podlich et al. 2004).

Using a slightly different simulation approach that studied only marker-assisted recurrent selection, Bernardo and Charcosset (2006) found that if large numbers (say 40 to 100) of QTL affected a trait, it was more advantageous to use only large-effect QTL and to ignore the small-effect QTL in selection, given the small population size typically used in marker-assisted recurrent selection. However, empirical evidence suggests that these large-effect QTL are fixed in early cycles while evidence from other studies (e.g., Openshaw and Frascaroli 1997) show that many of the genetic effects for traits such as yield are indeed small.

Thus, for most important breeding traits it is challenging to implement the large amount of QTL studies through marker-assisted selection to exceed the breeding efficiency of the conventional phenotypic selection.

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CHAPTER 5

AN INTEGRATED SYSTEMS APPROACH TO CROP IMPROVEMENT

G.L. HAMMER[#] AND D.R. JORDAN[##]

[#] *Agricultural Production Systems Research Unit (APSRU), School of Land and Food Sciences, The University of Queensland, Brisbane, QLD 4072, Australia.*
[##] *Hermitage Research Station, Queensland Department of Primary Industries, Warwick, QLD 4370, Australia.*
E-mail: g.hammer@uq.edu.au

Abstract. Progress in crop improvement is limited by the ability to identify favourable combinations of genotypes (G) and management practices (M) given the resources available to search among possible combinations in the target population of environments (E). Crop improvement can be viewed as a search strategy on a complex G×M×E adaptation or fitness landscape. Here we consider design of an integrated systems approach to crop improvement that incorporates advanced technologies in molecular markers, statistics, bio-informatics, and crop physiology and modelling. We suggest that such an approach can enhance the efficiency of crop improvement relative to conventional phenotypic selection by changing the focus from the paradigm of *identifying superior varieties* to a focus on *identifying superior combinations of genetic regions and management systems*. A comprehensive information system to support decisions on identifying target combinations is the critical core of the approach. We discuss the role of ecophysiology and modelling in this integrated systems approach by reviewing (i) applications in environmental characterization to underpin weighted selection; (ii) complex-trait physiology and genetics to enhance the stability of QTL models by linking the vector of coefficients defining the dynamic model to the genetic regions generating variability; and (iii) phenotypic prediction in the target population of environments to assess the value of putative combinations of traits and management systems and enhance the utility of QTL models in selection. We examine *in silico* evidence of the value of ecophysiology and modelling to crop improvement for complex traits and note that, while there is no definitive position, it seems clear that there is sufficient promise to warrant continued effort. We discuss criteria determining the nature of models required and argue that a greater degree of biological robustness is required for modelling the physiology and genetics of complex traits. We conclude that, while an integrated systems approach to crop improvement is in its infancy, we expect that the potential benefits and further technology developments will likely enhance its rate of development.

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INTRODUCTION

Progress in crop improvement depends on identifying favourable combinations of genotypes and management practices from among innumerable possible combinations. Available resources and variability in the target environments limit this search process. Crop improvement can be viewed as a search strategy on a complex adaptation or fitness landscape, which consists of the phenotypic consequences of genotype (G) and management (M) combinations in target environments (E) (Cooper and Hammer 1996). The phenotypic consequences of only a very small fraction of all possible G×M×E combinations can be evaluated experimentally. Hence, most of the fitness landscape remains hidden to its explorer, even if the experiments remain simple and measure only yield of as many combinations as resources allow, as in standard multi-environment trials. Despite this, conventional breeding strategies based on phenotypic selection and principles of statistical quantitative genetics (Lynch and Walsh 1997) have been able to achieve sustained levels of yield improvement (Duvick et al. 2004). But to maintain this rate of advance requires increasing resources. Can an integrated approach incorporating advanced technologies in molecular markers, statistics, bio-informatics, and crop physiology and modelling enhance the efficiency of crop improvement?

The complexity of the phenotypic fitness landscape arises from G×E, M×E, G×G and G×M×E interactions. Traits associated with genetic variation (e.g., maturity, tillering) may rank differently for yield depending on environment (Hammer and Vanderlip 1989; Van Oosterom et al. 2003); management interventions (e.g., row configuration, density) may rank differently depending on environment (Whish et al. 2005); and combinations of traits and management (e.g., maturity×density) may rank differently in different environments (Wade et al. 1993). In addition, the genetic architecture of the gene network underpinning complex multi-genic adaptive traits is likely to involve varying degrees of epistatic interactions. In such situations, trait expression is governed by context dependent gene effects, i.e. interaction with other genes (Podlich et al. 2004). Such G×G interactions add substantially to genetic architecture complexity, with major implications for G×M×E interactions and rate of progress in crop improvement (Cooper et al. 2005).

It has been over a decade since the 1994 international symposium at which Cooper and Hammer (1996) advanced the concept of crop improvement as a search strategy on a G×M×E adaptation landscape and outlined a general framework for an integrated systems approach to crop improvement. Their framework incorporated simultaneous manipulation of plant genetics and crop management and considered how crop-physiological understanding and modelling might add value to existing plant-breeding methodologies. Plant breeding requires prediction of phenotype based on genotype to underpin yield advance and this provided the logical entry for advances in quantitative functional physiology.

Since the 1994 symposium there has been considerable development in these concepts and methodologies. Advances in understanding the complexities of gene-to-phenotype and phenotype-to-genotype associations for traits, and the potential to use this knowledge in plant breeding, were the subject of a symposium at the most recent International Crop Science Congress (Brisbane, Australia, 2004: http://www.cropscience.org.au). Revised versions of invited papers to that symposium, which set out the current state of knowledge, have been published subsequently in a special issue of the Australian Journal of Agricultural Research introduced by Cooper and Hammer (2005). A number of other key review papers (Cooper et al. 2002; Hammer et al. 2002; Chapman et al. 2002; Tardieu 2003; Yin et al. 2004) cover developments in linking physiological and genetic modelling for crop improvement and in pursuing the G×M×E concept to enhance molecular breeding. In addition, there have been continuing advances in capacity for molecular genotyping and genomics approaches (Somerville and Dangl 2000; Jaccoud et al. 2001) and in statistics and bioinformatics (Van Eeuwijk et al. 2005; Verbyla et al. 2003). Such advances have enhanced the possibility for an integrated systems approach to crop improvement to link to genomic region level for complex traits. This is despite the limited progress of molecular breeding for complex traits to date due to gene and environment context dependencies (Podlich et al. 2004).

Here we consider the design and implementation of such an integrated systems paradigm for crop improvement. We assess progress from the initial concept construction in 1994 (Cooper and Hammer 1996) and focus on the linking role of crop ecophysiology and modelling to enhance the potential of molecular breeding and the efficiency of crop improvement in general. We use sorghum as a case study species, not only because it is the central focus of our crop improvement research, but also because there is advanced physiological understanding, well developed modelling capability, and a mature set of molecular technologies and genome resources, all linked to an operational breeding and crop improvement programme (Henzell and Jordan in press; Jordan et al. in press).

DESIGN OF AN INTEGRATED CROP IMPROVEMENT PROGRAMME

The central tenet of the integrated systems approach to crop improvement proposed is to change the focus from the paradigm of *identifying superior varieties* to a focus on *identifying superior combinations of genetic regions and packaging these regions into varieties*. Beyond this, it can change the focus from the breeding paradigm of *only developing superior varieties* to a crop improvement paradigm of *developing superior combinations of genetic regions and management systems to optimize resource capture and sustainability in particular cropping environments.* Key decisions in the integrated programme relate to selection of genotypes, management practices and test environments (Figure 1). A comprehensive information system supporting these decisions is the critical core of the approach.

The design of the programme involves a novel approach to integrating four relatively new technologies to enhance effectiveness in crop improvement:

- Enhanced marker technology – low-cost, high-throughput genotyping allowing all of the genotypes tested in a breeding programme to be genotyped with relatively high marker density (e.g., using DArT technology (Jaccoud et al. 2001)).

- Enhanced quantitative trait loci (QTL) detection methods – novel statistical approaches, pedigree-based methods and associative genetics to allow marker detection directly in breeding populations (Verbyla et al. 2003; Jordan et al. 2004; Van Eeuwijk et al. 2005).
- Enhanced gene-to-phenotype linkages – dynamic physiology and modelling frameworks to dissect complex traits to functional components to enhance association of phenotype with marker profiles (e.g., Leon et al. 2001; Reymond et al. 2003; Tardieu 2003; Yin et al. 2004; Messina et al. 2006).
- *In silico* evaluation – advanced modelling frameworks to characterize environments and to evaluate utility of trait and management combinations in target environments (Chapman et al. 2000a; 2000b; Hammer et al. 2005).

The proposed integration (Figure 1) provides the means to work across levels of biological organization from genetic regions to plant growth, development and yield while retaining the scale of a functional breeding and crop improvement programme. The physiology and modelling provides a ‘knowledge bank’ of process understanding. Modelling can generate benchmarks within the breeding-programme trialling system against which the degree of advance associated with new genetic recombinations and management systems can be assessed, despite genotype-by-management-by-environment interactions. Valuable novel combinations of regions can be identified and linked to dense marker profiles, which will be available across the breeding programme via the enhanced marker technology. The advanced statistical procedures will identify patterns of desirable genomic regions. The information accumulated in the breeding programme over time will enable

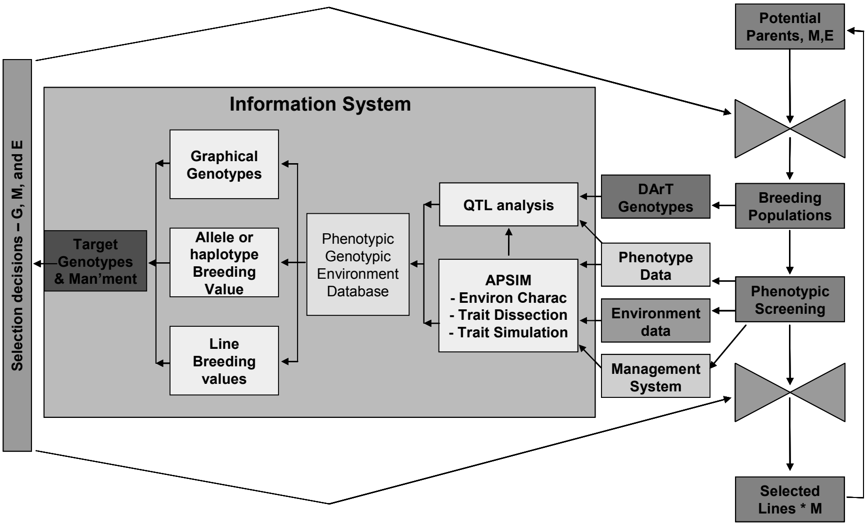

Figure 1. *Overview of an integrated crop improvement programme*

identification of key genomic regions and their value in breeding, as the genetic associations among lines will be known. Existing phenotypic information and populations generated in the breeding programme will be used in contrast to the conventional approach of developing populations specifically for mapping or validating markers. Key regions of unknown function can then be targeted for physiological analysis and modelling to build the information base. Modelling can be deployed to add value to conventional field testing by examining potential combinations of traits and management systems in a range of production environments (sites, soils, season types) via simulation analysis. Such projections of genotype and management combinations onto target environments contribute to the measures of breeding value.

ROLE OF ECOPHYSIOLOGY AND MODELLING IN INTEGRATED CROP IMPROVEMENT

There are three general areas in which crop ecophysiology and modelling can play a role in the integrated approach to crop improvement (Figure 1): (i) characterizing environments to define the nature and frequency of challenges in the target population of environments (TPE); (ii) understanding and dissecting the physiology and genetics of complex traits; and (iii) predicting phenotypes of G×M combinations in the TPE.

Environment characterization

Using modelling to characterize environments in the TPE can assist in unravelling G×E interactions in a manner that aids selection decisions and improves the rate of yield gain in crop improvement programmes. Muchow et al. (1996) demonstrated that a sorghum simulation model (Hammer and Muchow 1994) could be used to characterize water-limited environments more effectively than indices based only on climatic data. The time course of a relative transpiration (RT) index was derived from the dynamic interactions implicit in the model. It was used to define the nature of the water limitation experienced by the crop throughout the growing season. Chapman et al. (2000a) classified environments in the TPE for sorghum in Australia based on the time course of RT and identified three distinct environment types (Figure 2). They found that the frequency of environment types at specific locations correlated with patterns of discrimination among hybrids detected in multi-environment trials (MET) at those locations. When the same simulation and classification procedure was applied to the TPE using historical climate data (Chapman et al. 2000b) they noted that changes in frequency of environment types over time periods relevant to a breeding programme affected yield likelihood and generated differing patterns of G×E (Figure 3). They suggested that weighting genotype performance by the representativeness of the selection environment in each MET with respect to the TPE would be advantageous in breeding programmes in these variable environments. Podlich et al. (1999) used breeding-system

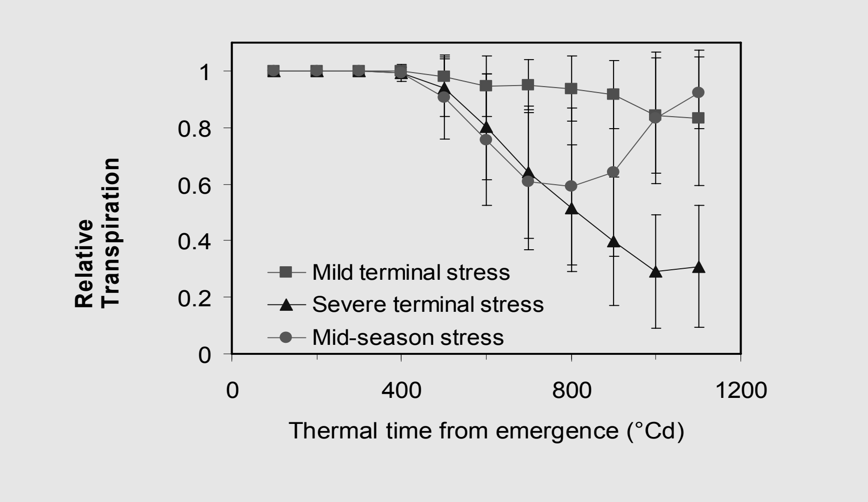

__Figure 2.__ Environmental characterization of sorghum production environments in Australia based on the time course of simulated relative transpiration throughout the crop life cycle (adapted from Chapman et al. 2000a)

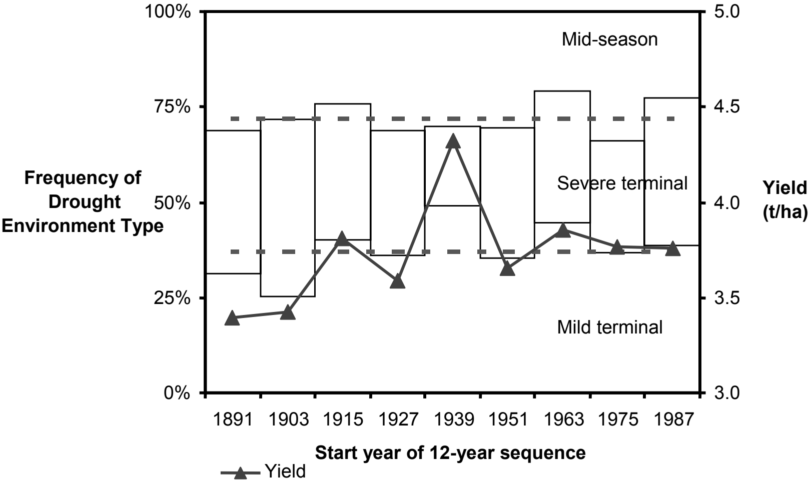

__Figure 3.__ Frequencies of environment types in consecutive 12-year periods during the 20th century for sorghum in Australia (adapted from Chapman et al. 2000b)

simulation to demonstrate the advantage of such a weighted selection strategy in variable environments, especially when G×E was high (Figure 4). Löffler et al. (2005) used a simulation-based environment classification of the TPE for the Corn Belt in the US to improve cultivar performance predictability for a maize-breeding programme.

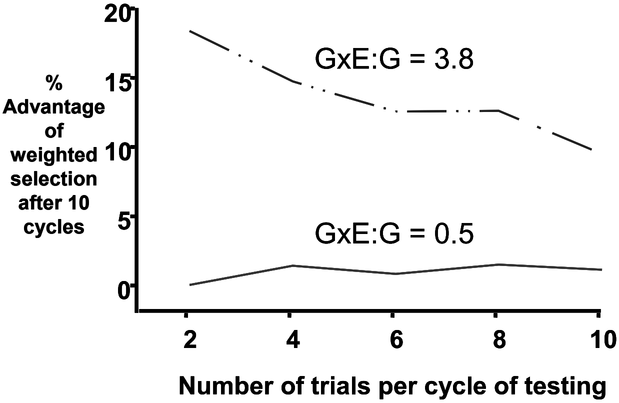

Figure 4. *Percent advantage of weighted selection versus number of trials per cycle of testing in a breeding programme with high or low G×E (adapted from Podlich et al. 1999)*

Complex trait physiology and genetics

A dynamic crop-modelling framework can aid understanding of the physiology and genetics of complex traits in a way that has potential to enhance efficiency in crop improvement programmes. The model provides an analytical framework to specify the functional basis underpinning phenotypic variation in a complex trait. The vector of coefficients that specify the functional and process control equations in the model is the basis for the link to genetic modes of action (i.e. QTLs or genes). The notion of using a virtual or *in silico* plant for this purpose has been discussed by Hammer et al. (2002; 2004), Tardieu (2003), Yin et al. (2004) and Dingkuhn et al. (2005).

Dissection of phenotypic variability in complex traits requires detailed experimental studies in controlled genetic backgrounds to unravel the functional biology underpinning the variability. In sorghum, studies on genotypes differing in their ability to retain green leaves during grain filling under terminal drought, known as the 'stay-green' trait (Borrell et al. 2000; 2001), have suggested that the trait arises as an emergent consequence of differences in underlying factors such as leaf size, specific leaf nitrogen, dry-matter partitioning, nitrogen uptake, and transpiration or transpiration efficiency. This understanding is being utilized in fine-mapping studies with near-isogenic lines (NILs) to isolate target genes in the genomic regions associated with the stay-green trait (Tao et al. 2000). In other studies on genotypes from a population differing in tillering (Kim et al. in press), size of leaves on the main culm and the consequent dynamics of internal plant competition for assimilate have been identified as a likely causal factor. This is consistent with the concepts presented by Luquet et al. (2006) in modelling morphogenesis and competition among sinks in rice.

To date, however, this modelling approach to trait physiology and genetics has only been demonstrated comprehensively at organ or component level for traits such as expansive growth of leaves (Reymond et al. 2003; Tardieu 2003) and crop development (Leon et al. 2001; Yin et al. 2005; Messina et al. 2006). In these cases, coefficients defining differences among lines in process responses to environmental influences have been linked with QTL analyses. Reymond et al. (2003) combined QTL analysis with an ecophysiological model of the response of maize leaf elongation rate to temperature and water deficit by phenotyping a population and conducting the QTL analysis on the fitted model parameters. Using the derived relationships between model coefficients and QTLs, they were able to predict responses of lines with novel combinations of QTLs in a range of environments. Messina et al. (2006) achieved similar results in predicting soybean development by linking temperature and photoperiod responsiveness coefficients of a photo-thermal phenology model to allelic variants at known regulating loci. They used a study on NILs varying at these loci to derive the relationships and then applied them successfully in predicting development of other genotypes in a range of environments.

It may be possible to use a modelling approach to link more directly with gene networks controlling growth and development processes (Welch et al. 2005). Knowledge is emerging rapidly from studies on model plants to support modelling frameworks based on experimental evidence for understanding the action of gene networks at the biochemical level (e.g., Blazquez 2000). For example, Koornneef et al. (1998) presented a working model for the genetic control of flowering time in *Arabidopsis* based on extensive molecular-genetic studies to dissect this process. These studies employed a large number of mutant genotypes of *Arabidopsis* varying in time to flowering. The genetic, molecular and physiological analyses have led to elucidation of components and pathways involved. Welch et al. (2003) adapted the qualitative understanding reported for *Arabidopsis* to a quantitative predictive model of transition to flowering using a genetic neural-network approach. Morgan and Finlayson (2000) have presented a similar qualitative model for flowering in sorghum, based on their extensive studies with mutant genotypes. Beyond this, Dong (2003) developed a dynamic flowering-time model of the gene network in *Arabidopsis* that simulated the temperature- and photoperiod-dependent dynamics of mRNA expression for key genes in the network. He used controlled-environment and gene expression studies for a range of mutants to develop the model and was able to predict successfully the transition to flowering for a far wider range of G×E combinations than used in model development.

The scientific insights gained from this approach at organ or component level could be connected to more conventional crop models to explore interactions between development and growth and yield processes, thus providing an effective bridge between genetic architecture and phenotypic expression. Messina et al. (2006) connected their prediction of development in soybean based on presence of specific genetic loci to cultivar performance in breeding trials. Van Oosterom et al.

(in press) connected a simplified gene network model for photoperiod control of transition to flowering in sorghum to the APSIM generic crop-modelling platform (Wang et al. 2002) to demonstrate that an input of allelic variability could generate G×E for yield as an emergent consequence of the model dynamics. The use of modelling technologies in support of understanding the consequences of alterations of specific genes, via validated QTL models linked to model coefficients or via direct linkages to gene networks where sufficient is known, provides one of the major opportunities to utilize modelling effectively in an integrated approach to crop improvement.

Phenotypic prediction in the TPE

Using modelling to project consequences of G×M combinations in the TPE can generate information that aids selection decisions and improves the rate of yield gain in crop improvement programmes. Numerous studies have approached this by exploring putative value of potential trait variation in a range of species using a diversity of crop models (e.g., Spitters and Schapendonk 1990; Muchow et al. 1991; Aggarwal et al. 1997; Boote et al. 2001; Sinclair and Muchow 2001; Asseng and Van Herwaarden 2003; Sinclair et al. 2005) or by exploring optimization of trait and management combinations (e.g., Hammer et al. 1996). Using this approach requires confidence in the adequacy of the crop model to simulate effects of trait variation. This aspect is discussed below in considering the nature of models required to support the integrated systems approach to crop improvement. It also requires rigorous specification of soil (e.g., water-holding capacity) and climate (e.g., daily temperature and radiation) conditions for relevant production zones of the TPE as input to the simulation analysis.

An example of a model-generated G×M×E interaction relates to manipulation of tillering (G) and row spacing (M) in dryland grain sorghum production systems in Australia (Figure 5). Canopy development and consequent demand for water are affected by extent of tillering (Kim et al. in press) and row configuration (Whish et al. 2005). Figure 5 shows the results of a 50-year simulation using historical climate data for Emerald in central Queensland, Australia with the sorghum model implementation in APSIM (http://www.apsim.info/apsim/)(Wang et al. 2002). The simulation was conducted for a medium-maturing hybrid planted in early January each year assuming 80 mm of available water in a 120-cm deep vertosol soil that held a maximum of 130 mm plant-available water. In wetter higher-yielding years, the greater cover associated with solid row configuration and tillering is advantageous. But in drier, low-yielding years, the lower cover associated with uniculm plants grown in a double skip row configuration is advantageous. There is a cross-over at a yield level of about 3.5 t ha^{-1} in the standard treatment (solid row configuration and no tillers). When a random-error component is added by assuming a coefficient of variation of 12% (as per Hammer et al. 1996) and three replicates stochastically generated, the resultant 50-year MET has a highly significant G (tillering) × M (row configuration) × E (year) interaction (data not shown). Hence, the interaction was an emergent consequence of the model dynamics generated by a change in one plant attribute and one management factor.

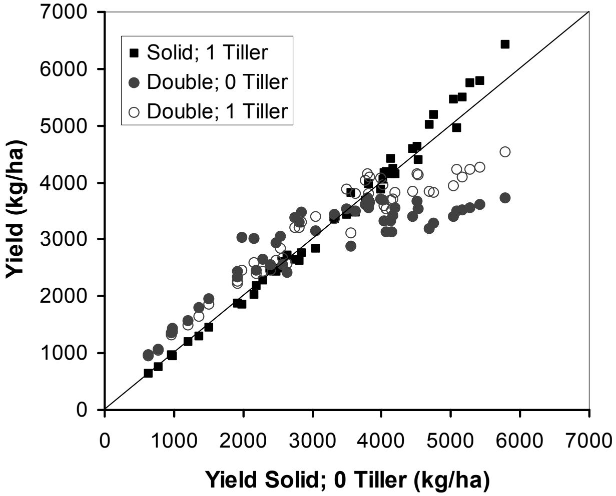

Figure 5. *Simulated yield of sorghum varying in tiller number (0 or 1) and row configuration (solid or double skip) for a 50-year simulation at Emerald, Australia (for details see text). Yield for each combination each year is plotted against yield of the standard treatment (plants with no tillers grown in a solid 1m row configuration) in that year*

VALUE OF ECOPHYSIOLOGY AND MODELLING IN INTEGRATED CROP IMPROVEMENT

As noted in the introduction, the key question to resolve is whether incorporating ecophysiological understanding and modelling in an integrated approach can enhance the efficiency of crop improvement. Is it possible to achieve a rate of yield improvement better than can be obtained by continued conventional empirical breeding based on phenotypic selection?

Beyond the demonstrated value of using models for environment characterization noted earlier, there is now some *in silico* evidence supporting a tentatively positive response to these questions in relation to crop improvement for complex traits (Cooper et al. 2002; Chapman et al. 2002; 2003; Hammer et al. 2005; Cooper et al. 2005). In those studies, sorghum phenotypes were simulated for a broad range of production environments in Australia based on assumed levels of variation in 15 genes controlling 4 adaptive traits. 'Virtual genotypes' were created by deriving combinations of expression states that depended on the number of positive alleles present for each trait. Expression states were then linked with crop model coefficients that quantified their physiological effects. By simulating a range of such virtual genotypes over a range of production environments, a data base of simulated phenotypes was generated. The data base of simulated phenotypes was linked to the QU-GENE breeding-system simulation platform (Podlich and Cooper 1998) to explore effects of cycles of selection on yield gain for a range of breeding strategies. When marker-assisted selection (MAS) breeding strategies were simulated, the inclusion of marker-trait associations based on physiological knowledge and marker weights based on simulated trait value in the TPE significantly increased average rate of yield gain over MAS strategies without such knowledge and modelling capability (Figure 6). This result was dependent on (i) the assumed existence of stable QTL models that linked regions to model coefficients; and (ii) the lessening of gene and environment context dependencies of the QTLs via inherent interactions in the model dynamics that allowed robust projection of consequences of combinations onto the performance landscape in the TPE.

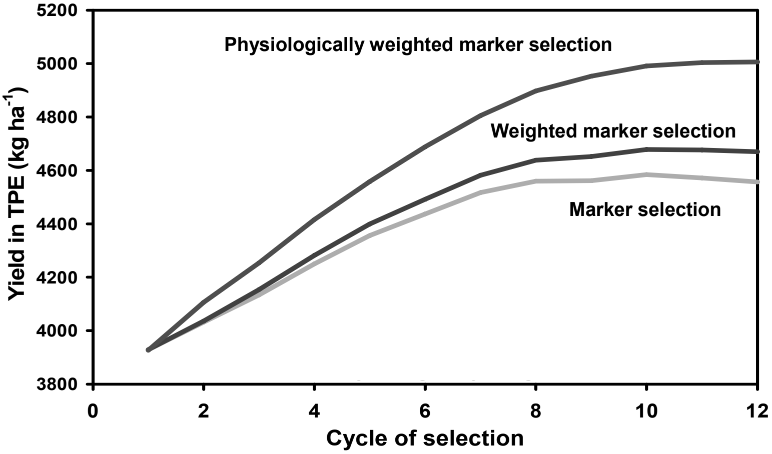

Figure 6. *Average yield in the target population of environments (TPE) over 12 cycles of selection for three marker-assisted selection (MAS) scenarios for a simulated sorghum crop improvement programme. The trajectories for marker selection and weighted marker selection represent the average result over individual breeding-system simulations based on a QTL analysis from single environments. Marker selection incorporates only detection of markers, whereas weighted marker selection includes the weighting associated with each marker in the single detection environment. The trajectory for physiologically weighted marker selection represents the average result over simulations where markers have been assigned to physiological traits and marker weights have been derived from the simulated value of that trait in the entire TPE (after Hammer et al. 2005)*

In a more comprehensive simulation analysis of response to breeding strategies, Cooper et al. (2005) examined a range of genetic models incorporating varying degrees of additive, epistatic and G×E effects that generated a spectrum of complexity in the resultant performance landscape. They quantified the qualitative expectation that response to phenotypic selection (PS) decreased as complexity of the genetic architecture of the trait increased. They also quantified the relative advantage of MAS over PS by simulating differences in response after 5 cycles of selection for the same range of genetic models. They placed the sorghum example above in the context of this diverse set of situations. While the performance landscape generated in that case demonstrated a relatively high level of complexity, their analysis indicated that G×E was the major component of genetic architecture influencing complexity, and that there was only a modest advantage of the MAS strategy proposed over PS. The previous analyses (Chapman et al. 2003; Hammer et al. 2005) had emphasized that value generated from the inclusion of physiological knowledge and modelling was generated by the enhanced ability to deal with environment context dependencies (i.e., QTL×E interaction) in the use of markers. However, an optimistic view of overall value may have been presented.

Hence, while there is as yet no definitive answer to our question, it seems clear that there is sufficient promise to warrant continued effort in pursuing approaches to using physiological knowledge and modelling to enhance crop improvement for complex traits.

WHAT IS THE NATURE OF THE MODELS REQUIRED?

Many conventional agronomic models are adequate and suitable for characterizing the abiotic-stress patterns experienced by crops. The major requirements for such environment characterization are reliable predictions of ontogeny, canopy dynamics and water use. This aligns with pressures on development of agronomic models where predictive capacity for growth and yield outputs has often been more to the fore than biological robustness or mechanistic rigour in components. Predicting ontogeny establishes the developmental time base relevant to perception and effects of stresses (e.g., water limitation around flowering; high temperature during grain filling etc). Predicting canopy dynamics well is important in capturing the patterns of potential water use throughout the season. Canopy leaf area and the associated cover, in conjunction with environmental factors (e.g., radiation, vapour pressure deficit), determine demand for water. Ability of the crop to meet this demand can be related to root depth, soil water content and extraction capacity in each soil layer occupied by roots. The sunflower model of Chapman et al. (1993) details a generic water supply/demand framework of this nature but numerous other models with varying approaches (see review of Hammer et al. 2002) would be adequate for environment characterization.

A greater degree of biological robustness is required for modelling the physiology and genetics of complex traits (Hammer et al. 2002). Dissection of the underlying components associated with function and control of complex traits and projection of their effects onto the TPE requires biological realism. Models need to be sufficiently detailed so that important physiological linkages and interactions are simulated implicitly. They should incorporate a hierarchy of physiological processes and input variables based on experimental analyses (Tardieu 2003). The phenotype becomes an emergent consequence of variation in system architecture and control and its interaction with the environment. This requires that (i) the physiological modes of action of the traits are understood and quantified; and (ii) the model is sufficiently detailed and robust to simulate realistically the interactions with crop growth and development generated by expression of the trait in any particular environment (Hammer et al. 1996). Tests of the integrative ability of the crop model to project consequences onto the TPE can range from qualitative sensibility testing of responses based on biological knowledge through to formal validation, where appropriate data are available (e.g., Messina et al. 2006). Robust models could add significant value to discussions on likely value of putative traits as breeding targets for indirect selection (e.g., Richards et al. 2002; Morgan et al. 2002) and to considerations of simpler targets/measures for the high-throughput phenotyping required for QTL modelling and forward selection in a breeding programme (e.g., Reynolds et al. 1998).

It is necessary to gain the understanding of the physiology and genetics of complex traits from studies in controlled genetic backgrounds. Tardieu (2003) used transgenic plants to link genetic responses to coefficients of a model of water flux through the plant. Messina et al. (2006) used near-isogenic lines varying at specific loci to derive coefficients for a phenology model that could then be estimated via linear functions of the alleles present. This contrasts with initial attempts to use agronomic crop models by optimizing a range of model coefficients to best fit observed phenotypic variation among sets of diverse genotypes, which had limited success (White and Hoogenboom 1996). The modest predictive capabilities found highlighted the need to understand better the physiological basis of the genetic variation involved via studies with controlled genetic backgrounds before seeking such predictive capability across diverse material.

The ability to generate stable associations between model coefficients and QTLs provides another criterion for model realism and adequacy to deal with physiology and genetics of complex traits (Welch et al. 2005). Reymond et al. (2003) were able to achieve stable QTLs for their ecophysiological model of leaf elongation rate in maize. Similarly, Messina et al. (2006) and Yin et al. (2005) found stable QTLs for photo-thermal phenology models for soybean and barley, respectively. However, Yin et al. (1999) were unable to find stable associations with QTLs for a study on specific leaf area (SLA $\text{cm}^2\ \text{g}^{-1}$) in barley. This suggested lack of validity with which the crop model architecture and associated coefficients captured and integrated the physiological basis of the genetic variation. The barley model used in their study simulated leaf expansion as the product of carbohydrate partitioning to the leaf and SLA. Tardieu et al. (1999) presented a modelling framework to explore whether leaf expansion was a consequence of specific leaf area or *vice versa*. They were able to conclude the latter and argued that leaf expansion should be modelled independently of the plant carbon budget and that it was largely driven by temperature. Despite now having this enhanced understanding of control of leaf expansion in cereals, many crop simulation models continue to use the SLA-driven approach erroneously. However, this would likely have few consequences when using such models for agronomic or environment characterization purposes.

Kitano (2004) discusses robustness as a fundamental feature of complex evolvable systems, like biological organisms. He notes that system controls and modularity are basic features providing system robustness and that system control is the prime mechanism for coping with environmental perturbations. Attention to these aspects is likely to be important in the progression to the type of models most suited to study of the physiology and genetics of complex traits. This notion accords with the separation of physical and control equations in plant models (Tardieu 2003) and with the motivation behind the on-going development of the APSIM modular generic crop routines (Wang et al. 2002). The latter is designed to capture advances in knowledge as they occur, while retaining parsimony in approach to the G×M×E modelling objective. Our current research is designed to test the ability of this type of model to generate more stable associations between model coefficients and QTLs.

CONCLUDING REMARKS

We suggest that an integrated systems approach to crop improvement that incorporates advanced technologies in molecular markers, statistics, bio-informatics, and crop physiology and modelling is likely to enhance the efficiency of crop improvement significantly. We discuss the design of such a system and consider the linking role of crop ecophysiology and modelling. A role of modelling in environmental characterization to support weighted selection is clear. It also seems clear that physiology and modelling will contribute significantly in the area of complex traits. The exact nature of this contribution is still emerging and is the focus of on-going research. Attention to biological robustness in modelling will likely assist in this regard. While an integrated systems approach is in its infancy, we expect that the potential benefits and further technology developments will likely enhance its rate of development. To this end, we are simultaneously pursuing the development and implementation of an integrated systems approach to crop improvement in the Australian sorghum programme.

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CHAPTER 6

CROP SYSTEMS BIOLOGY

An approach to connect functional genomics with crop modelling

X. YIN AND P.C. STRUIK

Crop and Weed Ecology Group, Wageningen University, P.O. Box 430,
6700 AK Wageningen, The Netherlands.
E-mail: xinyou.yin@wur.nl

Abstract. The response of the whole crop to environmental conditions is a critical factor in agriculture. It can only be understood if the organization of the crop system is taken into account. A popular view in modern science is that genomics (and other 'omics') will provide knowledge and tools to allow the characteristics of the crop to be altered for improved actual and potential crop yields, increased resource use efficiency and enhanced crop system health. As a result of this view, (molecular) plant systems biology has been considered as an approach to assist crop improvement for increased production, via modelling 'how things work' in (sub-)cellular units. However, phenotypes at the crop level, for example, as expressed in grain yields, are extremely complex, and not only achieved by molecular pathways but also through multiple intermediate metabolic and physiological processes. These processes are controlled by numerous genes whose effects and expression are highly dependent on environmental perturbations. Current prevailing initiatives for (molecular) plant systems biology so far have put little emphasis on bringing the 'omics' information to the crop level. Here, crop systems biology is presented as a complementary modelling approach to assist plant-breeding programmes to improve the yield and related resource use efficiencies of major crops. This crop systems biology approach honours the combined role of modern functional genomics and traditional sciences (such as crop physiology and biochemistry) in understanding and manipulating crop phenotypes relevant to agriculture. A stepwise routine for the development of crop systems biology models is proposed. Ultimately, these models should enable *in silico* assessment of crop response to genetic fine-tuning under defined environmental scenarios.

INTRODUCTION

The word 'systems' is not new in biological science. Ludwig Von Bertalanffy – a theoretical biologist – published a book, called General Systems Theory (Von Bertalanffy 1969), a compilation of his writings, some of which date back to the 1930s. In contrast to the reductionistic approach, he recognized the importance of 'wholeness' – the 'systems' of various orders not understandable by investigating

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their respective parts in isolation. This systems concept remains valid in biology as practised today.

Recently, a new scientific discipline – systems biology – was proposed (cf. Kitano 2002), in the wake of the need for instruments to summarize large amounts of experimental data from modern high-throughput technologies in functional genomics. Although the meaning of systems biology is still under debate (Kirschner 2005), systems biology generally aims to synthesize complex datasets from various genomic hierarchies (genome, transcriptome, proteome, metabolome) into useful mathematical models. It seeks to explain biological functioning in terms of 'how things work' in (sub-)cellular units. Similarly, plant systems biology was defined, in the 22nd Symposium on Plant Biology (summarized by Minorsky 2003), using computational modelling approaches to predict a plant cell(ome) from underlying genomic understanding.

However, (plant) systems biology should not be considered an entirely new field, or even a research paradigm shift (Bothwell 2006). Crop scientists have used systems analysis to investigate whole-crop physiology and crop ecology for decades. Dynamic crop growth models emerged in the mid-1960s with the pioneering work of De Wit and colleagues (e.g., De Wit 1965; De Wit et al. 1978), who introduced Von Bertalanffy's systems theory and the dynamic simulation method of Forrester (1961) into crop science. Crop systems modelling differs from empirical statistical analysis, just as systems biology differs from bioinformatics. In these crop models, constituting elements and processes are put together in mathematical equations. The rules by which the elements or processes interact give rise to systems behaviour and emerging properties illustrated by simulation, which may well be unexpected and even counterintuitive. This is model heuristics, which in turn enhances the understanding of individual processes and improves the next-round modelling of the crop. Dynamic crop systems models have been used to support theoretical research and applied activities.

There is no doubt that systems biology, as currently defined, is scientifically challenging. It will facilitate the development of functional genomics as a scientific discipline since, arguably, 'omics' has been driven more by emerging experimental technologies than by novel hypotheses. However, Hammer et al. (2004) argued that the current definition of plant systems biology not only largely overlooks the rich history of crop systems modelling, it also is probably not the best approach to solve the real-world problems towards crop improvement for increased production – the ultimate goal plant systems biology (Minorsky 2003) wants to achieve. We argue that new initiatives for plant-based systems research should first draw on the conventional science-based crop-modelling developments. At the same time, one should make use of modern genomics by parameterizing and redesigning some subroutines of crop systems models. To this end, the concept 'crop systems biology' is proposed; this concept is potentially more promising to fulfil real-world challenges in dealing with complex traits at the crop level, such as grain yield and resource use efficiencies. Concepts, rationales, methodology and future prospects of crop systems biology to resolve gene-to-phenotype relationships will be discussed.

THREE WAYS TO TACKLE COMPLEX TRAITS

Many crop traits related to agricultural production are quantitative and complex in nature, regulated by multiple interactive genes whose effects and expression may be highly dependent on environmental conditions and developmental times. To manipulate these complex traits, understanding their genetic basis is crucial. In terms of the linear causal framework: DNA → mRNA → protein → metabolite → phenotype, Miflin (2000) considered three basic types of approaches to understanding the genetic basis of crop performance: (i) starting at the beginning – genomics; (ii) starting at the end – trait analysis; and (iii) starting in the middle – metabolic analysis. In accordance with this classification, there have been three strategies or approaches for biological modelling: (i) bottom-up; (ii) top-down; and (iii) some combination of bottom-up and top-down as the 'middle-out' approach (Noble 2002; see also Struik et al. in press).

The first approach is relevant given that major efforts are undertaken to sequence genomes of a range of plant species, whereas the entire genomes of *Arabidopsis*, rice and poplar have already been sequenced. The next challenge is to analyse these sequences to infer functions for the genes. Functional genomics aims to discover the function of all genes, typically through high-throughput experimental studies in genome-wide transcriptomics, proteomics or metabolomics combined with bioinformatics tools for data analysis, but also by functional analysis using 'loss of function' mutant genotypes. Systems biology was proposed in the wake of the need for instruments to synthesize complex datasets from the 'omics' studies into explanatory mathematical models that help to elucidate 'how things work' in (sub-) cellular units. These 'bottom-up' studies will undoubtedly yield valuable information for gene functions, gene interactions and genetic regulatory networks. For example, Hirai et al. (2004) showed progress by combining genome-wide transcriptomics and metabolomics under deficiency of sulphur and nitrogen in the model plant *Arabidopsis*, an important step in linking genomic data with the function of metabolites in plants. Discovery of gene functions is a basic task in functional genomics; however, it is not sufficient for crop improvement and probably of little use for enhancing selection for quantitative traits such as crop yields (Bernardo 2001). The bottom-up approach also pays little attention to the modulation by (multiple) varying environments, as perceived by the whole crop and, therefore, is a long way from helping to explain the connections between multiple genes and complex phenotypes such as grain yield and quality traits in crop plants that are crucial for agriculture.

The second (top-down) approach has been an important tool to study the genetic basis of complex crop traits with the discovery of DNA-based molecular markers in the early 1980s. These markers are based on small base changes (deletions or additions of bases and base pair substitutions) or on variation in the number of repeats of short sequences, most likely in non-coding genome regions. These markers are naturally occurring, abundant in most species, and simply inherit as monogenic Mendelian factors. These markers are therefore suitable to construct a saturated marker map, which is essential for localizing quantitative-trait loci (QTL; genome regions conferring the variation of a quantitative trait). Typically, QTL analysis depends on making a cross between two individual genotypes that differ in the trait under study, and then deriving a segregating population consisting of a large number (mostly >100) of individual lines. Individual lines are both genotyped using a marker technology and phenotyped through experiments for the trait(s) under study, and then any close association between markers and a phenotype gives evidence of a QTL for the trait. This trait analysis is a 'shortcut' approach in connecting genome regions to phenotypes without needing any information for intermediate steps at transcript, protein or metabolite levels. It can be performed relatively independently of the pre-conception of the researchers, for example by physiologists (Prioul et al. 1997). Yin et al. (2000) introduced this approach to crop modelling, considering model-input parameters as a special type of quantitative traits, an approach which Yin et al. (2004a) later called 'QTL-based crop modelling'. Several subsequent studies (e.g., Reymond et al. 2003; Yin et al. 2005; Quilot et al. 2005) have indicated the added values of this combined QTL-model approach in resolving 'genotype-by-environment interactions', at least for the relatively simply quantitative crop traits studied so far. One drawback of QTL analysis using a bi-parental cross is the experimental setup in which only two parents are involved; therefore, it is unlikely that the total genetic variation present in the complete germplasm pool will be found. Linkage disequilibrium (LD) mapping, in which association between genotypes and phenotypes is scrutinized over a large germplasm collection, is increasingly becoming valuable for identifying loci contributing to quantitative traits (Remington et al. 2001). Especially when based on new, high-throughput, allele (haplotype)-specific markers, notably single-nucleotide polymorphisms, LD mapping allows whole-genome molecular-diversity characterization to localize favourable genes and alleles or haplotypes. Together with QTL mapping, this new development in association analysis may renew opportunities for gene-based crop modelling – the concept, as first put forward and empirically practised by White and Hoogenboom (1996), that aims to predict crop yield traits via linking underlying component traits with relevant known candidate genes.

Although breeders can make direct use of the results of the QTL and LD analysis (e.g., marker-assisted selection), the second approach treats the intermediate steps from genomes to phenotypes as a 'black box'. It could therefore be valuable to investigate some underlying mechanisms for the phenotype-marker association, for example by studying plant metabolisms and identifying genes involved in the metabolic pathways and their associated physiology – the 'starting in the middle' approach. Miflin (2000) has given examples for several aspects of this approach. From a broader perspective, this third approach has links with the second approach – targeting the identification of QTL by determining their contribution to biochemical or physiological components of macroscopic traits. Several studies have demonstrated that the combined QTL and biochemical or physiological analysis generated new insights into causal relationships between physiological traits that would have been difficult to obtain by conventional physiological approaches (e.g., Lebreton et al. 1995). The QTL approach has been applied to activities of metabolic enzymes (Prioul et al. 1999); apparent co-locations between QTLs for an activity and known enzyme structural-gene loci were found. Such a candidate-gene approach can facilitate the search for genes underlying QTL. Damerval et al. (1994) showed that the QTL approach could be applied to the variable proteins, and protein quantity loci were identified for 42 polypeptides. This may help in analysing metabolic and regulatory bases of the variation of general quantitative traits, thus providing a tool for characterizing QTL (De Vienne et al. 1999). Similarly, the LD-based association analysis could be applied to biochemical/physiological traits. A big advantage of this approach is that it allows the use of ample existing knowledge of conventional sciences such as biochemistry and physiology, which have already had a major impact on phenotypic characterization and thereby are very likely to impact on crop improvement programmes. To enhance the realization of this potential, combined examination of the crop-level phenotype with its associated underlying physiological components, biochemical traits and enzyme activities (cf. Hirel et al. 2001; Ashikari et al. 2005) is especially insightful.

NEED FOR A HOLISTIC APPROACH TO TACKLE THE COMPLEX TRAITS AT THE CROP LEVEL

The ultimate goal of crop scientists is to achieve increased crop production with given resource inputs to meet the demands of growing populations for sufficient food, feed, raw material and energy. Tremendous increases in crop yields have been achieved in some parts of the world over the last decades, especially since the first ‘Green Revolution’. For most cereal crops, major improvements in yield potentials have been attained from the introduction of a few genes (such as dwarfing genes and photoperiod-insensitivity genes) that lead to changes in mass partitioning in favour of harvested organs of the crop. However, empirical breeding (even when using the marker-assisted-selection approach) to manipulate partitioning further seems to approach its theoretical limits (Reynolds et al. 2000; Long et al. 2006). The possibility for improved incident-light use efficiency (LUE) via breeding should be explored; and selecting for super-high-yielding hybrid rice in China has already shown promises (cf. Normile 1999). To improve LUE and associated yield potential, a thorough understanding of the whole system of photosynthesis, assimilation and respiration based on insights from genes via metabolisms and physiology to crop yield is required to achieve the future long-term objectives of empirical breeding or genetic engineering (Lawlor 2002).

Systems simulation modelling has long been suggested as a powerful tool to understand crop yield formation and to assist crop improvement programmes. However, the lack of truly ‘mechanistic’ crop simulation models (which make use of biochemical information) is a major constraint to advance the understanding of crop yield traits (Lawlor 2002). Also the already proposed ‘plant systems biology’ modelling approach (Minorsky 2003) is probably not the best approach to increased global crop supply (Hammer et al. 2004). The approach (which is better specified as ‘molecular plant systems biology’) does not (yet) put emphasis on bringing the information from ‘omics’ to the crop level for understanding the complex traits such as grain yields, relevant to the real-world challenge for global food security. While the third approach mentioned in the previous section is well recognized in human biology (Noble 2002), especially in terms of connecting the various organizational levels, the biological modelling along the line with this approach is scarce in plant science (Giersch 2000). Yet, the need for such an approach is even larger in plant science than in human biology, given the specific nature of the interactions between organization levels in plants and the large influence of variable environmental conditions on primary production processes and phenology.

Nevertheless, there are studies using quantitative modelling to assess options of increasing LUE and yield potentials derived from qualitative knowledge of biochemistry and physiology. For example, actual measured photosynthetic quantum efficiency and light-saturated photosynthetic rate in C_3 crop species (including the world's major food crops: rice and wheat) under favourable environmental conditions are about only 50% and 25-35%, respectively, of their theoretical maximum values. Many biochemical and physiological factors (cf. Long et al. 2006) contribute to the gaps. An important factor is the loss due to photorespiration, as a result of O_2 competing with CO_2 for the catalytic sites of Rubisco. The C_4 metabolism is more efficient as it has a CO_2-concentrating mechanism via the integrated development of the Kranz leaf anatomy, the localization of C_3 and C_4 enzymes, and necessary membrane transporters. Modifying C_3 plants towards C_4 metabolism has been a long-held ambition of plant biochemistry. However, the success of the transformation for this entire mechanism seems an unlikely prospect for the near future (Leegood 2002). A simpler routine, viz., expression of C_4 enzymes in single mesophyll cells of C_3 crops, is not adequate in obtaining the full advantage of C_4 photosynthesis (Von Caemmerer 2003). An alternative approach is to express, in C_3 species, improved forms of Rubisco, notably those from non-green algae in which the relative specificity (Sc/o) for CO_2 compared to O_2 is higher than that of higher-plant Rubisco. Zhu et al. (2004) examined this opportunity, using the widely used biochemical model of C_3 photosynthesis (Farquhar et al. 1980) coupled with a canopy model, and concluded that a substantial increase (> 25%) in crop carbon gain could result if that specific Rubisco were successfully expressed in C_3 plants. Long et al. (2006) extended the analysis and suggested more potential routines of increasing LUE, including increased Rubisco Sc/o, improved canopy structure, increased rate of recovery from photoprotection of photosynthesis, increased Rubisco catalytic rate, and increased capacity for regeneration of RuBP. Collectively, these increases could bring potential crop yield to increase by up to 50%.

However, simulation studies of Zhu et al. (2004) and Long et al. (2006) were performed for one single day and for a crop canopy of a particular size (leaf area index = 3). Besides the neglect of the importance of sink capacity for yield determination, they did not consider the crop growth cycle, nor did they consider growth influencing factors other than light, CO_2 concentration ($[CO_2]$) and temperature, such as nitrogen availability. In fact, their hypothesis that increased photosynthesis results in increased yield stemmed from some experimental results that showed an increased growth of plants grown under elevated-$[CO_2]$ environments in comparison with those grown under the current atmospheric $[CO_2]$. Many experiments with a season-long $[CO_2]$ enrichment in C_3 plants showed photosynthetic acclimation to elevated $[CO_2]$ – the phenomenon that the early effect of increased photosynthesis in response to elevated [CO_2] will decline or even disappear when development progresses (Arp 1991). As a result, there is probably little grain yield advantage for plants grown continuously under elevated [CO_2] (e.g., Van Oijen et al. 1999), since carbon accumulated in the grain comes mostly from post-flowering photosynthesis. This acclimation can be ameliorated by nitrogen supply (Geiger et al. 1999). Sinclair et al. (2004) reasoned from their theory that a 33% increase in leaf photosynthesis may translate into an 18% increase in biomass, and only a 5% increase in grain yield or even a 6% decline in grain yield in the absence of additional nitrogen. Makino et al. (2000) also showed experimentally that, for a given leaf nitrogen content, a higher photosynthesis did not necessarily show greater biomass accumulation. Understanding the mechanisms of both carbon and nitrogen assimilation is essential to increase LUE (Lawlor 2002). Indeed, nitrogen assimilation in many species takes place simultaneously with CO_2 fixation in photosynthetic cells. There is a close interaction between nitrogen and carbon metabolism, both utilizing light energy, with some 10% of the chloroplast electron flux in photosynthesizing leaves used in nitrate assimilation (Foyer et al. 2001; Yin et al. 2006). Quantitative, integrated information on cellular production and utilization of ATP and reductants with regard to carbon and nitrogen metabolisms (cf. Noctor and Foyer 1998) may provide a first step to formulate a summary model for truly mechanistic approaches of yield trait analysis and prediction.

CROP SYSTEMS BIOLOGY AS AN APPROACH TO CONNECT FUNCTIONAL GENOMICS WITH CROP MODELLING

The modelling studies at the crop level using some knowledge of biochemistry are currently sporadic, modelling results published so far to analyse yield traits are inconsistent, and some models are based on untested hypotheses. We propose a more systematic modelling approach – ‘crop systems biology’ – to analyse complex traits at the crop level, not only with the aim of establishing close links with understanding at the gene or genome level but also in terms of its comprehensive reliance on the whole-metabolism biochemistry and physiology. Therefore, the proposed crop systems biology is a crop-level approach to modelling complex crop traits relevant to global food production and energy supply, via establishing the links between ‘omics’-level information, underlying biochemical understanding and physiological component processes.

Development of crop systems biology models certainly depends on what trait a research wants to target. Crop yield is a complex trait that most existing crop simulation models have attempted to predict. It may not be surprising that simulation of yield formation should be a first major trait in crop systems biology. In addition, if crop yield can be modelled accurately, underlying mechanisms for traits of resource use efficiencies (such as water use efficiency or nitrogen use efficiency) can be analysed accordingly. The question is how to achieve a crop systems biology model for the yield trait.

We propose a two-step strategy, largely following the earlier mentioned ‘middle-out’ approach. First, a widely used framework of many existing crop simulation models including processes such as photosynthesis, respiration and assimilate partitioning to calculate LUE implicitly can still be used, especially as a starting point. At the level of these processes, there are rich biological (physiological and biochemical) data and therefore the understandings are of the highest confidence. For this first step, crop systems biology models may not be necessarily more complex than existing crop simulation models in structure, nor is their computational requirement. However, model algorithms for individual processes are supposed to be more mechanistic. In many cases, a summary of a detailed biochemical model – e.g., the photosynthesis model of Farquhar et al. (1980) or its generalized form (Yin et al. 2004b) in order to deal better with stress conditions – can be incorporated as a sub-model. In other cases, direct results or stoichiometries from biochemical studies (e.g., examination of the biochemical pathways for production of protein, carbohydrates and lipids from glucose by Penning de Vries et al. (1974)) can be utilized. A prototype of crop systems biology models needs to be made available from this first step, in which physiological and biochemical information at the process level is assembled and then scaled up to the crop level in a way similar to temporal and spatial integrations as practised in conventional crop simulation models. In relation to crop improvement, a key element of the first step would be to identify the parts of mechanisms that are conservative in energy and water transfer and carbon and nitrogen metabolisms, and the parts of mechanisms that show genetic variation and are potentially amenable to selection and engineering. In case of grain yield, the prototype models should allow identification and quantitative assessment of specific parts of metabolic pathways and processes, which could be altered to achieve improvement of yield potential. The parts showing genetic variation can be identified by genetic analysis or the 'omics' approach in terms of the expressions of specific genes, proteins or enzyme activities.

After the first step is achieved, crop systems biology modelling could move to the second step as further progresses in understanding at the 'omics' level are being made, towards reaching down to lower organizational levels. For this, it is necessary to map the organization levels and the communication systems between these levels for the different key processes. Modelling for reaching down to the lower levels is most likely to be done in a manner of one-process-at-a-time; and in this respect, a modular design of the model is important to ensure that changes of a sub-model will not affect other parts of the model. Welch et al. (2003) have already developed a neural network model of *Arabidopsis* flowering-time control, based on studies on qualitative, genetic characterization of major flowering-time genes in this model plant species. Similar modelling studies could be performed for phenology of crop species. Further, existing modelling of metabolisms, such as the Benson-Calvin cycle of photosynthesis and the photorespiratory cycle (cf. Giersch 2000) and nitrogen assimilation in relation to the activity of key enzymes (e.g., nitrate reductase and glutamine synthetase), could also be added. With the rapid development of functional genomics in the wake of high-throughput technologies, combined studies of physiological components with gene expression profiles should illustrate the function of genes, biochemical pathways and cellular processes that are affected in a coordinated manner (Stitt and Fernie 2003). Such studies should lay the groundwork for elucidating regulatory networks and causal linkages among gene products, biochemistry and whole-plant physiology. Integrated molecular systems biology models for a particular metabolism or process are expected to become increasingly available. Such models can potentially be embedded into crop systems biology models. Clearly, different temporal, spatial and structural scales are required for different components, pathways and processes of the model; and ultimately, crop systems biology will develop into a highly computer-intensive discipline. Such coupled models should enable *in silico* assessment of crop response to genetic fine-tuning under defined environmental scenarios, thereby being powerful tools in supporting breeding for complex crop traits.

CONCLUDING REMARKS

It is understandable that people have different interpretations when a new science emerges. Systems biology is not an exception. Although a vast majority of existing systems biology reports are up to the level of a 'cell', systems biology should have a larger and more ambitious scope, and its definition should be anchored in the general systems theory put forth by Von Bertalanffy. For the case of plants, models of systems biology, if aimed to solve the real-world problems towards crop improvement for increased production (Minorsky 2003), should not be used in a predictive mode merely up to 'cell(s)'; instead, ultimately they should be applied to improving traits associated with agriculture (Gutiérrez et al. 2005). In this context, crop systems biology seems to be a more promising term and approach to fulfil real-world challenges in improving complex traits, to narrow gene-to-phenotype gaps, and to promote interaction and cooperation between modern and traditional disciplines. The authors believe that we, crop physiologists, should actively explore and involve modern plant-science research to find a niche that we deserve to help to establish the complete knowledge chain in plant biology. On the other hand, those working on modern plant sciences should not consider such a joint effort to be altruism from their side, as this effort has recently been argued essential for further success in crop improvement towards a second 'Green Revolution' (Wollenweber et al. 2005). To make genomics work, joint effort across the entire plant biology and crop science is probably the best way to develop a 'systems biology'. The most difficult part of the joint activity is the first step; but at least, plant and crop scientists share a common view that systems biology is a community effort.

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MODELLING GENOTYPE × ENVIRONMENT INTERACTIONS

CHAPTER 7

A MODELLING APPROACH TO GENOTYPE × ENVIRONMENT INTERACTION

Genetic analysis of the response of maize growth to environmental conditions

W. SADOK, B. BOUSSUGE, C. WELCKER AND F. TARDIEU

INRA – ENSAM, Laboratoire d'Ecophysiologie des Plantes sous Stress
Environnementaux, Montpellier, France.
E-mail: ftardieu@ensam.inra.fr

Abstract. Expansive growth of organs has a very large genotype × environment (G×E) interaction. Maximum leaf expansion rate observed in the absence of stress and of evaporative demand has a genetic variability which is usually smaller than environmental effects. The mechanisms driving the reduction in leaf growth rate under stress, namely changes in cell division rate, in cell-wall mechanical properties and in turgor, and their signalling pathways, interact in such a way that a bottom-up approach from genes to the G×E interaction cannot be envisaged. We propose an approach combining modelling and genetic dissection of model parameters. Three genotype-dependent parameters are considered for analysing the G×E interaction for leaf elongation rate of maize. The maximum leaf elongation rate per unit thermal time is stable during the night and over several nights, and it is repeatable for each genotype over several experiments. The responses of leaf elongation rate to evaporative demand and soil water status are linear and their slopes are reproducible over several experiments. Maximum elongation rate and slopes of the responses to evaporative demand and to soil water potential have been analysed genetically in three mapping populations. QTLs of maximum leaf elongation rate tended to co-localize with QTLs of leaf length under well-watered conditions, but also under water deficit. They also co-localized with QTLs of the Anthesis Silking Interval (ASI). In contrast, QTLs of response parameters did not co-localize with QTLs of length under water deficit. They are therefore 'adaptive' traits which cannot be identified otherwise. Each parameter of the ecophysiological model was computed as the sum of QTL effects, allowing calculation of parameters of new RILs known by their allelic values only. Leaf elongation rates of these new RILs were simulated and were similar to measurements in a growth-chamber experiment. This opens the way to the simulation of virtual genotypes, known only by their alleles, in any climatic scenario.

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INTRODUCTION

Expansive growth occurs from plant emergence to flowering. It determines the plant architecture and, indirectly, fundamental characteristics of plant functioning such as water and nutrient uptake by roots or light interception by leaves. In contrast to biomass accumulation, which is the result of numerous processes, expansive growth is the direct consequence of two main processes only, namely cell division and tissue expansion, which are largely synchronized (Fleming 2005). While the ability of genotypes to grow under favourable conditions has an appreciable genetic variability (e.g., 'intrinsic leaf elongation rate', Reymond et al. 2003; 'early vigour', Condon et al. 2004), environmental conditions usually have an overriding effect. Expansive growth is therefore one of the plant processes with highest genotype × environment interaction. Light availability determines tissue expansion in sink tissues such as roots or young leaves, in close relation with the local sugar concentration (Granier and Tardieu 1999; Freixes et al. 2002; Walter and Schurr 2005). In case of water deficit, a reduction in leaf expansion rate usually occurs before any reduction in photosynthesis (Boyer 1970; Saab and Sharp 1989). Numerous mechanisms can account for the changes in growth rate with environmental conditions, but all of them are still the object of contradictory experimental results and of some degree of controversy.

Cell division rate in leaves and roots is affected by restrictions in light availability (Muller et al. 1998; Granier and Tardieu 1999; Cookson and Granier 2006), probably with a signalling pathway involving local sugar concentrations. It is also affected by water deficit (Sacks et al. 1997; Granier and Tardieu 1999), possibly with a signalling involving abscisic acid (Wang et al. 1998). The reduction in cell division rate because of several environmental conditions is linked to the activity of a key enzyme of the cell cycle, the p34cdc2kinase (Schuppler et al. 1998; Granier et al. 2000), but the precise role of individual genes in the response to water deficit is insufficiently known to allow a predictive approach.

Cell wall stiffening is a major cause of the reduction in leaf growth in case of water deficit (Tang and Boyer 2002; Cosgrove 2005). Two gene families are the main molecular candidates for changes in cell wall properties with environmental conditions, namely expansins (Wu and Cosgrove 2000) and cell-wall-associated peroxidase (Bacon et al. 1997). Other families of proteins may also be involved, such as endoglucanases (Yuan et al. 2001). Each of these families can involve several dozens of genes whose individual effects are not known, and the interaction between families of genes is still less known.

Reduction in cell turgor has long been considered the cause of the decrease in leaf growth with water deficit (Zhang et al. 1999), implying that osmotic adjustment in growing tissues is not complete. This has been discussed in the last thirty years (Green et al. 1971) and, indeed, reductions in leaf elongation rate have been observed in response to soil water deficit in spite of an unchanged turgor pressure (Matthews et al. 1984; Westgate and Boyer 1985; Tang and Boyer 2002). However, turgor decreases in response to soil water deficit or evaporative demand have been observed in roots and leaves (Spollen and Sharp 1991; Bouchabke et al. 2006).

Abscisic acid (ABA) is widely believed to be a major contributor in the controls of plant transpiration and leaf growth, consistent with experiments in which the ABA biosynthesis pathway was affected (Iuchi et al. 2001; Borel et al. 2001) or in which artificial ABA was fed to plants (Zhang and Davies 1990; Ben-Haj-Salah and Tardieu 1997). However, the picture is more complex when the effect of ABA is dissected genetically. The signalling pathways of ABA and ethylene overlap (Beaudoin et al. 2000), and the same applies to ABA and sugars (Leon and Sheen 2003). Furthermore, ABA might promote the growth of droughted plants by restricting the biosynthesis of ethylene, instead of decreasing it as formerly believed (Sharp 2002).

In each of the four mechanisms presented above, the current state of knowledge appreciably differs from that widely accepted ten years ago. Both the categories of genes involved in the control of growth under fluctuating environmental conditions and the hierarchy of candidate mechanisms are the object of controversy. It seems therefore difficult to identify candidate genes from the literature. It is still more premature to elaborate a gene network model which would encapsulate all the gene regulations leading to reduced leaf growth under water deficit. If models of behaviour of genotypes are to be developed, they will be based on principles that differ from the gene-regulatory networks, at least in the next years or decades. The object of the following paragraphs is to present methods to deal with the genetic variability of the response of growth to environmental conditions.

GENETIC ANALYSIS OF RAW PHENOTYPIC TRAITS IN CONTRASTING ENVIRONMENTAL CONDITIONS

A different approach, which does not suppose that mechanisms are known, has been used by geneticists for the past 15 years. It consists in associating statistically gene alleles to phenotypes under abiotic stresses via quantitative trait loci (QTLs) identification in mapping populations (Prioul et al. 1997). This strategy has allowed identification of a large number of QTLs involved in the maintenance of yield or of related plant traits under abiotic stresses, and has had practical consequences in the elaboration of new genetic materials that tolerate water deficit (Bruce et al. 2002; Ribaut et al. 2002; Condon et al. 2004). A major interest of this strategy is that it helps interpreting correlations between traits and establishing a hierarchy of candidate mechanisms.

- Some associations between traits could be expected, such as the co-location of QTLs of maize yield and of those of the anthesis-silking interval (ASI) in case of water deficit (Ribaut et al. 1996; 2002), because ASI is phenotypically well correlated to yield. Expected associations between a complex trait in stressing conditions and enzyme activities have also been detected (Hirel et al. 2001; Consoli et al. 2002).
- Some co-locations were less expected, and may provide indications on the conditions in which experiments were carried out, rather than on genetic association *per se.* For example, Tuberosa et al. (2002b) found co-location of QTLs of field-measured yield under water deficit with QTLs of root growth in hydroponic conditions. This co-location suggests that deep rooting was a highly

favourable trait in the considered field, which is not always the case. Such results are only observed when there is a soil water reserve that is not exploited by roots (e.g., water table or deep soil). In cases where plants grow on a limited amount of water (e.g., shallow soil), improving the root system's ability to take up water is of little interest or even counter-productive (Richards and Passioura 1989). In the case presented by Tuberosa et al. (2002b), co-location of QTLs therefore provides an indication on the soil characteristics of the considered field rather than a widely valid association between root traits and yield.

- In some cases, the genetic dissection of traits provides results that could hardly have been expected. This is the case for a QTL of water use efficiency identified by measuring the leaf carbon-isotopic discrimination in a mapping population of *Arabidopsis thaliana* (Masle et al. 2005). When the underlining gene was cloned, it was found to be involved in the development of the inflorescence and not in the controls of stomatal conductance or photosynthesis.

Quantitative genetics is therefore an efficient way to identify mechanisms involved in the responses to environmental conditions and to propose a hierarchy of them. In all examples presented above, the phenotypic variables were analysed in individual experiments with or without the considered stress, and then the QTL × environment interaction was studied as in Van Eeuwijk et al. (2005). This method is efficient but faces conceptual problems. Each genotype senses differently its environment (e.g., genotypes with contrasting root systems or leaf area), and because each genotype affects its environment in its own way (e.g., they deplete soil water or nutrients at different rates), so treatments are not always well-defined. An alternative approach is developed by several groups, consisting of a dissection of the phenotype before any genetic analysis, in such a way that phenotypic measurements are stable characteristics of each studied genotype. This can be obtained either by fine-tuning environmental conditions during experiments in such a way that all studied genotypes sense the same environmental conditions (Granier et al. 2006), or by designing phenotypic variables that encapsulate the genotype × environment interaction (Tardieu 2003; Yin et al. 2004).

GENETIC ANALYSIS OF THE PARAMETERS OF A GROWTH MODEL

Metamechanisms at organ level can characterize a genotype

Crop modellers have long expressed phenotypic traits as a function of environmental inputs such as organ temperature, light intensity or soil water potential. Relatively simple equations are used, some of which are straightforward because they represent a physical process and have a known formalism (e.g., water or heat transfer). Other equations describe plant processes, e.g., the response of growth to an environmental condition or the progression of development of the plant. These control equations have no clear theoretical background but are based on reproducible behaviours such as that presented in Figure 1 for the response of leaf elongation rate to meristem temperature. Although the combination of molecular mechanisms which leads to the response to temperature is not known, leaf elongation rate is linearly related to meristem temperature, and the same response curve applies to plants grown in different experiments in the field, in the greenhouse and in the growth chamber, provided that the plant experiences no stress and a near-zero evaporative demand, during the night or during days with very low vapour-pressure deficit (VPD). The slope of this relationship is therefore a stable characteristic of the genotype and differs between genotypes (Figure 1C). In this example, it would be impossible to establish the gene regulatory network which controls the response of leaf elongation rate to temperature, but the quantitative analysis of the phenotype allows prediction of the response of a genotype in different environments and comparison of genotypes.

We have proposed that response curves, which are reproducible under different environments for each genotype, can be considered a ‘metamechanism’ at organ level, although we do not know all their genetic bases (Tardieu 2003). They can be dissected genetically, thereby allowing one to discover *a posteriori* their genetic determinisms, rather than *a priori*. As a ‘proof of concept’, we have proposed a method based on the genetic analysis of the parameters of response curves to environmental conditions (Reymond et al. 2003, Sadok et al. unpublished).

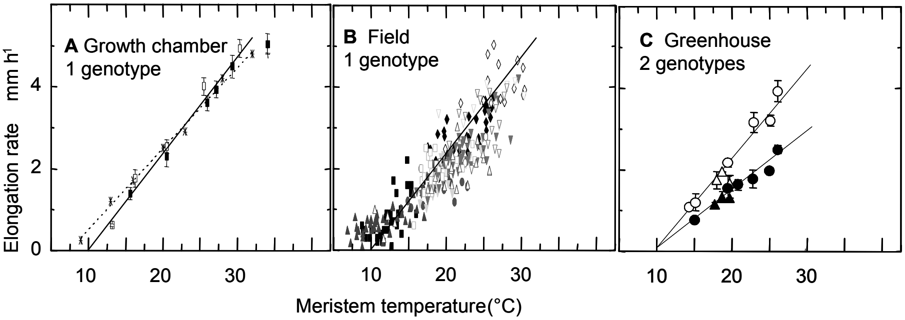

***Figure 1**. Relationship between meristem temperature and maize leaf elongation rate under low evaporative demand (night).* (**A** and **B**) *In a genotype (hybrid Dea), a single relationship applied to three experiments in the growth chamber and to 15 experiments in the field when plants were grown in the absence of evaporative demand (redrawn from Ben-Haj-Salah and Tardieu 1995). Each symbol, one coupled value temperature – elongation rate. Each type of symbol represents one experiment.* (**C**) *Two inbred lines with marked differences in slopes in two experiments each (redrawn from Reymond et al. 2003)*

Leaf elongation rate per unit thermal time has a genotype-dependent maximum value which is consistently observed in the absence of stress and of evaporative demand

Thermal time is used in crop modelling to take into account the effect of temperature on plant development (Bonhomme 2000). It is based on a linear formalism between rate and temperature, identified for instance for the rates of germination (Steinmaus et al. 2000), leaf development (Granier and Tardieu 1999) or leaf expansion (Ong 1983; Ben-Haj-Salah and Tardieu 1995). For monocot leaves, which have an essentially unidirectional expansion, this results in the linear relationship between elongation rate *LER* and meristem temperature *(T)* presented in Figure 1:

$$LER = \mathrm{d}L/\mathrm{d}t = a\ (T - T_0) \tag{1}$$

where L is leaf length, a and T_0 are the slope and x-intercept of the relationship between leaf elongation rate and temperature. If Equation 1 is acceptable, as suggested by Figure 1, it can be integrated to express leaf length at any time (t) as a function of the cumulated temperature above the threshold temperature T_0,

$$L = a \int_0^t (T(t) - T_0)\ \mathrm{d}t \tag{2}$$

where $\int_0^t (T(t) - T_0)\,\mathrm{d}t$ is thermal time (°Cd), termed t_{th} hereafter. The time course of leaf elongation rate can be expressed per unit thermal time (LER_{th}) which is temperature-independent if elongation is only limited by temperature (e.g., during the night, without water deficit and with a low evaporative demand):

$$LER_{\mathrm{th}} = \mathrm{d}L\ /\ \mathrm{d}t_{\mathrm{th}} = a. \tag{3}$$

Equation 3 implies that leaf elongation rate should be stable and characteristic of a genotype when plants are subjected to changes in temperature but to no other environmental constraint. In particular, this should be the case during the night in well-watered plants. Examples of temperature-independence of leaf elongation rate per unit thermal time are presented in Figure 2A for two maize genotypes.

To test the formalism of Equation 3, recombinant inbred lines (RILs) of three mapping populations were grown on a phenotyping set-up allowing one to measure the leaf elongation rate, the soil water status and the transpiration of 360 plants simultaneously, together with micro-meteorological conditions (Figure 3). A night plateau of leaf elongation rate was observed over a large number of time courses in the greenhouse and in the growth chamber. Although temperature fluctuated in the greenhouse, leaf elongation rate per unit thermal time was stable during the night and over up to 8 successive nights, corresponding to two phyllochrons (Figure 4). This plateau value was similar in the greenhouse (fluctuating conditions) and in the growth chamber (stable conditions) for each genotype, but differed between genotypes. Its heritability was 0.5 to 0.6 in three mapping populations (Reymond et al. 2003, Sadok et al. unpublished, Welcker et al. unpublished).

Genetic analyses of the maximum leaf elongation rate were carried out in three mapping populations, two with temperate and one with tropical origins (Reymond et al. 2004, Sadok et al. unpublished, Welcker et al. unpublished). In the three cases, QTLs were identified (Figure 5) and the QTL models accounted for about 50% of the genetic variance of parameter a in the three mapping populations. It was, therefore, possible to identify alleles associated with high or low maximum elongation rate in these three genetic backgrounds, either from temperate or tropical origin.

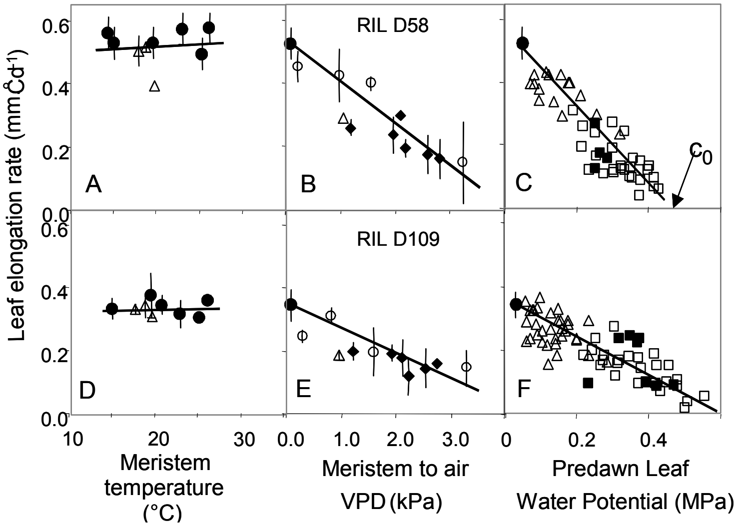

Figure 2.** Responses of leaf elongation rate per unit thermal time to meristem temperature* **(A, D), evaporative demand* **(B, E)** *and soil water deficit* **(C, F)** *in two different RILs.* **(A and D)** *Leaf elongation rate measured in the absence of evaporative demand plotted against meristem temperature. Individual results are pooled for better legibility.* **(B and E)** *Leaf elongation rate during day periods plotted against meristem to air VPD in well-watered plants. Night periods are regarded as having a VPD of 0, and individual results are pooled for better legibility.* **(C and F)** *Leaf elongation rate of night periods plotted against predawn leaf water potential. Individual values are presented. ○ Exp GC2 day values, ● Exp GC2 night values,♦ Exp FC2, Δ Exp GS1, □ Exp GS2, ● Exp GS2, second cycle of dehydration after rewatering*

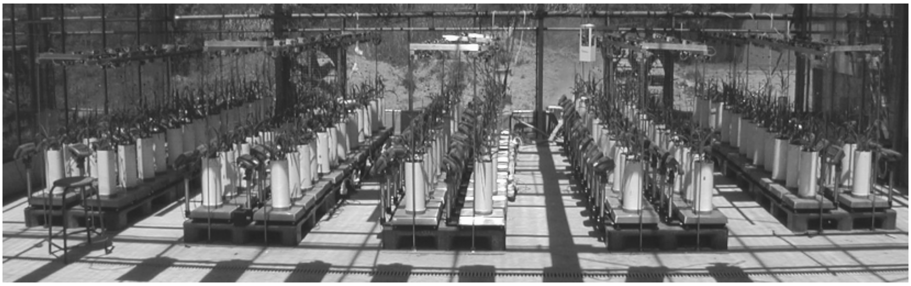

***Figure 3**. Phenotyping platform for continuous measurement of leaf elongation rate, soil water status and micrometeorological variables (up to 366 plants). Plants are grown in PVC columns and placed on balances. Each leaf is attached to a rotative displacement transducer. Environmental sensors (PPFD, vapour-pressure deficit, meristem temperature) are placed at plant level. All sensors are connected to data loggers with a time resolution of 15 min.*

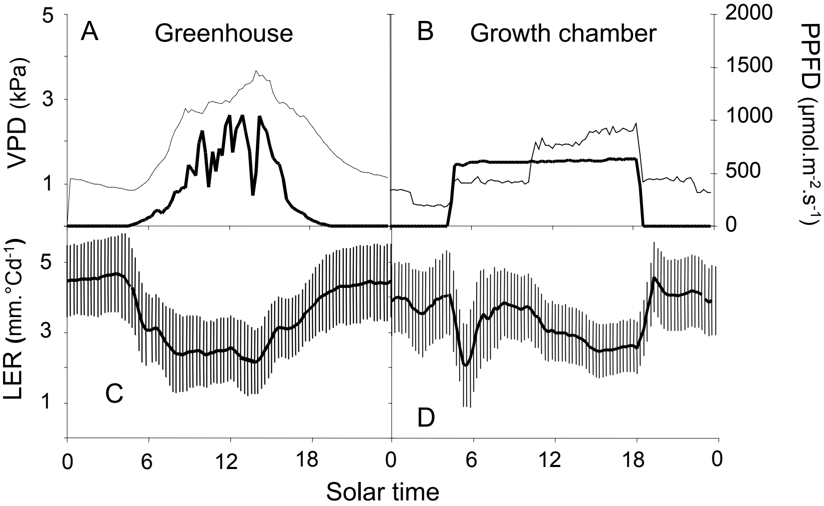

***Figure 4**. Meta-analysis of time courses of leaf elongation rate (LER) in the greenhouse (516 24-h time courses) and in the growth chamber (373 24-h time courses) for several days of experiments with well-watered plants subjected to fluctuating temperatures (greenhouse) or stable temperatures (growth chamber).* **(A, B)** *Leaf-to-air VPD (thin lines) and PPFD (thick lines) in the greenhouse* **(A)** *and the growth chamber* **(B)**. **(C, D)** *Mean and standard deviation of leaf elongation rate averaged over all time*

Responses of leaf elongation rate to evaporative demand and soil water status are characteristic of a genotype and can be dissected genetically

A clear effect of evaporative demand has been observed on leaf expansion rate of well-watered maize plants. A high and constant evaporative demand without soil water deficit reduced elongation rate, although predawn leaf water potential and the concentration of ABA in the xylem sap were close to 0 (Ben-Haj-Salah and Tardieu 1997). Consistently, a day-time depression of leaf elongation rate was observed every day in the meta-analyses presented in Figure 4, in which successive days had different temperatures but similar VPDs. LER_{th} decreased with increasing evaporative demand, closely following the transpiration rate. The morning decrease in LER_{th} occurred in less than 15 min. in the growth-chamber experiment, recovered in 1 h and followed afterwards the step changes in VPD. The morning decrease in LER_{th} was also rapid in the greenhouse, following a model with a negative linear effect of transpiration rate on elongation rate:

$$LER_{\mathrm{th}} = \mathrm{d}L \,/\, \mathrm{d}t_{\mathrm{th}} = a\,(1 - \mathrm{d}\,J_{\mathrm{w}}) \qquad (4)$$

where J_w is the transpiration rate per unit leaf area. Because transpiration cannot be measured in all experiments, we have proposed a simplified formalism (Reymond et al. 2003):

$$LER_{th} = dL / dt_{th} = a\,(1 - b\ VPD_{eq}) \qquad (5)$$

in which VPD_{eq} is the water vapour-pressure difference between leaves and air, corrected for the effect of light intensity. Relationships corresponding to different experiments analysed jointly are presented in Figure 2B,E. Equation 5 was applied to data of all RILs of three mapping populations (Reymond et al. 2003, Welcker et al. unpublished), and the slope *b* was calculated for each RIL by taking into account several experiments analysed jointly, some of them in the field, some in the greenhouse and some in the growth chamber. In spite of that, heritabilities of parameter *b* were high and QTLs were identified (Figure 5), accounting for about 40 to 50% of the phenotypic variance.

Soil water status affects leaf elongation rate in a reproducible way, in the same way as for evaporative demand. Reproducibility was only observed in the absence of evaporative demand, i.e., during the night. Common relationships applied to different experiments in the growth chamber and in the greenhouse, carried out over different years (Figure 2C,F):

$$LER_{th} = dL / dt_{th} = a\,(1 - c\ \Psi_{predawn}) \qquad (6)$$

where $\Psi_{predawn}$ is the predawn leaf water potential, an indicator of soil water status. The latter was indirectly estimated from soil water status, itself deduced from the weight of soil columns. Equation 6 was applied to all RILs of three mapping populations (Reymond et al. 2003, Welcker et al. unpublished). The slope *c* calculated for each RIL had high heritabilities and QTLs were identified (Figure 5), accounting for about 30 to 40% of the phenotypic variance.

DO GENETIC ANALYSES OF MODEL PARAMETERS PROVIDE DIFFERENT RESULTS COMPARED WITH QTL × ENVIRONMENT ANALYSES OF RAW PHENOTYPIC TRAITS?

It is commonly assumed that QTLs of constitutive traits are those which are observed in both well-watered and stressed conditions, while QTLs of adaptive traits are those observed in stressed treatments only (Prioul et al. 1997; Ribaut et al. 1996). The approach presented above provides another way of identifying constitutive versus adaptive traits. By definition, the maximum leaf elongation rate (parameter *a*) is a constitutive trait while the responses of leaf elongation rate to evaporative demand and to soil water status (parameters *b* and *c*) are adaptive traits. We have, therefore, compared both approaches by considering the co-locations between model parameters and the final leaf lengths measured either in well-watered conditions or in water deficit in the same sets of experiments (Reymond et al. 2004).

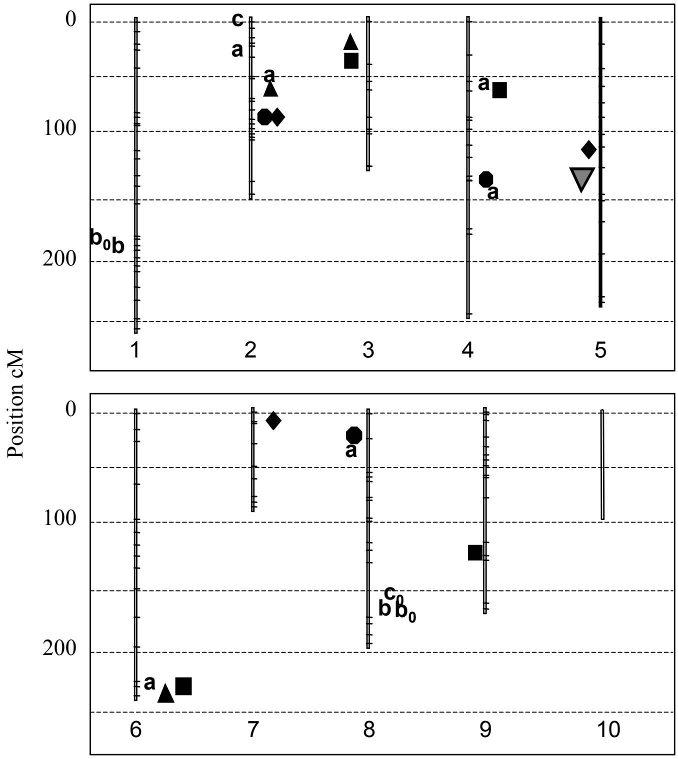

Figure 5. *QTLs of final leaf length (○, ♦, Δ and □ for 4 experiments) and QTLs of parameters of leaf elongation model (a, intrinsic elongation rate; b, slope of the response of leaf elongation rate to meristem to air VPD;* b_0*, x-intercept of the same relationship; c, slope of the response of leaf elongation rate to predawn leaf water potential;* c_0*, x-intercept of the same relationship). The QTL of leaf width common to the four experiments is also presented (●). Bars on chromosome indicate positions of markers. For leaf length and parameter a, symbols are located on the right-hand side of the chromosome if the allele F-2 increases the value of the trait. For parameters b and c, symbols are located on the right-hand side of the chromosome if the allele F-2 decreases the sensitivity of leaf elongation rate to the considered environmental condition (redrawn from Reymond et al. 2004)*

One QTL detection was carried out on length and width of leaf 6 in four experiments with either well-watered or stressing conditions in the field or in the greenhouse. The second QTL detection was carried out on parameters of response curves, following the method presented above. QTL of leaf length differed between experiments, but co-localized in 7 cases out of 13 with QTLs of the maximum leaf elongation rate, even in experiments with stressing conditions (Figure 5). QTLs of leaf length under water deficit were either alone or co-localized with those of maximum elongation rate (parameter *a*). They never co-localized with QTLs of responses to air or soil water conditions (parameters *b* and *c*). The same study was repeated in a mapping population with tropical parents (Welcker et al. unpublished), with similar conclusions. Several QTLs of leaf length under well-watered conditions and the most reliable QTL of leaf length in water deficit co-localized with QTLs of maximum elongation rate, while no QTL of leaf length under water deficit co-localized with QTLs of responses to water deficit or to evaporative demand (C. Welcker, unpublished).

A first interpretation of this result could be that we failed to detect QTL of parameters of response curves in loci where QTLs of leaf length of stressed plants were identified. However, (i) the clusters of QTLs of responses to soil water status or to evaporative demand did not correspond to QTLs of leaf length in stressed experiments; (ii) QTL detection on leaf length under water deficit often provided weak QTLs, in particular in the tropical mapping population in which no QTL of length under water deficit was detected in one year out of two, while QTLs of response to water deficit were detected. This may be due to the fact that each studied plant underwent slightly different scenarios of soil drying, which reduced the heritability of final leaf length but not of parameters of response. The classical method to identify QTLs of constitutive versus adaptive traits therefore did not apply to the experiments presented here. We suggest that identification of QTLs of parameters of response curves provide a promising alternative to deal with the genetic variability of adaptive traits.

HAVE THE EXPANSIVE GROWTHS OF DIFFERENT ORGANS A PARTLY COMMON GENETIC DETERMINISM?

The mechanisms which control the changes in tissue expansive growth with environmental conditions are essentially the same for several organs of the plant (see Introduction). The possibility is therefore raised that their genetic determinisms may be partly common. This possibility can be studied by considering co-locations of QTLs of growth of several organs. For instance, the QTL of parameter *a* on chromosome 2 (bin 2.04, Figure 4), which was also observed in the other two mapping populations (Sadok et al. unpublished; Welcker et al. unpublished), harbours a QTL of constitutive root characteristics (Lebreton et al. 1995; Tuberosa et al. 2002a). However, co-location of QTLs may be misleading because of the high probability of fortuitous co-locations when a large number of QTLs are considered.

We have considered the possibility that leaf and silk growth have common QTLs by analysing jointly QTLs of leaf growth parameters with QTLs of anthesis-silking interval (ASI), which depends on the growth rate of silks (A. Fuad and O. Turc unpublished data). ASI was measured in three and five fields under well-watered and water-deficit conditions, respectively, and QTLs of parameters of response curves were identified as presented above (Welcker et al. unpublished). The maximum elongation rate per unit thermal time (parameter *a*) was accounted for by five QTLs, among which three co-localized with QTLs of ASI in well-watered conditions. The responses of leaf elongation rate to evaporative demand and to predawn leaf water potential had partly common QTLs with ASI in water deficit. In all cases, the alleles conferring either high growth rate under favourable conditions or growth maintenance under water deficit were the same as those which conferred rapid silk growth (short ASI). This study therefore raises the possibility that different organs, involved in vegetative and reproductive developments, respectively, have partly common genetic determinisms.

TOWARDS VIRTUAL GENOTYPES WHOSE BEHAVIOURS COULD BE ANALYSED *IN SILICO* IN A VERY LARGE NUMBER OF CLIMATIC SCENARIOS

The QTL analysis of parameters presented above allows combining Equations 5 and 6 with expression of parameters as a sum of QTL effects:

$$LER_{\mathrm{th}} = \mathrm{d}L \,/\, \mathrm{d}t_{\mathrm{th}} = a\,(1 - b\; VPD_{\mathrm{eq}} - c\;\Psi_{\mathrm{predawn}}) \qquad (7)$$

$$a = \mu_{\mathrm{a}} + \Sigma\ \mathrm{QTLs_a};\;\; b = \mu_{\mathrm{b}} + \Sigma\ \mathrm{QTLs_b};\;\; c = \mu_{\mathrm{c}} + \Sigma\ \mathrm{QTLs_c} \qquad (8)$$

If Equations 7 and 8 apply, it should be possible to predict the behaviour of any RIL known by its alleles at QTLs, in any climatic scenario combining fluctuating temperatures, evaporative demand and soil water status. This possibility was tested on lines not involved in the construction of the QTL models and chosen to maximize the expected differences (Reymond et al. 2003). Leaf elongation rates measured in a growth-chamber experiment were compared with those predicted by the model, using measured temperature, VPD and soil water potential as inputs. Leaf elongation rate had similar time courses in modelled and observed data, and expected differences between RILs were observed.

CONCLUDING REMARKS

We propose that aggregating all the available knowledge about gene actions into a model is not feasible at the time being, and that this may well be the case for a long time. We therefore propose a different approach, in which the phenotype of a given genetic line is 'footprinted' via a vector of parameters of models. The genetic analysis of these parameters can be a useful avenue for modelling the genotype × environment interaction, but also to identify the genes involved in its controls.

The coupling of genetic and ecophysiological models presented here has now been tested in three mapping populations of maize with different origins, including tropical genetic material that could have been expected to have different behaviour compared with temperate material. The common analysis of anthesis-silking interval and of leaf growth parameters suggests that this approach could apply to different organs of a plant, with partly common genetic determinism across organs.

Three challenges are ahead of us. (i) The method presented in Equations 1 to 8 does not fully take advantage of the kinetic analysis presented in Figures 3 and 4. It is based on the use of averaged values over several hours, while kinetic parameters may provide new insights into the genetic variability. (ii) Three mapping populations have been used for testing the method. It is necessary now to deal with more complex genetic material, for instance collections of accessions. (iii) The phenotypic traits presented here were relatively simple, and will have to be combined with many others in order to predict the plant architecture, transpiration and biomass production. However, the combination of approaches proposed by Hammer et al. (2005) suggests that such an integration of mechanisms is possible and might allow one to evaluate plant-breeding strategies with crop models.

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CHAPTER 8

MODELLING GENOTYPE × ENVIRONMENT × MANAGEMENT INTERACTIONS TO IMPROVE YIELD, WATER USE EFFICIENCY AND GRAIN PROTEIN IN WHEAT

S. ASSENG[#] AND N.C. TURNER[##]

[#] *CSIRO Plant Industry, Private Bag 5, PO Wembley WA 6913, Australia.*
[##] *Centre for Legumes in Mediterranean Agriculture, M080, University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia.*
E-mail: senthold.asseng@csiro.au

Abstract. In breeding trials the complexity of interactions of genotypes (G) with their environment (E) requires extensive field experiments at many locations and seasons to test new traits. Management (M) treatments such as planting density and fertilizer supply are often neglected in such experiments. Moreover, several traits (e.g., increased specific leaf area and faster early root growth) are often changed simultaneously in new genotypes (e.g., early vigour lines) with the contribution of individual traits remaining uncertain.

Using a well-tested simulation model can assist in exploring the impact of new genotypes and the contribution of individual physiological traits on yield by simulating genotypes across many locations, seasons and a range of management options. Recent advances in simulating grain protein concentrations in wheat also allow the analysis of the impact of yield-related physiological traits on grain protein percentage. Simulation analyses have shown that trait effects on yield are location- and season-specific. The contribution of traits to yield varies with growing-season rainfall and soil types. In Mediterranean-type environments, traits of increased specific leaf area and faster early root growth improve grain yields and water use efficiency on low water-holding-capacity soils, but can be detrimental on better water-holding-capacity soils. Increased specific leaf area is often only expressed if N management is adjusted while the positive effect of faster early root growth in sandy soils diminishes with increasing N supply. The sum of individual traits incorporated in early vigour (that is, increased specific leaf area, faster early root growth, earliness, reduced radiation use efficiency) and increased transpiration efficiency is often not the same as the effects when the traits are combined. Another trait for an increased capacity for storing water-soluble carbohydrates can be beneficial for yield and water use efficiency in terminal-drought environments, but is not as effective when growth is limited by water or nutrients during anthesis.

The response of grain protein to yield-related trait modification (such as increased grain-filling rate) is usually negatively related to the response to grain yield. However, under combined water and N limitation, simulations indicated that this negative linear relationship can become non-linear, suggesting that a lower potential yield in such environments might improve grain protein concentrations without reducing the attainable yield.

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INTRODUCTION

In many environments, water supply is a major source of variability in crop yields (Ritchie 1983). The seasonal water use of a wheat crop consists of both crop transpiration and soil evaporation (French and Schultz 1984), with the latter varying between 14% (Angus et al. 1980) and 75% (Cooper et al. 1987) of total water use. Total water use or total evapotranspiration (ET) by a crop can vary substantially due to the limited water available from soil water storage or due to limited rainfall. It can also vary as a result of variation in crop transpiration resulting from management, such as nutrient supply (Shepherd et al. 1987) and sowing time (Connor et al. 1992), or from use of different species (Gregory et al. 1992) or cultivars (Richards and Townley-Smith 1987). For many years, specific traits have been sought to improve water use efficiency and grain yields in rain-fed agriculture (Turner 1986; Whan et al. 1991; Reynolds et al. 1996; Miralles and Richards 1999; Turner and Asseng 2005). However, incorporating new traits into a crop takes 10 to 12 years, and only then it will be known if a new trait has been effective in improving water use efficiency and yield in a specific environment. Simulation modelling can offer an alternative in testing traits in a computer-based experiment across a number of environments, soil types and growing seasons to evaluate the potential benefits from breeding for specific traits. To carry out such simulation experiments, a model needs to be comprehensive, must take into consideration the dynamics of crop-soil-weather interactions and modelled growth processes need to be based on sound physiological principles.

This chapter demonstrates how a crop-soil simulation model was used in evaluating traits in improving yield and water use efficiency (WUE in kg grain yield mm^{-1} ET) and grain protein concentration of rain-fed wheat in a Mediterranean-type climate.

APSIM-NWHEAT MODEL

The Agricultural Production Systems SIMulator (APSIM) (Keating et al. 2003) for wheat (*Triticum aestivum* L.) (APSIM-Nwheat version 1.55s) is a crop simulation model, consisting of modules that incorporate aspects of soil water, nitrogen, crop residues, crop growth and development and their interactions within a crop-soil system that is driven by daily weather data. A detailed description of APSIM-Nwheat is given by Asseng (2004).

APSIM-Nwheat has been tested extensively against field measurements in various studies under a large range of growing conditions (Probert et al. 1995; 1998; Asseng et al. 2000; 2004) and in particular in the Mediterranean climatic regions of Western Australia (Asseng et al. 1998a; 1998b; 2001b). The model was able to reproduce grain yields over the entire range of possible wheat yields from < 0.5 to $>$ 13 t ha^{-1} (Figure 1A).

Recent advances in simulating grain protein concentrations in wheat now allow the analysis of the impact of yield-related physiological traits on grain protein concentration. A comparison of simulated versus observed grain protein contents (in %) is summarized in Figure 1B.

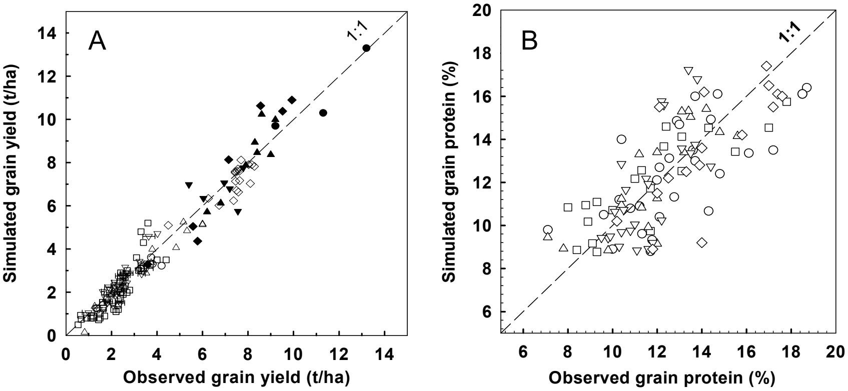

Figure 1. (**A**) *Simulated versus observed grain yield for Western Australia data set I (Asseng et al. 1998b), (▽); Western Australia data set II (Asseng et al. 2002), (□); New South Wales (Asseng and Van Herwaarden 2003), (○); Gatton, Queensland (Asseng et al. 2002), (△); Lincoln, New Zealand (Asseng et al. 2004), (◆); Xiangride, China (Asseng et al. 2002), (●); Arizona, USA (Asseng et al. 2004), (▲); Obregon Mexico (Asseng et al. 2004), (▼); Polder and Wageningen, The Netherlands (Asseng et al. 2000), (◇).* (**B**) *Simulated versus observed grain protein (%) for locations in Western Australia (○, ▽, ◇), New South Wales (□) and The Netherlands (△)(after Asseng et al. 2002)*

MEDITERRANEAN ENVIRONMENT

In the Mediterranean environment of Western Australia, rainfall is very variable, with between two thirds and three quarters of the total annual rain falling in the cropping season. Rainfall between April and October is considered to be the effective growing-season rainfall and ranges in average from 200 mm in the East to 400 mm in the West. The sowing date of wheat varies from late April to the end of July depending on when the first significant rainfall occurs after the dry summer. Depending on sowing date and phenology, wheat crops flower between September and October, and grain filling is often affected by high temperatures and terminal water shortage before maturity in November and December. In addition, soils in the Western Australian wheat belt have a low plant-available water-holding capacity (PAW) in the root zone (PAW: 50–120 mm). As a consequence, grain yields on average are about 2 t ha^{-1}, well below average grain yields from temperate environments (Turner 2004).

MODELLING GENOTYPE × ENVIRONMENT × MANAGEMENT (G×E×M) INTERACTIONS

A number of traits have been suggested to improve yields in the Mediterranean-type environment. A set of these are combined in early vigour as a combination of increased specific leaf area (SLA), faster early root growth, earliness and reduced radiation use efficiency (RUE) (when early vigour is achieved through increased SLA with less N per unit leaf area), which when linked with increased transpiration efficiency (TE) are considered to improve yields mainly through improved water use efficiency (Condon and Richards 1993). Another trait, an increased capacity to store water-soluble carbohydrate, has been suggested as being particularly efficient in improving yield in terminal-drought environments such as Mediterranean-type environments (Setter et al. 1998). A trait for increased grain filling rate, which has been suggested as increasing yields of lupin in terminal-drought environments (Palta et al. 2004), has also been simulated to investigate its impact on the grain protein content of wheat.

When modelling G×E×M interactions with APSIM-Nwheat, a specific trait was altered in the model and then compared with the unaltered crop across more than 80 seasons using contrasting soils appropriate to the Mediterranean-type region of Western Australia; a sand with 55 mm PAW and a clay with 109 mm PAW and a range of N management options. Trait effects were then analysed individually and in combination with other traits.

THE IMPACT OF SOIL TYPE, N SUPPLY AND RAINFALL ON TRAIT EXPRESSION

In a simulation study, Asseng et al. (2003) showed that on a sandy soil, doubling specific leaf area increased yields by 15%, but only when sufficient N was supplied. On a clay soil, doubling SLA reduced yield under low N supply, but was marginally beneficial with a high N supply. A trait for faster early root growth, increased grain yields by more than 15% on a sandy soil with low N input by assisting the crop to capture more N from deeper in the soil, but this trait became less important with high N input on a sand and gave little benefit on average on a clay (Figure 2A, C). Traits of earliness, increased transpiration efficiency (TE) and reduced radiation use efficiency all affected yield, but their impact varied with soil type and N supply. WUE usually changed in parallel to yields (Figure 2B, D) as the impact of these traits on ET was often less than on yield. For example, increased SLA stimulated growth and the increased crop water use was then offset by reduced soil evaporation due to the increased leaf cover of the soil.

When the traits of increased SLA, faster early root growth, earliness, reduced RUE and increased TE were combined, the effect on yield and WUE was greater than the sum of the individual yield effects due to non-linear interactions among the traits (Figure 2). For example, with low N on sand, the sum of the individual trait effects was 18% yield increase, while the combined trait effect was 32%.

On a clay soil, the simulated effect of the combined traits on yield in the low-rainfall region increased yields by 30% on average (Figure 3A). With high N input the average yield advantage remained at 30% as rainfall increased, but with low N input the average yield advantage was reduced to 10%. However, on a deep sand the average yield was increased by 20–30% by the combined set of traits with low N input and by about 20% when the N input was high across all rainfall environments (Figure 3B).

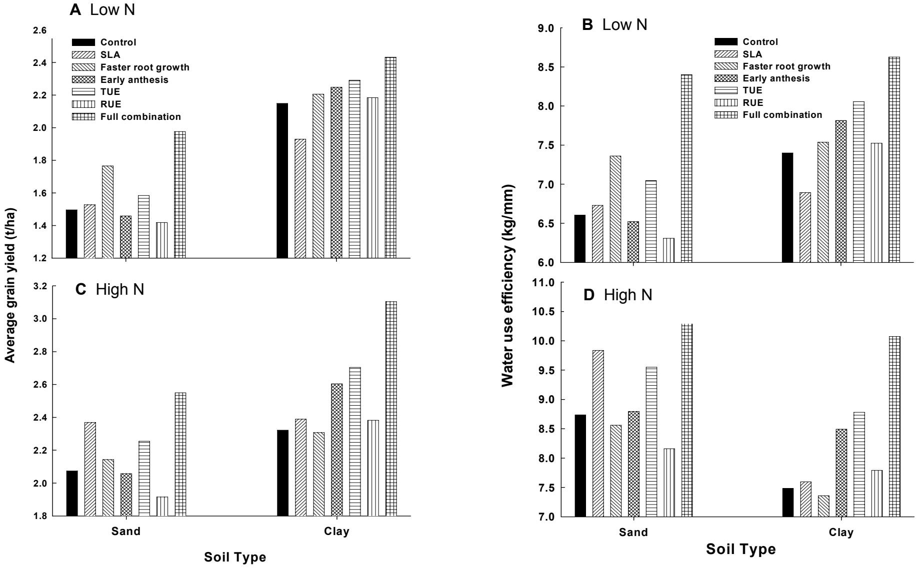

Figure 2. *Simulated average effects of individual traits (standard, increased SLA, faster early root growth, earliness, increased transpiration efficiency (TE), reduced radiation use efficiency (RUE) and all traits in combination) on* **(A, C)** *wheat yields and* **(B, D)** *WUE on a deep sand and a clay soil with* **(A, B)** *low N and* **(C, D)** *high N fertilizer input, for a medium-rainfall location in Western Australia (322 mm mean growing-season rainfall)*

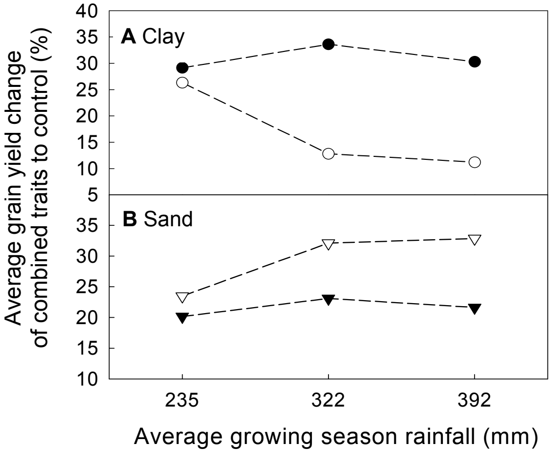

Figure 3*. Simulated relative average yield increases for wheat with the full compliment of traits associated with early vigour and high TE in three rainfall regions of Western Australia with low N (open symbols) and high N (filled symbols) fertilizer supply on* **(A)** *a clay soil and* **(B)** *a deep sand (after Asseng et al. 2003)*

THE IMPACT OF SEASONAL RAINFALL DYNAMICS ON GENOTYPE EXPRESSION

Increasing the capacity to accumulate assimilates in the pre-grain-filling period for later remobilization by 20% in a simulation experiment over 78 growing seasons, increased yields by up to a maximum of 12% in moderate seasons, but had little effect in poor or very good seasons. This was because several factors affected the amount of carbohydrates stored rather than the storage capacity itself, namely: (i) poor growth due to water or N deficits in the weeks prior to and shortly after anthesis (when most of the assimilates are stored for later remobilization) (Figure 4, Phase A); (ii) a small number of kernels due to little growth for most of the pre-anthesis season and hence poor sink demand by grains (Phase A); or (iii) a high photosynthetic rate during grain filling that is sufficient to fill grains (Phase C). Thirty-five of the 74 seasons shown in Figure 4 (note 4 seasons of the 78 simulated had no sowing opportunity due to lack of rainfall) resulted in > 5% yield increase due to the increased storage capacity. Further analysis of the simulated data revealed that 63% of >5% yield increases were in seasons with above average biomass accumulation at anthesis. Forty-nine percent of the yield increases > 5% were associated with late sowing opportunities (later than the simulated long-term average) that often resulted in a shorter grain-filling period due to water shortage and high temperatures arising from the delayed flowing. Thus, the simulation suggests that increasing the potential storage capacity for remobilization increases grain yields under conditions of terminal drought when the growing conditions up to the start of grain filling are favourable. Increasing the supply of N in Phase C in Figure 4 increased yields with an increased storage capacity, suggesting that the yields were N-limited, rather than water-limited.

Since water use was little affected by changes in the storage capacity of water-soluble carbohydrates, WUE reflected the simulated yield and yield changes (Figure 4B).

EFFECT OF GRAIN-FILLING RATE ON YIELD AND GRAIN PROTEIN CONCENTRATION

Using long-term historical weather records and simulating an increased yield potential by increasing the potential grain-filling rates resulted, as expected, in a large range of mostly negative but linear protein-yield relationships (Figure 5). A large proportion of these seasons had a combination of water and N limitations that gave the wide spread of lines. However, some non-linear relationships occurred in which, below a certain yield, grain protein decreased without a change in grain yield despite different potential grain-filling rates. In some cases, there was no change in simulated yield over the whole range of grain-filling rates but protein still declined. This unexpected result was caused by differences in dry-matter accumulation and N accumulation dynamics by the grain at the different potential grain-filling rates and a dependency of dry matter and N accumulation at extreme low and high rates (Asseng et al. 2002). In these years, the higher potential grain-filling rates initially

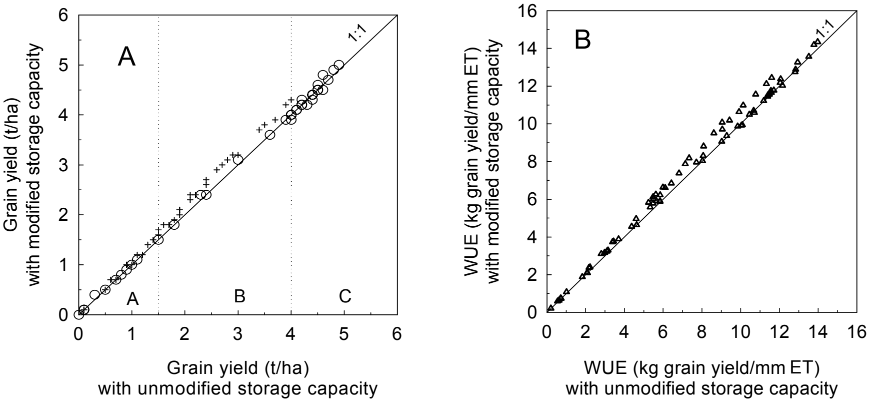

Figure 4. ***(A)*** *Simulated grain yields and* ***(B)*** *water use efficiency (WUE) with a 20% increased capacity to store pre-grain-filling assimilates for potential remobilization to the grain compared with an unmodified storage capacity at Barellan, New South Wales, with 90 kg N ha^{-1}, using weather records from 1915–1992. Yield change in* ***(A)*** *of >5% (+) and <5% (○). The diagonal shows the 1:1 line. In* ***(A)*** *the vertical lines separate different response scenarios. Phase A, dry years with yields of < 1.5 t ha^{-1}; Phase B, moderate years with a terminal drought and yields of 1.5–4.0 t ha^{-1}; and Phase C, wet years with yields of > 4 t ha^{-1} (after Asseng and Van Herwaarden 2003)*

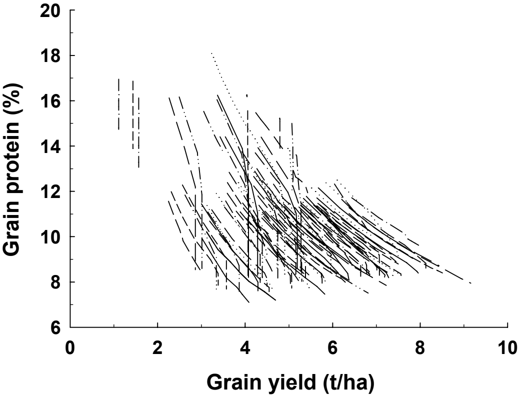

Figure 5. *Simulated relationship between grain protein percentage and grain yield with seven different potential grain-filling rates (rates increase with increasing yield or decreasing protein) for 104 years (lines connect the different potential rates, a range of different types of line is used for each year) at Kojonup (high-rainfall location), Western Australia, with 150 kg N ha^{-1} (after Asseng and Milroy 2006)*

allowed a higher rate of dry-matter accumulation in the grain, but had no effect on the rate of N accumulation because it was limited by N supply. The higher filling rate resulted in an earlier depletion of the available carbohydrates and the crop then relied solely on the supply of carbohydrates from current photosynthesis, which was also low by this stage. This led to brief restrictions in N accumulation because of the limits on the ratio of grain N to dry-matter accumulation in the model. The shortfall in N accumulation remained until maturity. With a low grain-filling potential, grain filling continued at the potential rate for longer. Thus the N accumulation, which proceeded at the same rate as in the plants with a high filling rate, could also continue for longer. Eventually the accumulated grain dry matter reached the same mass as in the high-potential-rate scenario, but N accumulation had been restricted less frequently by the low rate of dry-matter accumulation. The result was an altered protein content but the same grain yield.

DISCUSSION AND CONCLUSIONS

The analysis of trait effects on yield and WUE has indicated that yield and WUE of wheat crops in the Mediterranean-climatic region of Western Australia vary markedly depending on soil water-holding capacity, N management and rainfall amount. The degree of variation in yield and WUE is difficult to quantify from field experimentation alone. Thus, simulation modelling provides a powerful means of integrating all these factors, of linking these to traits, and when combined with long-term climatic data and regional soil information is able to markedly extend the interpretation possible from limited experimental studies. The results suggest that there is large potential for increasing yield and WUE through increased N application, in particular in the high- and medium-rainfall zones of Western Australia on soils with high water-holding capacity (Asseng et al. 2001a). Breeding for early vigour (increased SLA, faster early root growth, earliness, reduced RUE), together with increased TE, will further increase yields and WUE in this environment. The full combination of traits related to early vigour and increased TE can increase yields by up to 30% regardless of soil type. However, the different traits associated with early vigour and increased TE have been shown to have different impacts on yield depending on soil type, management and rainfall season. While the trait of faster early root growth could increase yields particularly with low N supply on a sandy soil, an increased SLA required high N fertilization for a positive impact on yield.

The simulation study with an increased capacity for storage of water-soluble carbohydrates highlighted that increasing the storage capacity may not always be effective in increasing grain yields even in a Mediterranean environment with frequent terminal water shortage. The analysis pointed out that the relative contribution of remobilization to grain yield depends primarily on source/sink interactions during grain filling in each environment and confirms results from shading experiments by Conocono (2002). If growth is restricted around anthesis due to lack of water or N resulting in a low grain number (Fischer 1985), then even if rainfall is low a small assimilate demand for total grain growth can be entirely met by photosynthesis during grain filling (e.g., Palta and Fillery 1995) and results in little water use by the crop during grain filling (Asseng et al. 2001a). A high N supply and high water use before anthesis can result in a high assimilate demand during grain filling, but the high pre-anthesis water use can reduce water availability after anthesis if the post-anthesis rainfall is low (Fischer 1979). Therefore, increasing the storage capacity will not be effective if (i) growth shortly before and after anthesis, when most assimilates are accumulated for remobilization (Conocono 2002; Schnyder 1993) is limited by periods of drought; or (ii) when yield is not limited by water supply and it is N supply which limits yield. Additional yield increases of more than 10% were simulated in some seasons and selecting for larger storage capacity of soluble carbohydrates appears to be worthwhile for terminal-drought environments. The simulation experiment indicated that assimilates stored prior to the main storage period around anthesis can also be important for grain yields in some seasons where growth rates are high for the first part of the growing season but low during the second part starting well before anthesis. While genetic variation for storage of soluble carbohydrates in the period up to 14 days after anthesis has been demonstrated (Nicolas and Turner 1993), it still needs to be shown that genotypic variation exists for the ability to accumulate significant amounts of assimilate well before anthesis for remobilization during grain filling.

The variation between different seasons in the protein-yield curves with the increased potential grain-filling rate indicated a large impact of rainfall, and consequently water and water by N interaction, on this relationship. The simulated change in protein content with no change in grain yield in some seasons was unexpected, but can be explained by the model through an intermittently restricted carbohydrate supply that occurred at different frequency with the different grain-filling rates. Hence N accumulation, which is constrained by periods of low grain dry-matter accumulation, was interrupted to varying extents. The frequency of changes in protein content without change in yield was higher at low N supply and at low-rainfall locations (Asseng and Milroy 2006). From these responses it is hypothesized that a higher genetic grain protein potential can be achieved via a lower genetic yield potential without compromising the *achievable* grain yield under water- and N-limited conditions. Cultivars with moderate yield potential might therefore be more suitable for growth-limited environments: attaining a higher grain protein but still achieving the environmental yield potential.

In summary, the G×E×M analysis has shown that using a well tested simulation model is a powerful tool in exploring the impact of new genotypes and the contribution of individual physiological traits on yield and WUE by simulating the responses of genotypes or traits across many locations, seasons, soil types and management options.

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CHAPTER 9

PHYSIOLOGICAL PROCESSES TO UNDERSTAND GENOTYPE × ENVIRONMENT INTERACTIONS IN MAIZE SILKING DYNAMICS

L. BORRÁS, M.E. WESTGATE AND J.P. ASTINI

Agronomy Department, Iowa State University, Ames, Iowa, USA.
E-mail: borras@iastate.edu

Abstract. Variation in maize yield across environments often reflects genotype-specific responses in crop-flowering dynamics. The most widely observed effect is the temporal separation of male (anthesis) and female (silking) floral maturity, referred to as the anthesis–silking interval (ASI). Many studies have shown that maize yield also is a function of crop growth rate around flowering. At present, however, the relationship between growth rate and flowering dynamics is not fully understood. In this chapter, we present a conceptual basis and experimental approach for quantifying and analysing maize female flowering responses to variation in plant growth. We show how this approach can be applied to resolve contrasting genotypic behaviour under a range of environmental conditions. Because maize canopies are composed of plants exhibiting a range of growth rates, understanding plant-to-plant variability is critical for evaluating genotypic and environmental effects on female flowering dynamics. We propose a simple model, based on well-established population dynamics, to capture intrinsic plant-to-plant variability within maize canopies. Specific genotype parameters were identified that integrate biomass production and partitioning into a framework to describe the flowering response of a particular genotype in a particular environment. These results have important implications for understanding yield formation in maize. They provide an approach to evaluate genotype × environment interactions, and a framework to evaluate genes regulating flowering dynamics.

INTRODUCTION

As in most extensive crops, variation in maize (*Zea mays* L.) yield is related more to the number of harvested kernels than to individual kernel weight. As such, the period of development when kernel number is defined has been referred as the 'yield critical period'. Numerous studies have shown that maize kernel number (and yield) is a function of crop growth rate around flowering (Early et al. 1967; Andrade et al. 1999). Environmental conditions that alter plant growth during this period affect specific aspects of flowering dynamics. The most widely observed effect is the

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temporal separation of male (anthesis) and female (silking) floral maturity, referred to as the anthesis-silking interval (ASI). The relationship between final grain yield and the ASI has been described in numerous studies (Woolley et al. 1962; Moss and Stinson 1961; Edmeades and Daynard 1979; Hall et al. 1982) and has attracted considerable attention in maize-breeding programmes (Bolaños and Edmeades 1996; Bruce et al. 2002; Bänziger et al. 2004; Campos et al. 2004). The relationships between plant growth and specific aspects of the flowering process, however, have not been fully resolved. Identifying the physiological mechanisms that regulate the visually observed changes in flowering dynamics has important implications for overcoming current limitations to grain yield in maize.

Maize is a monoecious plant, with staminate (male) flowers borne on an apical inflorescence (commonly referred to as a tassel) and with pistillate (female) flowers produced on one or more lateral branches, which develop into grain-bearing rachises (commonly referred to as ears). At the individual plant level, anthesis is defined as the beginning of pollen shed from the tassel, and is visually determined when at least one anther has dehisced and is liberating pollen. Appearance of the first pollen-receptive stigmas (commonly referred to as silks) from within the surrounding husks on the primary ear defines the silking date for each plant. As such, both flowering descriptors are qualitative traits that define a change of state. At any point in time, a plant either has or has not reached these flowering stages (anthesis or silking).

When these flowering processes are considered at the population level, anthesis and silking dates are set when a pre-determined proportion of plants in the population reach the stage. In general, anthesis or silking for a population is reported when 50% of the plants attain the stage. This simplification reflects the fact that all plants in a population do not achieve anthesis or silking at the same time. Rather, flowering throughout the population is recognized as a continuous (but finite) process. Thus, for the population, floral anthesis is a quantitative process; for individual plants, it is a qualitative process.

Using a mechanistic framework to analyse a biological phenomenon involving a qualitative process at the individual level and a quantitative process at the population level has met with considerable success. An excellent example is the prediction of seed-lot performance across contrasting environments from quantitative information of germination at the population level and a qualitative assessment of individual seed germination (Ni and Bradford 1992; Bradford 2002). As shown below, a clear understanding of the flowering process at the individual plant level is critical for resolving environmental effects on maize phenology at the population level. This is particularly evident when plant-to-plant variability within the population is large, as is often the case in maize crops, especially under stressful growing conditions.

BIOMASS PARTITIONING DURING FLOWERING AND SILKING DYNAMICS

Biomass partitioning and silking

In maize, biomass partitioning to the female reproductive structures varies with plant growth rate. Edmeades and Daynard (1979) used biomass partitioning to show that the tassels were a much higher priority sink than the ears at high population density, where individual plant growth is reduced. Figure 1, redrawn from Andrade et al. (1999), shows how ear growth varies over a wide range of individual plant growth rates during the 30 day period bracketing flowering. In this example, population densities were employed to alter radiation intercepted per plant, and consequently, individual plant growth rate around flowering. It is noteworthy that there was a positive ear growth only if aerial biomass increased at a greater rate than ca 1 g pl^{-1} d^{-1} during the 30-day period bracketing flowering. Above this threshold biomass partitioning to the growing ear was greatly enhanced (Figure 1). This example illustrates that biomass allocation to the maize ear is not a constant ratio of the total biomass produced around flowering. It is important to note that these analyses were made possible by testing plant responses across a wide range of population densities, and by examining the response of individual plants within each population rather than using population averages. Recent studies using this approach have exposed significant genotypic differences in the minimum threshold for ear growth and maximum ear growth rates at very high rates of plant growth (Echarte et al. 2004).

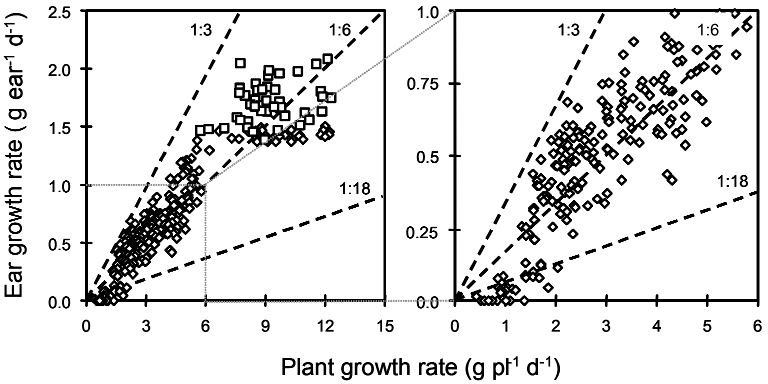

Figure 1. Relationship between ear growth rate and plant growth rate around flowering for individual plants. The variability in plant growth rate was achieved with population densities ranging from 2.2 to 16.9 plants m^{-2}. Adapted from Andrade et al. (1999). Dotted lines show constant biomass partitioning to the ear during flowering (1:3, 1:6, and 1:18 ratios)

Since the silking process is a function of ear expansion growth (Westgate and Boyer 1986; Cárcova et al. 2003), time to silking historically has been considered an indirect indicator of biomass allocation to the ear (Moss and Stinson 1961; Buren et al. 1974; Jacobs and Pearson 1991). Even though ears are growing continuously around flowering, there will be a finite ear biomass value at which silking occurs. Although it would seem intuitive that silking is a function of biomass allocation to the ear, examples illustrating this relationship are lacking. Therefore, we collected ears from a single genotype as it approached silking to determine if there was a critical ear biomass at this stage. As shown in Figure 2, ears of this genotype grown at 10 plants m^{-2} reached silking when accumulated ear biomass was around 0.75 g ear^{-1}. Effects of the environment on this value are currently unknown. But it is clear from Figure 1 that low plant growth rates around flowering that reduce the ear growth rate would increase the time for the ears to reach the critical ear biomass to achieve silking.

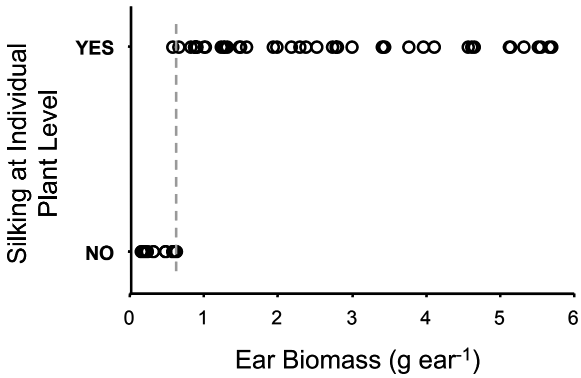

Figure 2. *Silking status of individual plants as a function of biomass accumulation by the primary ear. Data are for 63 plants sampled around silking. The dotted line was fitted by eye. The experiment was conducted in Ames, Iowa, in 2005*

Silking dynamics at the population level have been shown to be highly sensitive to reductions in plant growth rate caused by drought (Hall et al. 1982), shading (Moss and Stinson 1961) or defoliation (Yao et al. 1991) treatments around flowering. Phenology data presented in Figures 3A and 3B from Yao et al. (1991), for example, illustrate the impact of altering plant growth on the development of the female reproductive structures and resulting pattern of silking for the population. In this case, three maize populations were subjected to various levels of defoliation to decrease light interception and alter crop growth rate around flowering (confirmed from biomass measurements). The appearance of the male inflorescence was not affected by the decrease in resource capture (Figure 3A), while the silking pattern of the population was closely coupled to the source level for the crop (Figure 3B). As such, delayed silking at low plant growth rates was in accordance with a reduced biomass allocation to the ear at low plant growth rates, retarding the achievement of the needed biomass to reach silking. It is important to recognize that the defoliation treatments affected the time to silking of individual plants within the population (Figure 3). Therefore, plant-to-plant variability in ear development within each population is not a constant, and its impact on the silking dynamics of the population also must be considered.

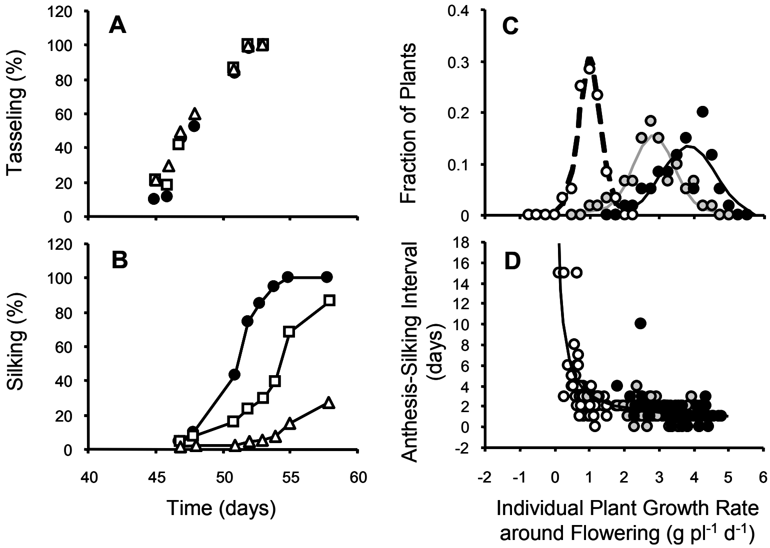

Figure 3. *Effect of reducing leaf area index of a maize canopy around the flowering period on the progress of tassel emergence* **(A)** *and silking* **(B)** *for the population. LAI = 2.3 (black circles, control), LAI= 0.6 (squares), LAI= 0.3 (triangles). Adapted from Yao et al. (1991). Description of variability in plant growth rate* **(C)** *and the anthesis–silking intervals* **(D)** *from a maize inbred line planted at 10 pl* m^{-2} *(grey circles), defoliated (ca 75% of the green leaf area removed) to reduce plant growth rate (white circles), or thinned (50% of the plants removed) to increase plant growth rate (black circles) about 15 days before anthesis. A total of 60 plants per treatment were sampled. A single power function was fitted to all 180 data points in* **D** *(*R^2 *= 0.52), as curve parameters where not different between treatments (*$P<0.05$*). The experiment was conducted in Ames, Iowa, in 2005*

Figures 3C and 3D illustrate individual plant growth and ASI for a maize inbred grown at three growth conditions. One group of plants was partially defoliated 14 days before anthesis (ca 75% of the green leaf area removed) to reduce plant growth rate. A second group was thinned to 5 pl m^{-2} at the same time, to increase individual plant growth. A third set left at 10 pl m^{-2} served as controls. Measured plant growth rates around flowering on average were 0.87, 2.63 and 3.70 g pl^{-1} d^{-1} for the defoliated, control and thinned treatments, respectively (Figure 3). The plant-to-plant coefficients of variations (CV) were 0.36, 0.26 and 0.20% for the defoliation, control and thinning treatments, respectively, which were in general agreement to previous observations (Edmeades and Daynard 1979; Vega and Sadras 2003). Time to silking and anthesis was monitored for each individual plant in each population. Plants with reduced plant growth were delayed in silking, resulting in an increase in ASI for these plants, which was most dramatic at very low plant growth rates (Figure 3D).

Developing a model to describe silking dynamics

Having described the basic relationships between plant growth rate around flowering, ear growth rate and time to silking (Figures 1, 2 and 3), we developed a model to predict the silking pattern of maize populations based on their plant growth. This model was based upon the understanding that:

- Biomass allocation to the ear is not constant, and varies with total plant growth around flowering. At low plant growth rates, the ear growth is reduced not only because of reductions in total plant growth, but also because partitioning of plant biomass growth around flowering changes. The proportion of biomass allocated to the ear decreases at low plant growth rates.
- Maize populations are composed of plants that grow at differential rates around flowering, impacting the time to silking for the various growing fractions of the population. At commercial stand densities, variability in plant growth rate around flowering is ca 30% (CV), and increases in stressful environments (Vega and Sadras 2003).
- Silking is a change of state at the individual plant level; this change in state can be related to accumulated ear biomass.
- At the population level, silking for each fraction of the population can be defined by their ear growth rates. Ear growth rate for each fraction of the population can be calculated from the mean plant growth rate for the population and the variability around this value.

The silking model parameters include: (i) the parameters that relate ear growth rate with plant growth rate using an hyperbolic function (Vega et al. 2001): the minimum plant growth rate that gives a positive ear growth rate (PGRb), the initial slope, and an attenuation coefficient; (ii) the minimum ear biomass at which individual ears reach silking; and (iii) two parameters describing an exponential growth pattern to calculate accumulated ear biomass. When all these parameters are considered on a unified framework, it is possible to simulate silking dynamics for a population or populations of plants. Figure 4 shows silking dynamics for individual plant growth rates within a population of plants (Figures 4A and B), and silking dynamics for three populations of plants differing in the mean plant growth around flowering (Figures 4C and D).

When predicting the silking dynamics of a maize population, the mean plant growth rate and the distribution of growth rates within the population have to be measured or estimated. These variables are specific to the genotype and its response to the environment (Glenn and Daynard 1974; Vega and Sadras 2003).

The mechanistic framework for predicting silking patterns was used for testing changes in specific parameters. Genotypic differences in silking patterns and yield performance under source-limited conditions around flowering have been well documented (Moss and Stinson 1961; Buren et al. 1974; Soriano and Ginzo 1975; Bruce et al. 2002). The physiological mechanism(s) underlying these differences in stress tolerance, however, remain obscure. At present, genotypic differences in rapid silking seem to be related more closely to differences in biomass partitioning than in plant biomass production around flowering (Edmeades et al. 1993; Chapman and Edmeades 1999; Monneveux et al. 2005). As such, we modelled the silking dynamics of two genotypes differing in the base plant growth rate at which higher plant growth rates give positive ear growth rates (1 vs. 2 g pl^{-1} day^{-1}) in two environments with contrasting mean plant growth rates (2 vs. 6 g pl^{-1} day^{-1}).

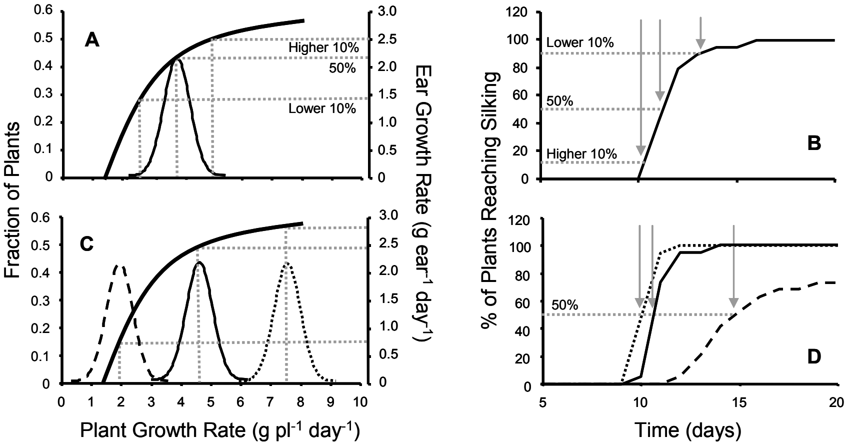

Figure 4. Schematic diagram relating silking dynamics to individual plant growth rates within a single population of plants (**A** and **B**)*, and silking dynamics for three populations of plants differing in the mean plant growth around flowering* (**C** and **D**). *Note that differences in plant growth rate among population fractions* (**A**) *or among population means* (**C**) *are not linearly related to differences in ear growth rate (bold line), and this has an impact on the silking pattern. In* (**B**) *arrows indicate time to silking for different fractions of plants within the same population (10, 50 and 90 %). In* (**D**) *arrows indicate time to silking for 50% of the plants from three plant populations*

Under favourable growing conditions, silking dynamics for the two genotypes were nearly identical (Figure 5). This would be expected because all plants of both genotypes had plant growth rates well above the minimum to support ear growth. Under less favourable growing conditions, however, silking dynamics were very different for these two genotypes. The one with a higher minimum plant growth rate to support ear growth showed a greater delay in silking and some plants failed to silk. This difference in genotype response reflected a higher proportion of plants with slow or zero ear growth. As such, this genotype was much more sensitive to reductions in plant growth around flowering than the other. The differential response of these two hybrids to a similar reduction in plant growth rate arose directly from the natural variation in plant growth within the population and the inherent genetic variation in partitioning to the ears.

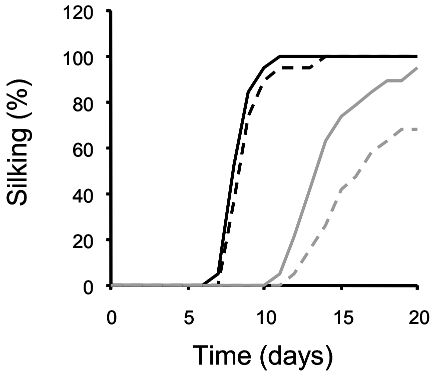

Figure 5. *Silking dynamics for two genotypes differing in the minimum plant growth rate to support ear growth (PGRb) and at two mean plant growth rates. Solid lines: PGRb =1 g* pl^{-1} day^{-1}*, dashed lines: PGRb = 2 g* pl^{-1} day^{-1}*. Black lines: mean plant growth rate = 6 g* pl^{-1} day^{-1}*, grey lines: mean plant growth rate = 2 g* pl^{-1} day^{-1}*. The CV of the populations was set at 30% for both genotypes and environments*

CONCLUSIONS

Considering maize-flowering dynamics as a quantitative trait at the population level and as a qualitative trait at the plant level enabled us to identify and integrate key genotypic coefficients needed to quantify silking behaviour. These factors are: (i) the relationship of plant growth rate and ear growth rate; (ii) the pattern of ear biomass accumulation during early growing stages; and (iii) the amount of accumulated biomass an ear needs to accumulate to reach the silking stage. We showed the value of using a population-based approach by taking into account the plant-to-plant variability to understand time to silking in maize crops. Methodologies, such as allometric models (Vega et al. 2000), are currently available to describe these plant-to-plant differences. For the first time, a framework to predict the silking dynamics of a maize population is presented that can explain environmental and genotypic differences affecting plant growth.

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CHAPTER 10

MODELLING THE GENETIC BASIS OF RESPONSE CURVES UNDERLYING GENOTYPE × ENVIRONMENT INTERACTION

F.A. VAN EEUWIJK, M. MALOSETTI AND M.P. BOER

Laboratory of Plant Breeding, Department of Plant Sciences, Wageningen University, P.O. Box 386, 6700 AJ Wageningen, The Netherlands
Biometris, Wageningen University, P.O. Box 100, 6700 AC Wageningen, The Netherlands
E-mail: fred.vaneeuwijk@wur.nl

Abstract. To increase tolerance to abiotic stresses in breeding programmes, typically families and collections of genotypes are evaluated in series of trials (environments) representing different levels of stress. The statistical analysis of the data from such trials concentrates on modelling the phenotypic behaviour of the genotypes across the set of environments. This phenotypic behaviour can be modelled in the form of genotype-specific linear and non-linear response curves in relation to environmental characterizations. Non-parallelism of the response curves indicates genotype × environment interaction. Identification of the genetic basis of the parameters determining the response curves will help in the development of breeding programmes for improving abiotic stress tolerance and understanding genotype × environment interaction. In this paper we present two strategies for locating quantitative trait loci for response-curve parameters and estimation of their allele effects. The procedures are illustrated by an application to drought stress in maize.

INTRODUCTION

Strategies for improving tolerance to abiotic stress in plant breeding almost invariably test collections of genotypes, whether segregating or not, across a series of trials chosen to represent as well as possible an environmental gradient relevant to the stress of interest. In such experiments, the genotypes will show differential performance across the stress gradient, as tolerant genotypes will do relatively better under stress conditions, whereas this advantage will disappear in the absence of stress. The differential performance of tolerant versus non-tolerant genotypes in relation to the severity of stress produces genotype × environment interaction (GEI),

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i.e., the phenomenon that differences between genotypes are environment-dependent. An adequate analysis of GEI is a prerequisite for success in breeding programmes for abiotic-stress tolerance. In plant breeding, GEI is mostly modelled within the context of analysis of variance models with emphasis on test procedures for detecting GEI. When GEI is found significant, the consequence is that genotypes that perform well under non-stress conditions, cannot automatically be recommended for stress environments. Of course, after the establishment of the existence of GEI, one may be interested to identify the genetic and environmental causes for the observed GEI. Various classes of statistical models have been developed that describe GEI in terms of differential genotypic sensitivities to environmental variables, where the models differ with respect to the character of the explanatory variables that are included (for reviews, see Van Eeuwijk et al. 1996; Van Eeuwijk 2006).

In plant breeding there is a tendency to describe GEI in terms of differences in linear responses to environmental variables, probably because restricted environmental gradients allow linear functions to give acceptable fits. In contrast, physiology-based approaches to modelling plant responses in relation to abiotic-stress gradients typically come in the form of non-linear functions. Whatever the chosen specification for the functional relationship between plant phenotype and stress intensity, an attractive option for studying the genetic basis of stress tolerance and GEI is the mapping of quantitative trait loci (QTLs) for the parameters underlying genotype-specific response curves. By treating estimated curve parameters as standard phenotypic traits, QTLs can be identified for the curve parameters and for the genetic basis of GEI. However, although this approach to the identification of the genetic basis of stress tolerance and GEI is straightforward and requires only standard QTL-mapping software, it has some drawbacks. These drawbacks include neglect of estimation error and correlations between parameters, which can lead to faulty inference on QTLs. A solution is given by an integrated modelling approach to GEI and QTL mapping.

In this paper, we present strategies to identify the genetic basis of GEI and response curves. We first give a brief general repository of QTL-mapping methods. Next, we look at a two-step QTL-mapping approach, in which in the first step the parameters are estimated, and in the second step, these estimates are treated as standard phenotypic traits. The following section describes an integrated modelling framework for GEI and QTL mapping. Finally, some of the methods are illustrated by an example on drought stress in maize.

QTL MAPPING FOR SIMPLE TRAITS BY REGRESSION AND MIXED MODELS

In the regression interpretation of QTL mapping, QTLs can be found by regressing the phenotypic response for genotype i, $\underline{P}_i$, on a quantitative or categorical variable, x_i, where x_i represents a function of QTL genotype probabilities (Haley and Knott 1992; Lynch and Walsh 1998). We will use the convention to underline random variables. As the QTL genotypes cannot be observed, but the marker genotypes can, QTL genotype probabilities are estimated from observed marker genotypes. To give an example, consider a population of doubled haploids. In such a population, at each locus there are only two possible, homozygous genotypes, say A and B, corresponding to the genotypes of the first and second parent, respectively. For a particular marker locus, x_i then takes the value 1 whenever the marker genotype is equal to A and –1 whenever B is the case. We can calculate for each marker a corresponding regressor, or better, genetic predictor, x_i, and then correlate this predictor with the phenotypic response $\underline{P}_i$. Everywhere where a test statistic, like the F-statistic, for the regression of $\underline{P}_i$ on x_i is significant according to some pre-set criterion, we can conclude that a QTL must be close, with the best estimate for the position depending on the maximum of the test statistic over a certain genome region.

We will elaborate these ideas more formally. We start by defining a model for the r-th phenotypic observation on genotype i, again underlining random variables, as

$$\underline{P}_{ir} = \mu + G_i + \underline{\varepsilon}_{ir} \qquad (1)$$

with $\underline{P}_{ir}$ the phenotypic observation, μ the general mean, G_i the underlying genotypic contribution as deviation from the mean, and $\underline{\varepsilon}_{ir}$ a random error. For the random variables, we will assume that they are normally distributed. Fitting a QTL model to the response $\underline{P}_{ir}$, merely means partitioning the genotypic effect, G_i, in a part due to regression on x_i, and a random residual $\underline{G}_i^*$:

$$\underline{P}_{ir} = \mu + x_i \alpha + \underline{G}_i^* + \underline{\varepsilon}_{ir} \qquad (2)$$

The parameter α represents a QTL effect for a putative QTL locus at the position corresponding to the genetic predictor x_i. Model (2) is a mixed model because it contains two random terms besides the fixed general mean and the QTL effect, α. Most general-purpose statistical packages, like SAS, SPSS, Genstat, S-Plus and R, have facilities to fit mixed models like Model (2) and other mixed models that will be mentioned below. A general test for the significance of fixed parameters, like α, in a mixed model, like (2), cannot be a standard F-test. An appropriate test for such a parameter in a mixed-model framework is the Wald test (Verbeke and Molenberghs 2000), which is produced by the packages just mentioned.

The use of Model (2) for a genome scan with genetic predictors calculated exclusively at marker positions is called marker regression, while Model (2) with genetic predictors calculated at and in between marker positions is called simple interval mapping (Lynch and Walsh 1998). To change Model (2) into a multiple QTL model with n_Q QTLs, we can write

$$\underline{P}_{ir} = \mu + \sum_{q=1}^{n_Q} x_{iq} \alpha_q + \underline{G}_i^* + \underline{\varepsilon}_{ir}. \qquad (3)$$

Model (3) shows that the building of a multiple QTL model can be interpreted as a subset-selection problem (Broman and Speed 2002), i.e., we want to find the set of genetic predictors that best explains the phenotypic response in terms of QTLs. As a strategy to identify such a subset, composite interval mapping has been developed. Analogously to the situation for simple interval mapping, in composite interval mapping, genome scans are performed by evaluating the effect of individual genetic predictors on a one-dimensional grid along the genome. However, to improve power, the effects of possible QTLs elsewhere on the genome are neutralized as much as possible by a set of so-called co-factors, a set of markers close to putative QTLs identified earlier, for example, in a simple interval-mapping genome scan (Lynch and Walsh 1998). A mixed model that can be used for composite interval mapping is

$$\underline{P}_{ir} = \mu + \sum\nolimits_{c \in C} x_{ic}\alpha_c + x_{iq}\alpha_q + \underline{G}_i^* + \underline{\varepsilon}_{ir}, \tag{4}$$

with C representing the set of co-factors appropriate for use in combination with x_q, the genetic predictor being tested for possible association with a QTL. The set of co-factors varies in relation to the genome position as individual genetic predictors, x_c, are dropped from the co-factor set when their position comes too close to that of x_q.

QTL MAPPING OF EARLIER ESTIMATED CURVE PARAMETERS

In the previous section, we presented mixed-model methodology for QTL mapping of simple phenotypic traits. For identifying the genetic basis of response curves, we can apply the above methodology without modifications when we are willing to interpret estimated curve parameters as if they were simple phenotypic traits. The consequence of the latter assumption is that we need to ignore the precision of the estimated parameters in subsequent analyses and this can lead to incorrect conclusions on QTL existence, location and effects, where the degree of inaccuracy will increase with the imprecision of the estimates. A very simple approach to QTL mapping of response curves, thus, consists of two steps. First, estimate curve parameters for individual genotypes by means of linear or non-linear regression. Second, treat the curve parameters of the first step as a phenotypic trait in its own right, and apply a QTL-mapping approach to this ‘trait’.

More important than the statistical-technical difference between linear and non-linear regression models is the type of environmental variables that is used in the set of explanatory environmental variables. We can distinguish two types of environmental variables or characterizations: those that refer to explicitly measured physical and biological variables, like temperature, rainfall and disease pressure, and those that are implicit in the sense that they are calculated from the phenotypic responses in the environment. For example, the mean response of all genotypes in an environment can serve as an implicit, integrated indicator of environmental quality.

Examples of implicit environmental descriptions occur in some well-known statistical models for describing GEI. A popular class of models for GEI describes

GEI by differential genotypic sensitivity to environmental characterizations that are themselves linear functions of observed phenotypes. In the regression on the mean model, probably better known as the Finlay-Wilkinson model (Finlay and Wilkinson 1963), the characterization for a particular environment, j, is just the average phenotypic performance across all genotypes minus the grand mean, denoted by E_j. The model for the phenotypic mean of genotype i in environment j, $\underline{\overline{P}}_{ij}$, reads

$$\underline{\overline{P}}_{ij} = \mu + G_i + \beta_i E_j + \underline{\delta}_{ij} \tag{5}$$

with μ as the general mean, G_i the average performance of genotype i across all environments given as a deviation from the general mean, a measure for wide adaptation, β_i the sensitivity of genotype i to the environmental characterization E_j, with $\overline{\beta_i}=1$, and $\underline{\delta}_{ij}$ the error attached to the mean for genotype i in environment j. The parameters G_i and β_i can first be estimated by linear regression and the estimates $\hat{G}_i$ and $\hat{\beta}_i$ can subsequently be introduced as ordinary phenotypic variables in a QTL-mapping procedure.

Model (5) can also be written in a form that better emphasizes the connection of the regression on the mean model with models for GEI by replacing the slopes β_i, which are on average 1, by the slopes b_i, which are on average zero,

$$\underline{\overline{P}}_{ij} = \mu + G_i + E_j + b_i E_j + \underline{\delta}_{ij} \tag{6}$$

The regression on the mean model, (5)/(6), has rather restricted versatility for modelling differences in phenotypic responses between genotypes across environments. A more flexible model, following the same philosophy, characterizing the environment on the basis of the phenotypic trait itself, is the 'additive main effects and multiplicative interactions' model (Gollob 1968; Mandel 1969; Gabriel 1978; Gauch Jr 1988):

$$\underline{\overline{P}}_{ij} = \mu + G_i + E_j + \sum_{k=1}^{K} a_{ki} b_{kj} + \underline{\delta}_{ij} \tag{7}$$

with hypothetical environmental characterizations b_{kj} that create maximum discrimination for the corresponding genotypic sensitivities, a_{ki}. The number of multiplicative terms necessary for an adequate description of the data is K. Various test procedures have been developed to assess K (Gollob 1968; Cornelius et al. 1996). Estimates for the genotypic sensitivities, $\hat{a}_{ki}$, and environmental characterizations, $\hat{b}_{kj}$, can be obtained by least-squares estimation procedures. Estimated genotypic sensitivities according to Model (7) can be mapped by ordinary QTL-mapping procedures.

The response Models (5) and (7) are attractive for plant breeders, because they do not require an explicit environmental characterization. However, when physical or biological descriptions of the environment are available, physiologically more attractive models would include explicit references to such descriptions. Factorial regression models are linear models with multiplicative terms for GEI that can model differential genotypic responses to one or more physical or biological environmental characterizations (Denis 1988; Van Eeuwijk et al. 1996). An example with one environmental variable, z_j, has the form

$$\overline{\underline{P}}_{ij} = \mu + G_i + E_j + b_i z_j + \underline{\delta}_{ij} \tag{8}$$

where z_j could be a function of temperature during a critical growth stage, radiation, nitrogen, water, etc., and b_i then is the corresponding genotypic sensitivity. Equivalently, z_j, can be a stress index obtained from a crop growth simulation. Model (8) can also model non-linear responses, for example by including polynomial terms,

$$\overline{\underline{P}}_{ij} = \mu + G_i + E_j + b_{1i} z_j + b_{2i} z_j^2 + \underline{\delta}_{ij} \tag{9}$$

where b_{1i} and b_{2i} represent the sensitivity of genotype i to the linear and quadratic term in the environmental variable z. Even the inclusion of response surfaces in various dimensions does not present statistical-technical problems, although the number of environments necessary for sufficiently precise estimation of the increasing number of regression parameters will not often be reached in plant-breeding programmes.

When good explicit environmental characterizations are available, it is often preferable to model the genotypic responses by parametric linear and non-linear regression functions based on physiological insights, control equations (Reymond et al. 2003; Tardieu 2003; Tardieu et al. 2005) or meta-mechanisms (Hammer et al. 2005), instead of working with polynomial approximations to these non-linear functions. A general expression for non-linear genotypic responses in one dimension is

$$\overline{\underline{P}}_{ij} = f(\theta_i, z_j) + \underline{\delta}_{ij} \tag{10}$$

with f representing a non-linear function in the parameter vector θ_i for genotype i and z_j, as before, the value for the environmental variable z in environment j. Model (10) is equally applicable in temporal contexts, with z related to time, as in spatial contexts, where z typically is related to soil and management conditions. When z is time related, the error term $\underline{\delta}_{ij}$ demands careful modelling of possible auto-correlations between observations at short intervals. Two illustrative examples of physiological modelling of response curves followed by QTL mapping of the estimated curve parameters are Reymond et al. (2003) for linear parameters and Yin et al. (2005) for non-linear parameters.

For all models discussed in this section, genotype-specific parameters can first be estimated and then subjected to a standard QTL analysis. This practice will work reasonably well as long as the hypothesized curves fit the observed data well across the full set of genotypes and the standard errors for the parameter estimates are relatively small in comparison to the parameter estimates themselves. Still, a better approach is to model the curve parameters directly as functions of underlying QTLs. The next section describes how to do this.

MODELLING GENOTYPIC RESPONSES AS FUNCTIONS OF QTLS

An integrated approach to the problem of mapping the genetic basis of response curves departs from the development of a multi-environment model for genotypic responses observed across a series of environments. A QTL model for the mean of genotype i in environment j with the possibility of the QTL effect being environment-specific is

$$\underline{P}_{ij} = \mu + E_j + x_i \alpha_j + \underline{\delta}_{ij} \tag{11}$$

with α_j standing for the environment-specific QTL effect in environment j. The generalization of Model (11) to a multi-QTL model would look like

$$\underline{P}_{ij} = \mu + E_j + \sum\nolimits_{q=1}^{n_Q} x_{iq} \alpha_{jq} + \underline{\delta}_{ij} \tag{12}$$

In Model (12) not necessarily each QTL needs to exhibit environment-specific expression. For some QTLs, the expression across environments may be more or less constant, so that a single, main QTL effect would suffice. A more correct QTL model for a multi-environment trial is then

$$\underline{P}_{ij} = \mu + E_j + \sum\nolimits_{q^*=1}^{n_{Q^*}} x_{iq^*} \alpha_{q^*} + \sum\nolimits_{q=1}^{n_Q} x_{iq} \alpha_{jq} + \underline{\delta}_{ij} \tag{13}$$

with the first set of QTLs, Q^*, just having constant expression across environments and the second set, Q, having environment-specific expression. The variance–covariance matrix for the residuals $\underline{\delta}_{ij}$ should be flexible enough to allow for heterogeneity of variance across environments and heterogeneity of correlations between environments due to genetic effects not modelled by the QTL part of the model (Piepho 2000; Verbyla et al. 2003; Malosetti et al. 2004; Piepho and Pillen 2004).

A QTL model in which QTL expression is modelled in direct dependence on environmental variables can be obtained from Model (11) by regressing the QTL effects, α_j, on an environmental variable z_j, $\underline{\alpha}_j = a_0 + a_1 z_j + \underline{\alpha}^*_j$:

$$\underline{P}_{ij} = \mu + E_j + x_i a_0 + x_i a_1 z_j + x_i \underline{\alpha}^*_j + \underline{\delta}_{ij} \tag{14}$$

where a_0 represents the QTL main-effect expression, the part that is constant across environments, while a_1 is a proportionality constant that shows how much the phenotype will change per unit change in the environmental variable z, this phenotypic change being conditioned on the QTL genotype information contained in x_i. The part of the QTL effect α_j that is not described by the regression on z determines a random residual QTL effect, $\underline{\alpha}_j^*$. Model (14) can be extended in obvious ways by the incorporation of further polynomial terms in z, and by incorporating different environmental variables for different QTLs.

For non-linear response curves as described in Model (10), a QTL model can be constructed by modelling each of the genotypic parameters in the parameter vector θ in terms of underlying linear (multi-)QTL models. The QTL model for the k-th genotypic parameter is then

$$\underline{\theta}_i^k = \mu^k + \sum_{q=1}^{n_{Q^k}} x_{iq} \alpha_q^k + \underline{\varepsilon}_i^k , \tag{15}$$

with the superscript k referring to the parameter within the vector θ, μ^k an intercept term, α_q^k the effect of the q-th QTL for the parameter θ^k, $q = 1...n_Q$, Q^k the set of QTLs underlying θ^k, and $\underline{\varepsilon}_i^k$ a residual term. The set of QTLs underlying a particular genotypic parameter can differ between parameters. Substituting Model (15) for each of the parameters θ^k in Model (10) will convert the latter phenotypic model into a QTL model for non-linear responses.

All QTL models treated so far, except the model for the non-linear responses, are linear mixed models, and parameter estimation and testing follow standard theory for this type of models (Verbeke and Molenberghs 2000). The QTL model for the non-linear responses is a non-linear mixed model (Davidian and Giltinan 2003; Malosetti et al. 2006). Estimation and testing for this class of models is more complex and requires special procedures. Such procedures are present in SAS and S-Plus/R.

None of the QTL models discussed so far is critically dependent on the use of segregating populations of offspring from crosses between inbred parents. The models are equally applicable to the analysis of arbitrary collections of varieties as in marker–trait association analyses. The difference between the former linkage and the latter linkage disequilibrium analyses resides mainly in the incorporation of extra random terms to correct for the varying level of genetic relatedness that characterizes arbitrary collections of varieties (Malosetti 2006; Yu et al. 2006).

EXAMPLE

To illustrate some of the concepts above, we reanalysed data from the CIMMYT maize-breeding programme on drought tolerance, consisting of yield evaluations for 211 F2-derived F3 families across eight trials with varying levels of water and nitrogen stress. Detailed descriptions and more analyses of these data can be found in Malosetti et al. (in press), Van Eeuwijk et al. (2001; 2002) and Vargas et al. (2006). In this chapter, we will present some results related to chromosome 1.

To model GEI in the maize data, we fitted a regression on the mean Model (5), an AMMI model (7) with two terms for interaction, and a number of factorial regression models (8) trying out a series of environmental variables, among which the minimum temperature during flowering and an environmental contrast between trial performance at the location of Poza Rica versus that at Tlaltizapán. The latter contrast is an example of a qualitative environmental variable. Figure 1 shows LOD profiles of simple interval-mapping scans with MapQTL (Van Ooijen 2004) for a selection of five parameters estimated in the GEI analyses. Figure 1 shows that there is some indication for a QTL main effect (intercept) in the region of 140-180 cM and no proof for QTLs related to the slopes in the regression on the mean model. For the AMMI-2 scores, the slopes for minimum temperature during flowering, and the genotypic contrasts for performance at Poza Rica versus Tlaltizapán, the LOD profiles look very similar, indicating a significant QTL for those parameters at 130-150 cM. All three parameters represent differential genotypic sensitivity to the same environmental contrast. The minimum temperature at flowering was higher at Tlaltizapán than at Poza Rica. The same environmental contrast between those two locations determined the second AMMI axis.

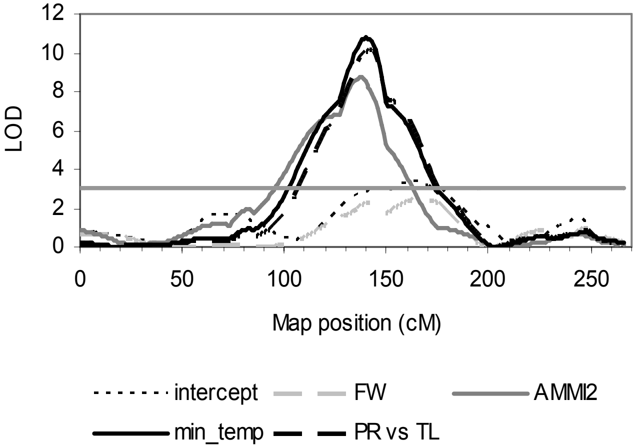

Figure 1. *LOD profiles for simple interval scans for chromosome 1 on different parameters characterizing phenotypic responses across environments for a set of F2-derived F3 lines in the CIMMYT drought-stress programme for maize. Intercept = genotypic mean across environments, FW = genotypic slope from the regression on the mean model, AMMI2 = genotypic score for the second multiplicative term in an AMMI model, min-temp = genotypic slope from factorial regression with minimum temperature during flowering, PR vs TL = genotypic contrasts for the average difference in performance between the locations Poza Rica and Tlaltizapán. Threshold for significance was chosen at an LOD score of 3*

We subsequently modelled the whole of the genotype × environment data by a mixed model with environment-specific QTLs, Model (11) and detected again a QTL in the region from 130 to 150 cM. The next step in a mixed-model QTL analysis of multi-environment data then consists of searching for environmental variables that can explain the differential QTL expression across environments. Figure 2 shows the regression of the QTL effects on the minimum temperature during flowering. With increasing temperature, the QTL allele coming from the high-yielding, drought-susceptible parent gives less advantage. At minimum temperatures around 10 °C, the temperature at Tlaltizapán, the QTL allele of the drought-susceptible, but high-yielding parent, still confers a yield increase of around 0.6 tons per hectare in comparison to the QTL allele from the drought-tolerant parent. At temperatures around 20 °C, the temperature at Poza Rica, the yield advantage of the QTL allele from the high-yielding parent has disappeared.

In the Sections *QTL mapping of earlier estimated curve parameters* and *Modelling genotype responses as functions of QTLs,* we discussed the step-wise QTL analysis on estimated parameters and the mixed-model QTL analysis of multi-environment data. Both types of QTL analyses for response curves produced similar conclusions for the example data. However, in general the multi-environment mixed-model QTL analysis would have our preference because of its more appropriate representation of variances and correlations and its more transparent statistical properties.

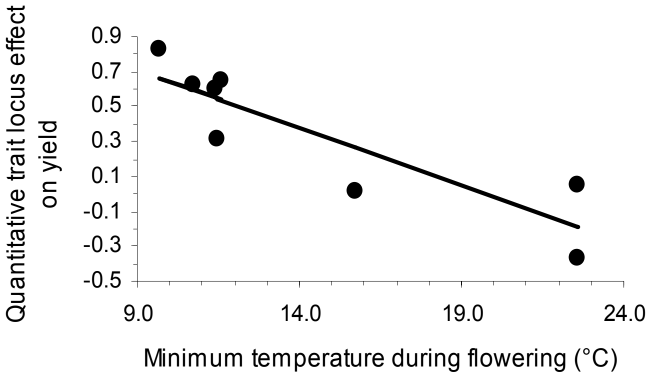

***Figure 2**. QTL effect at chromosome 1 as function of minimum temperature during flowering time*

ACKNOWLEDGEMENTS

This chapter was written as part of competitive project number 4 within the Generation Challenge Program: “An eco-physiological – statistical framework for the analysis of G×E and QTL×E as occurring in abiotic stress trials, with applications to the CIMMYT drought stress programs in tropical maize and bread wheat”. We thank Jean-Marcel Ribaut (GCP and CIMMYT) for permission to use the data in the example, and José Crossa and Mateo Vargas (CIMMYT) for preparation of the data and discussion.

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PHYSIOLOGY
AND GENETICS
OF CROP ADAPTATION

CHAPTER 11

PHYSIOLOGICAL INTERVENTIONS IN BREEDING FOR ADAPTATION TO ABIOTIC STRESS

M.P. REYNOLDS[#] AND R.M. TRETHOWAN[##]

[#] *CIMMYT, Int. Apdo. Postal 6-641, 06600 Mexico DF, Mexico.*
[##] *University of Sydney, Plant Breeding Institute, PMB 11, Camden, NSW, Australia.*
E-mail: m.reynolds@cgiar.org

Abstract. The physiological-trait-based breeding approach has merit over breeding for yield *per se* because it increases the probability of crosses resulting in additive gene action. While considerable investment in germplasm characterization is required, conceptual models of crop genotypes can be employed as research tools to quantify likely genetic gains associated with specific trait or in defining traits that may have generic value across different stresses. For example, deeper root growth that permits better access to soil water has obvious benefit under drought, while under hot, irrigated conditions permits leaf canopies to match the high evaporative demand associated with hot, low-relative-humidity environments, resulting in higher leaf gas-exchange rates and heat escape through evaporative cooling. Although improvement in adaptation to abiotic stress may occur as a result of transgressive segregation, exotic parents can be used to increase total allelic diversity for such traits. The bread-wheat-breeding programme at CIMMYT is exploiting new genetic diversity using inter-specific hybridization of the ancestral genomes of bread wheat. Novel genetic diversity is also being accessed more directly by crossing adapted germplasm with landrace accessions originating in abiotically stressed environments that have become isolated from mainstream gene pools. Through studying these genetic resources it has been possible to calculate the theoretical impact of combining their best values of trait expression into the check cultivar to gain some insight into which traits may hold most promise in terms of genetic enhancement. It was apparent that the genetic diversity found for water use efficiency offers the greatest and most consistent opportunity for increasing yield, while increasing stem carbohydrates and access to water at depth also shows some potential. Direct physiological interventions in breeding include (i) characterization of potential parents for more strategic crossing; (ii) early-generation selection; and (iii) evaluation of promising genetic resources in pre-breeding. The early-generation selection trait 'canopy temperature' (measured with an infrared thermometer) has been readily adopted since measurement is quick, easy and inexpensive. Although genetic markers are not currently used in selection for complex traits, as technology advances and combines with gene discovery approaches, more quantitative trait loci (QTLs) associated with adaptation to complex environments will emerge. A multi-staged approach to identifying molecular markers may be the best approach where QTLs for generic traits – i.e., valid across a range of environments – are identified in well controlled field environments and used to optimize germplasm. Subsequently, environment-specific models would be used to factor in additional traits commonly found in a specific region that may not be directly related to moisture stress, factors such as nematodes or microelement deficiency or toxicity that are exacerbated under drought.

J.H.J. Spiertz, P.C. Struik and H.H. van Laar (eds.), Scale and Complexity in Plant Systems Research: Gene-Plant-Crop Relations, 129–146.

INTRODUCTION

Since breeding for adaptation to abiotic stress at a global scale encompasses a range of target environments, an array of adaptive mechanisms must be considered for deployment accordingly. Research into the different physiological adaptations of crops to abiotic stress has been ongoing for decades (e.g., Fischer and Turner 1978; Blum 1988; Boyer 1996; Bruce et al. 2002), and molecular technologies have added a new dimension to the research (Xiong et al. 2002; Chaves et al. 2003; Wang et al. 2003). While a limited amount of research has been applied to crop improvement (Bolaños and Edmeades 1996; Condon et al. 2004; Rebetzke et al. 2002; Richards et al. 2002; Trethowan and Reynolds 2006), much has yet to be applied (Araus et al. 2002). Nonetheless, the physiological-trait-based breeding approach has merit over breeding for yield *per se* because it increases the probability of crosses resulting in additive gene action, although considerable investment in germplasm characterization is prerequisite. This paper discusses some of the ways that physiological intervention can assist in the breeding for abiotically stressed environments.

The use of conceptual models of genotypes is discussed. For example, candidate traits for increasing yield under abiotic stress can be grouped together such that physiological effects among groups are likely to be relatively independent genetically. Grouping traits in this way, while based on an incomplete knowledge of drought adaptation and its genetic basis, does help to establish a broad conceptual framework that can be used not only as a decision support tool in designing crosses between complementary parents but also in more strategic research.

The exploitation of new allelic variation related to abiotic-stress adaptation is discussed using examples from the International Maize and Wheat Improvement Center's (CIMMYT) wheat-breeding programme. The wild D-genome has been exploited for some time for disease resistance genes (Villareal et al. 1995). More recently, wide crossing has been applied to increase yield under drought (Trethowan et al. 2003; 2005) and there is already evidence for impact in drier regions world-wide based on data from recent international drought trials (Trethowan and Reynolds 2006). Collections of genetic resources including landraces originating in abiotically stressed regions are another important source of novel alleles that are relatively easy to work with from a breeding perspective.

The process of incorporating new genetic diversity can be accelerated with suitable early-generation selection tools that enable the best progeny to be identified before costly yield trials are run. For example, canopy temperature (CT) is now used in drought breeding at CIMMYT to select segregating populations with better access to water by roots (Trethowan and Reynolds 2006). Given the current lack of genetic markers for stress adaptation, physiological traits can be viewed as proxy genetic markers at least among locations where the level of genotype × trait interaction is relatively low. As our knowledge of the physiological and genetic basis of stress adaptation increases, it will become more biologically and economically feasible to apply molecular-marker-assisted selection for targeted breeding objectives. The main challenge to their application in breeding will be to determine the right combination of alleles to use, since conditions vary significantly from site to site and from year to year and significant quantitative trait locus (QTL) × environment interactions exist. A multi-staged approach to identifying molecular markers is discussed.

PHYSIOLOGICAL APPROACHES TO BREEDING

To achieve the maximum impact from using specific traits in breeding strategies, a detailed knowledge of target environments is prerequisite, especially in terms of building a developmental component into genotype models. Some of the most important factors to consider when defining abiotically stressed environments are outlined below. The potential benefits and examples of physiological breeding approaches are also discussed.

Abiotic-stress environments

Drought and temperature are the two most important abiotic stress factors influenced directly by climate; the former is discussed in more detail elsewhere (Reynolds et al. in press). One important characteristic of the drought environment includes the distinct types of water distribution profile in relation to evaporative demand, creating different combinations of pre- or post-anthesis stress and determining the relative value of drought-adaptive traits. Another is the presence of soil factors such as microelement deficiency or parasitic nematodes, whose effects on productivity are severely exacerbated under moisture deficit, confounding potential genetic gains associated with drought adaptation *per se*.

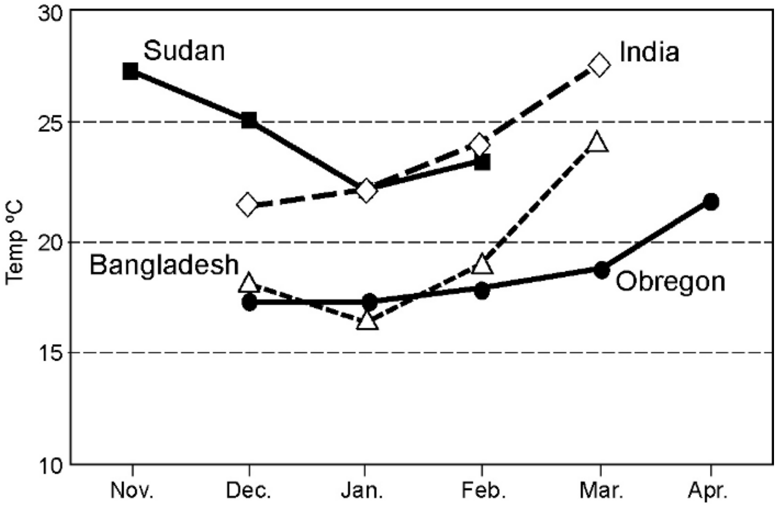

Figure 1. *Mean daily temperature for wheat crop cycle at four locations (Wad Medani, Sudan; Dharwad, India; Dinajpur, Bangladesh; Obregon, Mexico) of the International Heat Stress Genotype Experiment -IHSGE- (Reynolds et al. 1994)*

High temperature is detrimental to temperate cereals, defined (for wheat) as a mean average temperature of the coolest month higher than 17.5 °C (Fischer and Byerlee 1991). The heat-prone environment encompasses a range of temperature profiles when comparing different locations across a range of countries (Figure 1).

For breeding purposes CIMMYT defined two distinct sub-environments, one with high relative humidity and another with low relative humidity. This distinction is made primarily because of the fact that diseases are a severe problem in the humid environment, especially *Helminthosporium sativum*. Nonetheless, results of international yield trials (Reynolds et al. 1998) grown at over 40 environments (where disease incidence was controlled with fungicide application) confirmed that the main factor determining genotype × environment interaction (G×E) in hot climates was relative humidity (Figure 2). More recent and comprehensive analysis

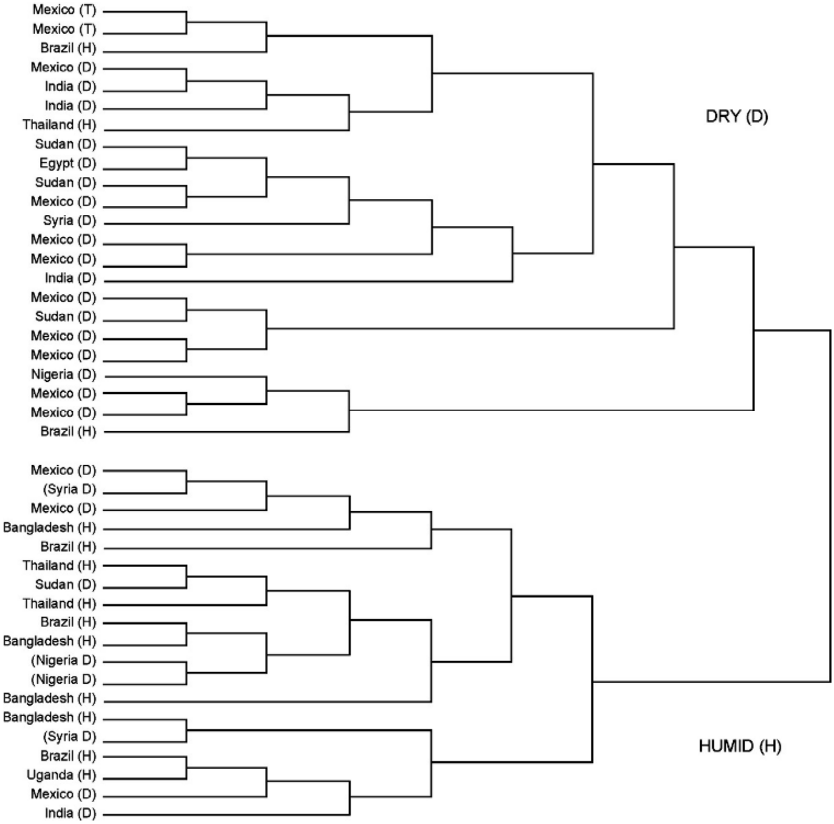

Figure 2. *Cluster analysis of hot wheat-growing environments internationally (Reynolds et al. 1998). Cluster analysis was based on cross-over interaction of genotypes as described by Vargas et al. (1998). The analysis indicates two main groups in which either hot, low-relative-humidity environments (upper group) or hot, high-relative-humidity (lower group) environments predominate. Temperate environments (T) are thus indicated; environments in parentheses indicate extreme low yield*

using CIMMYT international nursery yield data indicated that main genotype clusters correspond to three main types of environment, viz., temperate, continuous heat stress and terminal heat stress, and confirmed relative humidity as an important factor determining G×E within some of these clusters (Lillemo et al. 2005).

The other main abiotic stress factors associated with soils are discussed in detail elsewhere. For example, saline soils cover over 900 Mha of land area, and at least a third of the area under irrigation is affected by salinity (Ghassemi et al. 1995). While soil salinity is a global problem, it is especially so in arid or semi-arid climates where average rainfall is less than evapotranspiration. Other important soil stresses are associated with acid soils (see Hede et al. 2001) and micronutrient deficiency and toxicity (see Ascher-Ellis et al. (2001) for description of environments).

The merits of the physiological approaches to breeding

When breeding for defined target environments, whether broad or specific, the physiological approach has an advantage over empirical breeding for yield *per se* because it increases the probability of crosses resulting in additive gene action for stress adaptation. However, the corollary is that germplasm must be much more thoroughly characterized than for yield and agronomic type alone. Before they can even be characterized, individual traits must be conceptualized and defined in terms of (i) the stage of crop development at which they are pertinent; (ii) the specific attributes of the target environment for which they are adaptive; (iii) their potential contribution to yield over a range of crop cycles.

The physiological approach also has merit over the genetic approach for the very simple reason that there is a lack of in-depth understanding of the genetic basis of stress adaptation in crops in general. As a result, very few genes or gene markers exist that can be implemented in breeding for adaptation to abiotic stresses such as drought or heat (Snape 2004). On the other hand, physiological traits can be used to dissect stress adaptation into some of its components. Such physiological traits represent the closest approximation available to genetic markers, assuming they are applied to a restricted range of environments within which the traits show acceptable levels of heritability.

Application of a general conceptual model

A general model for drought adaptation of wheat was developed by physiologists and breeders at CIMMYT encompassing most of the traits for which evidence had been presented of a potential role in dry environments (Reynolds et al. 2000; 2005). The model is explained in detail elsewhere (Reynolds et al. 2005) and is summarized here. The many candidate traits for increasing yield under drought stress are grouped together such that physiological effects among groups are likely to be relatively discrete genetically. Grouping traits in this way, while based on an incomplete knowledge of drought adaptation and its genetic basis, helps to establish a broad conceptual framework. 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 rooting depth or intensity that would be expressed 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) 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) Photoprotection including energy dissipation (Niyogi 1999; Havaux and Tardy 1999), anti-oxidant systems (Mittler and Zilinskas 1994) and anatomical traits such as leaf wax (Richards et al. 2002).

The model is used to assist with breeding decisions permitting a strategic approach whereby drought-adaptive genes, for example, are more likely to be accumulated when parents with contrasting drought-adaptive mechanisms are crossed. In a subsequent section germplasm will be described that was generated using this approach. The conceptual platform can also be used as a decision support

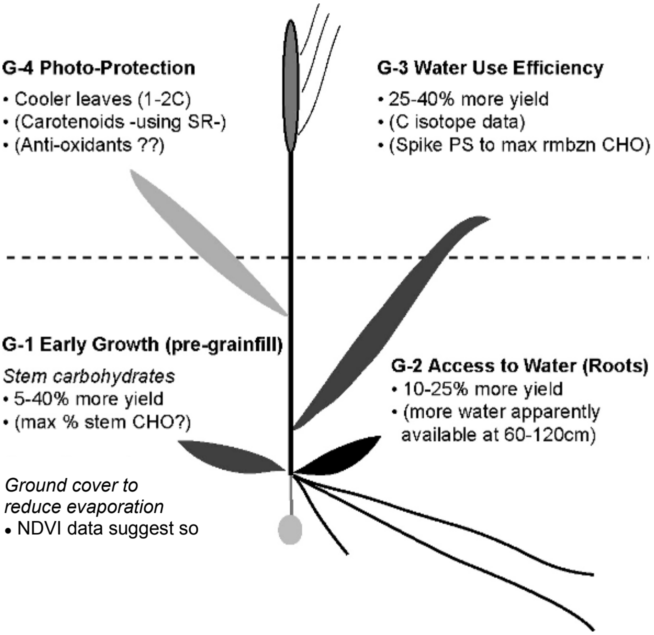

Figure 3. *Potential genetic gains associated with over-expression of drought-adaptive traits theoretically expressed in an elite check background, based on empirical data from controlled field studies involving 11 elite genetic-resource genotypes and the check cultivar. Potential genetic gains are expressed for the range of differences in trait expression (i.e. comparing the check with the best expression among all genotypes) across three drought cycles, NW Mexico (2002, 2004, 2005). Traits are grouped according to a conceptual model for drought-adaptive traits (Reynolds et al. 2005)*

tool for activities such as (i) defining suitably contrasting parents in development of molecular-mapping populations and subsequent gene discovery; (ii) quantifying the potential benefits of enhanced trait expression (Figure 3), and therefore indicating targets for exploration of genetic resources; and (iii) identifying common physiological bases between drought and other abiotic stresses such as high temperature.

For example, Figure 4 presents a generic conceptual model of a core-set of traits for adaptation to dry as well as hot, irrigated environments in wheat. It is clear when considering the groups of traits that a number of physiological mechanisms are likely to be of benefit in both situations. For example, rapid ground cover is a useful trait for avoiding the wasteful evaporation of soil water under pre-anthesis drought stress (Loss and Siddique 1994). The trait may also be of value under hot, irrigated conditions where rapid early ground cover could increase light capture and partially compensate for reduced tiller number associated with accelerated development rate at higher temperatures (Rawson 1986). Accumulation of stem carbohydrates and their subsequent remobilization in the post-anthesis period provide an extra source of assimilates for grain growth when either of these stress factors is experienced during the grain-filling stage (Blum 1998). Similarly, root growth that permits better

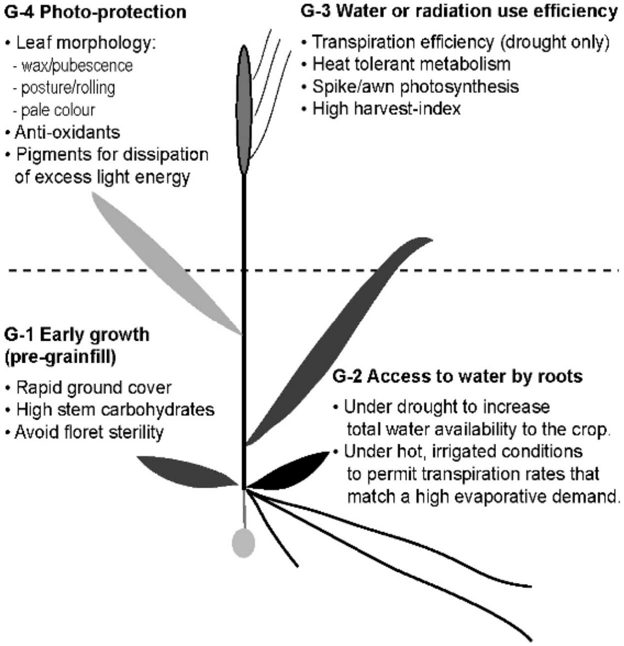

Figure 4. *Conceptual model for generic traits associated with adaptation to moisture-stressed and/or hot, irrigated environments (adapted from Reynolds et al. 2005; Reynolds and Borlaug 2006)*

access to soil water has obvious benefit under drought, while enabling heat-stressed canopies to match the high evaporative demand associated with hot, low-relative-humidity environments, resulting in higher leaf gas-exchange rates and heat escape through cooler canopies (Reynolds et al. 2000). There are also traits that will impact either WUE or radiation use efficiency (RUE) depending on the environmental conditions. For example, heat-sensitive metabolic processes such as starch synthesis (Keeling et al. 1994), photo-respiration and photo-inhibition (Osmond and Grace 1995) will impair performance when tissues reach supra-optimal temperatures, whether the cause is reduced evaporative cooling due to soil water deficit, the inability of the vascular system to match evaporative demand, or simply an ambient temperature or radiation load that precludes heat escape. Similarly, photo-protective mechanisms that either dissipate excess light energy (Niyogi 1999) or deactivate reactive oxygen species resulting from excess light (Mittler and Zilinskas 1994) are likely to be important under drought because there is insufficient water to permit full utilization of light energy, while under heat stress the metabolism may become impaired, leading to the same problem.

Using such an approach CIMMYT, in collaboration with CSIRO-Brisbane, has designed experiments using the Seri/Babax mapping population (Olivares-Villegas et al. in review; McIntyre et al. 2006) where QTLs for (i) stem carbohydrate accumulation and (ii) canopy temperature are being evaluated under both drought and hot, irrigated environments to establish whether there is a common genetic basis for the expression of either trait across environments.

INTRODUCING NEW ALLELIC VARIATION

While crosses among elite breeding lines may result in increased levels of trait expression due to transgressive segregation of alleles, exotic parents can be used to increase allelic diversity. The bread-wheat-breeding programme at CIMMYT is exploiting new genetic diversity using inter-specific hybridization of the ancestral genomes of bread wheat. Specifically tetraploid durum wheat is crossed with *Aegilops tauschii*, the ancestral donor of the D-genome to recreate hexaploid bread wheat (Mujeeb-Kazi et al. 1996). When elite wheat cultivars are crossed to these so called 'synthetic wheats', some of the progeny show considerable improvement in drought adaptation (Trethowan et al. 2005), though the physiological and genetic basis is not established. Novel allelic diversity can also be accessed more directly, for example, by crossing adapted germplasm with landrace accessions originating in abiotically stressed environments that have become isolated from mainstream gene pools (Reynolds et al. submitted). While landraces have been used for some time in breeding barley for adaptation to abiotic stresses (Ceccarelli et al. 2001), their use in bread-wheat breeding is less common.

Synthetic derived wheat

Although synthetic wheat possesses significant new variation for adaptation to moisture-limited environments (Trethowan et al. 2000; 2003; Villareal et al. 2003a;

2003b), the primary synthetics do not necessarily show better adaptation to drought compared with adapted modern cultivars. However, they sometimes carry complementary genes for drought adaptation that segregate transgressively in combination with modern materials. Table 1 shows yield advantages of up to 23% over the high-yielding adapted bread-wheat cultivar, Bacanora. This cultivar was crossed to drought-tolerant but low-yielding primary synthetic wheat and the resultant progeny were tested for yield under drought stress in northern Mexico. In association with their improved yield the synthetics maintain seed size under stress, an important quality characteristic in many cultures (Trethowan et al. 2003).

Table 1. *Mean yield (absolute and relative to that of Bacanora) of grain-derived lines based on crosses between Bacanora and primary synthetic wheat grown under drought stress in northwestern Mexico during 2000 (adapted from Trethowan et al. 2000)*

Pedigree	Yield (t ha^{-1})	Yield as % of Bacanora
Bacanora//Sora/*Ae. tauschii* (323) [b] CASS94600121S-1Y-2B-1PR-0B-0HTY	3. 838[a]	123
Bacanora//Sora/*Ae. tauschii* (323) CASS94Y00121S-1Y-2B-2PR-0B-0HTY	3.697[a]	118
Bacanora/Rabi//Gs/Cra/3/*Ae. tauschii* (895) CASS94Y00160S-40Y-7B-1PR-0B-0HTY	3.660[a]	117
Bacanora//Sora/*Ae. tauschii* (323) CASS94Y00121S-1Y-2B-3PR-0B-0HTY	3.536[a]	113

[a] Significantly different from Bacanora at $P < 0.05$.

[b] This line was evaluated in a separate trial with a different randomization.

Recent work compared two synthetic derived (SYN-DER) lines with their recurrent parents under moisture-stressed conditions for which a full description of methods and results can be found elsewhere (Reynolds et al. submitted). In summary, SYN-DER lines showed significant increases in yield (17 and 33%) and substantial increases in total biomass (45 and 66%) (Table 2). When considering water uptake characteristics, SYN-DER lines were more effective at removing water from the soil at all depth profiles, resulting in on average 11% increase in water use (Table 2). Despite this, SYN-DER lines showed a lower root:shoot ratio associated with less investment in root mass in the top 30 cm of the soil (Reynolds et al. submitted). Nonetheless, increased water extraction was not of sufficient magnitude to explain the increase in biomass of SYN-DER relative to recurrent parents. Using the SYN-DER value for WUE of 5.5 g m^{-2} mm^{-1}, an extra 26 mm of water would account for approximately 150 g m^{-2} of additional biomass. Mechanisms that may explain a larger WUE are increased transpiration efficiency associated with intrinsically low stomatal conductance and carbon-isotope discrimination (Condon et al. 2002; 2004; Rebetzke et al. 2002), and decreased losses of soil water early in crop establishment due to improved early ground cover (Richards et al. 2002). Although neither trait was estimated in this experiment, SYN-DER lines have been reported to display considerable early vigour and increased early ground cover

(Trethowan et al. 2005), which, along with higher values of above-ground biomass during tillering stage, suggests that this trait does play a role.

Table 2. *Growth and water-use parameters for two synthetic derived wheat lines and the recurrent parents under moisture stress (average two cycles) for two genetic backgrounds, NW Mexico, 2003-2005*

GENOTYPE	Grain yield ($g\ m^{-2}$)	Total biomass ($g\ m^{-2}$)	Root: shoot ratio	Water used (mm)	WUE (biomass) ($g\ m^{-2}\ mm^{-1}$)
CROC_1/AE.SQUARROSA (210)//2×EXCALIBUR	320	1125	0.09	252	4.46
EXCALIBUR (recurrent parent 1)	240	777	0.20	221	3.51
D67.2/P66.270//AE. SQUARROSA (320)/3/ CUNNINGHAM	350	1659	0.09	253	6.57
CUNNINGHAM (recurrent parent 2)	300	1000	0.14	233	4.29
Average effect	24%	57%	–46%	11%	41%
Least significant difference (LSD) ($P \leq 0.05$)	27	302.4	0.013	11.9	1.35

Quantifying the potential value of land races and other genetic resources

One of the benefits of interdisciplinary collaboration between physiology, breeding and genetic resources is the continuum provided by ready access to a vast range of genetic resources, the ability to evaluate germplasm in realistic field environments in comparison to up-to-date cultivars, and the opportunity to use and evaluate promising genetic resources in pre-breeding work. Through such collaboration, CIMMYT's wheat physiology programme has been assembling elite genetic resources and comparing them in controlled field environments with the best check cultivars in terms of expression for a number of stress-adaptive traits including early ground cover, the accumulation and remobilization of soluble stem carbohydrates, the ability to access water at different soil depths down to 120 cm, and apparent water use efficiency (calculated as the ratio of above-ground biomass and water used). Some of this work has already been reported and indicates that some of the best landraces collected in Mexico's driest regions have a significantly greater ability to extract water at depth compared to the elite checks, while other genetic resources including synthetic derived lines excel in WUE or the percentage of stem weight found as soluble carbohydrate at anthesis (Reynolds and Condon submitted;

Reynolds et al. submitted). A principal objective in assembling and comparing such materials was to calculate the theoretical impact of combining their best values of expression into the check cultivar to gain some insight into which traits may hold most promise in terms of genetic enhancement.

A rough quantitative assessment was made by identifying the highest expression of any trait amongst all genetic resources and comparing the value of its expression with that of the check cultivar, and estimating what the theoretical yield would be if the trait were expressed at the same level in the check cultivar. The calculations were performed for each of three years when water availability ranged from 175 to 300 mm; the range of potential benefits in terms of yield gain are presented in Figure 3 using the conceptual model developed previously as a frame of reference (Reynolds et al. 2005). It was apparent that the genetic diversity found for WUE offers the greatest and most consistent opportunity for increasing yield, while increasing stem carbohydrates and access to water at depth also show some potential. However the increased expression of a number of other traits studied could not be extrapolated directly to yield gains. For example, differences in early ground cover, estimated using spectral reflectance (Gutiérrez-Rodríguez et al. 2004; Babar et al. 2006), for which most genotypes were superior to the check, indicate a greater potential for early ground cover and thus reduced evaporation of soil moisture. Previous analysis using estimates of transpiration efficiency based on ^{13}C-isotope discrimination analysis and final biomass with a larger selection of genotypes estimated that genetic effects on water losses because of evaporation from the soil could vary from 20 to 40% (Reynolds and Condon submitted), indicating a substantial potential advantage associated with increased ground cover. Canopy temperature showed genetic effects associated with soil moisture extraction (Reynolds et al. 2005; submitted) and cooler leaves, and may also be associated with increased photo-protection.

DIRECT PHYSIOLOGICAL INTERVENTIONS IN BREEDING

Relatively few crop-breeding programmes have actively selected for genetically complex physiological traits due to expense and the time-consuming nature of their measurement. Exceptions would include selection for anthesis-silking interval (ASI) in breeding maize for drought adaptation (Bolaños and Edmeades 1996) and carbon isotope discrimination (Δ^{13}C), which provides an indirect measure of WUE (Condon et al. 2002), used to develop the Australian spring-wheat cultivar, Drysdale (Descriptions: *Triticum aestivum* 2002). The wheat-breeding programme at CIMMYT uses physiological interventions at three stages of the breeding process: (i) parental characterization; (ii) early generation selection; and (iii) pre-breeding. The first and second of these will be discussed subsequently, while the latter encompasses a combination of both, i.e., selection tools are used to screen large collections of genetic resources, and elite genetic resources are characterized and used as new sources of physiological traits for introgression into improved backgrounds.

Physiological characterization of parents

Potential parents are characterized for a range of physiological traits, thereby allowing plant breeders to combine these traits in a strategic manner in crosses (Reynolds et al. 2005). Table 3 shows an example of the range in physiological traits among key parental materials grown under drought stress in NW Mexico. These data were collected on lines using a managed drought-stress regime described in Trethowan et al. (2001). Quantitative analysis of physiological traits in a broad range of genetic backgrounds (including materials derived from inter-specific 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 at least 20–30% over current elite checks (Reynolds and Condon submitted; Reynolds et al. submitted). Many of the same traits have potential to improve yields under hot, irrigated environments (Figure 4), although quantitative data have yet to be analysed for this environment. This kind of information has been used for several years in breeding and pre-breeding at CIMMYT to design crosses (Trethowan and Reynolds 2006). These results show that it is possible to combine extreme expression of several physiological traits in one genetic background. However, the challenge remains to compile expression of all relevant stress-adaptive physiological traits into one genotype.

Table 3. *Physiological traits measured on parental materials at Ciudad Obregon 2003-2004 (from Trethowan and Reynolds 2006)*

Pedigree	Yield g m^{-2}	Biomass (Anthesis) g m^{-2}	CT[a] (Vegetative) °C	CT (Gr. fill) °C	Carbon isotope discrim.	Stem CHO[b] at anthesis % stem dry weight	Water extraction by roots % 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

[a] Canopy temperature; [b] carbohydrate.

Early generation selection

The difficulty of selecting for improved adaptation to abiotic stresses makes the use of indirect measures attractive to plant breeders. A good example is canopy temperature, for which measurement is quick (10 seconds), easy (aim and pull the trigger) and inexpensive. Previous studies have shown strong association between yield and canopy temperature (CT) in random-inbred lines (RILs) under drought, indicating the potential of the trait as an indirect selection criterion for achieving genetic gains in drought adaptation (Reynolds et al. 2000; Olivares-Villegas et al. in review). At CIMMYT, canopy temperature is evaluated in breeders' F4 populations to change gene frequency in favour of cooler lines (presumably better able to explore deep soil water profiles) and in combination with visual selection. The trait is measured during the late vegetative stage and again during grain filling, and bulks that are consistently cooler are selected (Figure 5) assuming they meet the visual criteria. The visually selected lines showing consistently warmer canopies are not thrown out; however, a greater number of plants are selected from the cooler bulks, thereby skewing gene frequency in the early generations in favour of these cooler materials (Figure 5). The trait could conceivably be measured as early as $F_{2:3}$ populations. Since CT has been shown to be well associated with ability to extract water from depth (Reynolds et al. 2005) selection for CT is most probably increasing gene frequencies for root-related traits.

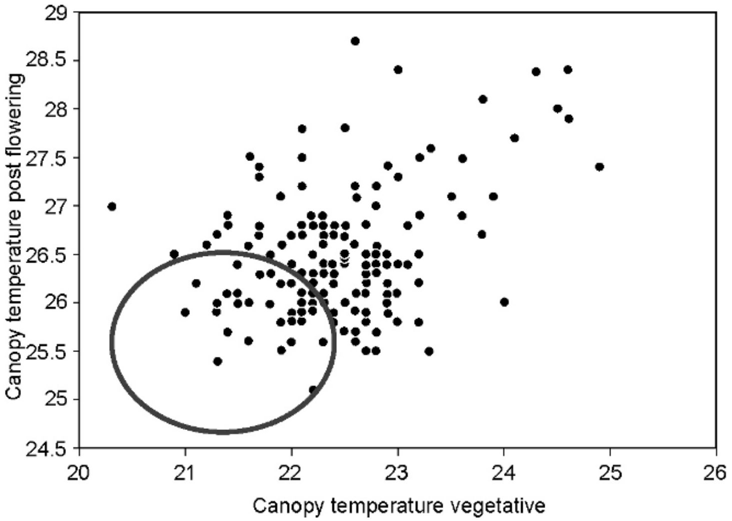

Figure 5. *Canopy temperature measured pre- and post-anthesis on visually selected F4 bulks grown under drought stress at Ciudad Obregon in 2004*

CT has also been shown to be associated with genetic gains in RILs under hot, irrigated conditions (Reynolds et al. 1998). As well as having application in early generation selection, CT was shown to be a powerful tool for selecting advanced lines within the breeding environment for performance at a number of international heat-stressed target environments (Reynolds et al. 1994; 1998; 2001). For example, when comparing the association of yield in wheat environments in target countries (Sudan, India, Bangladesh) with yield and CT measured in the selection environment (NW Mexico), it was found that both traits explained approximately equal amounts of variation in yield of 60 advanced lines, about 40% (Reynolds et al. 2001). However, CT was measured on plots of 2 m^2 instead of yield plots of 10 m^2, in about 10 seconds compared with several minutes to harvest and weigh a yield plot, and with an instrument that costs less than $200 compared with a small plot harvester. Although we are not suggesting that CT should replace yield estimates in a breeding programme, this example illustrates the point that indirect selection criteria like CT have a role to play in improving the efficiency of selection.

Another attraction of integrative traits like CT is that they may combine several important physiological mechanisms (Araus et al. 2002). When CT is measured on genetically diverse material during early generation selection, for example, under hot, irrigated conditions, cooler canopies would be found in lines that combine a number of the traits considered important (see Figure 4). These might include (i) a root system that can match evaporative demand at high vapour-pressure deficit; (ii) high intrinsic RUE; and (iii) photo-protective mechanisms that maintain RUE throughout the cycle. By measuring CT strategically, for example, at different phenological stages and times of the day, genotypes that are deficient in any of those three areas could be detected and eliminated while genotypes showing consistently cool CT would be advanced to the next generation. Similarly, under drought, a cool CT may – in certain environments – be related directly to genetic potential for root depth. In other environments, however, cooler genotypes would be found only for those lines that combine a number of relevant genes. This could be the case, for example, in environments where micro-element deficiency or soil-borne diseases are affecting root growth. Clearly the value of integrative selection tools such as CT can be augmented with a strategic use of selection environments, and conceptual models such as those described can play a role in developing such strategies.

Marker-assisted selection

As our understanding of the physiological basis of yield and how cultivars interact with environment accumulates, methods for manipulation of DNA such as marker-assisted selection (MAS) will become increasingly powerful (Snape 2004). For example, MAS is already applied in international wheat breeding to screen for a number of genetically simple traits (Trethowan and Reynolds 2006). Good examples are *Cre1* and *Cre3* (Lagudah et al. 1997) for cereal cyst nematode, genetic resistance being a key element of root health (Trethowan et al. 2005). A marker for tolerance to high boron (*Bo1*) is also routinely used; boron toxicity is frequently associated with soils in marginal environments world-wide, which exacerbates yield loss when water is limited (Ascher-Ellis et al. 2001). Diagnostic markers are available for the gibberellic-acid-insensitive Rht1 and Rht2 genes, and markers are either available (Rht8) or under validation for alternative gibberellic-acid-sensitive dwarfing genes such as Rht 12 and Rht 13 (Ellis et al. 2005). These gibberellic-acid-sensitive dwarfing genes can improve emergence characteristics due to longer coleoptiles when seed is sown in hot and dry environments (Rebetzke et al. 1999; Trethowan et al. 2005). The wheat-breeding programme at CSIRO, Canberra in Australia also make routine use of molecular markers for a large array of traits (Bonnett et al. 2005). Despite heavy investment, there has been significantly less success identifying markers for QTLs such as drought adaptation (Snape 2004). Given that

QTL analysis is essentially a statistical rather than a deterministic analysis of genes, the likelihood of identifying all of the genes associated with a complex trait decreases with the number of loci involved and the size of the individual effects. However, as marker technology advances and combines with gene-discovery approaches, more QTLs associated with adaptation to complex environments will emerge. Nonetheless, the main challenge to their application in breeding will be to determine the right combination of alleles to use, since conditions vary significantly from site to site and from year to year and significant QTL × environment interactions exist. A multi-staged approach to identifying molecular markers may be the best approach. For example, conceptual models for generic drought-adaptive traits (e.g., Figure 4) can be used to identify suitable crosses and populations that would be grown in well-controlled field environments so as to develop QTLs associated with performance under purely water-limited conditions. Generic traits for drought adaptation might include capacity of roots to access water deep in the soil, high intrinsic WUE, antioxidant systems for photo-protection, etc. (Reynolds et al. 2005). Once germplasm has been optimized for adaptation to moisture stress, environment-specific models would be used to include other factors commonly found in farmers' fields in a region that are not directly related to moisture stress, such as micro-element deficiencies/toxicities and soil-borne diseases. Environment-specific models could also be used to fine-tune QTLs related to rainfall distribution, temperature profiles and photoperiod etc.

DNA fingerprinting to identify key genomic regions associated with adaptation to abiotic stress

The CIMMYT wheat programme has generated an extensive data set of yield and disease performance collected from yield and screening nurseries over the past 3 decades. 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, for example, drought performance with specific genomic regions always present in materials performing well under drought stress. Wheat breeders could then ensure that these regions are present in their parental materials and could actively select for them in segregating populations. It would also be valuable to establish the traits associated with these regions to determine if they have been apparently optimized or indeed if they are associated with abiotic or biotic factors or a combination of both.

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CHAPTER 12

PHYSIOLOGICAL TRAITS FOR IMPROVING WHEAT YIELD UNDER A WIDE RANGE OF CONDITIONS

G.A. SLAFER[#] AND J.L. ARAUS[##]

[#] ICREA, the Catalonian Institution for Research and Advanced Studies, Department of Crop and Forest Sciences, University of Lleida, Centre UdL-IRTA,
Av. Rovira Roure 191, 25198 Lleida, Spain.
[##] Department of Plant Biology, University of Barcelona, Av. Diagonal 645,
08028 Barcelona, Spain.
E-mail: slafer@pvcf.udl.es

Abstract. A better understanding of relatively simple crop-physiological attributes that determine yield in a wide range of conditions may be instrumental for assisting future breeding. Physiological traits may be selected either directly or through the use of molecular-biology tools. Physiological and breeding literature frequently distinguishes between yield under potential, stress-free conditions and under the pressure of stress, mostly abiotic. Although the rationale behind the idea that the different physiological attributes contribute to yield under these contrasting conditions may be sound, in practice there is a large body of evidence pointing out the other way around. For instance, genotypes with physiological attributes conferring higher yield potential usually also perform better under stress conditions, at least when excluding extremely severe environments. As breeders normally need to release improved cultivars to be grown in different sites throughout several seasons and subjected to a wide range of management decisions, identifying physiological traits that may confer simultaneously high yield potential and constitutive tolerance to stress would be critical. These traits must allow the plants to capture more resources or to use them more efficiently. A well-known attribute conferring high yield potential and widely studied physiologically has been semi-dwarfism. Semi-dwarf cultivars normally yield better than tall ones in a wide range of stressful conditions (at least if seedling emergence is not a major inconvenience). This is because reducing height to a certain level does not alter the ability of the crop to capture resources, whilst improving markedly the efficiency with which the resources are used to produce yield. This trait is not further useful as modern cultivars possess already a stature within the ranges optimizing yield. Two other traits that may also be associated with improved performance in a wide range of conditions may be the discrimination against ^{13}C ($\Delta^{13}C$) and a lengthened stem elongation phase at the expense of previous phases. Although more research is needed before conclusions may be robust, physiological evidence supports the hypothesis that increasing $\Delta^{13}C$ and lengthening the stem elongation phase would result in an improved performance over a range of environmental conditions.

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INTRODUCTION

Brief description of the context

Wheat breeding for improved productivity has been noticeably successful during the second half of the 20th century (e.g., Calderini et al. 1999). In the future, breeding efficiency has to be increased in order to meet the constantly increasing demand of a still rapidly increasing population whilst facing a reduction, or at least a lack of expansion, of arable land if environmental sustainability is to be achieved (Reynolds et al. 1999; Cassman et al. 2003; Slafer et al. 2005). Thus, breeding to raise both yield potential and yield further under environmental constraints through improved adaptiveness will be of paramount importance (Slafer et al. 1999; Araus et al. 2002).

Do we need to breed for yield potential and adaptiveness separately?

There has been an active debate in literature whether to breed primarily for yield potential or for improved yield under the stressful environments that prevail in most wheat-growing areas worldwide. One can find examples of both views: (i) improving yield potential constitutively improves yield under stress conditions; and (ii) breeding for yield potential produces lines of poorer behaviour than landraces or lines selected for better performance under stress conditions.

Although recognizing that exceptions can doubtlessly be found, we believe that there is some agreement in literature on wheat and barley on:

a. Selecting for high yield potential does not necessarily bring about improved performance (Ceccarelli and Grando 1996; Slafer and Araus 1998), and should not be advised[1] for severely stressful environments, for instance, those with yields consistently below 1.5–2.0 Mg ha^{-1}.
b. Selecting for a higher yield potential in all other environments – from relatively harsh (e.g., yields ranging from 2.0 to 4.0 Mg ha^{-1}), through stressed (e.g., 4.0–6.0 Mg ha^{-1}) or mildly stressed (e.g., 6.0–8.0 Mg ha^{-1}), to unstressed conditions (yields > 8.0 Mg ha^{-1}) – will usually result in cultivars with constitutively improved adaptation to stress (i.e. performing in the stressed environment equally to, or better than, cultivars selected for performance in the prevalent environment, Slafer et al. 2005).

Thus, in most growing regions improved performance might be achieved by selecting for higher yield potential (i.e., selection under favourable conditions would even result in improved yields in less favourable environments, Richards 2000; Araus et al. 2002). There is clear empirical evidence supporting this generalized statement. Re-analysing data from studies conducted in different countries, Calderini and Slafer (1999) showed that modern wheats consistently yield more than their predecessors throughout a wide range of environmental conditions, a fact also recently shown from the comparison of yields of a modern and an older barley cultivar grown across a wide range of Mediterranean conditions (Tambussi et al. 2005). Other supporting evidence is provided by the parallelism in increase over the years in potential yield and in actual yields obtained by farmers (Evans 1993;

Abeledo et al. 2003; Slafer and Calderini 2005), which may mean that actual yields do not increase unless the genetic yield potential is improved.

The fact that we may trust that selecting for higher yield potential may bring about improved performance under a wide range of environmental conditions (though perhaps excluding extremely severe stresses) may be the good news. The bad news is that it does not seem to be easy to raise the yield potential further, because modern cultivars already possess a relatively high yield potential (as a positive consequence of successful breeding in the past). In fact, there is some evidence that in recent years genetic gains in yield potential have been far lower than what is required to keep pace with the increase in demand (e.g., Denison et al. 2003). We believe that further improvements need the integration of new tools and strategies to complement traditional breeding approaches. Two candidate disciplines that might contribute to complementing traditional breeding are molecular biology and crop physiology.

MOLECULAR BIOLOGY NEEDS SOUND CROP PHYSIOLOGY

Molecular biology can help in identifying and introgressing genetic factors responsible for traits in which breeders may be interested. When these traits are relatively simple the usefulness of these techniques is beyond questioning. However, when it comes to yield potential (or any other complex trait) the identification of a genetic basis (quantitative trait loci, QTLs) has proven of little empirical value. One example may be found by the fact that while literature is full of papers reporting QTLs for yield in wheat and barley, there are no examples of breeding programmes introgressing those QTLs and ending up with a consistent yield gain (Slafer 2003)[2]. As indicated by Goodman (2004), molecular biotechnologies will add little to actual breeding for improved yield until these technologies acquire capabilities to manipulate predictably complex traits.

If we were able to identify relatively simple traits putatively associated with yield potential, there would be a way to take advantage of the enormously powerful tools developed by molecular biology for improving yield potential. The whole effort would require joining the know-how of molecular biologists with expertise of conventional breeders and rigorous developments made by crop physiologists (Slafer 2003; Sinclair et al. 2004; Slafer et al. 2005; Wollenweber et al. 2005). In other words, these emerging technologies may have no value for improving yield potential without an improved ecophysiological assessment of relatively simple traits associated with yield under a wide range of conditions (Araus et al. 2003; Slafer 2003).

ARE THERE PHYSIOLOGICAL TRAITS CONTRIBUTING TO YIELD IN A WIDE RANGE OF CONDITIONS?

Most literature in crop physiology related to breeding does focus on either yield potential or yield under stressful conditions. This influences our perception on whether there might be a possibility to identify traits that being relatively simple would be mechanistically associated with crop yield, naturally under field conditions and for a wide range of conditions. By trying to identify such traits, it may seem that we are stretching too much the potential impact of physiological traits for future breeding. However, some traits may allow the crop to capture more effectively its most limiting resources, or may improve the overall efficiency of use of resources.

In this section, we will first illustrate that identifying traits combining both simplicity and virtually global impact is possible by describing the trait 'semi-dwarfism'. Then we will speculate on two other alternative traits that may also be selected to provide a yield advantage under a wide range of conditions: one is related to the use of more resources (increasing ^{13}C discrimination: $\Delta^{13}C$), and the other by exploiting more resources for accumulation of reserves by extending the duration of carbon allocation to stems and spikes.

Semi-dwarfism

Most genetic gains in wheat yield potential were mainly achieved by means of improvements in harvest index with marginal or no modification of biomass (see: Spiertz and De Vos 1983; Slafer and Andrade 1991; Feil 1992; Calderini et al. 1999), though recently some papers reported slight increases in biomass in spring wheat (Reynolds et al. 1999) and winter wheat (Shearman et al. 2005). Although trends in harvest index with the year of release of cultivars were slightly positive before the introgression of semi-dwarf genes, the incorporation of genes derived from Norin 10 (*Rht1* and *Rht2*) into wheat-breeding programmes has been decisive to increase harvest indices to a great extent (Gale and Youssefian 1985; Calderini et al. 1999). These genes reduce culm elongation, which is associated with an effect on the sensitivity to endogenous gibberellic acid.

The large and consistent (across genetic backgrounds) increase in yield potential has been physiologically explained by a relatively simple model. Briefly, semi-dwarf genes impose a genetic restriction to culm growth at the time when most assimilates are being competitively used for culm and spike growth, few weeks before anthesis. Compared with the tall genotype, more assimilates are available for spike growth resulting in higher weight in spike dry matter per unit land area at anthesis. Because of a consistent relationship between number of grains per unit land area and the spike dry mass at anthesis the impact of semi-dwarf genes is an increase of the number of grains per m^2 (e.g., Brooking and Kirby 1981; Gale and Youssefian 1985; Fischer and Stockman 1986; Youssefian et al. 1992; Miralles and Slafer 1995; Flintham et al. 1997; Miralles et al. 1998).

The increased yield potential due to the introgression of semi-dwarf genes was very consistent not only because it occurred in virtually all genetic backgrounds studied, but also because the yield advantage was still evident under a wide range of environments including stress conditions. With the exception of very severe stresses (say, yields < 2.0 Mg ha^{-1}), semi-dwarfism consistently combined improved yield potential with improved performance across a wide range of conditions (e.g., Laing and Fischer 1977; Richards 1992; Miralles and Slafer 1995). The natural consequence of improving yield potential and concomitantly actual yields in a wide range of agro-ecological conditions explains the huge success this characteristic had in most breeding programmes of the world. Nowadays, semi-dwarf wheats are cultivated virtually throughout the world.

Unfortunately, we cannot further reduce plant height, as the relationship between height and yield is parabolic and modern cultivars already possess a height that optimizes yield (Fischer and Quail 1990; Richards 1992; Miralles and Slafer 1995; Flintham et al. 1997). Semi-dwarfism constitutes a clear example that it is possible to find a relatively simple trait (controlled by major genes, expressed in most genetic backgrounds and environments) gaining yield improvements under a wide range of conditions. To further raise yield potential and constitutively increase actual yields, we must find alternative traits to improve spike growth before anthesis. These alternative traits should still be much simpler than the complex genes controlling ultimately yield itself under a wide range of conditions.

Increasing $\Delta^{13}C$

For C_3 species such as wheat, carbon isotope discrimination ($\Delta^{13}C$), when measured in plant tissues, constitutes an integrated record of the ratio of intercellular to atmospheric partial pressure of CO_2 (C_i/C_a) and therefore of the transpiration efficiency of the plant (A/T, the ratio of net assimilation to water transpired), over the period during which the dry matter is assimilated. Thus, $\Delta^{13}C$ measured in dry matter is widely accepted as an indicator of crop water use efficiency (WUE) of dry-matter production. Because $\Delta^{13}C$ is genetically correlated with grain yield and shows a higher broad-sense heritability than yield itself, it has been proposed as trait for breeding (Araus et al. 2002). As C_i/C_a is positively related with $\Delta^{13}C$ and negatively with A/T, then $\Delta^{13}C$ is negatively related with WUE (Farquhar and Richards 1984). Genetic gains in yield may then be expected by selecting for lower $\Delta^{13}C$ (and, thus, higher WUE) if the released cultivar is to be grown under severe water restrictions, such as when the crop grows primarily on water stored in the soil (e.g., Rebetzke et al. 2002).

However, in many environments in which water restrictions may limit yield, $\Delta^{13}C$ has been found positively correlated with grain yield (Condon et al. 1987; 1993; 2004; Richards 1996; Araus et al. 1998; 2002; 2003; Slafer and Araus 1998; Slafer et al. 1999; Voltas et al. 1999). This is likely because of the fact that a genotype possessing higher $\Delta^{13}C$ must have maintained a higher CO_2 conductance due to a better water status, the higher $\Delta^{13}C$ then likely reflecting avoidance to stress by either faster development or better access to soil water (Araus et al. 2002; Condon et al. 2004).

Several explanations may account for such positive correlation between $\Delta^{13}C$ and yields. Differences among genotypes in phenology may affect yield and also $\Delta^{13}C$, especially in drought-prone environments. For example, under Mediterranean conditions genotypes with fewer days from sowing to flowering show higher $\Delta^{13}C$ values (Richards 1996; Araus et al. 1998) probably because they attain grain filling with more water in the soil whereas the evapo-transpirative demand is lower. Nevertheless, in bread wheat (Sayre et al. 1997), durum wheat (Araus et al. 1998) and barley (Voltas et al. 1999) large genotypic variability in $\Delta^{13}C$, independent of phenology, has been reported.

Therefore, additional causes for such positive relationship may be envisaged. Positive relationships between $\Delta^{13}C$ and grain yield are mostly found under moderately to well watered conditions, whereas for severely stressed environments (characterized by yields below 2.0 Mg ha^{-1}) relationships are absent or negative (Voltas et al. 1999; Araus et al. 2003). In fact, a higher $\Delta^{13}C$ may be just the result of more water captured and used by the crop. Thus, a strong positive relationship between total water input during grain filling and grain $\Delta^{13}C$ has been reported (Araus et al. 1997c; 1999; 2003), which supports the fact that $\Delta^{13}C$ is also a good indicator of total crop water availability (Stewart et al. 1995; Araus et al. 2002; see also Blum 2005). Other causes for a positive relationship are less common and may arise usually under well-watered conditions due to miscellaneous causes, such as leaf structure (Araus et al. 1997a; 1997b), a higher transpirative cooling or a higher stomatal conductance associated with a larger photosynthetic sink (Richards 2000).

Summarizing higher $\Delta^{13}C$ has been proposed as a breeding criterion for increasing yield in wheat and other temperate cereals under a wide range of conditions, where yield formation is, at least in part, determined by in-season rainfall. The reason for the positive relationship between $\Delta^{13}C$ and yield is that a genotype exhibiting higher $\Delta^{13}C$ is probably able to maintain a better water status (Araus et al. 2002; Condon et al. 2004). Where additional water is not available to the crop (i.e., all the stored soil moisture is exhausted during the crop cycle), increased WUE appears to be an alternative strategy for improving crop performance (Araus et al. 2002; Blum 2005).

Lengthening stem elongation

An alternative approach recently hypothesized (and therefore not widely tested in many different environments and genetic materials) is to extend the duration of the stem elongation phase, from jointing to anthesis, at the expense of shortening of vegetative and early reproductive phases, with no major changes in time to flowering (e.g., Slafer et al. 1996; 2001).

In principle, the rationale for the hypothetical overall benefit of this characteristic is in line with the overwhelming evidence of the impact of introgressing semi-dwarfing genes: yield potential, and actual yields in a wide range of conditions, would be increased if we could further increase the spike dry weight, per unit land area, at anthesis. Lengthening the duration of the phase when spike growth takes place would result in higher spike dry matter at anthesis and subsequently more grains per m^2. In fact, artificially extending the duration of stem elongation by exposing the crop to different photoperiods did raise the number of grains (Miralles et al. 2000; González et al. 2003; 2005a).

It is clear that substantial genetic variation in duration of the stem elongation phase exists (Slafer and Rawson 1994; Kernich et al. 1997; Slafer 2003), though we need to identify specific genetic factors responsible for this variability. Main developmental traits that might be related to the variability in stem elongation duration are sensitivity to photoperiod (rate of development during stem elongation seems to be governed by photoperiod response, Slafer and Rawson 1997; Miralles and Richards 2000; González et al. 2002) or differences in earliness *per se* (genotypes may differ in their duration of stem elongation when photoperiod is long and plants were fully vernalized, Slafer 1996).

For the particular case of photoperiod sensitivity during stem elongation, it was suggested that this sensitivity would be independent from that of previous phases (Slafer and Rawson 1994; González et al. 2002), which is required if the total time to anthesis is to be maintained. However, we must learn much more on the genetic bases determining sensitivity to photoperiod during stem elongation before this information may be useful for practical breeding.

Most attempts to identify a clear and consistent genetic basis for this particular response have failed (González et al. 2005b). This is likely because we only know (and have worked with) few of the hypothesized genes for photoperiod sensitivity (Snape et al. 2001). There are now other approaches in ongoing projects attempting to determine what genes are down- or up-regulated when responses to photoperiod occur or to identify genes/QTLs for differences in length of different phases within mapping populations.

NOTES

[1] It must be noticed, however, that selecting for better performance under stressful conditions may likely be useful only when the type and intensity of the stress are rather homogeneous. In regions where stressful conditions vary temporally and/or specially in type, intensity and opportunity, the approach may not be useful: cultivars selected in particular stressful conditions may not behave well in other stressful environments, with a different combination (or periodicity) of interacting stresses (Cooper et al. 1997).

[2] Although some results, yet unpublished, presented in the 3rd Cereal Genetics and Genomics Workshop may provide empirical advantages of a QTL for yield under post-flowering drought conditions in pearl millet, the relatively little breeding of pearl millet as compared to that in wheat and barley should be taken into account before accepting simple extrapolations (Catherine Howarth, IGER-Aberystwyth, pers. comm. 2006).

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CHAPTER 13

IS PLANT GROWTH DRIVEN BY SINK REGULATION?

Implications for crop models, phenotyping approaches and ideotypes

M. DINGKUHN[#], D. LUQUET[#], A. CLÉMENT-VIDAL[#],
L. TAMBOUR[#], H.K. KIM[##] AND Y.H. SONG[##]

[#] *CIRAD, Département Amis, TA40/01 Ave. Agropolis,*
34398 Montpellier Cedex 5, France.
[##] *School of Agronomy and Horticulture, University of Queensland,*
Gatton Campus, Brisbane, QLD, Australia.
E-mail: dingkuhn@cirad.fr; y.song@uq.edu.au

Abstract. There is a new interest in plant morphogenesis and architecture because molecular genetics is providing new information on their genetic and physiological control. From a crop modeller's point of view, this requires particular attention paid to the regulation of sinks associated with organ development, as well as their interactions with assimilate sources. Existing agronomic and architectural crop models are not capable of simulating such interactions. A conceptual framework is presented for the analysis and simulation of crop growth driven by either assimilate source or sink dynamics, building on the assumption that meristems are the main sites in the plant architecture where sinks are initiated and adjusted to resources. Among the numerous sink–source feedbacks to be considered are sensing of the plant's resource and stress status by meristems (enabling adjustment of morphogenesis), as well as transitory reserves, organ senescence and end-product inhibition of photosynthesis (necessary for the plant to cope with acute imbalances). These feedbacks are to a large extent related to sugar metabolism and can be explained with recent molecular findings on the prominent place in plant development of sugar sensing and the regulation of sucrose cleavage at sink sites. A model integrating these phenomena in a simplified manner, called EcoMeristem, was developed and is being applied in phenotyping for functional-genomics studies on rice. Theoretical evidence and model sensitivity analyses suggested that sink regulation during vegetative growth has a strong effect on plant vigour and growth rate, even at given levels of leaf photosynthetic capacity. However, the usefulness of complex, whole-plant models such as EcoMeristem for heuristic phenotyping approaches remains to be demonstrated. Specific problems are related to the stability of process-based crop parameters across environments, as well as the measurement of such crop parameters that are inaccessible to direct observation. But it is argued that integrated, structural-functional models may be the only means to quantify complex traits, such as those governing adaptive morphology (phenotypic plasticity). Furthermore, such models may be well suited to develop improved plant type concepts *in silico*.

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INTRODUCTION

In recent years crop physiology has received a considerable boost from molecular genetics, and in particular from functional genomics. New advances in physiological theory are largely due to emerging information on causal linkages between processes at the molecular scale and at the plant scale. These linkages were inherently inaccessible to physiological research methodology, and are now being exposed by the identification of genes that are involved in them.

The clues provided by molecular genetics are redirecting the attention of physiologists to new or previously sidelined aspects. The physiology of phytohormones, which had exhausted its means of analysing ever smaller compartments and ever-increasing system complexity, is currently experiencing a revival through knowledge on direct causalities established by molecular genetics. On the other hand, the importance of developmental aspects, including the ontogenesis of architectural and morphological structure, has been emphasized by molecular findings and is receiving a prominent place in physiological research (Seki et al. 2002; Gazzarrini and McCourt 2003; Liu et al. 2005). The latter observation should not come as a surprise because genes, through physiological processes, build the plant apparatus in a continuous process of physical and biochemical differentiation. Plant functioning can therefore not be understood without the study of its (onto)genesis, and consequently, processes that happen in meristems – the tissues that are probably the least accessible to physiological study because of small cell size and hidden location within the plant.

Crop models have inadvertently reflected contemporary, physiological research priorities and accorded little attention to developmental biology. They generally give emphasis to environmental effects on source processes, such as photosynthesis, and mostly consider the size of sinks as perfectly adjusted ‘slaves’ of the incremental source. The type and relative weight of different sinks (e.g., leaves, roots, stems or inflorescences) is thereby commonly forced by empirical, phenology-dependent partitioning functions or tables (ORYZA2000: Bouman et al. 2001; APSIM: Wang et al. 2002; STICS: Brisson et al. 1998). The sometimes large discrepancy between predicted (potential) and observed growth is attributed (correctly or not but this cannot be ascertained) to biotic or abiotic constraints not simulated by the model. Generally, however, the possibility of sub-optimal regulation of developmental processes determining sink dynamics in the plant’s architecture is not considered (with the notable exception of reproductive sinks in cereals, which are frequently simulated through a resource-dependent, pre-dimensioning process after their initiation).

The present study, to a large extent conceptual, explores the hypothesis that sink dimensioning, as part of plant development, may act as a major driving force of plant growth. Furthermore, we will explore potential consequences of this hypothesis for model-assisted phenotyping, crop ideotype development and eventually, crop improvement strategies.

EVIDENCE FOR SINK-LIMITED VEGETATIVE GROWTH IN PLANTS

Any significant impact of sink dynamics on crop growth requires that growth is sink-limited in a significant range of situations and genotypes. Sink-limited growth, or excess production of assimilates, is a frequent phenomenon in perennial plants, which constitutionally have long lag phases between assimilate production and re-investment in growth processes. This involves large reserve compartments that buffer the asynchrony between supply and demand, particularly in temperate perennials that produce their foliage in spring with assimilates produced in autumn. Such asynchronies are also observed in tropical, seasonally defoliating perennials such as rubber tree (www.ppi-ppic.org/ppiweb/swchina.nsf/), as well as tropical orchard crops (Mango: Lechaudel et al. 2005). In oil palm, seasonal peaks of oil production probably draw from glucose and sucrose reserves stored in the trunk (Mialet, CIRAD, Montpellier 2005, unpublished); coconut trunks were found to maintain throughout the year a large reservoir of sucrose, little of which is utilized to buffer seasonal fluctuations in supply of, and demand for, assimilates (Mialet-Serra et al. 2005). Instead, it appears that assimilate production in coconut is down-regulated during periods of low demand (Mialet-Serra 2005).

It seems that the positive feedback of sink activity on leaf photosynthetic rates sometimes reported (Franck 2005) is probably due to the restitution of sub-maximal photosynthetic rates when the plant turns from sink-limited to source-limited conditions. Reductions of leaf photosynthesis by end-product inhibition (Sawada et al. 2001), associated with increased reserve storage, has been described for coffee (Franck 2005) and many other species. End-product inhibition of photosynthesis is under genetic control in *Arabidopsis thaliana*, and mutants were selected that show no such inhibition (Van Oosten et al. 1997). In other annual plants, such as cabbage, end-product inhibition of photosynthesis was caused by elevated ambient CO_2 concentration, but was less pronounced in genotypes that had greater assimilate storage capacity (Bunce and Sicher 2003). The same authors reported that end-product inhibitions could be predicted from weather, indicating that plants had a limited capacity to utilize assimilates exhaustively on sunny days.

Annual crops bred for rapid growth and maximal production, such as modern cereal varieties, probably have minimal lag periods between assimilate acquisition and their re-investment in structural growth. They are therefore unlikely to exhibit end-product inhibition of photosynthesis under stress-free conditions and ‘normal’ atmospheric CO_2 levels, although this may merit further investigation (Geigenberger et al. 2005). If such inhibitions exist, they are likely to occur in the afternoon on sunny days, and in fact stomatal conductance tends to decline during that time of day. To what extent this decline is caused by higher VPD (vapour pressure deficit) and/or saturation of carbon demand has not been studied explicitly. Leaves accumulate not only transitory starch but also soluble sugars in the afternoon (Munns et al. 1979). Furthermore, sugar concentrations in vegetative storage tissues, for example in leaf sheaths of rice, can be significant even during exponential growth (Luquet et al. 2005), and large quantities of non-structural carbohydrates are accumulated in stems during the month preceding heading (Samonte et al. 2001). Whether or not these phenomena indicate a general sink limitation of growth remains open. It is also possible that some of these reserves are not of short-term, transitory nature (spill-over reservoirs), but instead are the result of a specific storage sink located in stems.

As a last piece of (rather anecdotal) evidence, we would like to point out that hybrid vigour in rice, which is associated with both greater biomass and harvest index compared with high-yielding inbred lines of similar architecture, cannot be explained by higher leaf photosynthetic rates and its physiological determinants (such as N concentration or specific leaf area (SLA)), nor by different partitioning patterns among organs (Laza et al. 2001). The physiological basis of hybrid vigour remains a mystery, and open to the alternative hypothesis of a general stimulation of structural sinks. (If this hypothesis were true, hybrid vigour should be associated with low levels of transitory reserves during vegetative development.)

DIFFERENT WAYS OF MODELLING SINK DYNAMICS

In quantitative terms, the process of morphogenesis in plants may depend on carbon assimilation, but in terms of the resulting structure it is driven by the organogenetic activity of meristems. Organogenesis can thus be seen as the successive initiation of new structures that act as sinks during their expansion phase, and may eventually turn into sources in the case of leaves (Figure 1: organ development). If we assume that 'fresh' assimilates form a common pool available to all its sinks (an assumption made in most crop models, but wrong in the case of large, complex tree structures (Franck 2005)), it follows that many sinks compete with each other at any given time for the incremental pool of assimilates. The simplest possible model of this process attributes a fixed, relative sink force to each organ type at any given developmental stage (e.g., ORYZA2000, www.knowledgebank.irri.org). A slightly more complex model representing architectural detail would attribute such a relative value to each individual organ and provide it with an empirical temporal profile (e.g., GREENLAB: Yan et al. 2004; Guo et al. 2006). The latter solution already involves the notion of meristems providing organogenetic rhythms and initiating different organ types that represent metamorphoses of a basic entity. Both types of models, however, do not simulate resource or environment feedbacks on the organogenetic process because they assume a perfect match between demand and supply at all times. In other words, they do not allow for sink-limited, vegetative growth, with the exception of secondary forcing, such as temperature-limited leaf expansion (e.g., ORYZA2000). Both types of models also suppose that final organ weight remains open, or responsive to supply, until the end of organ growth.

In fact, final (potential) organ size is for many species and types of organs determined at an early stage of organ development, not only for fruits but also for leaves (*Arabidopsis* leaves: Cookson et al. 2005; maize leaves: Tardieu et al. 2000; grass leaves in general: Fiorani et al. 2000; seed of rice: Kobayasi et al. 2002). This point is crucial: if organ size is determined early on, the plant has to regulate its potential sink size before the sink becomes effective. This can be described as a physiological commitment on the basis of a 'best bet', or early assessment of available resources.

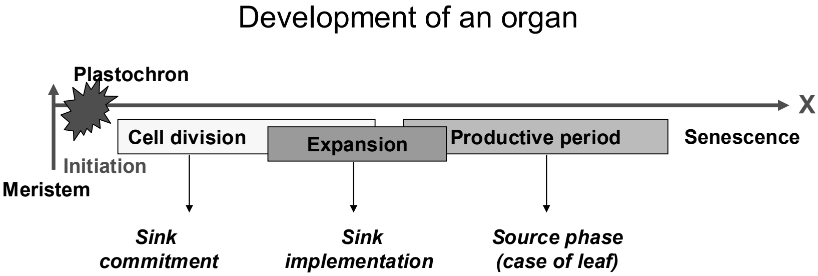

Figure 1. *Schematic diagram of functional phases of plant organ development, illustrating the hypothesis that sinks are initiated and pre-dimensioned ('committed') before becoming effective*

A model was recently reported that simulates such resource feedbacks on meristem behaviour (EcoMeristem: Dingkuhn et al. 2005; Luquet et al. 2006). According to this model, an index of internal competition (*Ic*) is calculated at each time step by dividing aggregate assimilate sources (supply) by aggregate sink activity (demand) (Figure 2). Via *Ic*, resources feed back on the rate of production of new organs (e.g., tillers, using a critical, genotypic value of *Ic* called *Ict*) and their potential size (down-sizing of sink if $Ic<1$). Since such adjustments of sinks involve a certain lag (time elapsing between organ initiation and expansion), temporary source/sink imbalances are inevitable, which result in reserve formation or mobilization or even organ death and recycling if *Ic* is very low.

SINK REGULATION VERSUS GROWTH AND PHENOTYPIC PLASTICITY

EcoMeristem demonstrates on a theoretical basis two phenomena that have so far received little attention in crop modelling. First, genotypes are conceivable that produce predominantly sink- or source-limited phenotypes, depending on whether organogenetic commitments made by the meristem are bold or rather conservative.

The bold (or source-limited) types would tend to use all available assimilates and frequently experience deficit situations, leading to low transitory reserve levels, large organ number and low organ longevity. The conservative (or sink-limited) types would tend to under-utilize available assimilates, produce fewer organs with greater longevity and accumulate more reserves. Interestingly, the conservative types show smaller biomass production even at given photosynthetic capacity (or radiation use efficiency (RUE)) because assimilates are not rapidly re-invested in new leaf area, and because of frequent end product inhibition of photosynthesis. This is illustrated in the sensitivity analysis of EcoMeristem to two crop parameters shown in Figure 3. The potential meristem growth rate (*MGR*), which sets the maximal rate of organ weight increase from one phytomer to the next, strongly increases not only plant height (which is evidently a function of organ size), but also plant biomass and leaf area. The increased organ size, however, is associated with

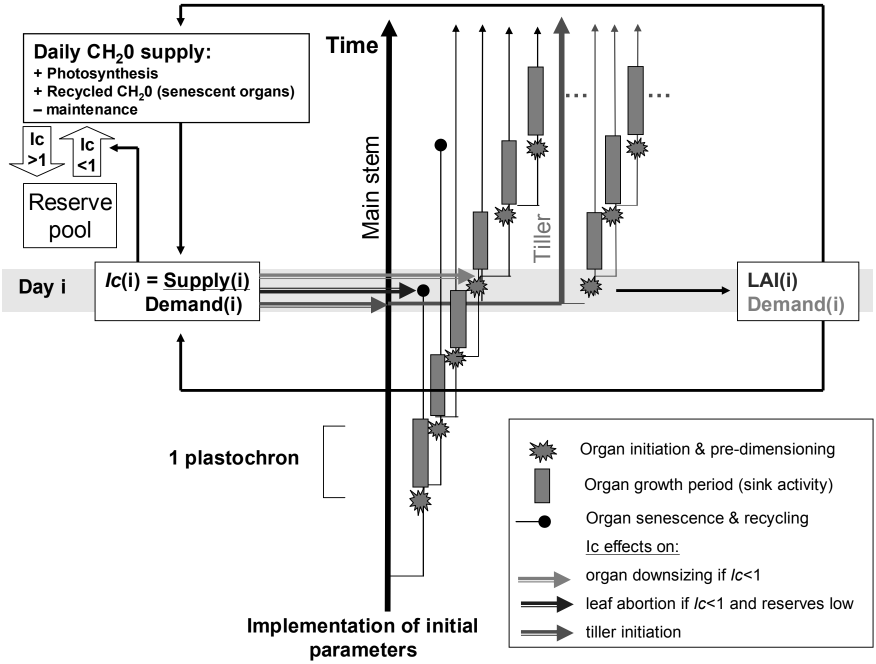

Figure 2. *Schematic diagram of the model EcoMeristem implementing feedbacks of an index of internal competition for assimilates (Ic) on transitory reserve dynamics and potential organ size, number and longevity. Adapted from Luquet et al. (2006)*

reduced tiller number and green-leaf number, whereas the fraction of dead leaves increases. In fact, if *MGR* is too high it results in excessive senescence and thus, reduced growth.

The critical *Ic* for tiller initiation (*Ict*), another morphogenetic crop parameter, has somewhat opposite effects. Increased Ict directly inhibits tiller production, and thus, number of leaves. Indirect effects include a slight, initial increase in leaf area (because reduced competition among tillers reduces leaf senescence), but a significant drop in leaf area at higher *Ict* (because the number of initiated leaves becomes limiting). Both *MGR* and *Ict* have previously been shown to differ strongly among genotypes (Dingkuhn et al. 2005) under given environmental conditions.

The predictions from sensitivity analyses should not be taken as quantitative but rather as trend information because the parameter variations tested exceed the ranges observed in true phenotypes. Also, not all possible combinations of parameter values will occur in reality. A real-world genotype producing large organs (high *MGR*) would probably have low tillering ability (high *Ict*), thus avoiding excessive organ senescence resulting from over-commitments (too many, too large sinks). As the sensitivity analysis of EcoMeristem shows, strongly over- or under-committing genotypes would still be able to thrive because of internal adjustments of organ size,

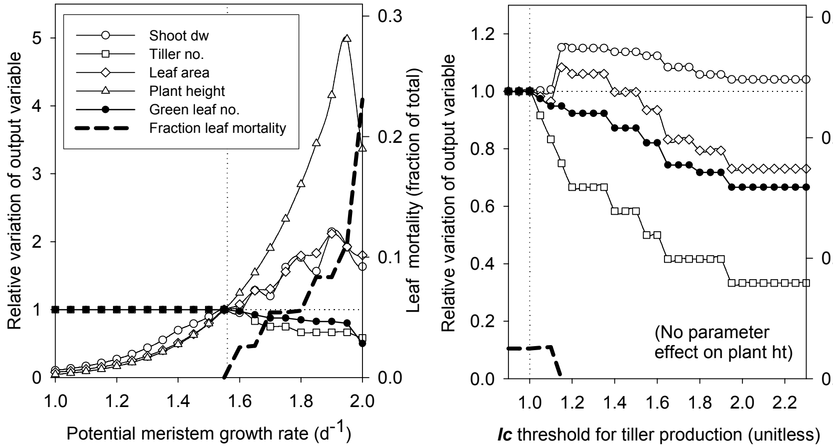

Figure 3. *Sensitivity analysis of EcoMeristem model output variables (vertical axes) to variation of two crop parameters. Dotted lines indicate reference values for IR64 rice. Adapted from Luquet et al. (2006)*

number and longevity; but they would not be efficient, and would probably not have survived any natural or man-made selection pressure.

The second biological phenomenon highlighted by EcoMeristem is resource-dependent, phenotypic plasticity (definition of term: Dewitt and Scheiner 2004). According to the model, branching rate (tillering), leaf appearance rate, organ size and assimilate-partitioning patterns are affected by internal competition for resources, and thereby lead to different plant architectures (Luquet et al. 2006). This type of phenotypic plasticity is of compensatory nature: it adjusts organ number and size on the plant, through modified rates of organogenesis and organ longevity, to variable carbon resources. The model was also used to interpret more specific effects of stresses, such as phosphorus deficiency, on rice plant morphology (Dingkuhn et al. 2006).

Phosphorus deficiency reduced shoot growth and stimulated, in relative terms, root growth. It thereby did not reduce RUE, but strongly reduced overall demand for carbon, resulting in sink-limited growth. The sink limitation, which led to a significant increase in carbohydrate reserves in leaf sheaths (Figure 4), was brought about by an inhibition of tillering and leaf extension rates (Dingkuhn et al. 2006). The lower leaf extension rates, combined with unaltered final leaf length, resulted in longer leaf extension duration and in reduced leaf appearance rates, probably by feedback. Root growth was stimulated by spill-over of excess assimilates from shoot to root, as evidenced by observations on sugar concentration levels and gradients within the plant.

This example of stress-induced phenotypic plasticity suggests that sink-limited growth situations, induced by the inhibition of specific, growth-related sinks, may

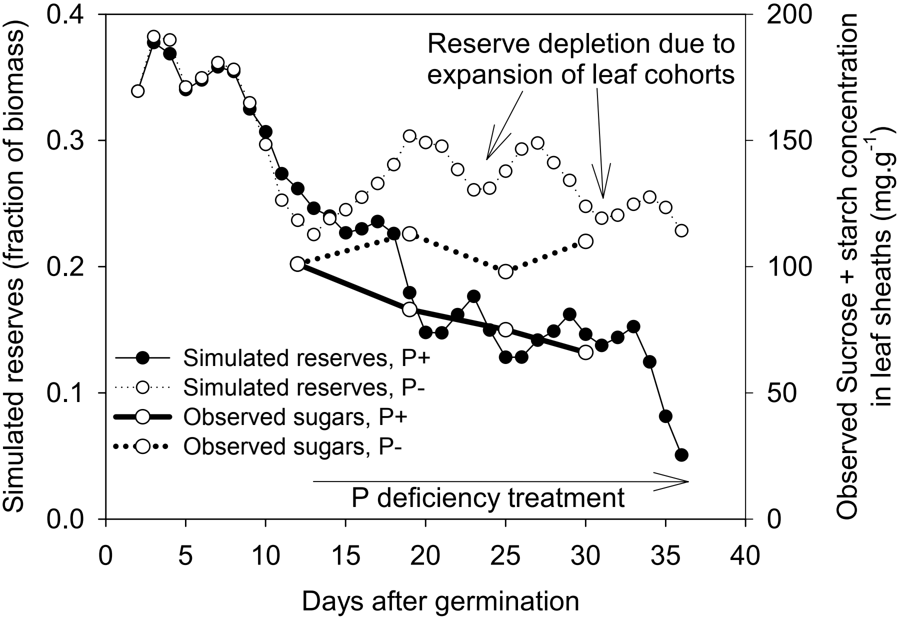

Figure 4. Kinetics of observed and simulated carbohydrate reserve concentrations in Aucena rice during vegetative growth under control and P-deficient conditions in controlled hydroculture environments. Initial reserve concentrations are high due to remains of seed reserves. Simulated oscillations of reserves are due to regular appearances of new organs (sinks). Adapted from Dingkuhn et al. (2006)

represent one possible strategy of plants to achieve adaptive changes in morphology and architecture.

PROCESSES INVOLVED IN THE REGULATION OF STRUCTURAL SINKS

A growing body of evidence points at a pivotal role of two types of acid invertases in source–sink relationships. The vacuolar invertase (Inv-V) is involved in tissue growth, including cell extension and reserve accumulation, whereas the apoplastic cell-wall invertase (Inv-CW) is involved in whole-plant sucrose partitioning, probably by controlling hexose supply to juvenile tissues such as meristems (Roitsch et al. 2000; Hirose et al. 2002). The Inv-CW is probably of particular significance for morphogenetic processes and phenotypic plasticity because it is controlled by both hormonal and sugar signalling (Black et al. 1995; Ji et al. 2005, Figure 5). Furthermore, tissue- or organ-specific control of Inv-CW activity is enabled by a family of genes coding essentially the same enzyme, called OsCIN 1 through 9 in rice (Ji et al. 2005; Cho et al. 2005). For example, OsCINx genes respond differentially to drought stress in flag leaves, panicles, anthers and peduncles of rice (Ji et al. 2005). Because of the close association of organ specific OsCINx expression with growth processes such as peduncle elongation under drought, the reversibility of its drought response and its sensitivity to GA (gibberellic acid) and ABA (abscisic acid) treatment, Ji et al. (2005) suggested that the hormonal regulation of OsCINx genes may be a promising intervention point for breeding strategies.

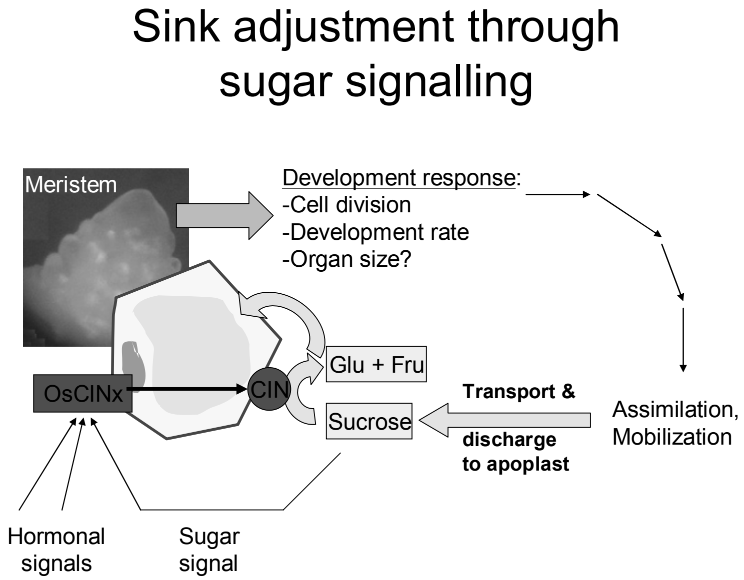

Figure 5. *Schematic diagram illustrating the theory of sugar signalling of plant assimilate resources to meristems via cell-wall invertases (Inv-CW) and their coding genes (OsCINx) in rice*

It is too early to conclude that cell-wall invertase genes are a generic, regulatory node linking hormonal with sugar sensing in developing tissues of the plant. However, if this should be the case, great scientific opportunities will arise for crop-physiological research and modelling (because Inv-CW regulation is pivotal for developmental biology, stress physiology and physiology of production), as well as for crop improvement (because Inv-CW regulation may hold the key for plant type traits and their phenotypic plasticity).

INCORPORATING SUGAR SENSING INTO CROP MODELS

It is evident that any crop model simulating sugar sensing by developing tissues (such as meristems) and the resulting effects on sink dynamics must include an explicit representation of organogenesis. This is the case for EcoMeristem (Dingkuhn et al. 2006; Luquet et al. 2006) and Greenlab (Yan et al. 2004; Guo et al. 2006), as well as for a number of other dynamic architectural models (e.g., GRAAL: Drouet and Pages 2003). Furthermore, the model must simulate carbon assimilation (including its dependency on key environmental and morphological variables) and conversion (including respiration) with sufficient detail to predict incremental sugar supply. This is the case for most agronomic models and EcoMeristem, but not for the architectural models currently available. Finally, simulation of both source- and sink-limited growth requires the consideration of transitory reserve compartments, provision for deficit-driven senescence (notion of recycling), and end-product inhibition of photosynthesis.

On the basis of this minimum set of processes, incremental, quantitative assimilate sink and source assessments can be done and the degree of sink or source limitation diagnosed. This is essentially what the plant seems to be doing via sugar sensing, although physiological reality may be far more complex than this model, for example because of sugar concentration gradients within the plant apoplast. (The notion of a single assimilate pool equally accessible to all sinks is convenient but ignores proximity effects and transport processes.)

It would be complicated to simulate at the scale of the whole plant the fluxes and local concentrations of inter-convertible sugars such as starch, sucrose and hexoses. As a possible simplification, one may assume that sugar sensing by meristem cells (which in itself remains a black box) effectively amounts to sensing of available assimilate resources (analogous to the *Ic* state variable in EcoMeristem). If this hypothesis is true, we can use the ratio of daily demand (aggregate sinks) and supply (aggregate sources) as indicator variable and define empirical sensitivity coefficients relating meristem response to this variable. A major difficulty, however, resides in the fact that these coefficients, or crop parameters, cannot be measured directly and thus need to be fitted statistically by optimization. This approach has been adopted for the EcoMeristem model. Proof of concept was provided by Luquet et al. (2006), but Dingkuhn et al. (2006) demonstrated that crop parameter values change when a physiological stress is applied, such as P deficiency. Similar observations were made under drought and low light stress (Luquet, Montpellier 2005, unpublished data), indicating that physiological stresses induce modified reaction norms in the plant. In fact, it is known that hormonal stress signals affect the expression of cell-wall invertase genes (Roitsch et al. 2000; Ji et al. 2005), and thus re-calibrate sugar-sensing mechanisms. More physiological information is needed to formulate a model whose morphogenetic parameters related to sugar sensing are sufficiently robust to be considered genotypic, or genetic – particularly in .the case of studies involving stresses.

HEURISTIC APPLICATIONS OF CROP MODELS FOR GENOMICS

What is the scientific and practical usefulness of models such as EcoMeristem – supposing their genotypic parameters can be stabilized in future versions? In terms of scientific gain, this modelling approach integrates emerging knowledge on the plant's developmental biology with established paradigms of crop physiology, thus explaining the genotype- and environment-dependent plasticity of morphology and productivity. Phenotypic plasticity of crops is poorly understood, and it is likely that much of the crop's capacity to adapt to variable environments is related to adaptive morphology, and not only tolerance mechanisms to physiological stresses.

In terms of practical applications, Dingkuhn et al. (2005) suggested that models of phenotypic plasticity can be used to assist phenotyping procedures, particularly with regard to process-based traits (or behavioural traits) that cannot be measured directly on the plant. Two recent examples show that model-assisted phenotyping by heuristics (Hammer et al. 2002) can provide quantitative trait loci (QTLs) that would be difficult to obtain with established phenotyping methods. First, Reymond et al. (2003; 2004) used a simple regression model to extract genotypic parameters from leaf elongation kinetics observed on maize. The experiment was designed to isolate soil water deficit, temperature and VPD as environmental variables while minimizing other sources of variation such as leaf rank, plant age or nutrition. The approach proved objectively superior to its conventional equivalent, namely, static measurements of leaf dimensions. In the second example, a simple phenological model was used to correct specific leaf area (SLA) observations on barley for bias caused by developmental stage (Yin et al. 1999; 2003). Here again, model-assisted phenotyping removed some genotype × environment (G×E) 'noise' from observations and, thus, gave more specific and significant information on QTLs.

In the two examples cited, extremely simple models were used that transformed observations on the basis of existing knowledge (heuristics), in order to remove unwanted bias from measurements. This may not be enough if behavioural traits involved in phenotypic plasticity are the target. As we have argued, such traits are expressed within a complex system of phenological and trophic interactions at the whole-plant level, and can only be extracted using a more holistic modelling approach. The principle, however, remains the same: the model is parameterized by adjusting its parameters to observation. The parameters are then considered species traits (ideally, genes) and the genotypic parameter values are considered varietal traits (ideally, alleles). The parameter values are then correlated with molecular-genetic information using either a QTL approach (multiple recombinants of 2 genotypes, infinite number of possible loci) or an association-mapping approach (polymorphisms of few candidate genes (=loci), infinite number of genotypes). The technical difficulty of measuring plasticity parameters must thereby be overcome by means of statistical parameter optimization against target files containing information on directly measurable traits. In the case of Ecomeristem, such parameterization methods are currently being tested with the objective to achieve high-throughput methodologies for phenotyping purposes.

Model-assisted phenotyping for complex traits is a new field of research. An attempt was made to calibrate some parameters of an agronomic yield model of barley with known QTL effects (Yin et al. 2000). Thus parameterized, the model was to some extent predictive of yield and biomass of recombinants, but errors remained large. Improved versions of Ecomeristem are currently being used for model-assisted phenotyping of rice populations using both QTL and association-mapping approaches, but results are not yet available. This applies also to an ongoing, model-assisted study on a rice KO mutant having modified architecture (Luquet et al. unpublished).

CONCLUSION

We tried to develop a conceptual framework for the analysis and simulation of crop growth driven by either assimilate source or sink capacity, depending on environmental conditions and morphogenetic processes at the plant scale. We found that such a framework needs to take into account feedback effects of the plant's resource status on meristem behaviour, as well as mechanisms to cope with temporary imbalances. These mechanisms include the management of transitory reserves, organ senescence and end-product inhibition of photosynthesis, and are generally related to sugar metabolism. This conclusion is in line with recent molecular findings on the prominent place of sugar sensing and the regulation of sucrose cleavage at sink sites. A model integrating these phenomena in a simplified manner, called EcoMeristem, was developed and is being applied to phenotyping objectives in the area of functional genomics of rice.

The effective usefulness of complex, whole-plant models in heuristic phenotyping approaches remains to be demonstrated. Specific problems are related to the stability of process-based crop parameters across environments, as well as the measurement of such crop parameters that are inaccessible to direct observation. On the other hand, models of the proposed type may be the only means to analyse quantitatively the traits governing phenotypic plasticity and to relate them to the behaviour of the tissues that are at their origin, the meristems. Furthermore, models of phenotypic plasticity may be much better suited than the available, agronomic models to develop *in silico* improved plant type concepts.

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CHAPTER 14

YIELD IMPROVEMENT ASSOCIATED WITH *Lr19* TRANSLOCATION IN WHEAT

Which plant attributes are modified?

D.J. MIRALLES[#,##], E. RESNICOFF[#] AND R. CARRETERO[#]

[#] University of Buenos Aires, Department of Plant Production, Av. San Martin 4453
(C1417 DSE), Ciudad de Buenos Aires, Argentina.
[##] CONICET (National Council of Scientific and Technical Research).
E-mail: miralles@agro.uba.ar

Abstract. Resistance to three rust pathogens (leaf rust, stripe rust and stem rust) is related to different resistance genes. Leaf-rust resistance gene *Lr19*, transferred to hexaploid wheat from *Agropyron elongatum*, appears to be a promising gene not only through the resistance to rust conferred by this gene, also because of yield increases produced in different backgrounds when alien chromatin carrying *Lr19* is introgressed in wheat. It was reported that *Lr19* was associated with increases in grain yield. Aerial biomass was also increased when *Lr19* was introgressed, although differences were not associated with improved light interception (indirectly measured) or radiation use efficiency (RUE). The physiological basis of the increased biomass and the mechanisms causing increased number of grains per spike, in terms of dynamic of floret development, are not completely understood.

The objective of this study was to determine the performance of a near-isogenic line (cv. Bourlaug) differing in *Lr19* in relation to: (i) the differences in grains per spike, by analysing the dynamics of floret primordia; and (ii) the dynamics of biomass partitioning between the spike and the rest of the vegetative organs pre- and post-anthesis.

Two field experiments were carried out during the 2001 and 2003 growing seasons; one near-isogenic line (cv. Bourlaug) was grown under potential conditions (i.e., without water and nutritional limitations). Also a check without *Lr19* was grown.

The results showed that *Lr19* was associated with increases in yield and a higher number of grains per unit area than the check. An increase in biomass was only observed in the 2003 growing season. Non-significant differences were observed in cumulative radiation intercepted between lines. Although RUE differed between growing seasons (i.e., 1.53 and 2.07 g MJ^{-1}), there was no significant difference between the *Lr19* and check genotype. In both years *Lr19* allocated more assimilates and nitrogen to the spike (14% and 50% more biomass and nitrogen, respectively), and this phenomenon was associated with more fertile florets per spike.

Summarizing the data, it can be concluded that the *Lr19* gene promotes the partitioning of assimilates to the reproductive organs and the nitrogen partitioning to the spike. This resulted in an increased number of fertile florets per spike and number of grains per unit area, without effecting number of spikes per unit area and crop development. Increases in biomass were not always evident.

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INTRODUCTION

Different genes are known to confer resistance to three rust pathogens (leaf rust, stripe rust and stem rust). Leaf-rust resistance gene *Lr19*, transferred to hexaploid wheat from *Agropyron elongatum* by Sharma and Knott (1966), appeared to be promising also for yield and biomass increases (Singh et al. 1998). Reynolds et al. (2001; 2005) using isogenic lines with the 7DL.7Ag translocation (containing the *Lr19* gene) demonstrated increases in yield of ca 9% across different backgrounds, explained by increases in radiation use efficiency (RUE) during post-anthesis, which were related to improved number of grains per unit land area (Reynolds et al. 2005).

Increased yields in near-isogenic lines with the 7DL.7Ag translocation were associated with a more favourable partitioning of assimilates to the spike and as a consequence a larger number of grains per spike (Reynolds et al. 2001; 2005). However, the mechanisms associated with increased number of grains per spike, in terms of dynamics of floret development have not been analysed yet.

The objective of this study was to evaluate the performance of near-isogenic lines (cv. Bourlaug) differing in *Lr19* allelic form to determine the dynamics of floret primordia development together with that of biomass accumulation and partitioning.

MATERIALS AND METHODS

Two field experiments were carried out during the 2001 and 2003 growing seasons at the experimental field of the Department of Plant Production, Faculty of Agronomy, University of Buenos Aires (35°35′ S, 59°29′ W; 25 m a.s.l.), Argentina. A pair of near-isogenic lines of cultivar Bourlaug (with and without the 7DL.7Ag translocation; from now on '+*Lr19*' and 'Check', respectively), kindly provided by Dr. M. Reynolds (CIMMYT), was sown on 29 June 2001 and 17 July 2003 at a density of 300 plants m^{-2} in plots of 9 rows, 0.15 m apart and 3 m long. Treatments were arranged in a randomized block design with three replicates. ANOVAs were performed in order to determine the impact of treatments, and significant differences among means were compared using least significant differences (LSD, $\alpha = 0.05$). The degree of association between variables was established by linear regressions.

Plots were irrigated to complement natural rainfall during the whole crop cycle maintaining soil near to field capacity. Urea was applied at sowing to reach a soil nitrogen availability of 150 kg N ha^{-1}. Phosphorus fertilizer was not applied as soil levels at sowing were higher than 20 mg kg^{-1}. Fungicides and insecticides were applied to prevent diseases and pest. Weeds were manually removed throughout the crop cycle.

Development stages at the beginning of stem elongation (DC30) and at anthesis (DC65) were determined. From emergence to anthesis, incident and transmitted global radiation were measured on clear days at noon. The percentage of intercepted radiation (IR%) was calculated considering the incident and transmitted radiation at ground level and the incident radiation measured over the crop canopy. The dynamics of IR% during crop growth was fitted by a sigmoid function. The cumulative intercepted radiation was estimated from the fraction of daily intercepted radiation and daily total incident radiation over time. To determine the dynamics of dry-matter accumulation (in spike and vegetative organs) from emergence to anthesis at least six samples of 50 cm in a central row of aerial biomass were taken from each treatment. RUE during pre-flowering was calculated as the slope of the relationship between cumulative biomass and cumulative global radiation intercepted by the crop. At maturity plant samples of 1 m in a central row of each plot were taken and biomass, yield and its components were recorded.

In 2001, the dynamics of floret development was also followed. For that purpose, two plants per plot were randomly sampled twice weekly and their spikes were dissected and the total number of floret primordia counted. In each sample the score of each floret within basal, central and apical spikelet was assessed following the Waddington et al. (1983) scale.

RESULT AND DISCUSSION

Crop development

There was no effect of the 7DL.7Ag translocation on phenological development. In fact, flowering date for both lines was the same (10 and 24 October in 2001 and 2003, respectively) and timing of the occurrence of the different pre-flowering stages was also unaffected by treatments (data not shown). These data confirm those reported by Reynolds et al. (2001), who did not find differences in phenology between +*Lr19* and the Check line in different backgrounds. Final leaf number and phyllochron (120 °Cd per leaf) was also the same in both lines.

Biomass and its physiological components

No significant differences were observed in biomass at anthesis, or in its physiological determinants (Table 1). However, in 2003 the 7DL.7Ag translocation was significantly associated with higher biomass at harvest, indicating an improved post-anthesis growth because of the introgression.

Yield and its components

The 7DL.7Ag translocation increased grain yield (23 and 35% in 2001 and 2003, respectively) and increased the number of grains per unit area without significant effects on average grain weight (Table 2). Since there were no significant effects on the number of spikes per unit area (although during 2003 +Lr19 established 13% more spikes per m^2 than the Check) the main effect 7DL.7Ag translocation on grains per unit area was associated with changes in the number of grains per spike. Thus, the number of grains per spike was in both years increased by ca. 18% if the line carried the translocation (Table 2). The data obtained in this study showed a similar increase in grains per spike (in relative terms) to those reported for different backgrounds by Reynolds et al. (2001; 2005).

Although the increase in grains per spike associated with the 7DL.7Ag translocation was a consequence of the higher number of fertile florets per spike in both years, the differences were only significant in 2001. In 2003 a slight difference was observed between genotypes, suggesting that grain setting (i.e., the proportion of fertile florets (FF) that form grains) could also be responsible for the differences in grain number between *+Lr19* and the Check (Table 3).

Since the 7DL.7Ag translocation did not modify crop development, the total number of spikelets was not affected. Thereby, differences in fertile florets per spike were mainly explained by changes in the number of FF per spikelet (2001) and by differences in the number of fertile spikelets per spike (2003). The difference in number of fertile florets per spikelet in the *+Lr19* respect to the Check observed in the 2001 growing season was evident in those spikelets placed in the central and apical position into the spike without significant differences in the basal spikelets (Figure 1).

Table 1. *Accumulated biomass (at flowering and at harvest) in main stems, tillers and total (main stems+tillers) and harvest index in both experimental years*
SE indicates the standard error of means

	Biomass at flowering ($g\ m^{-2}$)	Biomass at harvest ($g\ m^{-2}$)	Harvest index ($g\ g^{-1}$)
2001			
Check	627.2	754	0.34
+Lr19	712.9	774	0.40
SE	114.6	120.2	0.032
2003			
Check	812.9	934.4	0.47
+Lr19	844.7	1224.5	0.46
SE	113.8	119.4	0.0043

Table 2. *Plant height, grain yield and yield components*
SE indicates the standard error of means

	Height (cm)	Yield ($g\ m^{-2}$)	No. of grains (m^{-2})	Grain weight (mg)	No. of grains ($spike^{-1}$)	No. of spikes (m^{-2})
2001						
Check	62.3	248.8	7330	33.7	21.9	340
+Lr19	56.8	306.0	8383	36.5	26.0	342
SE	1.51	54.1	804.2	3.6	2.2	14.6
2003						
Check	84.5	424.3	11474	37.0	25.8	444
+Lr19	91.0	572.4	15186	37.5	30.3	502
SE	8.1	87.4	1826	1.6	2.4	17.5

Table 3. *Total and fertile spikelets per spike, fertile florets (FF) per spike and per spikelet for both genotypes (Check and* +Lr19*) in the 2001 and 2003 growing seasons*
SE indicates the standard error of means

	Number of spikelets		Fertile florets	
	Total	Fertile	spike^{-1}	spikelet^{-1}
2001				
Check	19.7	18.0	59.7	3.3
+*Lr19*	20.3	18.5	67.8	3.7
SE	0.7	0.6	4.7	0.1
2003				
Check	16.5	14.8	44.8	3.0
+*Lr19*	16.6	15.6	46.7	3.1
SE	0.8	1.1	4.1	0.3

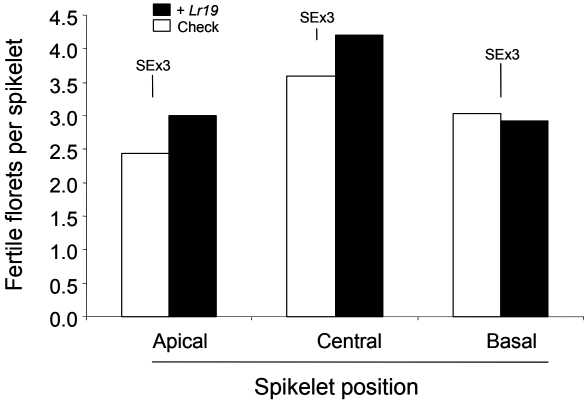

Figure 1*. Fertile florets per spikelet for different positions within the spike (apical, central and basal spikelets) in the* +Lr19 *(closed bars) and Check (open bars) lines. Vertical lines indicate three times the standard error. Data correspond to the 2001 growing season*

The analysis of the dynamics of floret primordia development showed that the increased spikelet fertility in the +*Lr19* was a consequence of a higher rate of development of the floret primordia (Figure 2). When floret development was plotted against thermal time, it could be observed that some florets at the same position on the spikelet progressed more in the +*Lr19* line than in the Check. For instance, considering the central spikelets into the spike, there was no difference in development for the first three floret primordia placed close to the rachis (F1 to F3), however, the 4th (F4) and 5th (F5) floret primordia reached a higher floret score in the +*Lr19* than in the Check (Figure 2).

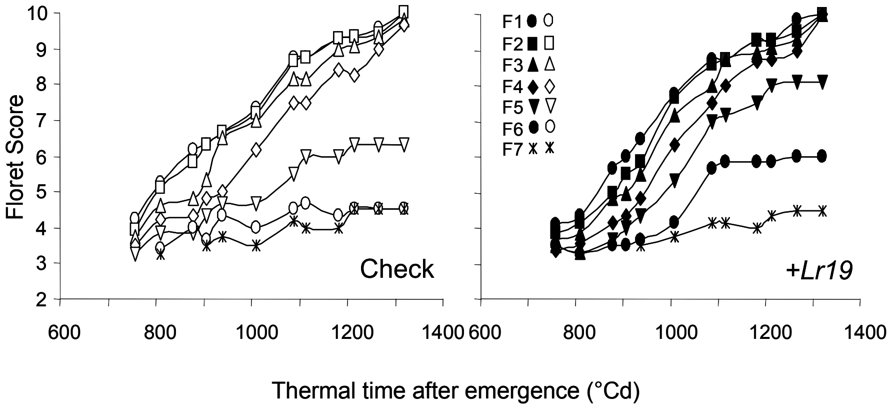

Figure 2. *Development of all florets primordia within the central spikelets in the* +Lr19 *and Check genotype in the 2001 growing season. Floret positions are indicated from F1 to F7, F1 being the floret closest to the rachis*

Reynolds et al. (2001) found that 7DL.7Ag translocation increased assimilate partitioning with ca. 13% to the spike. The results of this study showed that the dynamics of spike growth (mainly in 2001) were different between *+Lr19* and Check. The rate of biomass accumulation in the *+Lr19* was significantly higher than that observed in the Check between booting and flowering, resulting in a higher spike dry weight at anthesis (Figure 3). On the other hand, no significant differences were evident in the dynamics of shoot (stem+leaves) growth among genotypes.

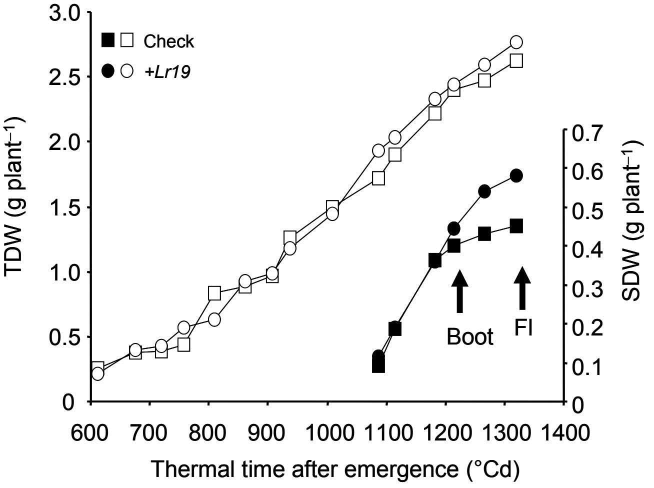

Figure 3*. Dynamics of total (TDW, open symbols) and spike (SDW, closed symbols) dry weight per plant in the* +Lr19 *and Check genotypes in the 2001 growing season. Black arrows indicate the stages of booting (Boot) and flowering (Fl)*

When the number of grains per unit area was plotted against the spike dry weight at flowering a positive association was found in both genotypes. However, the slope of the linear regressions was higher in the +*Lr19* (84 grains g^{-1}) than in the Check (66 grains g^{-1}), suggesting a more efficient investment of spike dry weight to produce grains in the +*Lr19* than in the Check.

In 2001, the spike nitrogen concentration was measured at anthesis. The data showed that, as was observed for spike biomass at anthesis, +*Lr19* also increased the nitrogen concentration in the spike. The percentages of N content in the spike at anthesis were 1.54 and 1.09% for the +*Lr19* and Check, respectively. Abbate et al. (1995) suggested a direct effect of nitrogen concentration on the number of grains per spike. Thus, the higher number of grains per spike observed in the +*Lr19* could be associated with either (i) an improved assimilates partitioning to the spike at anthesis or (ii) a more favourable nitrogen partitioning to the spike.

Although various studies highlighted the impact of assimilates availability during pre-anthesis on the number of fertile florets (e.g., Fischer and Stockman 1980; Fischer 1985; Reynolds et al. 2001), none of them have studied floret development. The results of this study show that the increased number of fertile florets associated with the 7DL.7Ag translocation was a result of a continued development of distal floret primordia within the spikelets, probably associated with a more favourable carbohydrate and nitrogen acquisition by the spikes at anthesis. It is important to highlight that even when a more favourable partitioning to the spike could be produced by different ways, as, for example: dwarfing-genes introgression (Miralles et al. 1998); altering the duration of the spike growth period (Miralles et al. 2000; Slafer et al. 2001; González et al. 2003b; 2003a); and introgression of alien chromatin carrying *Lr19* (Reynolds et al. 2001), the consequence on the number of fertile florets is exactly the same, i.e. increasing spike fertility. All this evidence suggest that independently of the mechanism involved, promoting a higher spike growth during pre-anthesis appears to be the common strategy to increase fertile-floret survival and thereby increase the number of grains per unit area and yield (Slafer et al. 2005).

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PHYSIOLOGY AND MODELLING OF CROP ADAPTATION

CHAPTER 15

SIMULATION ANALYSIS OF PHYSIOLOGICAL TRAITS TO IMPROVE YIELD, NITROGEN USE EFFICIENCY AND GRAIN PROTEIN CONCENTRATION IN WHEAT

P. MARTRE[#], M.A. SEMENOV[##] AND P.D. JAMIESON[###]

[#] INRA, UR874 Agronomie, Clermont-Ferrand, F-63100 France.
[##] Biomathematics and Bioinformatics, Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, UK.
[###] New Zealand Institute for Crop and Food Research Ltd., Private Bag 4704, Christchurch, New Zealand.
E-mail: pmartre@clermont.inra.fr

Abstract. The pressure of economic cost and environmental constraints dictates that farmers must optimize the use of nitrogen fertilizer. Industrial uses of new wheat varieties require specific and stable grain protein concentration, which needs accurate estimation of N demand during the crop cycle. Thus breeding for high N use efficiency (NUE) and yield, whilst maintaining high grain protein concentration, is of high priority for cereal geneticists. Here, the wheat simulation model *SiriusQuality*1 was used to analyse the effect of variation in physiological traits on wheat NUE, grain protein composition and concentration under variable climate and conventional and limited N supply conditions. Twenty-three of the 53 parameters of *SiriusQuality*1 were selected for sensitivity analysis based on a literature survey – four parameters were related to phenology and canopy development, seven to crop C assimilation and partitioning, eight to crop N uptake and assimilation, and four to grain development and C and N accumulation. Variations in weather and N treatments induced larger variations in NUE than most of the physiological traits considered. The simulations suggest that a single physiological trait is unlikely to break the negative correlation between the grain protein concentration and yield over a wide range of sites and seasons, especially under low N input environments. Increasing the N storage capacity of the leaves and stem and the allocation of N to non-structural proteins appeared as the more promising strategy to breaking the negative correlation between grain yield and protein concentration.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the major European arable crop with a total annual production of 212 Mt of grains. Nitrogen fertilization is an important component of

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wheat production and quality, and over 35% of the total annual N-fertilizer applications in the UK and France are given to wheat crops. These fertilizers have a direct economic cost to growers, but are also responsible for environmental impacts on groundwater quality through N leaching. The use of fossil fuels for their manufacture and application, plus N_2O emissions associated to denitrification, all potentially contribute to global warming. More efficient use of N fertilizers by wheat crops is thus particularly important.

Minimizing environmental impacts of applied N inputs requires N-efficient crops with lower fertilizer-N requirements. Crop N use efficiency (NUE) has been defined in different ways in the literature, depending on the objectives (Moll et al. 1982; Peng and Bouman in press). Here, our interest is to consider the productivity of N, regardless of its origin (added N fertilizer, inorganic soil N, organic soil N mineralized during the growing period), in order to define genetic traits that improve N utilization and minimize N losses. Therefore, we define NUE as the ratio of grain yield to total available soil N during the crop growth cycle. Using this definition, NUE can be decomposed into two components: the efficiency of apparent N uptake from the soil (NUpE, i.e., the ratio of crop N to the total amount of available N) and the utilization efficiency (NUtE, i.e., the ratio of yield to crop N content).

Proteins are the most important components of wheat grains governing end-use quality (Weegels et al. 1996), and variations in both protein concentration and storage protein composition significantly modify flour end-use quality (Wrigley et al. 1998; Lafiandra et al. 1999; Branlard et al. 2001). Storage proteins are divided into two broad fractions, which are the main contributors to the rheological and bread-making properties of wheat flour (Shewry and Halford 2002). Glutenins are mainly responsible for visco-elastic properties, and gliadins are important in conferring extensibility to dough (Branlard et al. 2001). The glutenin:gliadin ratio is a measure of molecular-weight distribution or protein size, and determines the balance between dough viscosity and elasticity independently of total protein concentration, and therefore affects dough rheological behaviour (Uthayakumaran et al. 1999).

Genetic improvement for traits such as yield or grain protein concentration is complicated. First, the complications arise because the traits result from several linked processes. Second, it is difficult to select for traits that are sensitive to environmental variations and show significant genotype × environment interactions. Quantitative-trait loci (QTLs) for grain protein concentration usually explain less than 10% of the observed variations and show low environmental stability (Blanco et al. 2002). One possibility for overcoming this difficulty is to link crop simulation models with genetic analysis. Simulation models relate a trait to various processes subjected to a range of environmental conditions, with parameters independent of the environment and characteristic of a genotype. Thus, simulation models specifically describe traits × traits and traits × environment × management interactions. Until recently, there have been only tentative relationships between these parameters and genotypes, and until very recently gene-based approaches to modelling have not received much attention (White and Hoogenboom 2003; Wollenweber et al. 2005). Simulation models often used empirical response curves (e.g., N dilution curve) and failed to link model parameters with physiological traits.

Current advances in the understanding of N uptake and redistribution and of wheat phenology allow us to model these processes more mechanistically, and it is now possible to link model parameters with physiological traits (Martre et al. 2006). In theory, such links allow the possibility of associating these model parameters with loci or genes (Yin et al. 2003; Quilot et al. 2005).

In the present study, we used the wheat simulation model *SiriusQuality*1 (Martre et al. 2006) to analyse the effect of different physiological traits on NUE, grain protein composition and concentration under variable climate and conventional and limited N supply conditions. Two sites where considered (Clermont-Ferrand, France and Rothamsted, UK). The effect of the climate at these two sites was assessed by running the model with over 30 years of observed climate data. Selection of physiological traits to increase NUE may involve antagonistic criteria, such as high grain yield, protein concentration and low N fertilizer use. Therefore, the effects of the identified physiological traits on the relationship between grain yield and protein concentration were investigated.

MATERIAL AND METHODS

The SiriusQuality1 *wheat simulation model*

The model used in this study is based on Sirius (Jamieson et al. 1998). It consists of sub-models that describe phenological and canopy development, biomass and N accumulation and partitioning, including responses to shortages in the supply of soil water and N. Canopy development is simulated as a series of leaf layers associated with individual main-stem leaves, and tiller production is simulated through the potential size of any layer (Lawless et al. 2005). The canopy intercepts light and uses it to produce biomass at an efficiency (radiation use efficiency, RUE) calculated from temperature, CO_2 concentration, water stress and the ratio of diffuse to direct radiation (Jamieson et al. 2000). The canopy radiation extinction coefficient, K, is assumed to be independent of N and water shortages (Robertson and Giunta 1994). The RUE in Sirius is also independent of N supply because a major assumption is that the specific leaf N concentration (SLN, N content expressed per unit green area) is constant (Grindlay 1997; Jamieson and Semenov 2000). Hence, shortage of N limits leaf area, and thus light interception, rather than RUE. Transfer of dry matter (DM) and N to grain after anthesis, and partitioning of grain N between gliadins and glutenins have been described in detail (Martre et al. 2006), as well as calculations of phenological development, evapotranspiration, soil water and N distributions (Jamieson et al. 1998). Sirius has been calibrated and evaluated for several modern wheat cultivars and tested in many environments and climates, including conditions of climate change (Jamieson et al. 1998; 2000; Jamieson and Semenov 2000; Martre et al. 2006).

Sites, N treatments, weather and cultivars

For this analysis, we selected two European sites with contrasting climates: Clermont-Ferrand, France (45°47' N, 3°10' E, 329 m elevation) and Rothamsted, UK (51°49' N, 0°21' W, 128 m elevation). Simulations were carried out over 32 and 40 years of daily weather records for Clermont-Ferrand and Rothamsted, respectively. We considered the same soil at both sites, with a rooting depth of 1.0 m, a plant-available water-holding capacity of 160 mm, 8 Mg ha^{-1} of organic N and 20 kg N ha^{-1} of inorganic N at sowing. Two N treatments were considered: a non-limiting N treatment (high N, HN) and a limiting N treatment (low N, LN). Nitrogen was applied as split dressings at the development stages described by the Zadoks scale (Decimal Codes (DC), Zadoks et al. 1974); in the HN treatment, three dressings of 50 kg N ha^{-1} at DC 21, 32 and 39, and one time 100 kg N ha^{-1} at DC 30. In the LN treatment two dressings of 40 kg N ha^{-1} were applied at DC 31 and 37.

In order to assess specifically the effects of climate, N fertilization and physiological traits on grain yield, NUE and protein concentration, only one set of cultivar-specific parameters, for the French winter bread-wheat cultivar Thésée, was used (Martre et al. 2006). For each year, the sowing date was held at November 1 for Clermont-Ferrand and October 10 for Rothamsted.

Observed weather for simulations consisted of 32 years for Clermont-Ferrand and 40 years for Rothamsted. Monthly average maximum daily temperature is 1.1 to 4.4 °C higher at Clermont than at Rothamsted all year around (data not shown). Monthly average minimum daily temperature is 0.5 to 2.6 °C higher at Clermont-Ferrand than at Rothamsted from March till October, but is ca. 1 °C lower at Clermont-Ferrand than at Rothamsted from November till February. Monthly total solar radiations show similar variations during the year at both sites, with Clermont-Ferrand receiving on average 80 MJ m^{-2} $month^{-1}$ more solar radiation than Rothamsted. Mean annual cumulated rainfall is 18% lower at Clermont-Ferrand than at Rothamsted (587 vs. 693 mm y^{-1}), and autumn and winter are drier in Clermont-Ferrand, whereas the spring and the summer are drier at Rothamsted.

PARAMETERS OF PHYSIOLOGICAL TRAITS

Below are physiological traits that were examined for improving wheat yield, NUpE and/or NUtE, and grain protein concentration. These parameters are defined in Table 1. They were modified independently in the model by + and –30% of their default values in 10% increments. This range of variations probably encompasses the genetic variability that could be expected for these different traits in wheat (Table 1).

Phenology and canopy development

Modifying the duration of crop photosynthesis and its timing in relation to seasonal variations of resource availability may have significant effects on DM and N yields (Akkaya et al. 2006; Richards 2000). Past increases in wheat yield have been associated with shortening the duration of vegetative development phases (Calderini et al. 1997; Donmez et al. 2001). The rate at which the leaves appear, determined by

Table 1. *Symbols, definitions, and units of the most important parameters of the SiriusQuality1 wheat simulation model*

Parameter	Definition	Default value	Unit	Genetic variability		
				Nb of cultivars	Min–max	References
Phenology and canopy development						
P	Phyllochron	97	°Cday	4	78–110	Asseng et al. (2002)
				20^{a}	93–119	Mosaad et al. (1995)
				8^{b}	91–101	Giunta et al. (2001)
$P_{\mathrm{flag}}^{\mathrm{anth}}$	number of P between flag leaf ligule appearance and anthesis	3	phyllochron			
α_{max}	maximum potential size of biggest leaf layer (including the ear)	60	cm^{2} mainstem^{-1}	8	22.1–39.7^{c}	Shearman et al. (2005)
				8	34.0–50.3^{c}	Fischer et al. (1998)
				10	41.3–87.6^{d}	Motzo and Giunta (2002)
P_{sen}	fraction of P for leaf senescence	1.8	phyllochron			
Crop C assimilation and partitioning						
K	radiation extinction coefficient, based on intercepted PAR	0.45	dimensionless	3	0.63–0.76	Yunusa et al. (1993)
				5	0.36–0.57^{e}	Green (1989)
				3^{f}	0.48–0.78	Miralles and Slafer (1997)
				7	0.37–0.46^{g}	Calderini et al. (1997)
				8	0.45–0.70	Shearman et al. (2005)
				3	0.66–0.76	Muurinen and Peltonen-Sainio (2006)
RUE	radiation use efficiency, based on PAR	2.46	g DM MJ^{-1}	3	1.5–2.4	Yunusa et al. (1993)
				3^{f}	2.27–2.79^{h}	Miralles and Slafer (1997)
				7	1.96–2.5^{h}	Calderini et al. (1997)
				8	2.33–2.64	Shearman et al. (2005)
				3	1.69–2.04	Muurinen and Peltonen-Sainio (2006)
SLW	specific leaf mass	45	g DM m^{-2}	8	40.8–54.0	Shearman et al. (2005)
				8	45.1–53.9^{g}	Fischer et al. (1998)
				4^{i}	32.2–45.0	Duggan et al. (2005)

Table 1 (cont.)

Table 1 (cont.)

Parameter	Definition	Default value	Unit	Genetic variability		
				Nb of cultivars	Min–max	References
D_{eg}	fraction of $P_{\mathrm{flag}}^{\mathrm{anth}}$ for ear growth	0.25	dimensionless			
μ	fraction of biomass increment allocated to ear during the ear growth period	0.5	dimensionless	6 5	0.16–0.58 0.28–0.33	Slafer et al. (1990) Abbate et al. (1998)
σ	Ratio of grains number to ear DM at anthesis	100	grains g^{-1} ear DM	5 8	62–106 73–129	Abbate et al. (1998) Shearman et al. (2005)
γ	fraction of crop DM at the end of the endosperm cell division stage remobilized during grain filling	0.25	dimensionless	6	0.13–0.36	Austin et al. (1977)
Crop N uptake and assimilation						
$RVER$	rate of root vertical extension	0.001	m $(°\mathrm{Cday})^{-1}$			
$N_{\mathrm{max}}^{\mathrm{uptake}}$	maximum N uptake rate at anthesis	0.4	g N m^{-2} day^{-1}			
k_{sen}	scaling parameter modifying the rate of root N uptake decrease after anthesis	1	dimensionless	-	-	-
$[N]_{\mathrm{max}}^{\mathrm{stem}}$	maximum stem N concentration	10	mg N g^{-1} DM	3^{j} 21 3	10–26 7.6–14.9 6.7–9.5	Kim and Paulsen (1986) Triboï and Ollier (1991) Ma et al. (1996)
SLN	specific leaf N concentration	1.5	g N m^{-2}			
$[N]_{\mathrm{stru}}^{\mathrm{leaf}}$	leaf structural N concentration	6	mg N g^{-1} DM	6	3.5–6.9	Halloran (1981)
$[N]_{\mathrm{stru}}^{\mathrm{stem}}$	stem structural N concentration	3	mg^{-1} N g^{-1} DM	15^{b} 6	1.7–5.1 2.1–4.0	Desai and Bhatia (1978) Halloran (1981)
β	scaling parameter modifying the rate of crop N remobilization during grain filling	1	dimensionless	-	-	-

Table 1 (cont.)

Table 1 (cont.)

Parameter	Definition	Default value	Unit	Genetic variability		
				Nb of cultivars	Min–max	References
Grain development and C and N accumulation						
D_{gf}	thermal time from anthesis to end of grain filling	750	°Cday	14	600–820	Loss et al. (1989)
				16	717–870	Robert et al. (2001)
				16	719–875	Akkaya et al. (2006)
				194[k]	514–737	Charmet et al. (2005)
D_{cd}	duration of the endosperm cell division stage	250	°Cday	5	176–330	Gleadow et al. (1982)
$\alpha_{N:C}$	grain structural C to N ratio	0.02	$(°Cday)^{-1}$			
k_{cd}	relative rate of accumulation of grain structural C	0.0084	$(°Cday)^{-1}$	11	0.0144–0.0161[l]	Darroch and Baker (1995)

[a] spring wheat cultivars grown under greenhouse conditions with 12 h photoperiod

[b] durum wheat cultivars

[c] flag leaf area

[d] ear plus flag leaf area for awned and awnless isogenic lines of durum wheat

[e] values reported based on intercepted solar radiation and adjusted to intercepted PAR basis by multiplying by 1.3 (Abbate et al. 1998)

[f] tall, semi-dwarf, and dwarf isogenic lines of bread wheat grown under field conditions

[g] not significantly different among cultivars

[h] values reported based on intercepted solar radiation and adjusted to intercepted PAR basis by dividing by 0.48.

[i] pairs of isogenic lines of spring bread wheat differing for the presence of the tillering inhibition gene (*tin*) grown outdoor in tubes

[j] tall, semi-dwarf, and dwarf isogenic lines of winter bread wheat grown in nutrient solutions in a controlled environmental chamber

[k] recombinant inbred lines

[l] use a base temperature of 5 °C

the phyllochron (P), will influence the rate at which the canopy develops and soil N and water are depleted – increasing this rate will shorten the duration of the vegetative development phase. Consistent with P, the duration of the period between the appearance of the flag leaf ligule and anthesis, $P_{\text{flag}}^{\text{anth}}$, was varied in these simulations. Preliminary simulations have shown that vernalization and photoperiod responses have a small effect on the simulated duration from sowing to anthesis or on final leaf number. These parameters were not included in this study.

Increasing the green-area index (GAI) at anthesis may delay the senescence of the canopy and increase the duration of grain filling, and therefore grain yield, especially under limiting N supply (Austin 1999). In the model, the potential size of the culm leaves is scaled based on α_{max}. Changes in α_{max} simulate a change in the leaf area development and the overall potential in the crop GAI at anthesis. Maintaining green leaf area longer, particularly after anthesis, is another mean to increase crop yield, and possibly crop N if N uptake is also maintained (Austin 1999; Triboï and Triboï-Blondel 2002). In *SiriusQuality*1 the ontogenic rate of leaf senescence is constant and is determined by the parameter P_{sen}.

Canopy architecture also becomes important once leaf area index exceeds about three (Reynolds et al. 2000), and has been used to improve light distribution within the canopy and optimize canopy carbon gain (Long et al. 2006). The radiation extinction coefficient (K) represents an integrative measure of the canopy architecture in term of light interception, and is a potential target for increasing RUE. Significant genetic variability for K has been reported for wheat (Abbate et al. 1998), although Shearman et al. (2005) did not find any genetic difference in K for the eight UK wheat cultivars they studied.

The contribution of the stem to grain N content at maturity is close to that of the leaves (Spiertz and De Vos 1983). One possibility for increasing NUpE and grain protein concentration would be to select genotypes with larger stem mass for N storage and subsequent translocation to the grain. Shearman et al. (2005) reported a linear increase of stem (including leaf sheaths) biomass at anthesis with the year of release of UK winter wheat cultivars. An increase in the stem in comparison to the leaf might increase the N storage capacity and slightly decrease the carbon input so that an overall shift to an increase in plant N:C ratio might be achieved. In *SiriusQuality*1, stem DM is calculated as the excess DM after leaf DM has been calculated assuming a fixed specific leaf mass (SLW). SLW is less than its maximum only early in life of the crop, if there is insufficient biomass for leaf tissue of that thickness. Thus decreasing SLW in the model induces an earlier stem growth and increases the stem:leaf ratio at anthesis and the N storage capacity of the stem. In *SiriusQuality*1, the stem includes the ear, and thus changes in stem DM due to SLW variations reflect variations of the biomass of the true stem or/and of the ear.

Past gains in crop yield have often been associated with increases of grain number per ground area unit (Reynolds et al. 1999; Donmez et al. 2001; Shearman et al. 2005). However, this relationship may reflect more the adjustment of the sink size to the capacity of the source to fill them, than a causal relationship (Sinclair and Jamieson 2006). Although, within the Sirius framework, accumulation of grain DM and N is calculated independently of ear growth and grain number (Jamieson et al.

1998), in *SiriusQuality*1 grain number is a coupling variable between DM and N supplies, defined at the crop scale, and the grain demand for structural/metabolic DM and N, defined at the grain scale (Martre et al. 2006). Thus, in *SiriusQuality*1 grain DM and N are not fully independent of ear growth and grain number. Grain number per unit area is also needed to partition storage protein between gliadin and glutenin fractions (Martre et al. 2003). Grain number is computed as in ARCWHEAT1 (Weir et al. 1984), where it is calculated from the ear mass at anthesis assuming a constant number of grains per unit ear dry mass (σ). Ear biomass is assumed to accumulate during a fraction D_{eg} of the thermal time between the appearance of the flag leaf ligule and anthesis. During that period of time, a fraction μ of the biomass accumulated each day is allocated to the ear. These parameters have been considered here, because the allocation of biomass to the ear may have feedback effects on crop biomass and N. Genetic variability for these parameters has been reported, especially for σ, which has been associated with genetic gain in yield in Argentina (Abbate et al. 1998). Ear to stem DM ratio at anthesis has also increased in modern cultivars (Yunusa et al. 1993). In the model, this ratio is determined by μ and D_{eg}.

Finally, increasing the fraction of pre-anthesis crop DM remobilized during grain filling (γ) may also be a way to increase the DM harvest index and thus crop NUE. However, this trait may have a negligible effect on N dynamics, and may thus contribute to grain N dilution.

Crop N uptake and assimilation

A critical question regarding the increase of N accumulation by wheat plants is the role of roots in limiting N uptake. Are there critical features in the roots that are constraining the rate of N uptake by crops? While physiological research indicates that roots have a very high capacity for N uptake (Oscarson et al. 1995), in whole-plant studies there appears to be a maximum uptake by cereal crops of 0.5 to 1.0 g N m^{-2} d^{-1} (Spiertz and Ellen 1978; Sinclair and Amir 1992; Asseng et al. 2002). Is N uptake constrained by shoot activity and overall plant growth rates, or are there processes in the roots that can be altered to increase N uptake? In *SiriusQuality*1, before anthesis crop N uptake is driven by the potential expansion of green area to maintain a constant SLN, and is limited only by the capacity of the stem to store accumulated N (Martre et al. 2006), but under limiting N supply it can also be limited by the capacity of the roots to explore deep soil layers. In the model, roots are characterized by their vertical extension in the soil profile. They are assumed to extend downward at a constant rate (RVER) until they reach the soil-dependent maximum depth or anthesis, whichever occurs first (Porter 1993). Here shoot demand was modified by changing phenological and canopy development as well as vegetative storage parameters. The root capacity to explore deep soil layers was changed through RVER.

The higher grain protein concentration of high grain protein concentration isogenic lines of durum wheat results partly from increased N uptake during grain filling (Kade et al. 2005). Similarly, increased grain N content in wheat hybrids compared with their parents (Oury et al. 1995), or in maize and sorghum stay-green mutants (Rajcan and Tollenaar 1999; Borrell et al. 2001) is largely due to increased N uptake during grain filling. Maintenance of root activity after anthesis has been suggested to extend the C and N supply period as well as overcoming the trade-off between N remobilization and senescence (Richards 2000). In *SiriusQuality*1, after anthesis root N uptake is co-limited by the capacity of the stem to store N and by the activity of the roots, which decreases linearly with accumulated thermal time after anthesis to reach zero at the unconstrained end of grain filling (Martre et al. 2006). In this study, the potential maximum rate of N uptake ($N_{\mathrm{max}}^{\mathrm{uptake}}$) and the storage capacity of the stem ($[N]_{\mathrm{max}}^{\mathrm{stem}}$) were modified, and a parameter (K_{sen}) was introduced to scale the rate of root activity decrease during grain filling.

Increasing the N storage capacity of the crop may allow N to be taken from the soil more quickly, and therefore reducing potential N losses by leaching or denitrification. If this extra N can then be transferred to the grains, increasing N storage capacity might increase both NUE and grain protein concentration. In these simulations the storage capacity of the crop was modified through $[N]_{\mathrm{max}}^{\mathrm{stem}}$ and SLN. Alternative hypotheses regarding the relationship between RUE and SLN were implemented in the model: (1) RUE is independent of SLN (the assumption used in the Sirius framework), and (2) RUE depends on SLN according to the relationship given by Sinclair and Amir (1992). The former hypothesis assumes that the efficiency of the Rubisco carboxylase reaction can be engineered so that it is increased as the Rubisco concentration per unit of leaf area is decreased (Long et al. 2006).

In general, N harvest index, the ratio of grain N to total shoot N, decreases with increasing N supply (Ugalde 1993). Increase of N remobilization efficiency during grain filling, may increase both NUE and grain N content in wheat (Kichey et al. 2006), maize (Gallais and Hirel 2004) or barley (Mickelson et al. 2003). Here, increase of N remobilization efficiency was simulated by modifying the structural N concentrations of leaf ($[N]_{\mathrm{stru}}^{\mathrm{leaf}}$) and stem ($[N]_{\mathrm{stru}}^{\mathrm{stem}}$). In *SiriusQuality*1, N is supplied to grain assuming that all non-structural shoot N is available for transfer to grain. At the end of the cell division phase, the daily flux of N transferred to grain is set daily so that all of the non-structural crop N would be transferred by the unconstrained end of grain filling (Martre et al. 2006). The importance of the rate of N remobilization during linear grain filling was assessed by introducing a scaling parameter (β) to modify proportionally the rate of N remobilization.

Grain development and C and N accumulation

Increasing the length of the grain-filling period has been also suggested as a putative trait for increasing grain yield in wheat (Evans and Fischer 1999), and low, but significant, genetic variability has been reported for this trait (Robert et al. 2001; Charmet et al. 2005). The potential size and storage capacity of the grain are determined during the initial phase of endosperm cell division. Increase of the growth rate or/and duration of this phase is another proposed trait to increase grain yield and NUE. These traits were manipulated in the simulations by changing the duration of grain filling (from anthesis to end of grain filling, D_{gf}), the duration of the cell division phase (D_{cd}), the potential relative rate of accumulation of structural C (k_{cd}), and the grain structural N:C ratio ($\alpha_{\mathrm{N:C}}$). It is worth noting, that in *SiriusQuality*1, modifying D_{cd} or D_{gf} also modifies the rate of DM and N remobilization after the end of cell division phase, which are scaled based on ($D_{\mathrm{gf}} - D_{\mathrm{cd}}$). Parameters affecting the partitioning of grain protein have not been considered here, because they have no effects on grain yield, protein concentration or crop NUE.

RESULTS AND DISCUSSION

Effects of climate, sites and N treatments

Variations in NUE and yield due to climate were substantial (Figure 1A). NUE was closely related to grain yield variations, because total available soil N during the cropping period was fairly constant. The range of NUE variations was similar for both N treatments, whereas for yield it was two times higher for HN than for LN. In good agreement with experimental results (Le Gouis et al. 2000), for most of the years, NUE was higher for LN than HN treatments. NUtE was largely independent of N supply, and as reported by Dhugga and Waines (1989), NUtE became more important than NUpE in determining NUE as N supply increased.

Average yield and grain protein concentration were ca. 35% lower for LN than for HN treatments (Table 2). Grain yield was negatively correlated with grain protein concentration for both N treatments ($r = -0.51$ for both LN and HN; Figure 1B). In good agreement with observed data (Triboï et al. 2006), N deficit lowered both yield and grain protein concentration, but the negative genetic correlation was conserved and, although not statistically significant, the slope was steeper (more negative) for HN than for LN treatments.

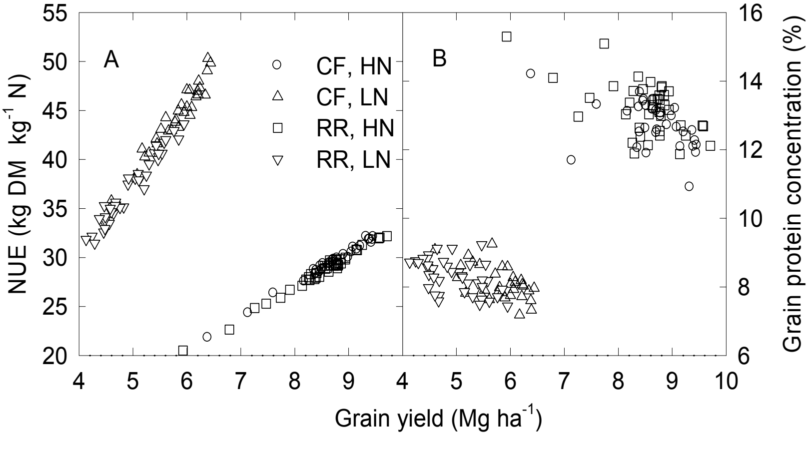

Figure 1. *Simulated N use efficiency* **(A)** *and grain protein concentration* **(B)** *versus grain yield at high (HN) low (LN) N supplies for 32 and 40 years at Clermont-Ferrand (CF) and Rothamsted (RR), respectively*

Although the weather was significantly different at the two sites, using the same cultivar and soil for HN treatments average yield was not significantly different (Table 2). Under these conditions, NUpE and NUtE, and grain protein concentration and composition were also similar at the two sites. As expected, N harvest index and NUE components were higher for HN than LN (Table 2). At low N supply, average yield was 0.8 Mg ha^{-1} higher at Clermont-Ferrand than at Rothamsted. For the LN treatment, NUE was also higher (18%) at Clermont-Ferrand than at Rothamsted, but average grain protein concentration and composition were similar at both sites.

However, the simulated gliadin and glutenin percentages and the glutenin:gliadin ratio showed an inter-year variability two times higher at Rothamsted than at Clermont-Ferrand (Table 2). In *SiriusQuality*1, grain protein composition is determined at the individual-grain level, whereas total yield and grain protein content are determined at the crop level. The higher variability of grain protein composition at Rothamsted reflects the higher variability of single grain size and protein content at this site compared with Clermont-Ferrand.

Table 2. *Average simulated grain yield, N harvest index, N use efficiencies, grain protein concentration, gliadin and glutenin percentages, and glutenin:gliadin ratio at low and high N supplies for 32 and 40 years at Clermont-Ferrand and Rothamsted, respectively. Numbers in parenthesis are the coefficients of variation (%)*

	Clermont-Ferrand		Rothamsted	
	Low N	High N	Low N	High N
Grain yield (Mg ha^{-1})	5.80 (7.9)	8.67 (7.7)	4.97 (10.0)	8.51 (8.4)
N harvest index (g g^{-1})	0.68 (3.6)	0.78 (1.6)	0.64 (4.4)	0.78 (2.2)
NUpE (kg N kg^{-1} N)	0.92 (3.6)	0.84 (6.2)	0.84 (5.1)	0.85 (5.6)
NUtE (kg DM kg^{-1} N)	48.0 (6.2)	35.1 (6.1)	44.2 (5.8)	33.7 (7.3)
NUE (kg DM kg^{-1} N)	44.3 (7.7)	29.4 (7.4)	37.1 (9.2)	28.6 (8.1)
Grain protein concentration (%)	8.11 (6)	12.72 (5.3)	8.30 (5.9)	13.21 (5.8)
Gliadins (% of total grain N)	11.5 (28.8)	27.7 (8.5)	7.2 (54.7)	27.0 (11.2)
Glutenins (% of total grain N)	28.8 (16.6)	45.5 (3.1)	21.2 (34.7)	44.8 (4.3)
Glutenin:gliadin ratio	2.56 (12.8)	1.64 (5.4)	3.23 (26.9)	1.66 (7.0)

Effects of physiological traits

As for the effect of climate variability and N treatments, the different physiological traits had fairly similar effects at both sites and only the results for Clermont-Ferrand are reported here.

At low N supply, none of the parameters had a significant effect (i.e., > 5%) on N uptake efficiency (NUpE; Table 3), because crop N uptake was primarily limited by soil N availability.

Under non-limiting N conditions, reducing the duration of the vegetative phase of the crop cycle, either through the rate of leaf appearance and canopy development (*P*) or after the canopy has reached its maximum size ($P_{\text{flag}}^{\text{anth}}$), increased NUpE

Table 3. *Average simulated changes in NUpE and NutE in response to a 30% increase or decrease of parameters values for 32 years at Clermont-Ferrand at low and high N supplies*

Parameter	Changes in yield (%)				Changes in NUpE (%)				Changes in NUtE (%)			
	Low N		High N		Low N		High N		Low N		High N	
	–30	+30	–30	+30	–30	+30	–30	+30	–30	+30	–30	+30
Phenology and canopy development												
P	0.8	-11.7	-11.5	1.7	-2.4	1.9	-21.2	12.5	7.0	-15.2	11.3	-10.6
$P_{\mathrm{flag}}^{\mathrm{anth}}$	11.5	-10.0	1.0	-0.5	-1.6	1.3	-7.1	6.9	13.8	-11.3	9.3	-7.2
α_{max}	2.0	-1.3	-3.8	0.2	0.0	0.0	-14.0	9.2	2.0	-1.3	12.5	-8.2
P_{sen}	-0.6	0.6	-0.3	0.2	0.0	0.0	0.1	0.1	-0.6	0.6	-0.4	0.1
Crop C assimilation and partitioning												
K	-8.6	6.0	-8.7	4.4	0.5	-0.5	-5.9	3.7	-8.4	5.9	-2.5	0.6
RUE	-15.9	-0.6	-29.5	26.6	-1.2	2.2	-16.2	15.5	-15.7	-0.8	-15.7	9.9
SLW	1.6	-1.3	0.3	-0.5	-0.1	0.1	6.5	-6.3	1.6	-1.3	-5.8	6.3
D_{eg}	-3.6	3.8	-0.1	0.2	0.3	-0.2	0.2	-0.2	-3.6	3.8	-0.3	0.4
μ	3.7	-10.3	0.1	-0.3	-0.7	1.0	-0.7	0.5	11.2	-10.2	1.2	-0.6
σ	11.2	-10.3	0.7	-0.3	-0.7	1.0	-0.7	0.5	11.2	-10.3	1.3	-0.6
γ	-9.4	9.2	-9.0	8.9	0.0	0.0	0.0	0.0	-9.4	9.2	-9.1	9.0
Crop N uptake and assimilation												
RVER	0.6	0.0	0.1	-0.1	-0.1	0.1	0.1	-0.2	0.8	-0.1	0.1	0.1
$N_{\mathrm{max}}^{\mathrm{uptake}}$	-0.1	0.1	-1.1	1.8	-0.2	0.1	-7.3	2.2	0.6	0.0	4.4	-0.4
K_{sen}	0.0	0.0	-0.6	1.4	0.0	0.0	-0.2	0.1	0.0	0.0	-1.9	1.3
$[N]_{\mathrm{max}}^{\mathrm{stem}}$	0.1	0.0	-2.6	1.5	-0.1	0.0	-9.5	9.0	0.1	0.1	7.7	-6.9
SLN (con. RUE)	4.9	-6.4	1.8	-3.9	-0.7	0.5	-17.8	12.9	8.0	-6.6	24.9	-14.8
SLN (var. RUE)	0.8	-4.2	-7.8	3.8	-2.3	0.7	-27.1	18.1	-0.4	-4.4	18.4	-11.9
$[N]_{\mathrm{stru}}^{\mathrm{leaf}}$	5.0	-5.5	0.7	-0.8	-0.3	0.3	-0.1	0.1	5.0	-5.5	0.7	-0.8
$[N]_{\mathrm{stru}}^{\mathrm{stem}}$	3.3	-4.1	-1.2	1.1	-0.2	0.2	0.3	-0.3	3.4	-4.2	-1.4	1.4
β	8.2	-17.1	8.2	-17.7	-0.3	0.9	-0.8	0.5	31.9	-17.0	30.8	-17.7
Grain development and C and N accumulation												
D_{gf}	-18.7	20.1	-24.3	24.0	2.3	-0.3	1.2	-1.1	-18.5	20.1	-24.2	23.9
D_{cd}	19.9	-22.9	9.0	-7.4	-0.7	1.3	-0.2	0.3	20.0	-22.8	9.0	-7.4
$\alpha_{\mathrm{N:C}}$	6.8	-3.7	4.9	-2.7	0.1	0.0	0.0	0.0	6.8	-3.7	4.9	-2.7
k_{cd}	16.0	-24.3	0.9	-1.3	-1.0	4.4	-0.3	0.4	16.0	-24.3	1.0	-1.4

(Table 3), but this possible effect was overwritten by the reduction of NUtE, and overall had no effect on NUE and yield. However, grain N and protein concentration increased by 5 to 7% in response to a 30% increase of P or $P_{\mathrm{flag}}^{\mathrm{anth}}$. In contrast, under limiting N conditions, increasing these two parameters by 30% produced a 10 to 15% decrease in mean NUtE, NUE, yield, and grain protein concentration.

The potential size of the culm leaves (α_{max}) had no significant effect on NUpE, and its effect on NUtE depended on N supply (Table 3). For both N treatments neither α_{max} nor P_{sen} had a significant effect on yield, NUE or grain protein concentration. Surprisingly, P_{sen} had no effect on both NUpE and NUtE.

The effect of K on yield and NUE was largely independent of N supply, and mean yield and NUE increased by 4 to 6% in response to a 30% increase of K. Increasing RUE by 30% benefited (+27%) mean yield and NUE only at high N supply. None of these two parameters was able to sustain grain protein concentration as yield increased. However, under low N supply cultivars with a low K or RUE had similar yield but a higher grain N and therefore grain protein concentration compared with cultivars with a high K value (Figure 2).

For both N treatments, SLW had a small effect on yield and NUE, but for the HN treatment, mean grain N increased by 8% in response to a 30% decrease in SLW, which resulted in an increase of mean grain protein concentration from 12.7% to 13.6% (Figure 2).

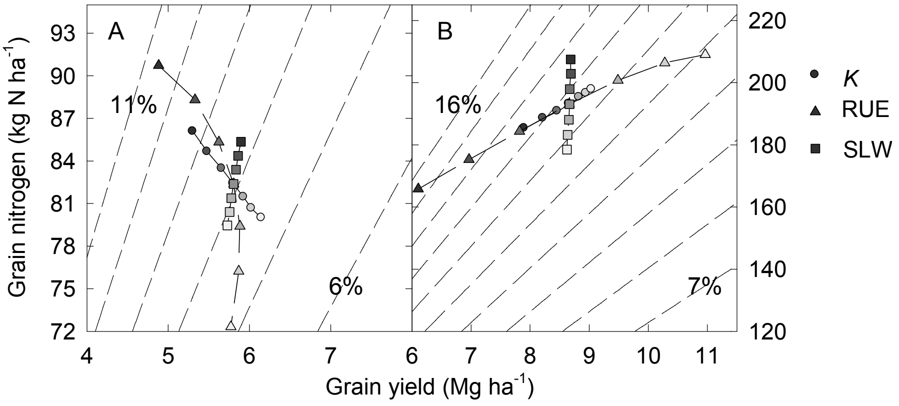

Figure 2. *Grain N versus yield in response to variations of K, RUE and SLW for 32 years at Clermont-Ferrand at low* **(A)** *and high* **(B)** *N supplies. The grey intensity of the symbols decreases as the value of the parameters increases by 10% increments from –30% to +30% of their default value. Dashed lines are grain protein concentration isopleths in 1% increments*

For HN treatment, the parameters related to ear growth (D_{eg} and μ) and grain number to ear DM ratio (σ) had no significant effect on yield and NUE components (Table 3). However, under limiting N supply, increasing μ or σ by 30% reduced mean yield and NUtE by ca. 10%. These parameters had no significant effect on crop or grain N dynamics. The fraction of crop DM remobilized during grain filling (γ) had no effect on grain N either (data not shown). However, a 30% increase in γ

increased mean yield and NUE (NUtE) by 9% independently of N supply (Table 3), because of a higher DM harvest index, which increased from 0.39 to 0.43 and from 0.48 to 0.52 for LN and HN, respectively.

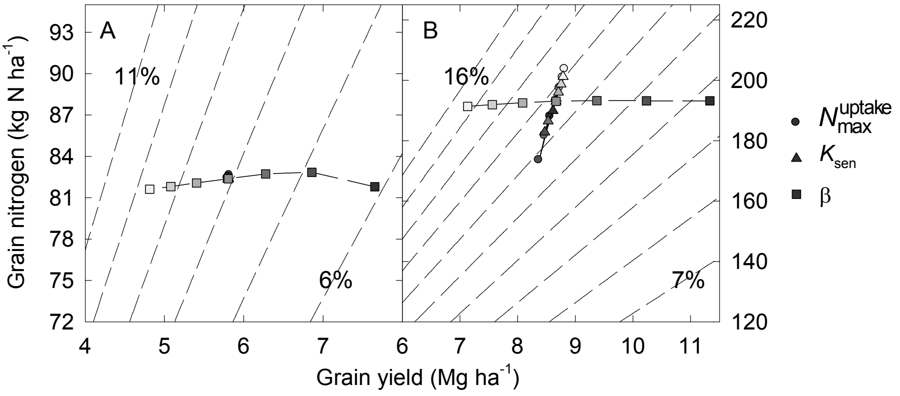

Figure 3. *Grain N versus yield in response to variations of* $N_{\max}^{\text{uptake}}$*,* K_{sen} *and* β *for 32 years at Clermont-Ferrand at low* **(A)** *and high* **(B)** *N supplies. The grey intensity of the symbols decreases as the value of the parameters increases by 10% increments from –30% to +30% of their default value. Dashed lines are grain protein concentration isopleths in 1% increment*

For both N treatments, RVER had no effect on mean NUE components, grain yield or N. Similarly changes in potential maximum rate of root N uptake at anthesis ($N_{\max}^{\text{uptake}}$) or in the rate of root senescence (K_{sen}) during grain filling had no significant effect on mean NUE or yield (Table 3). However, for HN treatment, both $N_{\max}^{\text{uptake}}$ and K_{sen} were positively associated with grain N and protein concentration (Figure 3). In contrast with earlier suggestions (Richards 2000), delaying leaf senescence by reducing the rate of N remobilization during grain filling (b) had no effect on final grain N content, but allowed the crop to assimilate more C; therefore, grain yield and NUE increased, but grain protein concentration decreased (Figure 3). Interestingly, lowering of the rate of N remobilization (β) by 30% increased mean NUtE by ca. 31% at both N supplies (Table 3). Increasing the efficiency of N remobilization during grain filling through changes in $[N]_{\text{stru}}^{\text{leaf}}$ and $[N]_{\text{stru}}^{\text{stem}}$ had small effects on yield and NUE (Table 3). For LN, decreasing $[N]_{\text{stru}}^{\text{leaf}}$ and $[N]_{\text{stru}}^{\text{stem}}$ by 30% produced a 3 to 8% increase in mean grain yield and N, and therefore yield was increased while grain protein concentration was maintained. For HN, decreasing $[N]_{\text{stru}}^{\text{leaf}}$ and $[N]_{\text{stru}}^{\text{stem}}$ by 30% had no significant effect on grain yield, but increased mean grain protein concentration from 12.7% to 13.0% and 13.3%, respectively.

Under non-limiting N supply, increasing $[N]_{\max}^{\text{stem}}$ and SLN by 30% increased mean NUpE between 9% and 18%, but this was partly cancelled by a decrease (between 7 and 15%) of NUtE (Table 3). Overall, the improvement in yield and

NUE due to N storage capacity of the crop was surprisingly low (between 1 and 5%); but for HN treatment, leaf and stem storage capacity had large effects on grain protein concentration (Figure 4). The same conclusions were reached in terms of yield and NUE improvement with the two hypotheses regarding the relationship between SLN and RUE. However, assuming that RUE increases non-linearly with SLN (Sinclair and Amir 1992), which is the most likely hypothesis, under non-limiting N supply, increasing SLN by 30% resulted in a 4% and 11% increase of grain yield and protein concentration, respectively (Figure 4).

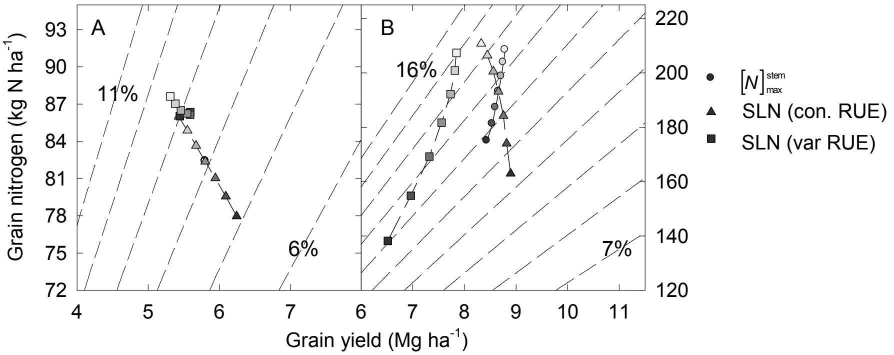

Figure 4. *Grain N versus yield in response to variations of* $[N]^{\text{stem}}_{\text{max}}$ *and SLN for 32 years at Clermont-Ferrand at low* **(A)** *and high* **(B)** *N supplies. The grey intensity of the symbols decreases as the value of the parameters increases by 10% increments from –30% to +30% of their default value. Dashed lines are grain protein concentration isopleths in 1% increment*

The parameters related to grain development (D_{gf} and D_{cd}) and structural N and C accumulation ($\alpha_{\text{N:C}}$ and k_{cd}) had no significant effect on NUpE (Table 3). Rather they modified C and N grain demand, and therefore NUtE. Increasing D_{gf} by 30% produced a 20 to 24% increase in yield and NUE, independently of N treatments; D_{gf} had a much lower effect (less than 7%) on grain N, and grain protein concentration decreased as D_{gf} increased. Similar results were obtained with D_{cd}, but its effects on yield, NUE and grain protein concentration were larger under limited N supply (data not shown). The ratio of structural grain N to C ($\alpha_{\text{N:C}}$) had no effect on grain N (data not shown). For the two N treatments a decrease in $\alpha_{\text{N:C}}$ of 30% resulted in an increase in mean yield and NUE of 7% (Table 3). Under low N supply, the grain demand for structural C (k_{cd}), had a symmetrical effect on yield and NUE and grain N compared with D_{gf}. Under non-limiting N supply, k_{cd} had no effects on grain yield and NUE, and slightly increased (+3%) grain N when increased by 30%.

CONCLUSION

Crop simulation models provide the best approach for integrating our understanding of complex plant processes as influenced by weather, soil and management. As such they provide a powerful tool in guiding the direction of future research by providing quantitative predictions and highlighting gaps in knowledge. The effect of changing a single plant characteristic on crop performance can be determined in a field experiment assuming that suitable plant material is available. But the crop response will also depend on weather and environmental conditions. So determining how a range of plant characteristics, either individually or in combination, will affect crop performance under a range of growing conditions becomes a daunting practical task.

Some cautions are required when using crop simulation models, since the ability of a model to predict subtle traits × environment × management interactions depends on the simplifications and assumptions made in the model (Boote et al. 2001). On the other hand, simulation models allow us to focus on the most important aspects of the physiology of the crop. Simulation models also show complex interactions that are not intuitive. For example, in this study delaying leaf senescence by reducing the rate of N remobilization during grain filling (β) had a large effect on C assimilation and final yield but had no effect on final grain N content, and thus resulted in a large decrease of grain protein concentration. This result is in good agreement with experimental results, where stay-green mutants for durum wheat had a higher NUE than the wild type but grain N content was the same for the mutants and the wild type, and therefore the grain protein concentration was lower for the mutants than for the wild type (Spano et al. 2003).

Increasing the N storage capacity of the leaves and stem and the allocation of N to non-structural proteins appeared as the more promising strategy to break the negative correlation between grain yield and protein concentration. Moreover, it may also reduce the risk of N losses by leaching, denitrification and volatilization. The analysis of 21 genotypes of bread wheat revealed a significant genetic variability of stem N storage capacity, ranging from 7.6 to 14.9 mg N g^{-1} DM (Triboï and Ollier 1991). In *SiriusQuality*1, the default value for $[N]_{\mathrm{max}}^{\mathrm{stem}}$ lies in the middle of this range; we could thus expect a larger increase of $[N]_{\mathrm{max}}^{\mathrm{stem}}$ than what has been considered here. In good agreement with our results, a recent survey of UK winter-wheat cultivars revealed a positive association between grain yield and stem N concentration (Shearman et al. 2005). We are not aware of any study reporting the observed range of genetic variability for wheat canopy SLN.

Martre et al. (2006) showed that in *SiriusQuality*1, in most cases the end of grain filling occurred before D_{gf}, and was triggered by leaf senescence, which was driven by N remobilization and not by the ontogenic rate of leaf senescence (P_{sen}). Thus, we expected that increasing root N uptake after anthesis would delay N remobilization and leaf senescence. However, increasing the rate of post-anthesis root N uptake had not effect on N remobilization and grain-filling duration or grain yield, but it promoted grain N content and protein concentration. Overall, crop N accumulation appeared to be more limited by the shoot demand and its capacity to store newly assimilated N temporarily, than by the capacity of the roots to extract N from the soil.

Intuitively, one can consider that increasing RUE might be more important under limiting N conditions, where the leaf area and the canopy duration are reduced. However, our results suggest that for low-N-input environments, it might be more interesting to select cultivars with low RUE. Under low N, RUE had a relative small effect on grain yield, but a decrease in RUE by 30% increased mean grain protein concentration from 8.1% to 10.6%.

From our simulations it clearly appears that the importance of a physiological trait for crop breeding is largely dependent on the objectives in terms of quality. Crop N requirement and NUE cannot be used independently to identify favourable traits. For example, the effects of P and $P_{\mathrm{flag}}^{\mathrm{anth}}$ on NUE were similar and depended largely on N supply, but only P allowed breaking the negative correlation between grain yield and protein concentration for both N treatments.

In agreement with theoretical and experimental considerations, plants exhibiting a similar capacity of growth show a low genetic variability for NUtE among C_3 species (Greenwood et al. 1991). In this study, changes in NUtE were closely correlated with changes in grain yield, and none of the physiological traits considered had an effect on NUtE except the trivial effect of plant mass *per se*. More surprisingly, under low N conditions, none of the parameters had a significant effect on NUpE. This may be due to a severe N shortage, but it may also point at a lack of understanding of root system development and functioning under limiting N supply (Jamieson and Ewert 1999).

In this study, variations in weather and N treatments induced larger variations in NUE than most of the physiological traits considered. These simulations suggest that a single physiological trait is unlikely to break the negative correlation between the grain protein concentration and yield over a wide range of sites and seasons, especially under low-N-input environments. In a recent study, using the APSIM–NWHEAT simulation model Asseng and Milroy (2006) reached the same conclusion. Much of the genetic gain in complex traits such as yield or grain protein concentration is believed to be due to the accumulation of genes with small additive effects (Blanco et al. 2002). Similarly, our simulation results support the idea that breaking the negative relationship between grain yield and protein concentration will require pyramiding physiological traits, and with opposite effects for some of them.

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CHAPTER 16

AN ARCHITECTURAL APPROACH TO INVESTIGATE MAIZE RESPONSE TO LOW TEMPERATURE

K. CHENU[#,##], C. FOURNIER[##], B. ANDRIEU[##]
AND C. GIAUFFRET[#]

[#] UMR SADV, INRA-USTL, Estrées-Mons, BP136, 80203 Péronne, France.
[##] UMR EGC, INRA-INAPG, 78850 Thiverval-Grignon, France.
E-mail: chenu@ensam.inra.fr; giauffre@mons.inra.fr

Abstract. In maize, grain yield is highly associated with light interception and photosynthetic activity during grain filling. In Europe, this period typically occurs when solar radiation is already decreasing and water availability may be limiting. The improvement of cold-tolerance is a major challenge for maize production because earlier sowing would allow a better fit between crop cycle and availability of natural resources.

Low temperatures have a major impact on (i) radiation interception through the modification of foliage development and (ii) radiation use efficiency (RUE) through the reduction of leaf photosynthetic activity. Little is known about the specific contribution of each of these traits to the lower biomass production under cold conditions and their genetic variability.

A field experiment with two planting dates was carried out on four maize inbred lines from temperate or highland-tropical origin, chosen as source of genotypic and phenotypic variability for cold tolerance. Biomass production was measured over time and analysed with respect to the amount of radiative energy received by the plant to quantify the radiation use efficiency of the different genotypes. The major impact of early sowing was found through the reduction in leaf dimensions. Early sowing affected leaves growing both during and after the cold period. Less striking effects were observed for plant developmental rate, final leaf number, RUE and leaf inclination. Virtual plants simulating the architecture of the genotypes both in early and normal sowings were generated and used to evaluate the effect of individual traits on light capture.

The results presented here are a first step to provide a phenotyping tool of plant response to low temperatures based on virtual plants. Such a tool should help to assess structural (light interception) and functional (RUE) traits that could then be used in segregating populations for genetic studies.

INTRODUCTION

Despite its subtropical origin, maize (*Zea mays* L.) has become a major crop in northern latitudes during the last 50 years. However, suboptimal temperatures occurring during spring affect seedling establishment and photosynthetic activity

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(Stirling et al. 1991; Leipner et al. 1999) so that productivity and yield stability are reduced (Carr and Hough 1978; Stamp 1986). Low temperatures also preclude early sowing, so that in high-latitude environments (> 45° N), silking and grain filling occur when both radiation and temperature are declining, which affects the yield potential (Otegui and Bonhomme 1998). Furthermore, in mid-latitude environment, water deficit may occur around flowering, during the period critical for grain set (Hall et al. 1981). Therefore, improving cold tolerance would allow earlier sowing, resulting in a better fit between crop cycle and availability of natural resources.

During the last decades, many studies have focused on the temperature effects on plant development (e.g., Tollenaar et al. 1979; Padilla and Otegui 2005) and processes involved in photosynthetic activity (e.g., Fryer et al. 1995). However, it is still poorly understood how these processes interact during canopy development in a realistic range of low temperatures and affect yield. A modelling approach could help to identify the key processes involved in above-ground biomass accumulation during the vegetative period, and thus to determine the traits important for plant breeding. Biomass accumulation mainly results from light interception and radiation use efficiency (RUE). The aim of this work was to study the impact of plant architecture and functioning on biomass production. A field experiment was carried out with two planting dates to analyse cold response in terms of plant development, architecture, radiation interception and use efficiency. The 3D architectural model developed by Fournier and Andrieu (1998) for maize was coupled with a radiative model (Chelle and Andrieu 1998; 1999) to quantify the contribution of specific responses of plant architecture in terms of light intercepted by the crop.

We used four maize inbred lines that originate from temperate and highland-tropical environments. Temperate and highland-tropical cultivars are know to have contrasting responses to low temperatures in terms of photosynthetic activity (Hardacre and Greer 1989), seedling biomass and leaf area development (Eagles et al. 1983).

MATERIALS AND METHODS

A field experiment was carried out in Estrées-Mons, France (49° N, 3° E, 85 m elevation) in 2005. Four maize inbred lines from temperate (F2 and F286) or highland-tropical (F334 and F331) origins were cultivated at a density of 10 plants m^{-2}. They were sown on 11 April (early sowing), and 2 May (normal sowing), resulting in contrasting temperatures for the period of early development (Figure 1).

Incident photosynthetically active radiation (PAR), air and apex temperatures were measured over time using a PAR sensor (Quantum Sensor SKP215, Skye Instruments Ltd, Llandrindod Wells, UK), a thermohygrometer (50Y, Campbell Scientific Ltd, Shepshed, UK) and thermocouples (copper–constantan), respectively. An equivalent thermal time expressed in degree-days (°Cd) was calculated using the equation of Yan and Hunt (1999), adjusted to a base temperature of 9.8 °C in the linear part of the response. Intercepted radiation by the canopy was determined from measurements of incident PAR at the top of the canopy and at ground level. The efficiency of radiation interception was estimated as the ratio between intercepted and incident PAR. Crop radiation use efficiency (RUE) was calculated from above-ground biomass accumulation and accumulated intercepted PAR from plant emergence. Plant growth, development and architecture were determined from measurements of above-ground biomass accumulation, number of appeared and liguled leaves, dimensions (area, length and width) and angles of laminae, lengths of sheaths and internodes.

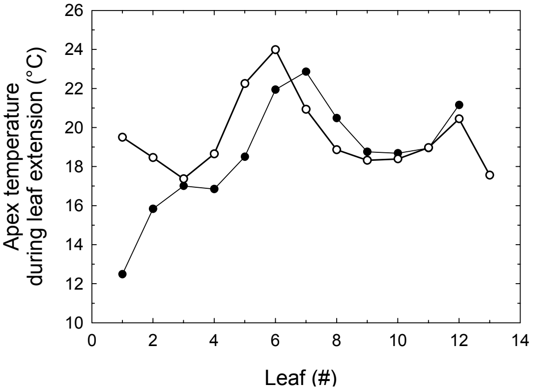

Figure 1. *Growth temperatures for the different leaves for one line (F2) in early (closed dots) and normal (open dots) sowings. Temperatures were estimated as the apex temperature averaged for the period of leaf expansion (from tip to ligule appearance of the leaf)*

RESULTS

Early sowing affected development, growth and spatial distribution of organs

Early sowing reduced the biomass production for all the studied genotypes from the early stages of development (data not shown). Plant development differed among the genotypes (Figure 2). For F2, the early sowing increased phyllochron (thermal time interval between the emergence of two successive leaves) and reduced final leaf number despite a slight increase in the duration of the vegetative period, estimated here by the date of tassel emergence (Figure 2, inset). F286 had a development unaffected by the sowing date with similar phyllochron, final leaf number and vegetative period duration. In the highland-tropical lines (F334 and F331), early sowing increased both phyllochron and the duration of the vegetative period so that the final leaf number was not modified. Finally the three-week difference between the sowing dates led to a tassel emergence occurring in early sowing from one week before (F286) to one week after (F331) the date for the normal sowing (data not shown). Interestingly, the increase in the length of the vegetative period of F334 and F331 allowed some compensation in terms of biomass production: these two genotypes had an above-ground biomass reduced, respectively, by 62% and 53%

when their leaf 11-12 emerged, whereas the reduction was only 29% and 33% at tasselling. Conversely, the biomass reduction in F2 and F286 was maintained over time (data not shown).

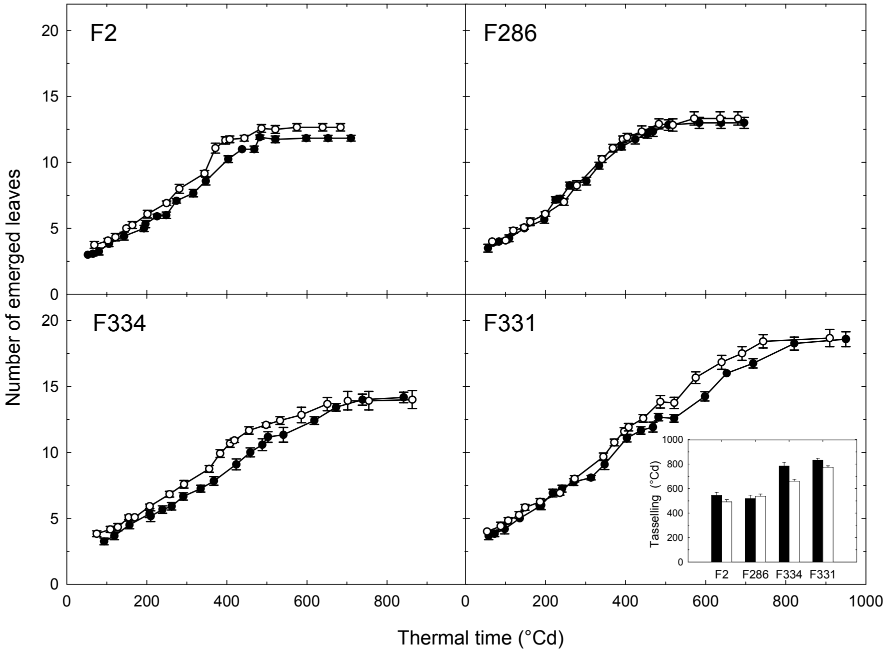

Figure 2. Number of emerged leaves over thermal time from plant emergence for lines F2, F286, F334 and F334 in early (closed dots) and normal (open dots) sowings. Inset, duration until tassel emergence at early (black bars) and normal (white bars) sowings. Error bars indicate confidence limits at P=0.03

Early sowing did not affect the dimensions of leaves 1 and 2, but reduced the area, length and width of all other leaves (Figure 3). Since only the first leaves extended during the cold period (Figure 1), the reduction in dimension of the upper leaves seems to have resulted from a propagation of the initial effect, more than from a direct effect of cold temperatures on the behaviour of the leaf-growing zone. On the other hand, some compensation occurred in some lines (e.g., F331), with similar dimensions (length, width and area) of the top-most leaves in both treatments.

Mean inclination of laminae differed among genotypes (Figure 4). Genotypes also showed contrasting variation of leaf inclination with leaf rank. For instance, in F334 all leaves were more or less planophile whereas in F2 leaf angle increased with higher leaf position on the stem (data not shown). Early sowing slightly reduced the inclination of all laminae (Figure 4). The maximal effect on light interception was observed for F2 and corresponded to a 15% increase in surfaces projected on soil.

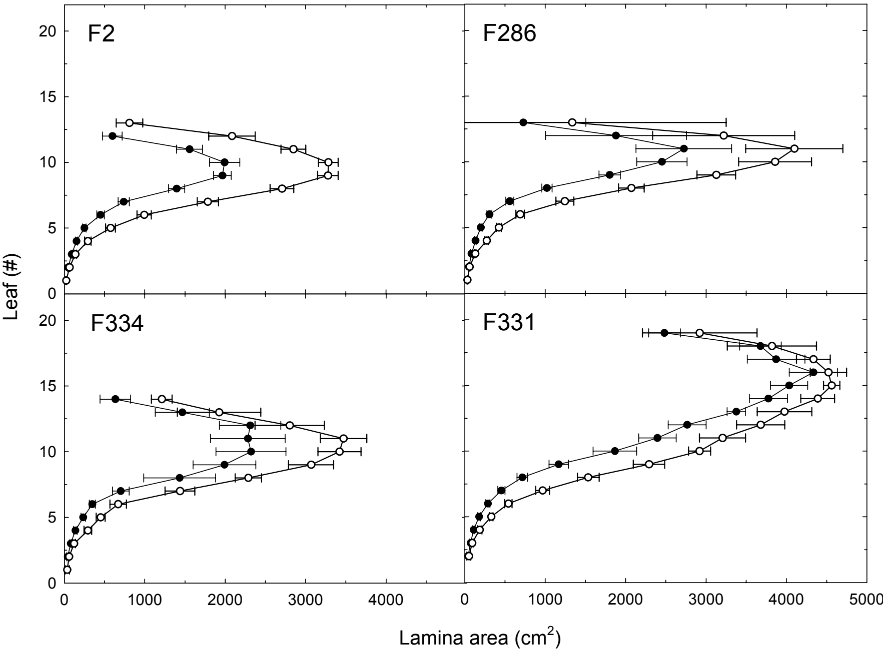

Figure 3. Final lamina area of successive leaves along the shoot. Data for lines F2, F286, F334 and F331 in early (closed dots) and normal (open dots) sowings. Error bars indicate confidence limits at P *= 0.05*

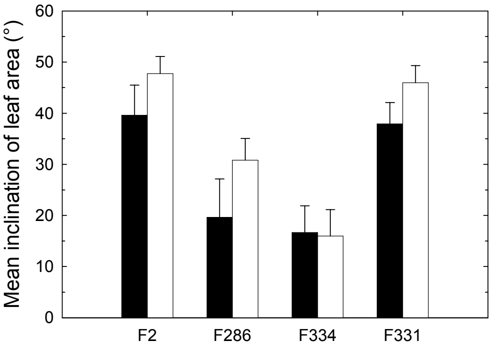

Figure 4. Mean inclination of surface of fully expanded leaves for lines F2, F286, F334 and F331 in early (black bars) and normal (white bars) sowings. Error bars indicate confidence intervals at P *= 0.05*

Early sowing reduced efficiency of light interception with no drastic effect on the radiation use efficiency for the sowing–flowering period

Efficiency of light interception was lower in early sowing from the first stages of plant development (Figure 5). Interestingly, the high number of leaves produced in F331, associated with a high degree of leaf size compensation for the top-most leaves, allowed its light interception to reach similar values at silking, in early and normal sowings.

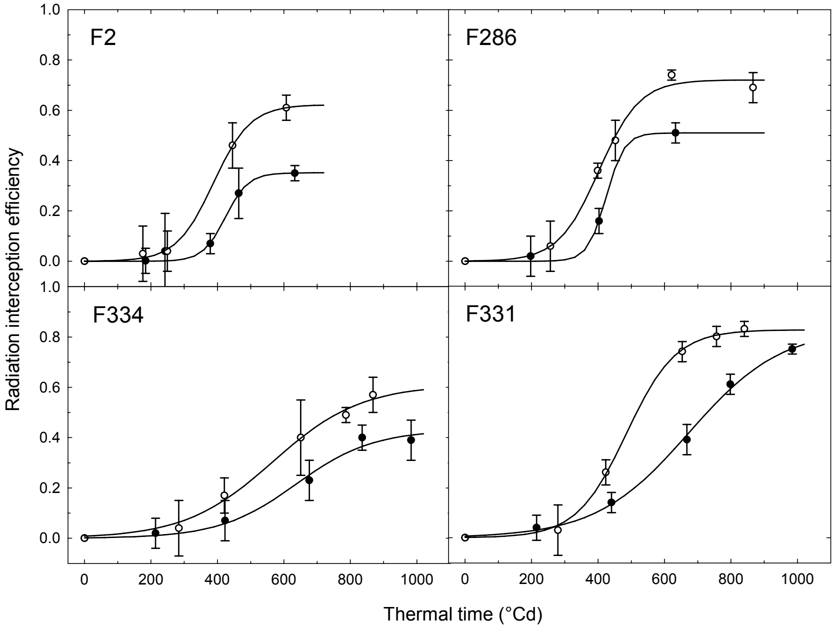

Figure 5. *Efficiency of radiation interception over thermal time for lines F2, F286, F334 and F331 in early (closed dots) and normal (open dots) sowings. Error bars indicate confidence limits at* P *= 0.05*

RUE for the sowing–silking period differed among genotypes but was weakly affected by the date of sowing (Figure 6). Effects of sowing date on RUE were significant in the temperate lines but not in the highland-tropical ones. Similar results were observed in a previous field experiment carried out in 1999 (C. Giauffret et al. unpublished data). RUE tended to be negatively linked to genotype earliness for the sowing–silking period. This was not the case when measurements were done at the same calendar date for all the genotypes (e.g., around silking of the earliest flowering genotype in the 1999 experiment). The genotypic variation observed for the RUE averaged through the sowing–silking period could thus result from change in RUE over plant development or from photosynthesis dependence on temperature (Giauffret et al. 1991; Stirling et al. 1993), as measurements were done about one month later for the late-flowering lines than for the early-flowering ones.

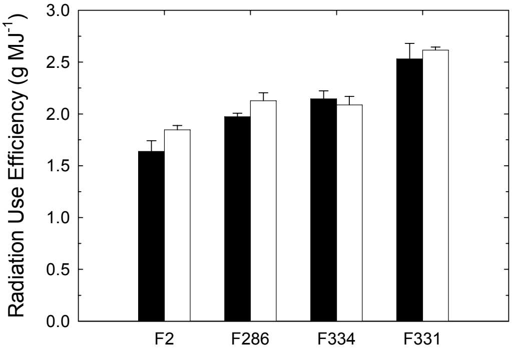

Figure 6. *Radiation use efficiency (RUE) for the sowing–flowering period, for lines F2, F286, F334 and F331 in early (black bars) and normal (white bars) sowings. Error bars indicate confidence limits at* P *= 0.05*

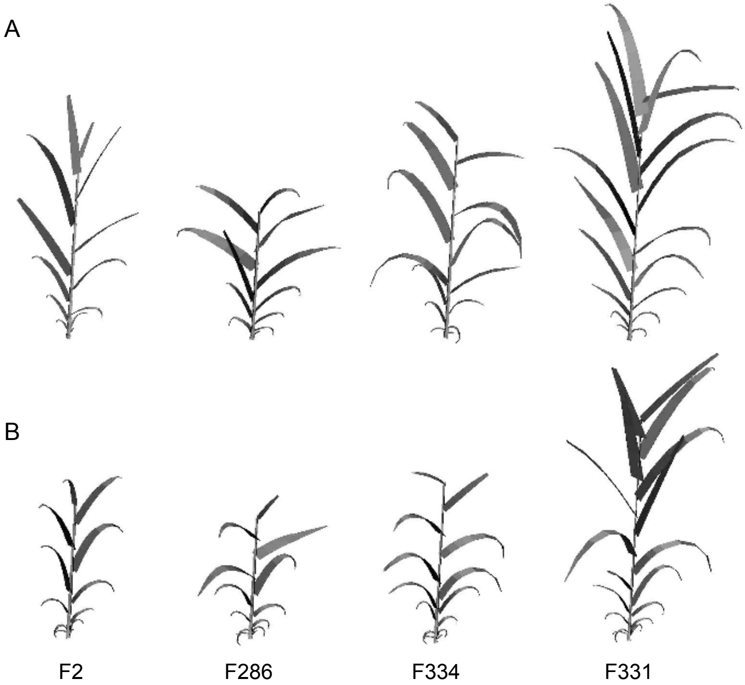

Figure 7. *Representation of 3D virtual plants for the four inbred lines in normal* **(A)** *and early* **(B)** *sowings. Examples are given for median plants at silking*

3D virtual plants to dissect the contribution of architectural traits to light interception

ADEL maize (Fournier and Andrieu 1998; 1999) was adapted here to the different genotypes and sowing-date treatments (Figure 7). Efficiency of light interception was simulated for each situation. Ground cover simulated with virtual plants was estimated for vertical viewing with a field of view of 25°, consistent with field measurements with photographs. Simulated ground cover matched the measured one with a good accuracy ($y=0.88x$, $r^2=0.88$), thus showing the ability of the method to assess the consequence of architecture on light capture. There was, however, a small bias in the model, as simulated ground-cover values were slightly lower than observed ones. This could partly be explained by the fact that the tassel was not represented in virtual plants.

Virtual plants combining characteristics of normal and early sowing treatments were generated to estimate how specific changes in architectural variables affected light interception. Decrease in organ dimension (leaves and internodes) had a major impact on ground cover, with a reduction up to 29.2% at flowering time. Response in leaf inclination slightly increased the light-capture efficiency with a change in ground cover at flowering time of up to 6.4%.

DISCUSSION

Some initial leads to drive genetic studies on cold tolerance

The cold period was limited to the first stages of plant development; however, it affected the organ growth and development during the whole plant cycle. Such an effect of early planting has already been reported for leaf appearance rate or leaf elongation rate (Giauffret et al. 1995). Improvement of cold tolerance thus needs to focus not only on the direct effect of sub-optimal temperatures (e.g., Jompuk et al. 2005), but also on the processes involved in propagation of these effects. This propagation could be because of trophic effects, as light interception was decreased in plants affected by cold. Many studies have shown that light affects leaf initiation and expansion rates (e.g., Granier and Tardieu 1999; Chenu et al. 2005). Another hypothesis concerns the coordination of organ growth. Strong correlations have been found between the dimensions of successive organs (lamina and sheath) on the stem, for different types of environments (e.g., Andrieu et al. in press).

The response of the rate and duration of leaf initiation to temperature could also be a target for the improvement of cold tolerance. Indeed, leaf appearance rate and final leaf number are highly associated with silking date, light interception and grain filling (Tollenaar et al. 1979). Furthermore, a higher leaf number is often correlated with higher individual leaf areas (Dwyer et al. 1992; Fournier and Andrieu 1998). A way to improve yield could then be to find genotypes that produce a large number of leaves with a high initiation rate in early sowing conditions, in order to obtain both early flowering and high leaf area index. Genetic variability exists for the response of such traits to temperature (Figure 2, Lafitte et al. 1997; Padilla and Otegui 2005) and could therefore be exploited.

Contribution of 3D virtual plants to study the genetic basis of cold tolerance

We propose two major reasons to use 3D virtual plants to investigate genotype response to low temperature.

First, dynamic 3D plant models, when coupled with a radiative model (Chelle and Andrieu 1998; 1999) allow the estimation of light interception for different genotypes and environments, without multiplying the number of radiative sensors. This method also makes it possible to estimate light interception accurately during the early stage of growth, when physical measurements are difficult due to the small size of the plants and the high spatial variability. Such an approach would thus improve the estimates of radiation interception and RUE during the cold period.

Second, architectural models are useful to dissect the impact of different architectural variables on light interception (Chenu et al. 2005). These models can be used to quantify the expected benefit resulting from some improvements in the response to low temperatures, concerning developmental rate, organ dimension, leaf angle or RUE. With sufficient understanding, they can be used to define ideotypes depending on the existing genetic variability and the prospected climate. We have shown here that early sowing had a major impact on light interception through a reduction in organ dimension, whereas the response of leaf inclination had smaller impact for the studied genotypes. These results are a first step to build a phenotyping tool to simulate 3D virtual plants with a limited set of measurements. Such an approach could help to assess light interception in large populations. This would allow studying the genetic controls (quantitative-trait loci) of cold tolerance for integrative traits such as light interception and RUE.

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CHAPTER 17

A 3D VIRTUAL PLANT-MODELLING STUDY

Tillering in spring wheat

J.B. EVERS AND J. VOS

Crop and Weed Ecology Group, Plant Sciences, Wageningen University,
P.O. Box 430, 6700 AK Wageningen, The Netherlands.
E-mail: jochem.evers@wur.nl

Abstract. Tillering in wheat (*Triticum aestivum* L.) is influenced by both light intensity and the ratio between the intensities of red and far-red light. The relationships between canopy architecture, light properties within the canopy, and tillering in spring-wheat plants were studied using a 3D virtual plant-modelling approach. The advantage of virtual plant models is that each element in the architecture of the canopy is given an explicit 3D representation, which enables simulation of processes at the level of individual organs.

The model used, called 'ADELwheat', was calibrated for spring wheat. The model was validated for ground cover and leaf area index, using an independent dataset. Experimentally, it was shown that new tillers ceased to appear when the fraction of light intercepted by the canopy exceeded 0.4. That threshold was independent of plant population density, shading, developmental stage of the plants and rank number of the tiller. At the time tillering ceased, the red/far-red ratio (R:FR) was fairly similar across population densities. Cessation of tillering in ADELwheat was therefore made dependent on thresholds of light properties. A light model ('nested radiosity') was coupled to ADELwheat and was used to calculate both PAR interception and R:FR at the level of the individual organ while employing a series of different thresholds. The simulation results show that the virtual plant-modelling approach is useful to simulate global effects of local stimuli. The study demonstrates that the virtual plant-modelling approach can provide insight into the factors that determine the developmental plasticity of wheat in terms of tillering.

INTRODUCTION

This chapter presents a 3D virtual plant-modelling study of tillering in spring wheat. A virtual plant (Room et al. 1996) is a three-dimensional representation of the development of a plant or crop, i.e., the geometrical and topological properties of the plant are taken into account. Therefore, in cases in which the research question benefits from analysis at the level of the individual organ, the virtual plant approach has an advantage over less detailed modelling methods.

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The subject of this modelling exercise is the phenomenon of tillering (analogous to branching in dicotyledons) in spring wheat, and its determinants. Next to nitrogen availability, which was not taken into account in this study, light properties greatly influence the pattern of tillering in wheat and other Gramineae (Bos and Neuteboom 1998). Two properties of light are especially important:

a) The intensity of photosynthetically active radiation (PAR). It had been hypothesized by Bos (1999), that for tiller bud outgrowth, the PAR intensity incident on the parent leaf of the bud (which is the leaf on the same phytomer as the bud) is an important determinant of bud outgrowth, through its effect on the assimilate production of the parent leaf.
b) The ratio between the respective intensities of red and far-red light (red/far-red ratio, R:FR). It had been shown that a reduction in R:FR, resulting from the differential scattering properties of plant tissues for red and far-red light (Holmes and Smith 1977), severely reduces tillering in Gramineae (Casal et al. 1987; Casal 1988; Davis and Simmons 1994). Changes in R:FR are perceived by vertically oriented organs such as sheaths and elongating leaves; in general the base of the plant is a site of R:FR perception, especially at early stages of development (Cordukes and Fisher 1974; Skinner and Simmons 1993).

The local nature of the responses to these two determinants of tillering (at the parent leaf and at the base of the plant, respectively) made this problem an ideal candidate to be analysed using a virtual plant-modelling approach. The objectives of the study were (a) to construct and parameterize an architectural model of spring wheat; (b) to evaluate model performance using independent data for contrasting growing conditions; (c) to formulate hypotheses on tiller bud outgrowth in relation to light based on experimental data; and (d) to incorporate these hypotheses into the model and evaluate simulation output.

MODEL CONSTRUCTION AND PARAMETERIZATION

The first objective was to design an architectural model of spring wheat, which would be able to produce a 3D description of a wheat canopy (Figure 1), for the cultivar and growing conditions as prevalent in spring wheat cropping seasons in The Netherlands (Evers et al. 2005). In the initial model, the occurrence of tillers was not dependent on light conditions, as this was a feature to be implemented after the correct functioning and performance of the wheat model had been evaluated. The model, which was implemented in the plant-modelling language CPFG (Měch 2005), was based on an existing architectural model of wheat, called ADELwheat (Fournier et al. 2003). This model, based on the L-system formalism (Lindenmayer 1968a; 1968b; Prusinkiewicz and Lindenmayer 1990), contained explicit descriptions of rates of initiation and extension of organs and final organ dimensions, leaf-geometrical properties (base angle, curvature, azimuth), and tillering kinetics. The model was initially parameterized for winter wheat. Reparameterization therefore provided the opportunity to compare parameters and functions for winter and spring wheat. The reparameterization was based on an outdoor experiment using spring-wheat cultivar Minaret, grown in a regular grid in containers of 70 × 90 cm, at a low plant population density (100 plants m^{-2}) to induce extensive tillering. The following sections describe a selection of the components that were reparameterized.

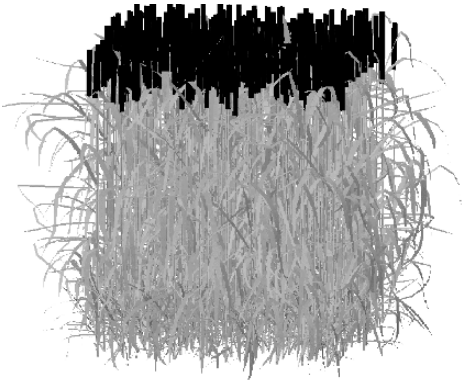

Figure 1. *An example of the visual output of a simulation run for spring wheat at a population density of 100 plants m*$^{-2}$

Leaf appearance

The elapsed time between the appearance of two consecutive leaves (the phyllochron), was found to differ between main stem and tillers. This difference was caused by a high rate of appearance (i.e., a short phyllochron) of main-stem leaves one to four, whereas higher main-stem leaves had phyllochron values similar to those of tiller leaves. A similar distinction between early and late leaves was made by Jamieson et al. (1995) for wheat and by Abeledo et al. (2004) for barley. Therefore, the model was parameterized with two values for phyllochron: one for main-stem leaves one to four (52 °Cd) and one for all other leaves (92 °Cd).

Relative phytomer number (RPN)

In the original winter-wheat parameterization (Fournier et al. 2003), several properties of tiller organs could be directly derived from those of the main stem using the concept of relative phytomer number (RPN). These properties included leaf blade and sheath dimensions, internode length and final leaf number (see next section for their specific parameterization).

The RPN value of a phytomer is the sum of the rank number of the phytomer on the shoot to which it belongs, and a phytomer shift value. The latter is specific for a particular tiller. For example, if tiller 2 would have shift value 2.7, phytomer 3 on tiller 2 would have an RPN value of 5.7. The components of this phytomer would then have properties similar to an imaginary phytomer 5.7 on the main stem.

The RPN concept appeared applicable to the spring-wheat cultivar used in our study. The shift values differed only slightly between spring and winter varieties.

Organ dimensions and final leaf number

Final length and width of the leaf blade and final length of the leaf sheath were parameterized by fitting appropriate functions. For blade length, a linear relationship with RPN was used for main-stem leaves one to five, and the Lorentz Peak Distribution function (Buck-Sorlin 2002) was used for all other leaves (Figure 2A). For blade width, a linear relationship of sheath length data with RPN appeared appropriate (not shown). For sheath length, a logistic relationship with RPN was used (Figure 2B). The functions were all slightly different from the ones used for winter wheat, and were discussed to be more applicable for Gramineae in general in Evers et al. (2005).

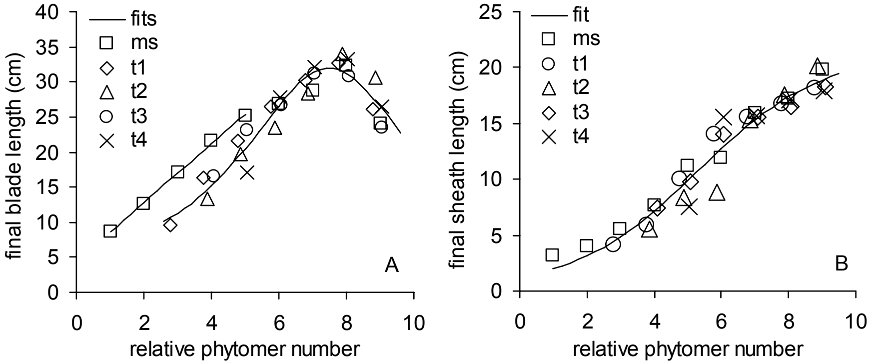

Figure 2. *Final blade* **(A)** *and sheath* **(B)** *length vs. relative phytomer number of the main stem (ms) and primary tillers t1 to t4. The lines indicate in* **(A)** *the linear and Lorentz Peak Distribution fits, and in* **(B)** *the sigmoidal fit. N = 33 in both cases*

Leaf blade geometry

To parameterize the geometrical properties of leaf blades, a Polhemus Fastrak magnetic digitizer was used (Polhemus, Colchester, USA). This method records the coordinates (x, y, z) of a point in space relative to a reference point. For each leaf blade, several points along the midrib were digitized. From these data, the base angle and the curvature of the leaf blades were derived (based on a model by Prévot et al. 1991) as well as the azimuth of the leaves (the angle between consecutive leaves, when viewed from the top). These were all stochastic components in the model: during a simulation, for each individual leaf the coefficients defining its base angle, curvature and azimuth were drawn from observed distributions. This stochasticity reflected the variation as experimentally observed.

MODEL EVALUATION

To evaluate the parameterization and the performance of ADELwheat, a second outdoor experiment was performed. Three plant population densities were used (100, 262 and 508 plants m^{-2}, square grid), and the plants were subjected to two light regimes (0% and 75% shading). To obtain additional data, an indoor (growth chamber) experiment was conducted with plants grown at 100 plants m^{-2}; light intensity was set at ca. 425 μmol m^{-2} s^{-1} at the top of the canopy.

Various components of the model parameterization were evaluated. Generally, phyllochron, tiller appearance delay and the final number of produced leaves were not significantly affected by population density in the full-light treatments. Shade generally increased phyllochron by 13–46% and reduced final number of leaves by 4–25%. The relationships of final blade length and width, sheath length and internode length with RPN were basically similar to the parameterized relationships. However, the coefficient values of these relationships depended on the light regimes and plant population density. The phytomer shift values were similar to those obtained from the parameterization experiment.

The performance of ADELwheat was evaluated using the (logistic) time courses of both ground cover (Figure 3) and gLAI (gross leaf area index, i.e., leaf death not taken into account) as test variables. Both are global characteristics of leaf production and their values integrate effects of several important model parameters and functions such as phyllochron and blade dimensions. ADELwheat appeared capable of simulating development of wheat in growth conditions for which the model was not calibrated (Evers 2006); however, refitting some key coefficients accounting for the effects of population density and shading yielded still better results. A sensitivity analysis of changes in blade length, width, phyllochron and tiller appearance delay showed that phyllochron needs to be parameterized accurately as small changes can have significant effects on the model output. A full description of the model validation and sensitivity analysis can be found in Evers (2006).

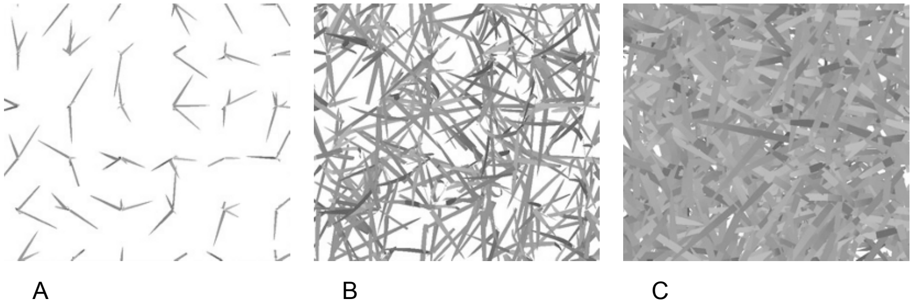

Figure 3. *Top view of ground cover of simulated wheat plots at a population density of 100 plants m^{-2}, at* **(A)** *183 °Cd,* **(B)** *365 °Cd,* **(C)** *620 °Cd after emergence*

ANALYSIS OF TILLERING BEHAVIOUR

The outdoor experiment that was used for model evaluation was also used to analyse the mechanisms that determine the tillering pattern of spring wheat (Evers et al. 2006). To this end, data were gathered on the tillering dynamics of the plants grown in the different treatments; simultaneously, changes over time were measured in the fraction of PAR intercepted by the canopy and R:FR (both measured at soil surface).

Both population density and shading affected the time course of the number of tillers per plant: a higher population density resulted in fewer tillers per plant than a lower population density, and shading dramatically decreased the number of tillers per plant (Table 1). Tiller appearance was hardly affected by population density, but shading delayed tiller appearance by 0.52 phyllochrons on average. The maximum number of tillers produced per plant differed between treatments, as was the stage of development at which this maximum number was reached (i.e., cessation of tiller appearance). However, the fraction of PAR intercepted by the canopy at the moment of cessation of tiller appearance was identical in five out of six treatments, independent of the rank number of the last emerging tiller, the population density or the shading treatment. Also R:FR at soil level at the moment of tillering cessation was independent of the rank number of the last emerging tiller and the population density, but differed between light regimes. The probability of a bud to grow out was shown to be related to the leaf mass per unit leaf area (LMA, in mg cm^{-2}) of its parent leaf: a low LMA was related to bud dormancy, and a high LMA to bud outgrowth.

It was concluded that cessation of tiller appearance was primarily regulated by the fraction of PAR intercepted by the canopy, and/or R:FR (taking into account that these two variables are highly correlated) rather than the absolute amount of intercepted light by the parent leaf, confirming suggestions by Simon and Lemaire (1987) and Lafarge and Hammer (2002), and corroborating the results of Sparkes et al. (2006). Only the appearance of the first primary tiller (the coleoptile tiller) seemed related to absolute PAR intensity. A threshold value of fraction of PAR intercepted (0.40) or R:FR (0.32 in full-light situations and 0.51 in shade) was considered to be the trigger of tiller bud outgrowth. It was hypothesized that the relation between bud dormancy and LMA was caused by the photomorphogenetic effects R:FR has on both bud outgrowth and LMA of the parent leaves.

Table 1. *Maximum and final number of tillers reached per plant, for plants grown at 0% or 75% shade, at a population density of 100, 262 or 508 plants* m^{-2} *(D100, D262 and D508, respectively)*

	0% shade			75% shade		
	D100	D262	D508	D100	D262	D508
Maximum tiller number	8.90	5.65	3.65	2.95	1.25	0.65
Final tiller number	3.80	1.90	0.10	2.55	1.00	0.06

SIMULATION OF TILLERING PATTERN UNDER THE INFLUENCE OF LIGHT PROPERTIES

The light conditions for suppression of tiller bud outgrowth, described above, were adopted for use in the virtual plant model. ADELwheat was interfaced with a light model called Nested Radiosity (Chelle and Andrieu 1998), using the L-systems communication functionality (Měch and Prusinkiewicz 1996; Měch 2005). The Nested Radiosity model is capable of calculating PAR interception and R:FR perception at the level of the individual organ, and was therefore highly suitable for our purpose. ADELwheat was modified to make tiller bud outgrowth dependent on the fraction of PAR intercepted by the bud's parent-leaf blade (analogous to the hypothesis by Bos (1999) mentioned above) and on the R:FR perceived by the tube of sheaths (the pseudostem); sheaths are known to act as an R:FR sensor (Cordukes and Fisher 1974; Skinner and Simmons 1993). Simulations were done using a threshold of fraction of PAR intercepted by the parent leaf for tillering cessation of 0.2, 0.4, 0.6 and 0.8, or using a R:FR threshold of 0.32 with either the sheaths or the parent-leaf blades as the sites of R:FR perception.

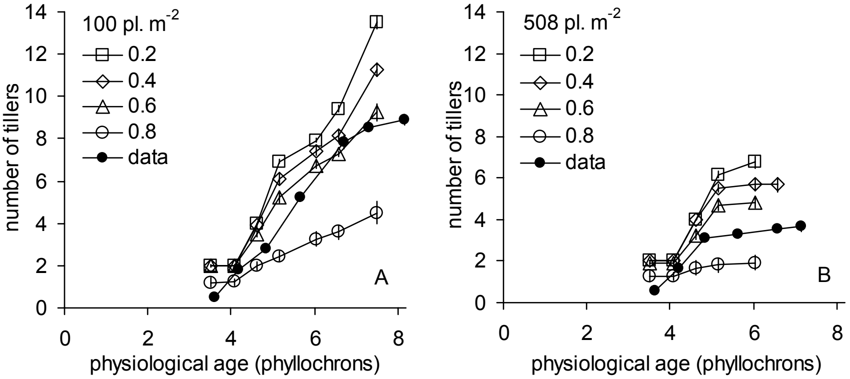

Figure 4. *Number of tillers per plant versus the physiological age of the plants for population densities of* **(A)** *100 and* **(B)** *508 plants m^{-2}, from simulations using threshold values for PAR intercepted by the parent leaf of 0.2 (squares), 0.4 (diamonds), 0.6 (triangles), 0.8 (circles), and from experimental data (dots). Error bars show 2 × SE*

Plant population density affected the degree of tillering in accordance with expectations: a higher population density resulted in fewer tillers produced per plant. A higher threshold value for PAR interception resulted in reduced tillering (Figure 4) and a lower production of leaf area (not shown). When compared to experimental data, the fraction of PAR intercepted at the parent leaf blade appeared to be a good indicator for outgrowth of tillers of a low rank, but outgrowth of tillers of a high rank was overestimated. Perception of R:FR by the pseudostem resulted in an overestimation of tiller production at any of the three population densities. These overestimations suggested that photo-morphogenetic effects alone may not be sufficient to predict tiller production; introduction of photosynthesis and carbon distribution through the plant may enhance model performance in terms of appropriate simulation of tiller production. Nevertheless, the study shows that the L-system approach is a powerful tool to analyse crop-morphological/ecological research questions in which the determinants act on the level of the individual plant organ.

CONCLUSIONS

Overall, this study has shown that (a) most of the functions in the spring-wheat parameterization in ADELwheat can be regarded as generic for Gramineae; (b) in our experiment, cessation of tiller appearance occurred at fixed light conditions within the canopy; and (c) hypotheses on local stimuli affecting global characteristics of crop development can be tested using a 3D virtual plant-modelling approach.

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CHAPTER 18

USE OF CROP GROWTH MODELS TO EVALUATE PHYSIOLOGICAL TRAITS IN GENOTYPES OF HORTICULTURAL CROPS

E. HEUVELINK[#], L.F.M. MARCELIS[##], M.J. BAKKER[#]
AND A. VAN DER PLOEG[#]

[#] *Horticultural Production Chains Group, Wageningen University, Marijkeweg 22,*
6709 PG Wageningen, The Netherlands.
[##] *Plant Research International, P.O. Box 16, 6700 AA Wageningen,*
The Netherlands.
E-mail: ep.heuvelink@wur.nl

Abstract. Quantifying the relevance of different plant traits for yield and quality under different growth conditions can improve the efficiency of a breeding programme. Crop models are powerful tools to give guidance to breeding, because model calculations enable the analysis of many different situations (sensitivity analysis and scenario studies). Three case studies of using crop growth models to evaluate physiological traits potentially used in breeding programmes are presented. The models used are explanatory models, with several submodels; e.g., for light interception, leaf photosynthesis, organ formation and biomass partitioning.

Case study 1: It is hypothesized that yield improvement of cut chrysanthemum can be obtained by a higher specific leaf area (SLA) or a higher light-saturated leaf photosynthetic rate ($P_{g,max}$). Model calculations showed that for a winter planting, a higher SLA has more impact on yield than improving $P_{g,max}$, whereas for a summer-grown crop $P_{g,max}$ and SLA are of equal importance for yield.

Case study 2: Regarding the yield of tomato, it is hypothesized that new genotypes, with two leaves in between trusses, may improve yield. In tomato cultivars generally there are three leaves in between two trusses. The formation of fewer leaves favours dry-matter partitioning towards the fruits, but it also decreases leaf area index (LAI), resulting in less light interception. Model calculations showed that a genotype with two instead of three leaves between trusses indeed will improve yield. To maximize the benefit of this trait it is important to keep the LAI sufficiently high.

Case study 3: It is hypothesized that modified tomato genotypes that show a shade avoidance response will result in higher yields as they can be grown at higher planting densities. Model calculations for tomato showed that this modification would hardly influence total yield. Standard conditions already result in a high light interception, which can hardly be improved by a higher planting density. Hence, it may be questioned whether for tomato developing genotypes with suppressed shade avoidance response for yield improvement is worthwhile.

J.H.J. Spiertz, P.C. Struik and H.H. van Laar (eds.), Scale and Complexity in Plant Systems Research: Gene-Plant-Crop Relations, 223–233.

In conclusion, crop growth models are powerful tools to evaluate the impact of differences in crop characteristics under different growth conditions. Such quantitative evaluations are important to focus breeding programmes and to ideotype genotypes for different environments.

INTRODUCTION

Plant breeding may have multiple objectives, e.g., enhancement of disease resistance or the improvement of yield or product quality. With regard to a complex trait such as yield, a better insight in underlying physiological and morphological parameters is most important to obtain a superior genotype (G). Furthermore, the importance of those parameters may depend on environment (E) and management (M). Crop growth models can improve the efficiency of a breeding programme (Boote et al. 2001), as model calculations (sensitivity analysis and scenario studies) enable a quantitative analysis of the crop (phenotypic) response to altering genetic traits under a range of growing conditions. Such studies have been published for several crops, e.g., cucumber (Marcelis 1994), tomato (Heuvelink 1999), soybean (Boote et al. 2003) and cut chrysanthemum (Carvalho et al. 2003).

Explanatory crop growth models are a powerful method to represent and combine knowledge in a generic way (Challa et al. 1994). In contrast to the more common empirical research, explanatory models enable a scientific approach to agricultural problems by incorporating knowledge of underlying processes. Development and use of explanatory models in agricultural sciences started some 40 years ago with the pioneering work of, among others, Prof. C.T. de Wit in Wageningen, The Netherlands. A review of model development by groups of Wageningen University and Research Centre is given by Bouman et al. (1996) and Van Ittersum et al. (2003). Yin and Van Laar (2005) presented a new approach in the GECROS (Genotype × Environment interaction on CROp growth Simulator) model, which can be used for examining responses of biomass and protein production of arable crops to both environmental and genotypic characteristics. In recent studies (Yin et al. 2000; Reymond et al. 2003; Messina et al. 2006) it is shown that explanatory crop models can be helpful in quantitative-trait loci (QTL) analyses of complex traits, thereby improving breeding efficiency and enhancing breeding by design.

To illustrate the importance of using explanatory models three case studies are addressed with simulation studies.

Case study 1: As an example of the relative importance of crop characteristics, it is studied whether breeding for yield improvement in cut chrysanthemum should focus on increasing specific leaf area (SLA) or light-saturated leaf photosynthetic rate ($P_{g,max}$). A higher SLA will result in more leaf area per unit of leaf weight, and hence more light interception and crop growth. A higher $P_{g,max}$ will also increase yield, because of increased leaf photosynthesis. Genotypic differences in SLA have been reported for many crops, e.g., tomato (Smeets and Garretsen 1986) and chrysanthemum (De Jong and Jansen 1992). A higher content of Rubisco protein, measured in sun leaves compared to shade leaves and coinciding with a higher $P_{g,max}$ (Murchie et al. 2005), may be a way to obtain a higher $P_{g,max}$ by breeding. However, photosynthesis is a very complex process and these effects would only be expressed in plants where Rubisco content is the limiting step in light-saturated photosynthesis. Without a quantitative analysis it is not obvious which of the two parameters is more important, and whether the effect depends on the season.

Case study 2: A reproductive tomato plant usually forms sequentially three leaves followed by a truss of fruits. A crop growth model is used to determine whether a tomato genotype with two instead of three leaves between two trusses would improve yield. On the one hand, fewer leaves and internodes between trusses would favour partitioning to the trusses resulting from a higher fruit/leaf ratio (Figure 1), but on the other hand fewer leaves means a lower leaf area index (LAI), resulting in less light interception and hence a lower total biomass production. As tomato yield can be seen as the product of total biomass and the fraction partitioned to the fruits, the impact on yield of such a genotype under contrasting conditions is not clear and may well depend on crop management.

Case study 3: The topic of shade avoidance response in tomato is presented. Plants grown closely together will elongate stems and petioles (among other responses), a strategy known as shade avoidance. Many authors have found that a shade avoidance response is detrimental to yield (Ballare et al. 1997; Robson and Smith 1997). When this response would not occur, plants could be grown at higher densities, and this is often suggested to improve yield. One mechanism by which the response to shade can be reduced is by constitutive over-expression of phytochrome genes. Over-expression of oat phyA in tobacco indeed resulted in shorter internodes and a higher harvest index in tobacco (Robson et al. 1996). In our crop growth models no shade avoidance response is implemented. Hence, we could study the impact of planting density on yield of a 'modified' tomato genotype in which shade avoidance was eliminated.

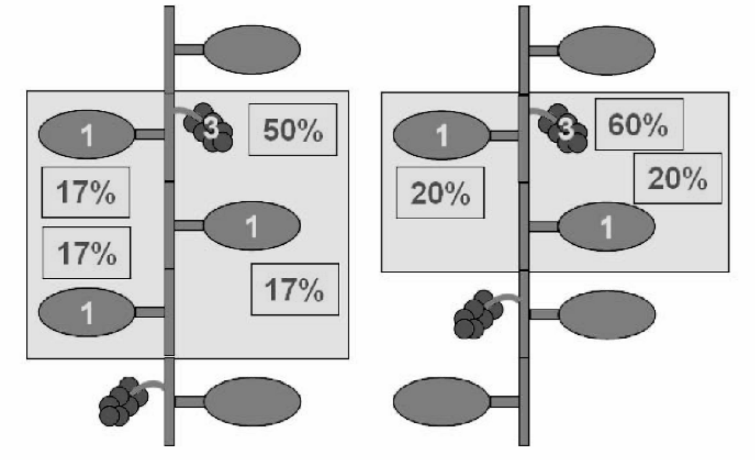

Figure 1. *Schematic presentation showing the effect of leaf:truss ratio on assimilate partitioning between leaves and trusses in tomato. Numbers inside organs represent sink strength for a specific day. Percentages represent partitioning on that day, resulting from these sink strengths*

MATERIALS AND METHODS

General information on the crop models used

The models used in this chapter are CHRYSIM1.0 (Lee et al. 2002b) for chrysanthemum and TOMSIM (Heuvelink 1999) and INTKAM (Gijzen 1994) for tomato. These models consist of modules for greenhouse radiation transmission, radiation interception by the crop, leaf and canopy photosynthesis, dry-matter production and dry-matter partitioning among plant organs (roots, stem, leaves and trusses of fruits for tomato, or flowers for chrysanthemum). For tomato also modules for fruit harvest and leaf picking are included.

Interception of radiation and canopy gross photosynthesis are calculated for a multi-layered uniform canopy (Goudriaan and Van Laar 1994). Leaf gross photosynthesis is calculated with the biochemical model of Farquhar et al. (1980) in the shade avoidance study, whereas in the other two studies a summary version of that model was used (Goudriaan et al. 1985).

Net assimilate production results from the difference between canopy gross photosynthesis and maintenance respiration. Maintenance respiration is calculated as a function of dry weights of the different plant organs, temperature and crop relative growth rate according to Heuvelink (1999). For tomato, assimilate partitioning between vegetative parts and individual fruit trusses is simulated on the basis of relative sink strengths (Marcelis 1994). In this concept the fraction of assimilates partitioned into an organ is calculated as the ratio between its potential growth rate (sink strength) and that of all plant parts. Appearance rate of new sections and trusses depends on temperature solely (De Koning 1994). In the standard setting, leaves from a section are removed when the corresponding truss above this section has reached developmental stage 0.9, which means at 20°C about 6 days before the truss is harvest-ripe. All trusses are assumed to have seven fruits. For chrysanthemum, partitioning is determined by fixed ratios as determined by crop developmental stage (Lee et al. 2002b). Computation of leaf area increase follows the approach given by Gary et al. (1995), as explained in Heuvelink et al. (2005).

Daily global radiation was model input and taken from Breuer and Van de Braak (1989), representing average data for De Bilt (52 °N, The Netherlands), but with natural variation. A greenhouse transmittance for diffuse radiation of 70%, 71% or 75.6%, respectively, was assumed in the three simulation studies. Hourly values of greenhouse temperature and CO_2 concentration were also model input.

Impact of SLA and $P_{g,max}$ on cut-chrysanthemum yield

Chrysanthemum is a qualitative short-day plant. In greenhouses, blackout screens and lamps provide the means for day-length control and year-round production. Cultivation of chrysanthemum starts with a rooted cutting, which is grown in about three months to a plant with a harvestable shoot; the harvestable shoot represents about 90% of the total above-ground plant biomass. In the simulations we used the standard seasonal pattern for SLA (Lee et al. 2002b); however, a positive effect of temperature on SLA was implemented.

The following model input was based on an experiment. The use of supplementary assimilation light (49 µmol m^{-2} s^{-1}) was dependent on the incoming radiation (switch on at 200 W m^{-2} and off at 300 W m^{-2}). Mean 24h greenhouse temperature varied between 19°C in winter (22 December – 5 March; 73 days; short day (SD) started on 7 January; planting density 48 plants m^{-2}) and 21°C in summer (29 June – 1 September; 64 days; SD started on 8 July; planting density 48 plants m^{-2}). CO_2 concentration ranged from 400 ppm in summer to 1000 ppm in winter. Day length was 20h for LD and 11.5h for SD period.

Simulation of a tomato genotype with two leaves per truss

Two leaves per truss were simulated by reducing the sink strength of each vegetative section by one third (Figure 1). The simulation started at flowering of the first truss (10 January) and continued until 26 November. Temperature was chosen at 19°C day and night. CO_2 concentration was 400 ppm and planting density was 2.5 plants m^{-2} with one stem per plant. Besides simulation of standard cultivation practices, a delay in removal of old leaves by one week was simulated; so, leaves from a vegetative section were removed one week after the corresponding truss was harvest-ripe.

Elimination of shade avoidance in tomato

Simulations run from 11 December to 30 November of the next year and were conducted for three different planting densities: 2.5, 3.0 and 5.0 plants m^{-2}. Temperature set-point was 19°C day, 17°C night and CO_2 set-point ranged from 1000 ppm at day when the ventilators were closed to 300 ppm at night. Actual temperatures and CO_2 concentrations were calculated with KASPRO (De Zwart 1996). For 50% of the plants an extra shoot was allowed after 50 days. All shoot tops were removed after 260 days.

RESULTS

Evaluation of plant traits for yield improvement

For a chrysanthemum crop grown in summer simulating a 20% higher SLA resulted in a 5% higher biomass production (Table 1). A 20% higher light-saturated photosynthetic rate ($P_{g,max}$) had the same effect on biomass production. However, for a winter-grown crop, a 20% rise in SLA resulted in a stronger increase in biomass production (+11%), whereas a 20% higher $P_{g,max}$ gave a smaller increase in biomass production (+4%). Hence, for a winter-grown crop improving SLA is more relevant than improving $P_{g,max}$, whereas for a summer-grown crop $P_{g,max}$ and SLA are of equal importance for yield.

Table 1. *Total simulated crop dry weight at harvest for cut chrysanthemum grown in two seasons. Results for a standard set of parameters, or a 20% higher SLA, or a 20% higher* $P_{g,max}$*. Relative values given in brackets*

	Dry-matter yield (g m^{-2})	
	Winter	Summer
Standard	315 (100%)	737 (100%)
SLA + 20%	351 (111%)	777 (105%)
$P_{g,max}$ + 20%	328 (104%)	778 (106%)

A tomato genotype with two leaves between trusses

The simulation showed that the fraction of dry matter partitioned into the fruits is favoured in a tomato genotype with only two leaves and internodes between trusses (Table 2).

Integrated over the whole cultivation period, 74% of biomass was partitioned to the fruits, whereas for the standard genotype this was 66%. However, of the new genotype average LAI was only 2.1, compared to 2.8 in the standard. This resulted in a reduced biomass production. Hence, the favourable effect on partitioning was partly counteracted by a reduced total biomass production and as a result the predicted yield improvement was rather small. When the old leaves were removed one week later than standard, average LAI of the new genotype increased from 2.1 to 2.6, reduction in biomass production was only 2% and fruit yield improved by 10% compared to the standard. In conclusion, the simulations showed that a tomato genotype with two instead of three leaves between trusses may improve yield. To maximize the benefit of this trait it is important to keep the LAI sufficiently high.

Table 2. *Simulated fraction partitioned to the fruits, total dry matter, fruit dry weight and average LAI, for a tomato crop planted on 10 January and ended on 26 November*

	Fraction to fruits	Dry matter (kg m^{-2})		LAI_{av} (m^2 m^{-2})
		Total	Fruits	
Standard genotype	0.66	4.08	2.63	2.8
New genotype[1]	0.74	3.82	2.77	2.1
New genotype[1] + delayed leaf removal	0.74	4.01	2.91	2.6

[1] Two leaves between trusses instead of three; vegetative sink reduced by 33%.

Elimination of shade avoidance in tomato

In plants without shade avoidance response, as represented in the crop growth model, simulating an increased planting density had only a limited influence on yield. Simulated tomato fruit dry-matter yields were 3.9, 4.0 and 4.2 kg m^{-2}, for 2.5, 3.0 and 5.0 plants m^{-2}, respectively. Doubling the planting density resulted in a 6% yield increase. This resulted from a 6% increase in total biomass production, which was caused by a higher light absorption because of a higher LAI (Figure 2). Averaged between day 150 and day 300 after planting, LAI was 3.2, 3.8 and 5.9, and fraction absorbed light was 0.82, 0.84 and 0.88 for 2.5, 3.0 and 5.0 plants m^{-2}, respectively.

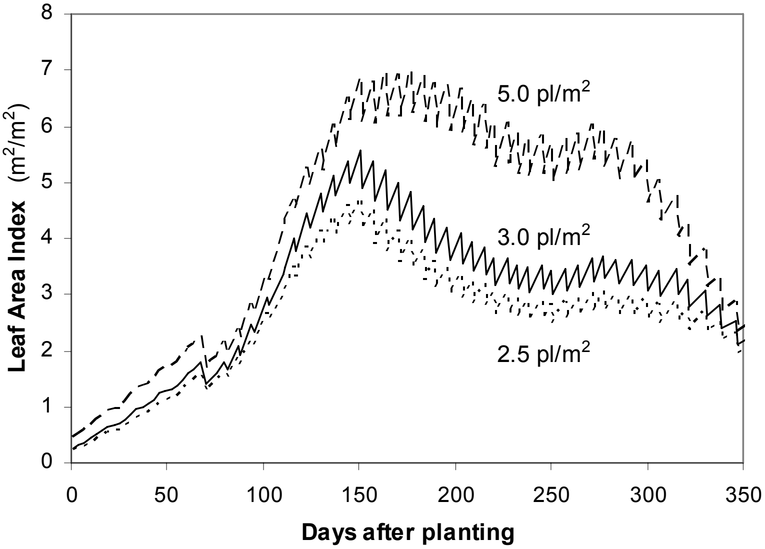

Figure 2. *Simulated effect of planting density on LAI for a tomato crop planted on 11 December and continued until 30 November (planting densities 2.5, 3.0 and 5.0 plants m^{-2}). For 50% of the plants an extra shoot was allowed after 50 days. All shoot tops were removed after 260 days*

DISCUSSION

Impact of SLA and $P_{g,max}$ on cut chrysanthemum yield

Explanatory models provide the opportunity to evaluate the impact of physiological characteristics on complex plant traits such as yield. It may seem inadequate to express yield of cut chrysanthemum in terms of dry mass per m^2. However, when dry mass is increased by 5% and the same individual final plant weight as before is considered to be acceptable, a 5% higher planting density could be allowed (Langton et al. 1999; Lee et al. 2002b). This would indeed result in more harvestable stems, so a 5% yield increase.

The higher impact of SLA on dry-mass yield in winter than in summer (Table 1), may be explained by the lower average LAI in winter; so, an increase in LAI because of a higher SLA has more impact on light interception. $P_{g,max}$ is more important in summer than in winter, as average light intensities are much higher in summer and $P_{g,max}$ is especially determining leaf-photosynthetic rates at high light intensities, whereas at low light intensities it is mainly the initial light-use efficiency influencing leaf photosynthesis.

Focusing on one parameter for yield improvement may not be very helpful, because of covariance between traits and feedback mechanisms. For example, in tomato a strong negative correlation has been reported between SLA and net photosynthetic rate (NAR) (Smeets and Garretsen 1986). Thin leaves are more susceptible to mechanical damage and maybe also to diseases. In such cases the challenge is to find ways to counter such associations (Goudriaan et al. 1985).

When comparing genotypes in winter, a genotype with a 20% higher SLA would be higher-yielding than a genotype with a 20% improved $P_{g,max}$. However, when compared in summer, no difference in yield between both genotypes would be observed. Hence the comparison of the genotypes depends on the environment (G×E interaction). Furthermore, the outcome of the comparison between breeding for improved $P_{g,max}$ or improved SLA will also depend on management measures such as planting density or the use of supplementary lighting.

A tomato genotype with two leaves between trusses

The potential of yield improvement for a tomato genotype with only two instead of three leaves and internodes between trusses was also reported by Xiao et al. (2004). Besides modelling, these authors conducted a greenhouse experiment where one out of three young leaves was removed. This resulted in plants with two leaves between trusses (however, still three internodes), and indeed an improved partitioning to the fruits was found. These authors also observed a reduction in average LAI, resulting in a reduced total biomass production and, therefore, a yield reduction by 5% (not statistically significant). To counteract the negative effect that two leaves between the trusses have on LAI and total biomass production, old leaves can be retained longer, as was done in our simulations (Table 2). Alternatively, a higher planting density could be maintained, as was demonstrated by Xiao et al. (2004).

Whether breeding can realize the predicted extra yield for a genotype with two leaves between trusses is not clear. Tomato genotypes with only two leaves between trusses do exist, but this plant characteristic seems to be linked to a determinate growth pattern (W.H. Lindhout, pers. comm.), whereas for greenhouse cultivation plants with indeterminate growth pattern are needed.

Elimination of shade avoidance

The model has been validated, and it was shown that predicted planting-density effects (2.9–4.8 plants m^{-2}) on biomass production were in agreement with measurements (Heuvelink 1999). The present simulation results show that for tomato cultivation a higher planting density hardly improves yield. This is explained by the high LAI already obtained for a standard tomato crop (Figure 2). Starting at a density of 2.5 plants m^{-2}, and retaining one side shoot on 50% of the plants from March onwards, hence 3.75 stems m^{-2}, resulted in an average LAI of 3.1. Therefore, increased planting densities only slightly improve light interception. Furthermore, the predicted yield increase of 6% is an overestimation, as the model assumes seven fruits per truss for all trusses, whereas a higher planting density will negatively influence fruit set (Papadopoulos and Ormrod 1991). This will reduce partitioning to the fruits and yield.

For crops that have already a high LAI, e.g., tomato (Heuvelink et al. 2005, Figure 2), sweet pepper (Dueck et al. 2006), cut chrysanthemum (Lee et al. 2002a) or rose (Kool 1996) no substantial yield improvement will result from increased planting densities, as the fraction of absorbed light is already close to its maximum. Improved yield expectations because of higher planting densities after elimination of shade avoidance are in these cases not realistic. If elimination of the shade avoidance response would mean that crops grown at their current densities partition less biomass to the stems and petioles, it is expected to improve yield, comparable with semi-dwarf cultivars in Gramineae (Cooper 1979). However, in crops such as tomato or cucumber, assimilate partitioning to the stems and petioles is already small (< 10%); so, even from a further reduction by as much as 50%, only an improvement of the yield by 5% can be expected. It should also be considered that for determining the economically optimum planting densities also costs of extra plant material and plant handling (e.g., removing side shoots, guiding plants) must be taken into account.

CONCLUSION

Crop growth models are valuable tools to evaluate differences in plant-physiological characteristics of horticultural crops under different growth conditions. Such quantifications are important in focusing breeding programmes and in ideotyping for different environments. Our case studies have shown that (1) breeding for higher SLA in cut chrysanthemum has more impact on yield than breeding for higher light-saturated leaf photosynthesis when grown in winter, but not in summer; (2) a tomato genotype with two instead of three leaves between trusses would improve yield, and even more so when cultivation is adapted to keep LAI high enough; and (3) elimination of shade avoidance response would hardly affect tomato yield.

The use of crop models would add value to breeding programmes if model parameters could be linked to genetic information (e.g., QTLs). The first steps in this direction are currently made.

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DIVERSITY, RESOURCE USE AND CROP PERFORMANCE

CHAPTER 19

ROLE OF ROOT CLUSTERS IN PHOSPHORUS ACQUISITION AND INCREASING BIOLOGICAL DIVERSITY IN AGRICULTURE

H. LAMBERS AND M.W. SHANE

School of Plant Biology, Faculty of Natural and Agricultural Sciences,
The University of Western Australia, 35 Stirling Highway, Crawley, WA 6009,
Australia.
E-mail: hans.lambers@uwa.edu.au

Abstract. Soils in the south-west of Western Australia and South Africa are among the most phosphorus-impoverished in the world, and at the same time both of these regions are Global Biodiversity Hotspots. This unique combination offers an excellent opportunity to study root adaptations that are significant in phosphorus (P) acquisition. A large proportion of species from these P-poor environments cannot produce an association with mycorrhizal fungi, but, instead, produce 'root clusters'. In Western Australia, root-cluster-bearing Proteaceae occur on the most P-impoverished soils, whereas the mycorrhizal Myrtaceae tend to inhabit the less P-impoverished soils in this region. Root clusters are an adaptation both in structure and in functioning; characterized by high densities of short lateral roots that release large amounts of exudates, in particular carboxylates (anions of di- and tri-carboxylic acids). The functioning of root clusters in Proteaceae ('proteoid' roots) and Fabaceae ('cluster' roots) has received considerable attention, but that of 'dauciform' root clusters developed by species in Cyperaceae has barely been explored. Research on the physiology of 'capillaroid' root clusters formed by species in Restionaceae has yet to be published. Root-cluster initiation and growth in species of the Cyperaceae, Fabaceae and Proteaceae are systemically stimulated when plants are grown at a very low P supply, and are suppressed as leaf P concentrations increase. Root clusters in Proteaceae, Fabaceae and Cyperaceae are short-lived structures, which release large amounts of carboxylates, briefly, at a particular stage of root development. The rates of carboxylate release are considerably faster than reported for non-specialized roots of a wide range of species. Root clusters play a pivotal role in mobilization of P from P-sorbing soil. Because the world P reserves are being depleted whilst vast amounts of P are stored in fertilized soils, there is a growing need for crops with a high efficiency of P acquisition. Some Australian and African native species as well as some existing crops have traits that would be highly desirable for future crops. The possibilities of introducing P-acquisition-efficient species in new cropping and pasture systems are explored. In addition, possible strategies to introduce traits associated with a high P-acquisition efficiency into future crop species are discussed.

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INTRODUCTION

Phosphorus (P) is an essential inorganic nutrient for all living beings. After nitrogen (N), P is quantitatively the most important inorganic nutrient for plant growth. Phosphorus is a non-renewable resource, unlike N, which can be assimilated from N_2 into NH_3 by free-living and symbiotic N_2-fixing micro-organisms or converted into NH_3, NO_3^- or urea industrially. Global P reserves are rapidly being depleted; current reserves will be halved (relative to the reserves at the turn of the 20th century) by 2040–2060 (Steen 1998). Whilst global P reserves are being depleted, P levels in many P-sorbing, agricultural soils are accumulating (Parfitt 1979), because 80–90% of P applied as fertilizer is sorbed by soil particles, rendering it unavailable for plants without specific adaptations to access sorbed P (Parfitt 1979; Jones 1998). With decreasing global P reserves, P-fertilizer prices are bound to increase. There is an urgent need to develop crops that are more efficient in acquiring P from soil and/or in using P more efficiently. Equally, it is becoming increasingly important to use crops that reduce the off-site effects of P fertilization, thus reducing the risks of pollution of streams and rivers. This chapter focuses on traits associated with efficient P acquisition.

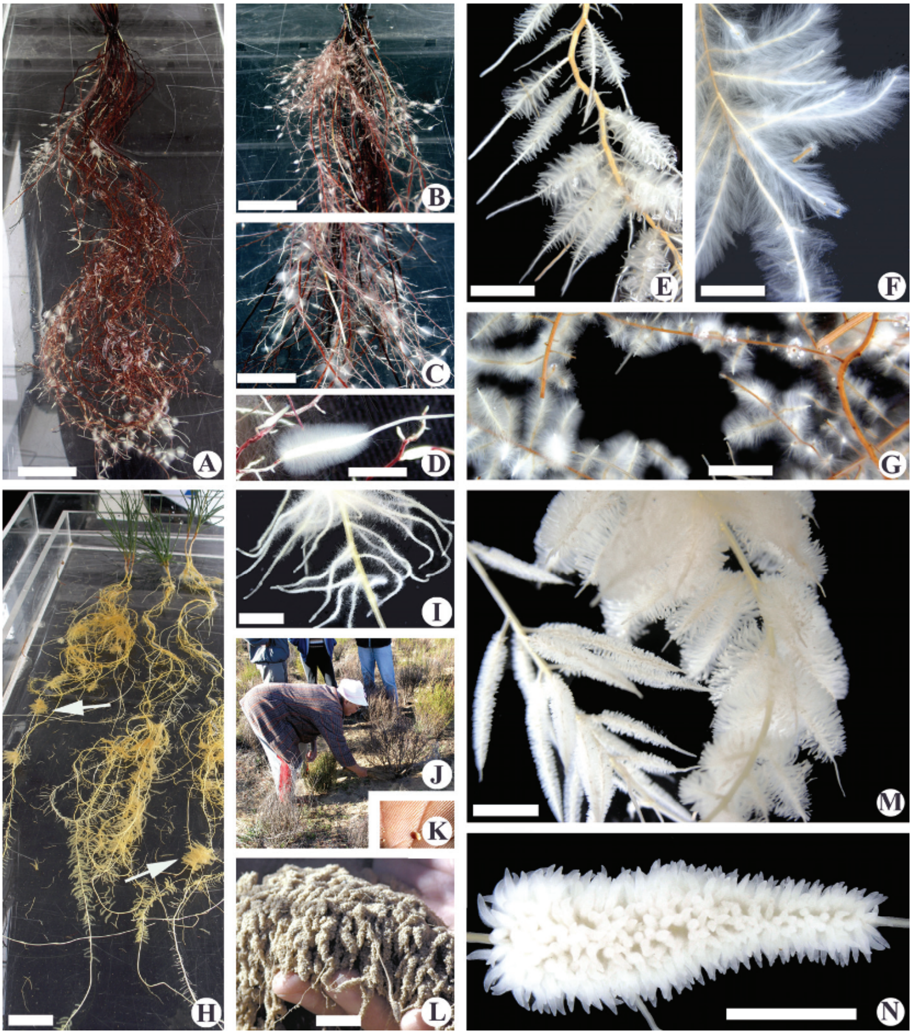
Unlike nitrate, which readily moves in soil towards the roots via both mass flow and diffusion, phosphate is highly immobile in soil. Mass flow typically delivers as little as 1–5% of a plant's P demand, and the amount intercepted by growing roots is only half of that (Lambers et al. 1998). The rest of all required P has to reach the root surface via diffusion; diffusion coefficients for phosphate in soil are typically very low, compared with those for other nutrients: $0.3–3.3\ 10^{-13}\ m^2\ s^{-1}$ (Clarkson 1981). Diffusion is particularly slow in dry soil (e.g., Turner and Gilliam 1976; Bhadoria et al. 1991). Increasing P delivery to roots via mass flow can be achieved by enhanced transpiration rates, but this cannot have a major effect, and would be at the expense of a plant's water-use efficiency. Root interception of P can be increased by root proliferation, increased frequency and length of root hairs, a root architecture that leads to enhanced root growth and root foraging in upper soil horizons (where nutrients are often relatively enriched), and mycorrhizal symbioses (Lambers et al. 2006). When the P concentration in soil solution is very low, an effective mechanism to increase acquisition of P is to enhance P diffusion. In dry soil, this can be achieved by increasing the moisture content of the soil, involving 'hydraulic redistribution'; it can also be driven effectively by increasing the concentration of inorganic P in the rhizosphere (Lambers et al. 2006). This review focuses on specific structural and functional root traits that enhance P acquisition from soil(s) with restricted availability of P. In particular, we discuss the traits of naturally occurring species taken from two of the world's 25 hotspots of biodiversity, the south-west corner of Western Australia and South Africa. These two regions have soils that are nutrient-impoverished, and the soils in Western Australia are exceptionally ancient and deeply weathered. Consequently, a host of species have evolved in these two regions with very efficient root adaptations to acquire sparingly available soil P. We further explore possibilities these traits may offer to future crop plants, and include pertinent information on several crop species that form root clusters.

***Table 1**. Families with root clusters (Shane and Lambers 2005a; Lambers et al. 2006) and examples of their present and potential commercial use (numerous sources)*

Family	Genus	Species	Type of root cluster	Commercial use
Betulaceae	*Alnus*	*incana*	simple	timber
Casuarinaceae	*Allocasuarina*	*huegeliana*	simple	bio-energy
		luehmannii	simple	timber, fodder
	Casuarina	*cristata*	simple	fodder for browsing sheep
		cunninghamiana	simple	timber
	Gymnostoma	*papuanum*	simple	timber
Cucurbitaceae	*Cucurbita*	*pepo*	cluster-like	vegetable
Cyperaceae	*Carex*	*flava*	dauciform	fodder
		flacca	dauciform	fodder
	Caustis	*blakei*	dauciform	ornamental
Elaeagnaceae	*Hippophae*	*rhamnoides*	simple	fruit juice, health tea, source of flavonoids and vitamin C
Fabaceae	*Aspalathus*	*linearis*	simple	tea
	Hakea	*oleifolia*	simple	pulpwood
	Lupinus	*albus*	simple	protein
	Viminaria	*juncea*	simple	pulpwood
Moraceae	*Ficus*	*benjamina*	cluster-like	indoor plant
Myricaceae	*Myrica*	*cerifera*	simple	bayberry candles
		esculenta	simple	fruit
		gale	simple	insect repellent
Proteaceae	*Banksia*	*coccinea*	compound	flowers
	Grevillea	*robusta*	simple	timber
		leucopteris	simple	fine paper, pulpwood
	Leucadendron	*L. laureolum* × *L. salignum.* cv. safari sunset	compound	cut-flower
	Protea	*Eximia* × *susannae* cv. sylvia	simple	cut-flower
	Macadamia	*integrifolia*	simple	nuts
	Telopea	*speciosissima*	simple	flowers
Restionaceae	*Chondropetalum*	*tectorum*	capillaroid	ornamental plant, thatch
	Calopsis	*paniculata* (silk koala)	capillaroid	ornamental
	Desmocladus	*flexuosus*	capillaroid	restoration
	Baloskion	*tetraphyllum*	capillaroid	ornamental, landscaping

ROOT-CLUSTER STRUCTURE AND CARBOXYLATE RELEASE

Root clusters are formed by species belonging to eight families of dicotyledonous plants and also by species in two families of monocotyledonous plants (Table 1, Shane and Lambers 2005a; Lambers et al. 2006). Root clusters were first described in Australian Proteaceae (therefore termed 'proteoid' roots, Purnell 1960) but 'cluster' root is now a preferred general term following their subsequent identification in the other families (Lamont 1982). In terms of their structure, cluster roots can be identified as belonging to one of two broad types, i.e., 'simple' or 'compound' (Figure 1A–N). Many species in the families listed in Table 1 form the



simple root cluster that has a distinct bottlebrush-like appearance (e.g., *Leucadendron meridianum*, Figure 1N). There are several quite distinct morphologies of simple cluster roots among the species, and especially between species of the Proteaceae (e.g., *Leucadendron*) and those in other families, e.g., Fabaceae species (e.g., *Aspalathus linearis* (rooibos), Figure 1H and 1I). This difference likely reflects differences in anatomical structure related directly to the number of primary xylem poles as described for *Hakea prostrata* (Proteaceae) and *Lupinus albus* (Fabaceae). The number of longitudinal rows of short lateral roots (rootlets) developed within each cluster root reflects the number of xylem poles, of which there are more in *Hakea prostrata* (6 to 8) than in *Lupinus albus* (2 poles) (Lamont 1982; Watt and Evans 1999; Shane and Lambers 2005a). Within the Proteaceae, a few genera also produce (alone or in combination with the simple type) a 'compound' type of cluster roots (Table 1, *Protea* sp. Figure 1M), which are essentially 'branched simple cluster roots'. The compound type of cluster root is known for a couple of Australian genera (e.g., *Banksia*) and several South African genera (e.g., *Leucadendron* and *Protea*) (Lamont 1982; 1983). It is not clear what the significance is for some species to form preferentially the compound type of root cluster. Lamont (1983) has suggested that compound cluster roots are perhaps ontogenetically and phylogenetically more advanced than simple cluster roots, but whether or not these two types of cluster root preferentially access specific fractions of soil nutrients (e.g., inorganic and organic P) has yet to be determined.

Root clusters have been described in the monocotyledonous plant families Cyperaceae (sedges) and Restionaceae (rushes). In the Cyperaceae 'dauciform' root clusters were first described by Russian plant scientists (Selivanov and Utemova 1969, and references cited therein). They were subsequently found in cyperacean species around the world (Lamont 1982; Shane et al. 2006; Lambers et al. 2006). Lamont (1974) coined the term 'dauciform', because of the carrot shape of these

Figure 1. *Roots and root systems of South African species of Cyperaceae, Restionaceae, Fabaceae and Proteaceae grown under conditions of low P availability.* (**A-D**) *sedge roots,* Tetraria *sp.* (**A**) *whole root system; bar is 45 mm.* (**B** and **C**) *higher magnification showing cotton-ball-like dauciform roots; bars are 36 and 24 mm, respectively.* (**D**) *specialized 'dauciform' root showing abundant, long root hairs; bar is 4 mm.* (**E-G**) *capillaroid roots of rush species,* (**E**) Mastersiella digitata*; bar is 15 mm.* (**F**) Thamnochortus fraternus*; bar is 8 mm, and* (**G**) Chondropetalum tectorum*; bar is 9 mm.* (**H-L**) Aspalathus linearis *(Fabaceae, rooibos),* (**H** and **I**) *roots were from plants grown in hydroponics,* (**H**) *whole root system containing cluster roots (arrows); bar is 35 mm.* (**I**) *single cluster root; bar is 3 mm.* (**J**) *seed being collected from beneath wild rooibos plants.* (**K**) *very small (ca. 2 mm length) rooibos seed in the palm of a hand.* (**L**) *soil, tightly bound to mature rootlets of root cluster from field-grown rooibos plants; bar is 23 mm. Images of proteoid roots in* **M** *and* **N** *are from the species in the genus* Leucadendron. (**M**) *abundant proteoid-root development in* L. *var.* chameleon (L. laureolum × L. salignum), *groups of ca. 15 individual proteoid roots on the left side of the image are at an earlier stage of development than the group of proteoid roots on the right side of the image; bar is 6 mm.* (**N**) *individual proteoid root of* L. meridianum *(limestone conebush), with hundreds of growing rootlets that had yet to develop root hairs; bar is 10 mm*

root clusters (e.g., South African *Tetraria* sp. Figure 1A–D). It is apparent that dauciform roots are developed in two tribes of the Cyperaceae (i.e. Cariceae and Rhynchosporeae) (Lamont 1982). Dauciform root clusters occur in groups of up to 20 to 30 but their most remarkable external feature is the very dense formation of long root hairs over the carrot-shaped axis (Figure 1D). The Restionaceae (the 'Southern Hemisphere rushes') are mostly distributed in Australia and South Africa (as are Proteaceae). Root clusters in this family are termed 'capillaroid' and are characterized by dense numbers of rootlets densely covered with long root hairs (Figure 1E, F and G). The term 'capillaroid' stems from their sponge-like properties on holding soil water (Lamont 1982).

In terms of the functioning of root clusters for nutrient acquisition we summarize the literature concerning root exudation of carboxylates (e.g., citrate), and the reader is referred to Lambers et al. (2006) for more information about the other exudates, such as phosphatases released by root clusters. The cluster roots of Fabaceae (*L. albus*, Watt and Evans 1999) and Proteaceae (*Hakea prostrata*, Shane et al. 2004) release citrate in a developmentally programmed exudation event that lasts for a brief time (ca. 1 to 2 days) once the roots mature. The dense 'root mats' in the field developed by the compound-cluster-root-forming Proteaceae also release carboxylates (Grierson 1992; Roelofs et al. 2001), but there are no reports on the exact time course of carboxylate exudation in these root-mat-forming species. Pate and Watt (2002) calculated that citrate in the cluster-root rhizosphere of *Banksia prionotes* (Proteaceae) accumulates to levels of 35–72 μmol per gram soil. Such concentrations are sufficiently high to mobilize P. The authors calculated that the clusters mobilized 44% of the total P in the soil trapped by their clusters, or 250% of the 'available' P.

Though morphologically and anatomically very distinct, dauciform roots function in a way very similar to proteoid roots (Davies et al. 1973; Lamont 1974; Shane et al. 2005; 2006; Playsted et al. 2006). That is, dauciform-root formation is suppressed when plants have a relatively high P status (Shane et al. 2005; Playsted et al. 2006) and carboxylates (e.g., citrate) are released during a brief interval once the dauciform root has matured (Figure 1D, Shane et al. 2005; 2006). This brief time interval when large amounts of carboxylates are released from the roots is considered important to mobilize P before microbial activity builds up. Microbial activity in the rhizosphere of root clusters of *L. albus* is slowed down by rhizosphere acidification and by exudation of flavonoids that promote fungal sporulation (Weisskopf et al. 2006). Finally, release of anti-fungal cell-wall-degrading enzymes (chitinase and glucanase) prior to the release of carboxylates would inhibit fungal growth (Weisskopf et al. 2006). We have yet to assess whether the capillaroid roots of species within the Restionaceae function like proteoid roots, but based on our own preliminary observations, we hypothesize that they do.

In summary, root clusters differ greatly in their anatomy and morphology. Proteoid root clusters and dauciform root clusters release carboxylates in a developmentally programmed, brief exudative event, a pattern that is considered vital for their functioning (Lambers et al. 2006).

EFFECTS OF PLANT P STATUS ON DEVELOPMENT AND FUNCTIONING OF ROOT CLUSTERS

Root clusters in species of the Casuarinaceae (Racette et al. 1990; Reddell et al. 1997), Cyperaceae (Shane et al. 2005; Playsted et al. 2006), Fabaceae (Gardner et al. 1983; Keerthisinghe et al. 1998), Myricaceae (Louis et al. 1990) and Proteaceae (Lamont 1982; Aitken et al. 1992; Shane et al. 2003a; 2003b) are suppressed when adding P to the root environment. Feeding P to leaves also suppresses cluster-root formation in *L. albus* (Marschner et al. 1986; Gilbert et al. 2000; Shane et al. 2003a), showing that the signal(s) that leads to suppression of cluster-root initiation and growth originate in the shoot, most likely in young leaves (Keerthisinghe et al. 1998).

Root clusters are relatively short-lived (approx. 1 to 3 weeks) and intermittent development of new root clusters produces spacing between old and new cluster roots (see Figure 1 in Shane and Lambers 2005a), which are typically separated by unbranched regions along the root axis. In experiments using plants with a split-root system, where one root half received a low and the other a high P supply, depending on species, cluster roots are either produced equally on both root halves (i.e., *L. albus*, Shane et al. 2003b; *Grevillea crithmifolia*, Shane and Lambers 2006), or predominantly on low-P root halves (*H. prostrata*, Shane et al. 2003a). This suggests that there are local signals as well as systemic ones. Therefore, there is evidence for both systemic and local signals controlling root cluster formation and functioning (Watt and Evans 1999; Shane and Lambers 2005a). Local signals may be stronger for some processes (exudation) and may also vary among species.

Since auxin-transport inhibitors suppress root-cluster formation in P-deficient *L. albus* plants (Gilbert et al. 2000), whereas auxins stimulate root-cluster formation in P-sufficient *L. albus* (Gilbert et al. 2000; Skene and James 2000), it is very likely that auxin is a component of the signal-transduction path between plant P status ('P-sensing') and cluster-root formation. Shane et al. (2003a) showed that various processes in cluster roots of *Hakea prostrata* (Proteaceae) have different sensitivities to plant P status, with carboxylate exudation from cluster roots being the most sensitive, followed by cluster-root growth, and then cluster-root initiation. Interestingly, application of auxins leads to cluster-root formation in P-sufficient *L. albus* plants, but does not lead to carboxylate exudation from those clusters (Hocking and Jeffery 2004). This suggests that, whilst systemic signals account (in part) for production of root clusters in *L. albus*, exudation of carboxylates from cluster roots in this species may be controlled by (additional) local signals. Root-produced cytokinins probably play an antagonistic role in the transduction pathway (Neumann et al. 2000).

Liu et al. (2005) showed that sugars are related to P-deficiency-induced gene expression in *L. albus*. Interruption of phloem supply to P-deficient roots resulted in a rapid decline in accumulation of gene products induced by P deficiency. Regulation of P-deficiency-induced genes appears to be conserved across plant species and sugars are crucial for P-deficiency signal transduction.

Much remains to be discovered about signalling molecules and signalling pathways involved in the development and functioning of cluster roots. Systemic and locals signals involved in nodule formation may guide us towards a model for cluster-root formation (e.g., Ferguson and Mathesius 2003). Auxins and other phytohormones play a role in nodule initiation and development; nitrate locally inhibits nodule formation. Figure 2 summarizes our current thinking; it has components that are firmly established (auxins, cytokinins) as well as aspects that are speculative (direct P effects). What is currently lacking is a sound understanding of specific genes that are responsible for the synchronous development of numerous rootlets that form a root cluster. Nothing about their development appears to be cluster-specific; what makes the process special is the synchronization of rootlet development and metabolism. Identification of the gene(s) controlling that synchronous development would be a major step in the direction of future crops with root clusters.

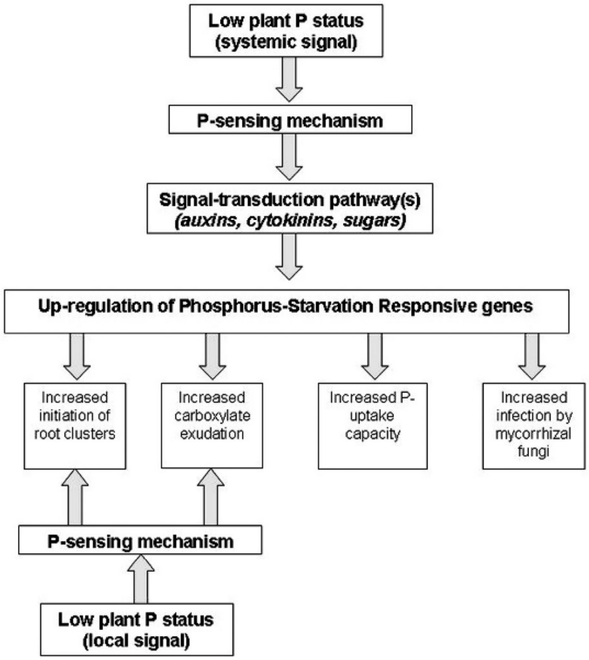


Figure 2. *Plant responses to P limitation. A low external P availability decreases the plant's internal P status. When the plant senses a low P status, P-starvation responses are induced. P-starvation responses, depending on the species, include increased root-hair formation, root-cluster initiation and development, carboxylate exudation, P-uptake capacity, and mycorrhiza formation. Apart from systemic signals, most likely originating in young leaves, there are local signals. Systemic and local signals may interact*

EXISTING CROP PLANTS WITH A HIGH P-ACQUISITION EFFICIENCY

Many species occurring on severely P-impoverished soil have the capacity to produce root clusters that enhance the availability of P in the rhizosphere. At one stage, a plant's capacity to produce root clusters was considered an alternative to the mycorrhizal habit. For example, proteacean (Purnell 1960), cyperacean (Powell 1975) and *Lupinus* (Trinick 1977) species are non-mycorrhizal (or weakly at most, Shane and Lambers 2005a). However, it has since been discovered that there are also many species that can produce both root clusters and mycorrhizas (reviewed in Lambers et al. 2006).

Root clusters combine adaptive structures with adaptive functioning. Root clusters occur in a large number of species belonging to 10 families (Lambers et al. 2006). Root-cluster-bearing plants include several species used for the production of food, fodder, fibre, timber, tea or bayberry candles: *Grevillea* and *Macadamia* species (Proteaceae), *Aspalathus linearis* (rooibos) and *Lupinus* species (Fabaceae), *Myrica cerifera* (wax myrtle) (Myricaceae), *Carex* species (Cyperaceae), *Allocasuarina* and *Casuarina* species (Casuarinaceae), *Cucurbita pepo* (Cucurbitaceae) and *Hippohae rhamnoides* (sea buckthorn) (Elaeagnaceae). Many are also used for horticultural purposes (Betulaceae, Cyperaceae, Elaeagnaceae, Fabaceae, Moraceae, Proteaceae, Restionaceae) and in restoration. Considering that P reserves are rapidly being depleted (Steen 1998), whilst vast amounts are present in soils that have been fertilized for decades (Singh and Gilkes 1991), we should consider options for incorporating root clusters in new crop species or cropping systems.

There are several advantages of a large capacity to mobilize P in the rhizosphere, especially by root clusters, and the downsides are limited (Lambers et al. 2006). Provided the risks of enhanced cadmium uptake and eutrophication are carefully managed, P-acquisition-efficient new crops, especially high-exuding, cluster-bearing crops, offer tremendous potential. To apply information gleaned from the study of native plants for cropping and pasture systems, we should be willing to consider new crop species. Equally, we should consider new cropping systems where combinations of species in intercropping systems and ideal rotations are used to maximize the acquistions of P from low-P soils (Lambers et al. 2006). These approaches should lead to more sustainable cropping systems with less off-site risks of eutrophication of streams and rivers.

PERSPECTIVES FOR EXISTING AND FUTURE P-ACQUISITION-EFFICIENT CROP PLANTS

Root clusters allow plants to grow in soils where the total amount and availability of P is restricted (Neumann and Martinoia 2002). Root clusters can mobilize sparingly available P, and hence support plant growth where mycorrhizas are less effective (Lambers et al. 2006). As such, the cluster-root-bearing habit contributes to the biodiversity in natural systems, allowing cluster-root-bearing species to compete successfully on the poorest soils, whilst being less competitive on slightly less P-impoverished soils. Do nearest neighbours of root-cluster-bearing plants in natural systems benefit from the P-solubilizing ability of their neighbours? There are no hard data available to answer this question, but there is information from pot experiments on growth-enhancing effects of root-cluster-bearing *L. albus* plants on neighbouring *Triticum aestivum* plants (Horst and Waschkies 1987; Cu et al. 2005). In this section, we explore how the cluster-bearing habit might be valuable in new cropping and pasture species and systems.

Biodiversity in western agricultural systems has been reduced dramatically over the last few decades, but is now increasingly considered important for a variety of reasons: it provides ecosystem services beyond production of food, fibre, fuel and income (Altieri 1999; Van Elsen 2000). Enhanced biodiversity may also allow more efficient acquisition of P from P-sorbing soils. The results from pot experiments with cluster-root-bearing *L. albus* and low-carboxylate-exuding *T. aestivum* (Horst and Waschkies 1987; Cu et al. 2005), as cited in the preceding paragraph, are promising, but the experiments need to be followed up using more realistic root densities under field conditions. Intercropping is common practice in large parts of China (Zhang and Li 2003), and some combinations greatly enhance the efficiency of nutrient acquisition (Zuo et al. 2000), but so far no combinations include the use of cluster-root-bearing species. The cluster-root-bearing proteacean tree, *Grevillea robusta*, is frequently intercropped with *Zea mays* (e.g., Smith et al. 1999; Smith and Roberts 2003), but Radersma and Grierson (2004) concluded that it is unlikely that the extent of P mobilization by *G. robusta* will benefit adjacent crop plants, unless crop roots actually share the rhizosphere with tree roots. However, Kumar et al. (1999) found that *G. robusta* enhanced ^{32}P uptake by *Cocos nucifera* (coconut) when the two species were interplanted in coconut plantations. Since cluster-root-bearing plants mobilize not only P but also micronutrients (Shane and Lambers (2005b) and references cited therein), intercropping also has beneficial effects on Mn uptake (Gardner and Boundy 1983). Since cluster roots can also be induced by Fe deficiency (Arahou and Diem 1997; Hagström et al. 2001), it is envisaged that Fe uptake might also be enhanced by intercropping with a cluster-root-bearing species. Generally speaking, it is anticipated that species with complementary nutrient-acquisition strategies will do well in intercropping systems (Zhang and Li 2003); for example, the combination of a monocotyledonous species that mobilizes Fe in calcareous sol and a legume that fixes dinitrogen symbiotically.

Beneficial effects of cluster-root-bearing species with a large capacity to mobilize soil P are not restricted to increased P uptake by neighbouring plants, but may extend to enhanced P acquisition and growth by the following crop (Kamh et al. 1999). Little et al. (2004) showed that Olsen-extractable P in plots 8 weeks after sowing potatoes was enhanced after growing *L. albus* or a combination of *L. albus* and *B. napus* as a cover-crop relative to that after *Avena sativa* or *B. napus* alone. These results provide evidence that cover-crops containing the cluster-bearing *L. albus* potentially enhance the P availability for the following crop. Similarly, other fast-exuding plants can have a beneficial effect on the following crop (Lambers et al. 2006).

Incorporation of root-cluster-bearing species into cropping, pasture and forestry systems need not be restricted to the introduction of new species. Interspecific crosses between root-cluster-bearing *Lupinus* species and congeneric species without root clusters might be a valuable approach that has yet to be explored. Combined with molecular-marker-assisted breeding, this might allow the development of new lupin crops that are excellent at acquiring P from P-sorbing soils. Alternatively, a better understanding of the genes and molecular events involved in root-cluster formation might lead to the isolation of genes that could be introduced in existing crop species that lack root clusters.

There are risks associated with the introduction of new crop species. One of these risks is the potential for any introduced species to become an invasive weed. Some of the highly P-acquisition-efficient proteacean species, e.g., *Hakea drupacea*, *H. gibbosa* and *H. sericea*, are serious weeds outside Australia, e.g., in South Africa (e.g., Dyer and Richardson 1992). However, we do not know if this is accounted for, in part, by their P-acquisition strategy. Another risk of P-mobilizing species is that the mobilized P might leach and reach the groundwater and then streams and rivers, contributing to their eutrophication (Djodjic et al. 2004). This risk should be managed by measuring both agronomic and environmental soil P saturation, and fertilizing accordingly (Maguire and Sims 2002).

FUTURE RESEARCH

P-acquisition-efficient plants offer potential as future crops and cropping systems. Future research should aim at identification of the genes involved in the development and functioning of root clusters, in an attempt to transfer these to other species. Equally, the potential of ‘new’ species should be explored, e.g., Australian cluster-root-bearing *Kennedia* species (Adams et al. 2002) for introduction as food (Rivett et al. 1983) or pasture plants (Cohen and Wilson 1981; Cocks 2001).

Much still needs to be learned about how P is made available for the subsequent crop. Beneficial effects on P acquisition have been found on several occasions, but the mechanism that accounts for these beneficial effects is not known. It is highly unlikely that released carboxylates are still present when the next crop is growing. Rather, the effects may be via P-containing crop residues (Nuruzzaman et al. 2005), but this needs further investigation.

CONCLUDING REMARKS

Global P reserves are rapidly being depleted, whilst agricultural soils that have been fertilized for decades contain substantial amounts of P that cannot be accessed by plants lacking specific root adaptations. To acquire soil P more efficiently, new crops need to be developed, and there should be a strong focus on species with root clusters, as these represent a combination of plant root form and function that is highly desirable in a world where P will be harder to obtain. There is still much to be learned on the role of root clusters in natural systems, and it is envisaged that new knowledge based on investigations of such systems will further enhance our potential to develop new crops and cropping systems that use P more efficiently.

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CHAPTER 20

PROSPECTS FOR GENETIC IMPROVEMENT TO INCREASE LOWLAND RICE YIELDS WITH LESS WATER AND NITROGEN

S. PENG AND B.A.M. BOUMAN

International Rice Research Institute, DAPO Box 7777, Metro Manila, Philippines.
E-mail: s.peng@cgiar.org

Abstract. Increasing yield potential of lowland rice remains to be the top priority in rice genetic-improvement programmes, because rice farmers' primary concern is still grain yield and world rice production has to increase by 1% annually in the next 20 years to meet the demand of the growing population. Improvements in yield potential of irrigated lowland rice were achieved under ample supply of water and nutrients. Water use efficiency (WUE) and nitrogen use efficiency (NUE) were seldom included in the breeding objectives for irrigated lowland rice. Scarcity of freshwater resources has threatened the production of the flood-irrigated rice crop, and excessive use of N fertilizer is causing environmental concerns. We have to increase lowland rice yield with less water and N. Newly developed crop management strategies have proven to be effective in increasing WUE and NUE. Several water-saving technologies such as alternate wetting and drying (AWD) and aerobic rice system have been developed to increase the water productivity of rice. However, yield penalty occurred when these water-saving technologies were practised with current varieties. New varieties have to be developed to reduce the yield loss under AWD and aerobic rice system in order to increase WUE further. Direct selection for WUE under flood-irrigated lowland conditions may have a negative impact on grain yield under water-saving strategies. Optimizing the timing and rate of N application to synchronize supply and demand of N by the crop has resulted in a great reduction in fertilizer-N input without yield loss and greater NUE. Genetic improvement of NUE has not been achieved in rice. Genotypic variation in NUE has been reported in many studies. Plant traits that are associated with high grain yield and high NUE should be identified so that breeders are able to use these traits easily as selection criteria in the breeding programme to develop N-efficient varieties without sacrificing rice yield potential. New breeding techniques such as development of F_1 hybrids, marker-aided selection, transformation and genetic engineering should be combined effectively with the empirical breeding method in order to increase rice grain yield with less water and N.

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INTRODUCTION

World rice production has to increase by 1% annually to meet the demand of the growing population (Rosegrant et al. 1995). Most of this increase must come from higher yields on existing cropland to avoid environmental degradation, destruction of natural ecosystems, and loss of biodiversity (Cassman 1999). Irrigated lowland contributes more than 75% of total rice production, although it accounts for about 55% of total rice area. In the past, improvement in the yield potential of irrigated lowland rice varieties has contributed greatly to the steady growth of world rice production. Most progress in the improvement of yield potential of irrigated lowland rice varieties was achieved when water and nutrients were amply supplied. Because of water scarcity and environmental pollution caused by over-application of fertilizers, the challenge is to increase rice yield of irrigated lowland with less water and less fertilizer, especially N fertilizer.

Irrigated lowland rice receives 24–30% of global developed freshwater resources (Bouman et al. in press), while in Asia it receives more than 45% of total freshwater used (Tuong and Bouman 2003). However, scarcity of freshwater resources now threatens the production of the flood-irrigated lowland rice crop (Tuong and Bouman 2003), mainly caused by the increasing competition for freshwater resources from urban and industrial sectors. By 2025, 15 out of 75 million ha of Asia’s flood-irrigated lowland rice crop are predicted to experience water shortage. At the field level, flood-irrigated rice requires two to three times more water than other cereal crops such as wheat and maize. In the past, crop, land and water management practices were the main considerations to increase rice’s water productivity (defined here as the amount of grains produced per unit water supplied by irrigation and rainfall). Several water-saving technologies have been developed that aim to reduce non-beneficial water flows from rice fields during crop growth, namely seepage, percolation and evaporation (Bouman and Tuong 2001): saturated soil culture (Borrell et al. 1997), alternate wetting and drying (Tabbal et al. 2002), groundcover systems (Liu et al. 2005) and aerobic rice (Bouman et al. 2002). In addition, reducing the duration of land preparation and shallow tillage significantly reduce the total water input for wetland preparation. However, very little effort has been devoted to improve the water productivity of irrigated lowland rice at the plant level by developing varieties with intrinsic higher water use efficiency (transpiration efficiency) or by developing varieties that maintain a high yield potential under non-flooded conditions. There is limited information on morpho-physiological characteristics of varieties that are required for superior performance under water-saving crop management.

Crop yields world-wide have continuously increased, partly because of the increase in fertilizer nutrient input, especially N fertilizer (Cassman 1999). To maximize grain yield, farmers often apply more N fertilizer than the minimum required for maximum crop growth (Lemaire and Gastal 1997). Global use of N fertilizer increased over sevenfold from 1960 to 2002 with an average growth rate of 5% per year (FAO 2005). About 60% of global N fertilizer is used for producing the world’s three major cereals: rice, wheat and maize (Ladha et al. 2005). Rice production accounts for about 20% of global N consumption. Nitrogen use efficiency is relatively low in irrigated lowland rice because of rapid N losses from ammonia volatilization, denitrification, surface run-off and leaching in the soil–floodwater system (De Datta and Buresh 1989). These N losses are much greater under excessive N use and cause severe environmental consequences, such as groundwater contamination (caused by nitrate leaching from soil), eutrophication of lakes and rivers (because of surface run-off and seepage of N from rice fields), and acid rain (caused by ammonia volatilization). Denitrification contributes to global warming by emitting greenhouse gases such as nitrous oxide (N_2O).

Further increase in rice production has to be achieved with less N fertilizer by improving N use efficiency (NUE) through better N fertilizer management and new rice varieties. Research on improving NUE of the rice crop has been focused on the development of fertilizer management strategies in the past three decades. Great progress has been achieved to reduce N losses by new application methods and modified N sources. Another important research area is optimizing the timing and rate of N application for better synchronization between the supply and demand of N by the crop (Cassman et al. 1998). Some efforts have been devoted to germplasm improvement in NUE, but the impact has not been as great as for the first two approaches.

GENOTYPIC IMPROVEMENT FOR WATER PRODUCTIVITY

Transpiration efficiency (TE) is defined as the ratio of photosynthesis (A) to transpiration. Whole-plant water use efficiency (WUE) can be expressed as the ratio of total biomass or grain production to the amount of water transpired. A close relationship between TE at the single-leaf level and whole-plant WUE was observed in winter wheat (Heitholt 1989) and grain sorghum (Peng and Krieg 1992). Farquhar et al. (1982) developed a theoretical relationship that demonstrated a negative correlation between ^{13}C discrimination (Δ) in C_3 plants and the ratio of A to stomatal conductance (g) through the ratio of intercellular and ambient CO_2 partial pressures (p_i/p_a). A negative relationship between Δ and whole-plant WUE has been reported in many crops, including upland rice (Dingkuhn et al. 1991).

Peng et al. (1998) reported that improved tropical *japonica* rice lines had 25–30% higher TE at the single-leaf level than *indica* varieties when grown under flooded conditions. This was because *indica* varieties had a higher transpiration rate than the tropical *japonica* lines whereas the differences in A between the two types were relatively small and inconsistent across growth stages and years compared with the differences in transpiration rate. A smaller Δ in a tropical *japonica* line than in an *indica* variety suggested that the improved tropical *japonica* rice may have greater whole-plant WUE than the *indica* rice. Yeo et al. (1994) observed large differences among *Oryza* species in TE at the single-leaf level. *Oryza australiensis* had significantly higher TE than *Oryza sativa* at the same photosynthetic rates. The potential for exploiting this trait, however, has not been investigated. Varietal differences in TE at the single-leaf level and whole-plant WUE measured by gravimetric determinations of growth and water loss from individual plants were reported in rice by Flowers et al. (1988). However, the high WUE was associated with the non-dwarfed habit and therefore it may not be useful to incorporate this trait into commercial varieties to increase water productivity. Increase in waxiness of rice leaves was proposed to reduce non-stomatal transpiration but the impact on WUE has not been demonstrated (Lafitte and Bennett 2002).

Transforming the C_3 rice plant into a C_4 plant by genetic engineering of photosynthetic enzymes and required anatomic structures was suggested as another approach to improve TE. High-level expression of maize phosphoenolpyruvate carboxylase (PEPC) and pyruvate, orthophosphate dikinase (PPDK) and NADP-malic enzyme (NADP-ME) in transgenic rice plants has been achieved (Agarie et al. 1998). Ku et al. (2000) reported that PEPC and PPDK transgenic rice plants had up to 30 to 35% higher A than untransformed plants. However, this increased A was associated with enhanced stomatal conductance, which reduces the potential for increasing TE by the development of C_4 rice plants. The mechanism underlying the maintenance of higher stomatal conductance by the transgenic plants is unknown.

Development of early maturing and high-yielding varieties of flood-irrigated lowland rice during the Green Revolution in the 1960s and 1970s has increased the average rice yield and reduced crop duration significantly. This has contributed to a three-fold increase in water productivity with respect to total water inputs (Bouman et al. 2006). Hybrid rice varieties have a 9% higher yield potential than inbred varieties with comparable growth duration when grown under flood-irrigated conditions in the tropics (Peng et al. 1999). This yield advantage offers another opportunity to increase the water productivity of flood-irrigated lowland rice (Guerra et al. 1998).

Breeders and crop physiologists in Australia selected specifically for high TE in wheat using carbon isotope discrimination techniques (Richards 2004). Wheat varieties with a 2–23% increase in grain yield over check varieties have been successfully developed for water-limited conditions. Blum (2005) pointed out that high yield under water-limited conditions is generally associated with reduced WUE mainly because of high water use. Plant traits such as small plants (small leaves and reduced tillering) or short growth duration are associated with low yield potential and high WUE because of reduced water use. Therefore, selection for high WUE by using carbon isotope discrimination techniques in a breeding programme will result in smaller or earlier flowering plants that use less water but have low yield potential at the same time (Blum 2005). The challenge is to develop water-efficient genotypes that produce higher yields with limited water supply, and equal or greater yields than current varieties under favourable growth conditions without stress.

Bouman et al. (in press) stated that the possibility of increasing water productivity of irrigated lowland rice is much greater by improving crop, land and water management practices than by developing rice varieties with high TE or whole-plant WUE. Because photosynthesis and transpiration rates are generally proportional, there is only a small difference in TE among rice varieties at the single-leaf level when grown under flooded and aerobic cultivation (Singh and Sasahara 1981). However, developing rice varieties with superior performance under water-saving technologies such as alternate wetting and drying (AWD) and aerobic cultivation could result in a significant improvement in water productivity of irrigated lowland rice.

GENOTYPIC VARIATION IN RESPONSE TO WATER REGIMES

In AWD, irrigation water is applied to achieve intermittent flooded and non-flooded soil conditions. The frequency and duration of non-flooding can be determined by re-irrigating (to achieve flooded conditions) after a fixed number of non-flooded days, when a certain threshold of soil water potential is reached, when the ponded watertable level drops to a certain level below the soil surface, when cracks appear on the soil surface or when plants show visual symptoms of water shortage. AWD is a mature technology that is widely practised in irrigated lowland rice in China (Bouman et al. 2006). A yield increase under AWD is rare. Bouman and Tuong (2001) summarized 31 field experiments on AWD and reported that yield reductions of 0 to 70% were observed in AWD treatments compared with continuously flooded controls in 92% of the experiments. The large variability in the performance of AWD was caused by differences in the irrigation interval, soil properties and hydrological conditions across the experiments. In fact, varieties that were developed for the traditional continuously flooded-irrigated rice system may not be suitable for AWD conditions. In all the experiments, AWD increased water productivity with respect to total water input because the yield reduction was smaller than the amount of water saved.

Bouman and Tuong (2001) re-analysed the yields of a variety evaluation under AWD conditions by De Datta et al. (1973a; 1973b), who grew 30 different cultivars with irrigation applied when soil water tension in the root zone reached 500 mbar in either the vegetative or reproductive stage. There were six cultivars that had higher yields with AWD in the vegetative stage than in the continuously flooded control. More recently, Virk et al. (2003) evaluated seven hybrids and 37 inbred varieties under AWD and continuously flooded control during the 2003 dry season at IRRI. For the entire growing season, there were six non-flooded soil periods with the first one initiated 21 days after transplanting. With irrigation, 5-cm ponded water depth was imposed, and the field was re-irrigated after the ponded water level had receded to 25 cm below the soil surface. Overall, AWD saved 17% of the water used in the continuously flooded control. Yield losses under AWD ranged from 3 to 23% for hybrids and from –6 to 26% for inbreds. Three out of seven hybrids and 18 out of 37 inbreds did not show any significant yield decline because of AWD. Three hybrids and six inbred varieties that were adapted to AWD conditions were identified. These varieties demonstrated higher water productivity under AWD than under continuously flooded conditions. Both studies, De Datta et al. (1973a; 1973b) and Virk et al. (2003), suggest that genetic variability in tolerance for relatively mild water stress during the vegetative phase exists in both hybrids and inbreds. This trait can be used for the development of new varieties that are more suitable for AWD conditions.

Aerobic rice is high-yielding rice grown under non-flooded conditions in non-puddled and unsaturated (aerobic) soil. It is responsive to high inputs, can be rainfed or irrigated, and tolerates occasional flooding (Bouman and Tuong 2001). In this chapter, aerobic rice refers to rice crops grown in non-flooded and non-puddled lowland soil with supplemental irrigation. Aerobic rice promises substantial water savings by minimizing seepage and percolation and greatly reducing evaporation

(Bouman et al. 2002). Experimentally growing the high-yielding lowland rice varieties under aerobic conditions has shown great potential to save water, but with severe yield penalty. In the early 1970s, De Datta et al. (1973a; 1973b) tested the lowland variety IR20 in aerobic soil under furrow irrigation at IRRI. Water saving was 55% compared with flooded conditions, but the yield fell from about 8 t ha^{-1} under flooded conditions to 3.4 t ha^{-1} under aerobic conditions. However, large varietal differences in grain yield exist under aerobic conditions. George et al. (2002) reported that a lowland hybrid variety (Magat) yielded 7.8 t ha^{-1} compared to 2.1 t ha^{-1} for a traditional upland rice variety (Lubang Red) when grown under aerobic conditions in the first season with supplemental irrigation. Magat's high yield was because of a harvest index (HI) of 0.43 in contrast to 0.31 of an improved upland rice variety (Apo) and 0.17 of Lubang Red. George et al. (2002) concluded that rice can be highly productive in aerobic soils if crop management is optimized for varieties with high HI and adapted to aerobic conditions. Aerobic rice is grown commercially in highly acidic soil of Brazil, producing about 5 t ha^{-1} with adequate supply of fertilizers and irrigation in rotational systems (George et al. 2002). In China, aerobic rice yield potential approaches 7 t ha^{-1} in farmers' fields in the Huang-Huai-Hai Rice Plains (Wang et al. 2002). Yields up to 8.7 t ha^{-1} were reported for aerobic rice in Jiangsu province, which was only 11% less than the yield under continuously flooded conditions with the same variety (Chu et al. 2004). However, in field experiments around Beijing, maximum aerobic rice yields were only 5.7 t ha^{-1} (Yang et al. 2005). Still, there is a yield penalty for current varieties when grown under aerobic soil conditions.

In the dry season of 2004, we compared the performance of four varieties under continuously flooded and aerobic conditions at the IRRI farm. The field was grown with flooded lowland rice in previous seasons and it was the first season to grow aerobic rice. The four varieties were lowland *indica* inbred (PSBRc80), lowland *indica* hybrid (Magat) and improved upland rice (Apo and UPLRi7). Flooded plots were puddled and kept continuously flooded from transplanting until two weeks before harvest. The aerobic plots were dry-ploughed and harrowed but not puddled during land preparation. One day before transplanting, the aerobic plots were soaked with irrigation water to facilitate transplanting. Afterwards, the aerobic plots were flash irrigated with about 5 cm water when the soil moisture tension at 15 cm depth reached –30 kPa. Around flowering, the threshold for irrigation was reduced to –10 kPa to prevent spikelet sterility. Twenty-days-old seedlings from wet-bed nurseries were transplanted at the rate of three seedlings per hill and at a spacing of 25 × 10 cm for both aerobic and flooded rice plots. Adequate P, K and Zn were applied and incorporated in all plots one day before transplanting. Fertilizer N was applied in three equal splits of 50 kg ha^{-1}, one day before transplanting and at 25 and 45 days after transplanting. Insects, diseases and weeds were adequately controlled to achieve high yields. A maximum of 7.2 t ha^{-1} was produced by PSBRc80 under aerobic conditions, which was only 8% less than when grown under flooded conditions (Table 1). A large difference in aerobic rice yield existed among the four varieties. The varietal difference in yield was associated with HI, not with biomass production.

Table 1. *Comparison of yield parameters among four varieties under continuously flooded and aerobic conditions at the IRRI farm in the dry season of 2004*

Variety	Growth duration (d)	Plant height (cm)	Harvest index (g g^{-1})	Grain yield (t ha^{-1})
Continuously flooded conditions				
PSBRc80	117	97	0.48	7.84
Magat	113	99	0.49	8.12
Apo	117	137	0.47	7.78
UPLRi7	117	121	0.38	6.33
Aerobic conditions				
PSBRc80	124	91	0.47	7.22
Magat	117	85	0.49	6.68
Apo	117	117	0.45	6.32
UPLRi7	124	108	0.42	5.13
LSD (0.05)		5	0.03	0.71

Breeding rice varieties for adaptation to lowland aerobic cultivation is relatively new compared with development of drought-resistant varieties for upland and rainfed lowland environments. Several promising aerobic rice varieties have been developed in China (Wang et al. 2002), but the mechanism of their aerobic adaptation is not clear. Apparently, short plant height associated with high HI should be an important target. However, not all semi-dwarf varieties have high HI and high yield under aerobic condition. For example, short-stature IR72 produced only 4.9 t ha^{-1} with a HI of 0.28, which was lower than that of the tall upland variety Apo (George et al. 2002). Apparently, IR72 does not have the same aerobic adaptation as PSBRc80 and Magat, although they are all semi-dwarf varieties and produce similar biomass under aerobic conditions. The physiological basis of the yield gap between aerobic and flooded rice has not been studied in detail. Furthermore, it is not clear what physiological traits contribute to aerobic adaptation. One possible candidate is reduced sensitivity of grain filling to mild water stress during the grain development phase. Bouman et al. (2006) suggested that the relatively high yields of aerobic rice cultivars developed in China may be attributed to their capacity to maintain a high HI through high spikelet fertility under aerobic conditions. In IRRI's aerobic rice-breeding programme, crosses are being made between lowland semi-dwarf *indica* and upland tall varieties for achieving aerobic adaptation (Atlin et al. 2006). Identifying the physiological and morphological traits associated with aerobic adaptation is vital to support the selection and breeding of high-yielding aerobic rice varieties. In addition, aerobic rice-breeding nurseries should be managed at optimum level to avoid stresses other than those imposed by aerobic culture so that aerobic yield potential can be fully expressed.

Yield decline under monocropping of aerobic rice has been reported by George et al. (2002) and Peng et al. (2006). The causes of yield decline in the continuous aerobic rice system remain unclear. It could be related to the build-up of nematodes and soil pathogens under aerobic conditions (Ventura et al. 1981), changes in soil mineral nutrients (Lin et al. 2005) or growth inhibition by toxic substances from root residues (Nishio and Kusano 1975). Crop management strategies to mitigate or prevent the yield decline of continuous aerobic rice can only be developed when the causes of yield decline are clearly identified. New aerobic rice varieties with a minimum yield gap compared with flooded rice, and crop management strategies that can prevent the yield decline of continuous aerobic rice must be developed before the aerobic rice technology can be widely adopted in large areas of the irrigated tropical lowlands.

GENOTYPIC VARIATION IN NITROGEN USE EFFICIENCY

Nitrogen use efficiency can be separated into different component indices (Ladha et al. 2005). All indices that have been used for studying NUE of rice genotypes can be divided into three groups: uptake efficiency, utilization efficiency and NUE-related traits (Table 2). Different NUE indices can be used for different purposes. Apparent uptake efficiency of fertilizer-N (RE_N) is the percentage of fertilizer-N recovered in above-ground plant biomass at the end of the cropping season. Both the N-difference and ^{15}N-dilution method can be used to quantify RE_N but the estimate is typically higher with the difference method than with the ^{15}N-dilution method (Ladha et al. 2005). There are many ways to define N utilization efficiency. The most widely used for comparing different genotypes is the N utilization efficiency for grain production (NUE_g). The quantification of NUE_g does not need a zero-N plot. Agronomic N use efficiency (AE_N) and partial factor productivity of applied N (PFP_N) are mainly used by agronomists to evaluate the NUE of different crop management practices. The determination of AE_N requires a control plot to estimate the grain yield without fertilizer N application. Compensation takes place among different components of NUE because of the interactions among traits related to NUE. Indigenous N supply capacity and N fertilizer input rate have a large influence on NUE. Growing conditions that govern plant growth and crop yield will also affect NUE. There is no common standard system to evaluate NUE of different genotypes, making it very difficult to compare results across studies.

In rice, genotypic variation in NUE has been reported by many researchers. Broadbent et al. (1987) studied NUE_g of 24 genotypes with and without fertilizer-N application. They estimated NUE_g using the ratio of panicle weight to total N uptake (WP/TN) and reported that there were significant differences in WP/TN among the genotypes. WP/TN was well correlated with other NUE-related parameters and provided genotypic rankings that did not differ greatly from multiple-parameter rankings. Tirol-Padre et al. (1996) compared NUE_g of 180 genotypes without fertilizer N application. There were significant differences in NUE_g among the 180 genotypes, ranging from 38 to 82 kg kg^{-1}. They also confirmed the presence of genetic variability within a maturity class for N acquisition. Singh et al. (1998) compared the N responses of 20 genotypes under fertilizer N rates of 0, 50, 100, 150 and 200 kg ha^{-1}. They identified N-efficient genotypes that produced high grain yield at both low and high levels of N application, N-inefficient genotypes that produced low yields at low N levels but responded well to N application, and

N-inferior genotypes that gave low yields at both low and high N levels. Although 75% of the variation in grain yield was explained by total N uptake, genotypic differences in NUE_g were observed. Inthapanya et al. (2000) determined the differences in NUE_g among 16 genotypes grown under rainfed lowland conditions at three locations with and without N application. There was a significant effect of genotype and an insignificant effect of genotype × location interaction for NUE_g. Mean NUE_g of the 16 genotypes ranged from 55 to 84 kg kg^{-1}. Koutroubas and Ntanos (2003) studied NUE_g of five cultivars with N application under Mediterranean conditions. They reported that *indica* cultivars generally had higher NUE_g than *japonica* cultivars. In a recent study (Samonte et al. 2006), NUE_g of Lemont, Teqing and 13 advance recombinant inbred lines obtained from a Lemont × Teqing cross was determined under high N application level. There was a significant variation in NUE_g among the 15 genotypes, ranging from 25 to 64 kg kg^{-1}. The large genotypic variation in NUE_g was probably caused by some genotypes with low yield potential. When only high-yielding varieties were compared, the genotypic variation in NUE_g could be much smaller.

Table 2. *Components of nitrogen use efficiency (NUE) and their related traits*

No.	Parameter	Symbol	Calculating equation	Unit
N uptake efficiency				
1[a]	Apparent uptake efficiency of fertilizer N	RE_N	100 × N uptake from fertilizer N / Fertilizer N input	%
N utilization efficiency				
2	N utilization efficiency for grain production	NUE_g	Grain yield / Total N uptake	kg kg^{-1}
3[b]	Fertilizer N utilization efficiency for grain	$FNUE_g$	Grain yield increase / N uptake from fertilizer N	kg kg^{-1}
4	N utilization efficiency for biomass production	NUE_b	Total dry matter / Total N uptake	kg kg^{-1}
5[b]	Fertilizer N utilization efficiency for biomass	$FNUE_b$	Total dry-matter increase / N uptake from fertilizer N	kg kg^{-1}
6[b]	Agronomic N use efficiency	AE_N	Grain yield increase / Fertilizer N input	kg kg^{-1}
7	Partial factor productivity of fertilizer N	PFP_N	Grain yield / Fertilizer N input	kg kg^{-1}
8	Photosynthetic N use efficiency	PNUE	Photosynthetic rate / Leaf N concentration	μmol g^{-1} s^{-1}
NUE-related traits				
9	N harvest index	NHI	Grain N content / Total N uptake	
10	N translocation ratio	NTR	Grain N content / Plant N content at flowering	
11[b]	N productivity index	NPI	Grain yield at zero N × NUE_g	
12	Harvest index	HI	Grain yield / Total dry matter	

[a] Requiring zero-N plot to estimate indigenous N supply or ^{15}N labelling technique to estimate N uptake from fertilizer N.

[b] Requiring zero-N plot to estimate indigenous N supply, grain yield and total dry matter without fertilizer N application.

PHYSIOLOGICAL UNDERSTANDING OF NITROGEN USE EFFICIENCY

Several factors can cause the differences in N acquisition among rice genotypes (Ladha et al. 1998). Genotypic differences existed in N uptake from various soil depths (Kundu and Ladha 1995). Rice genotypes differed in their ability to stimulate plant associative N_2 fixation (Ladha et al. 1998). Rice genotypes had different rhizosphere effects on the extent and pattern of soil N mineralization (Kundu and Ladha 1997). These differences could be related to differences in rooting characteristics such as root surface area, root mass, root depth, root number and root length density (Kirk and Bouldin 1991).

On the other hand, many physiological processes affect N utilization efficiency for biomass production (NUE_b) and NUE_g (Ladha et al. 1998). NUE_b is largely affected by critical concentrations (internal N requirement) for expansion and organ formation, N partitioning between leaves and stems, vertical N distribution in a canopy, efficiency of N use in converting CO_2 to carbohydrate through photosynthesis, rubisco activity and leaf senescence. Grain N concentration, sink capacity, unproductive tillers, HI and the ability to remobilize the absorbed N from straw to grain determine N harvest index (NHI) and NUE_g.

Leaf N plays a major role in biomass production through photosynthesis. Leaf N content is closely correlated with single-leaf photosynthetic rate (Peng et al. 1995). Canopy photosynthetic rate is affected by leaf N through leaf area expansion. High plant N content delays leaf senescence and therefore increases photosynthetic duration (Makino et al. 1984). Increasing leaf N content and delaying N efflux from leaf (i.e., delaying leaf senescence), especially the flag leaf, could improve NUE_b if the ratio of photosynthesis to respiration was not decreased. A large proportion of radiation is intercepted by the top layers of the canopy, especially in the rice crop with high leaf area index. Matching vertical N distribution and light distribution in the canopy is another approach to achieving high NUE_b. In the leaf, N is concentrated in the chloroplasts, mainly as the enzyme protein rubisco. Rubisco accounts for more than 50% of total soluble protein and over 25% of total N of leaves (Makino et al. 1984). Therefore, leaf is a major storage organ for N. The major source of N for developing leaves of mature rice plants is the N mobilized from older, senescing leaves. Out of total N translocated from vegetative tissues to the panicle, 64% was from leaf blades, 16% from leaf sheaths and 20% from stems (Mae and Ohira 1981). Therefore, efficiency in N remobilization from old to new leaf and from straw to grain will affect both NUE_b and NUE_g.

Unproductive tillers could reduce NUE_g, especially when mutual shading occurs. Unproductive tillers capture solar radiation and absorb soil N during early stages of growth. Genotypic variation in tillering capacity and unproductive tiller percentage exists in rice (S. Peng, unpublished data). The N and carbon of unproductive tillers can be mobilized to the productive tillers before they die (Thorne and Wood 1987), although the efficiency of such transport needs to be quantified. If the majority of N in unproductive tillers could be mobilized before they die, the negative impact of unproductive tillers on NUE_g can be minimized.

PLANT TRAITS RELATED TO NITROGEN USE EFFICIENCY

Although genotypic variation in NUE_g has been observed for a long time, improving NUE in rice has not been an objective in plant breeding (Samonte et al. 2006). This is partially because of the lack of simple and quick methods for estimating NUE_g, which could be used for screening large numbers of progenies in the breeding programme. The G × E interaction for NUE_g also makes the improvement of NUE more difficult. Furthermore, there is a danger of selecting for plants with low total N uptake and low grain yield if the selection is solely based on NUE_g. Rice breeders should develop new rice varieties that not only produce high yields but also use N efficiently. In order to achieve these objectives, plant traits that are associated with high grain yield and high NUE should be selected concomitantly, and breeders should be able to use these traits easily as selection criteria in the breeding programme.

Many plant traits have been studied to improve the mechanistic understanding of NUE_g. Genotypes with higher NUE_g had a lower percentage of straw N (Ns%) at maturity (Tirol-Padre et al. 1996; Singh et al. 1998; Koutroubas and Ntanos 2003). Low grain N concentration (Ng%) was also associated with high NUE_g (Tirol-Padre et al. 1996; Singh et al. 1998; Inthapanya et al. 2000; Koutroubas and Ntanos 2003). Grain N concentration decreases by about 0.1% as NUE_g increases by 7 to 10 kg kg^{-1} as grain N concentration decreases by about 0.1% (Tirol-Padre et al. 1996; Koutroubas and Ntanos 2003). Genotypes with high HI were more efficient in N use (Inthapanya et al. 2000).

Mathematically,

$$NUE_g = \frac{1}{(\frac{1}{HI} - 1) \times Ns\% + Ng\%} \tag{1}$$

This equation indicates that HI, Ns% and Ng% determine NUE_g. At HI=0.5, Ns% and Ng% contribute equally to NUE_g (Inthapanya et al. 2000). Rice generally has greater NUE_b and NUE_g than some other C_3 crops such as soybean and wheat, because of low Ns% and Ng%. Further decreasing Ng% could contribute to efficient N utilization theoretically. Genotypic variation in Ng% is relatively small compared to the variation caused by management practices. Tirol-Padre et al. (1996) studied genotypic variation in Ng% of 180 lines grown without fertilizer-N input. About 93% of genotypes had Ng% of 0.95 to 1.20. It may not be feasible to improve NUE_g by reducing Ng% using conventional breeding because grain N concentration is affected more by environments than by genotypes (Ladha et al. 1998).

Koutroubas and Ntanos (2003) reported that difference in NHI explained genotypic variation in NUE_g. There was a significant positive correlation between NUE_g and N translocation ratio (NTR), which was calculated as the ratio of grain N content at maturity to plant N content at flowering (Samonte et al. 2006). Crop growth duration affects NUE_g through influencing crop yield and N uptake. Medium-duration genotypes had higher NUE_g than short-duration genotypes

(Broadbent et al. 1987; De Datta and Broadbent 1990). Longer duration could result in a decrease in NUE_g (Samonte et al. 2006).

Koutroubas and Ntanos (2003) argued that using single selection criteria for improving NUE may have negative implications on grain yield because of interaction among NUE-related traits. Broadbent et al. (1987) stated that the evaluation of NUE of different genotypes should be based on multiple parameters rather than just one parameter. The sum of Z-transformed values of grain yield, NUE_g, panicle weight, panicle weight/total N uptake and total dry matter/total N uptake, putting equal weights on each parameter, was used to rank genotypes (Broadbent et al. 1987; De Datta and Broadbent 1988; 1990). Singh et al. (1998) developed the N productivity index (NPI), which is the product of grain yield at zero N and NUE_g for evaluating NUE of genotypes. Quantifying and ranking N-efficient genotypes based on NPI was most consistent, whereas NUE_g, AE_N and RE_N had biases either toward soil N or fertilizer-N supply (Singh et al. 1998). However, determination of NPI requires field evaluation of genotypes at low N (or zero fertilizer-N input) and high N levels. The high N level should not be too high to induce lodging, pest and disease damages, which affect the crop N response and NUE (Tirol-Padre et al. 1996; Singh et al. 1998).

MOLECULAR APPROACHES TO IMPROVE NITROGEN USE EFFICIENCY

Recent developments in molecular biology provide a new opportunity to improve NUE through crop improvement. Transforming C_3 rice plants into C_4 rice plants has resulted in 10 to 35% increase in grain yield compared with control plants (Ku et al. 2000). The C_4 plant is assumed to have higher NUE than the C_3 plant (Ladha et al. 2005). Obara et al. (2001) used a backcross inbred-line population to detect putative quantitative-trait loci (QTLs) associated with the contents of cytosolic glutamine synthetase (GS1) and NADH-glutamate synthase (NADH-GOGAT). GS1 is a key enzyme in the mobilization of N from senescing leaves, and its activity in senescing leaves is positively related to yield. NADH-GOGAT is important in the utilization of N in grain filling, and its activity in developing grains is positively correlated with yield. Seven chromosomal QTL regions for GS1 and six for NADH-GOGAT were detected. Some of these QTLs were related to N recycling from senescing organs to developing organs. A structure gene for GS1 on chromosome 2 was co-located in the QTL region for seed weight. A structure gene for NADH-GOGAT on chromosome 1 was co-located in the QTL region for soluble protein in developing leaves. Yamaya et al. (2002) developed transgenic lines overproducing NADH-GOGAT, and two of the transgenic lines showed an increase in grain weight. These studies suggest that genotypes, obtained from genetically manipulated populations or genetic resources, with high GS1 in senescing leaves and high NADH-GOGAT in developing grains should promote N remobilization from straw to grain and consequently improve NUE_g (Andrews et al. 2004).

SUMMARY

The emphasis on increasing rice grain yield of irrigated lowland rice when water and N are amply supplied will gradually diminish in importance as concerns mount about reduced water availability for irrigated rice and the environmental impact from N losses. The new challenge is to sustain the needed increase in rice yields while reducing the requirements for water and N per unit of grain production. Newly developed crop management strategies have been very effective in maintaining rice yield with reduced input of water and N, resulting in great WUE and NUE. However, it is always more difficult for farmers to adopt a new crop management technology than planting new varieties. Research on genetic improvement of rice varieties for high WUE and NUE should receive more attention in the near future. For water, new varieties that produce high grain yields under water-saving technologies such as AWD and the aerobic rice systems should be developed. Small yield penalty is acceptable when they are grown under water-saving technologies compared to flooded conditions. For N, genotypic variation in NUE has been reported in many studies. Plant traits that are associated with high grain yield and high NUE should be identified so that breeders are able to use these traits easily as selection criteria in the breeding programme to develop N-efficient varieties without sacrificing rice yield potential. New breeding techniques such as development of F_1 hybrids, marker-aided selection, transformation and genetic engineering should be combined effectively with the empirical breeding method in order to increase rice grain yield with less water and N.

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CHAPTER 21

EXPLOITING DIVERSITY TO MANAGE WEEDS IN AGRO-ECOSYSTEMS

L. BASTIAANS[#], D.L. ZHAO[#,##], N.G. DEN HOLLANDER[#],
D.T. BAUMANN[#,###], H.M. KRUIDHOF[#] AND M.J. KROPFF[#]

[#] *Crop and Weed Ecology Group, Department of Plant Sciences, Wageningen University, P.O. Box 430, 6700 AK Wageningen, The Netherlands.*
[##] *International Rice Research Institute, Los Baños, Philippines.*
[###] *Swiss Agricultural Research Station, Agroscope FAW Wädenswil, Switzerland.*
E-mail: lammert.bastiaans@wur.nl

Abstract. Weed management systems that rely less on chemical control are needed globally. Next to curative chemical weed control, there are other ways of tackling weed problems, such as (a) reduction of the weed seed bank in the soil, (b) reduced recruitment of weed seeds from the soil seed bank, and (c) strengthening the relative competitive ability of the crop. A number of case studies are presented in which diversity is used as a basis for improved weed management. In the first case study, diversity refers to genetic variation within a crop species, which is utilized in breeding programmes aiming at the development of more competitive cultivars. In the other case studies, diversity refers to the reinforcement of weakly competitive crop species through the addition of a second species that contains a strong weed-suppressing function. Here a distinction is made between intercropping, where the species are grown simultaneously, and sequential or rotational cropping, where a cover crop is introduced in the cropping interval in between two main crops.

Weed-competitive cultivars, intercropping and rotational cover cropping all have potential to contribute significantly to the weed management of agro-ecosystems. Rather than making curative control completely redundant, they allow the regular curative control measures to be applied at a lower dose or in a less frequent manner. The weed-suppressive effect was largely determined by the combined effects of genotype (or species) and management. Obtaining a sufficient level of weed suppression while maintaining the yielding ability is a major issue in the development of weed-competitive cultivars and the design of intercropping systems. In both cases, competition models showed to be useful tools to analyse and optimize systems. Opportunities and potential obstacles for implementation of the proposed strategies are discussed.

INTRODUCTION

Weeds have always been a major disturbing factor in agricultural production systems. If left uncontrolled, weed plants compete with crop plants for resources

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essential for plant growth, thereby reducing crop yield and quality. To minimize the negative consequences of weeds on crop production, curative actions to remove or kill weed plants shortly after their establishment are often undertaken. In spite of intensive control activities in most agricultural systems, the loss in yield because of weed competition was still estimated to be 10% (Oerke et al. 1994). Weed management is largely herbicide-dominated. The widespread concern about environmental side-effects of herbicides combined with fear for public health has called for a reduced use of herbicides. These concerns have already led to the banning of several herbicides in various countries (Matteson 1995). At the same time, the release rate of new herbicides has decreased. Particularly for some minor crops this has caused situations where farmers are forced to rely on other weed control technologies. The development of herbicide-resistant biotypes is another mechanism through which the vulnerability of herbicide-dominated systems is increased. Despite the need for systems with a reduced use and reliance on herbicides, widely applicable alternative solutions are still lacking. This is most clearly illustrated in organic farming systems, where the application of herbicides is excluded and weed management often develops into a high-priority issue.

A number of directions have been suggested for minimizing the use of herbicides. A first strategy is to make a more efficient use of herbicides through technological solutions, such as an improved application technology, improved application timing, factor-adjusted dosages and spot spraying. A second strategy is to focus more on alternative curative weed control options such as mechanical weed control. A third strategy to minimize the use of herbicides is to develop methods other than direct weed control measures (Bastiaans et al. 2002). This is illustrated in Figure 1, where a hyperbolic curve is used to relate the yield loss of the crop to weed plant density. A second x-axis, representing the seed bank density, is added to illustrate that most weed plants evolve from seeds that are stored in the weed-seed soil bank. In Figure 1A curative weed control is represented. Weed seedlings are killed through, e.g., a herbicide treatment or a mechanical weed control intervention. Reducing the number of weed plants decreases the competitive pressure of the weed population on the crop and consequently the yield loss of the crop is diminished. Bearing in mind the life cycle of weeds, alternative weed management could be based on the following principles: (a) a reduced recruitment of seed or vegetative reproduction organs (Figure 1B); (b) alteration of crop–weed competitive relations to the benefit of the crop (Figure 1C); and (c) a gradual reduction of the weed infestation level in the soil (Figure 1D).

One way to achieve weed management based on alternative principles is through the exploitation of diversity, schematically represented in Figure 2B-D. A first option is to breed for weed-competitive genotypes. Then diversity refers to the heterogeneity within a plant species, and exploitation of diversity occurs through breeding rather than through crop management. Large variation within plant traits exists and breeding is directed towards accumulating favourable traits, such as weed-suppressive ability, in a single genotype. Another option for exploiting diversity is by combining two or more species with the purpose of strengthening the weed-suppressing function. A distinction can be made between intercropping, where the species are simultaneously present for at least part of the growing season, and sequential cropping, where the cover crop is introduced to fill up a crop-free period in between two main crops.

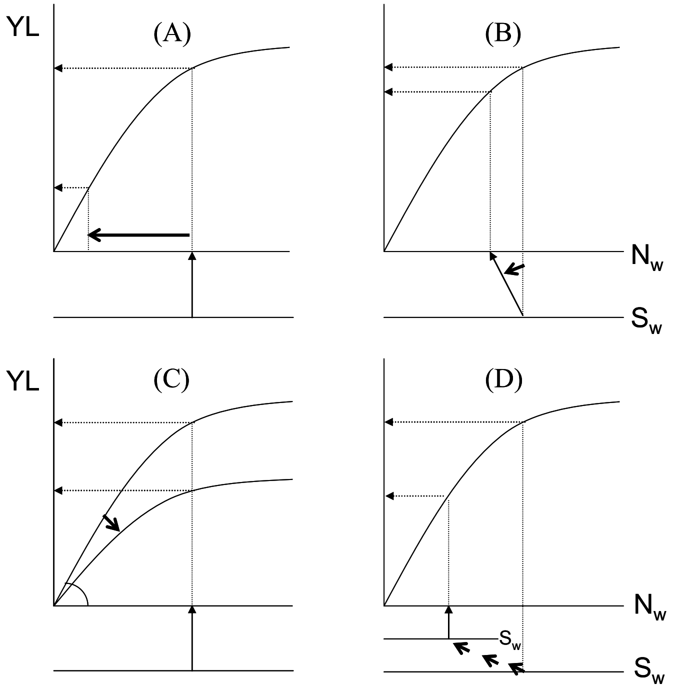

Figure 1. *The hyperbolic yield loss (YL) – weed density relation used to illustrate various principles for reducing yield loss due to weeds.* (**A**) *Killing or removal of weed plants;* (**B**) *reduced recruitment of weeds from the seed bank;* (**C**) *alteration of crop–weed competitive relations;* (**D**) *gradual reduction or depletion of the weed seed bank. Thick arrows represent the major effect of a specific intervention. Weed density is expressed in two ways: as weed plant density (N_w) and as seed bank density (S_w) (after: Bastiaans et al. 2002)*

A number of case studies will be presented. The case studies have in common that they were initiated to explore the potential of improving weed management through the utilization of diversity. The case studies either deal with the development of weed-competitive genotypes, intercropping or sequential cover cropping. All case studies were conducted at, or in connection with, the Crop and Weed Ecology Group of Wageningen University. Main findings and important aspects that were encountered during developing the conceptual frame works and the research process are presented. In a final section the various options and strategies are compared and attention is given to aspects such as effectiveness with regard to weed suppression, consequences for yielding ability, relevance of management, and opportunities for systems optimization and implementation.

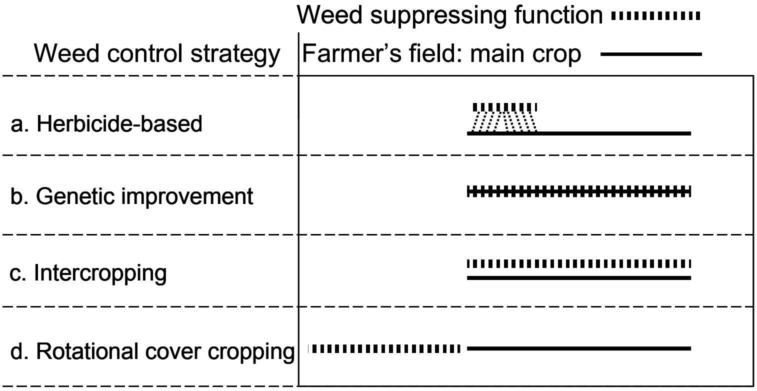

Figure 2. *Schematic representation of alternative weed management strategies based on the utilization of diversity. Solid line represents the growing season of the main crop. Dotted line represents the basis for the weed-suppressing function (a. herbicides; b. genes introgressed from weed-competitive germplasm; c. weed-competitive companion crop; d. weed-competitive cover crop grown in the cropping interval between two main crops)*

WEED-COMPETITIVE GENOTYPES

Weeds: an increasing problem in rice production

In traditional irrigated rice systems, the weed problems are relatively small. Transplanting favours the crop in its competition against weeds, as it provides rice a 2- to 3-week head-start relative to the weeds. Also the presence of a water layer after transplanting is beneficial, as it effectively suppresses the emergence and growth of most of the weed flora. Therefore, irrigated lowland rice is a good system in terms of ease and cost of weed control (De Datta and Baltazar 1996). This system, however, is seriously under pressure. Firstly, the high labour cost coupled with the shortage of on-farm labour causes a rapid shift from transplanting to direct seeding (De Datta 1986; Erguiza et al. 1990). Secondly, the growing water scarcity is threatening this rice production system (Tuong and Bouman 2003). Water consumption per kg of rice ranges from 1000 to 3000 litres, which is about 2 to 3 times more than is needed to produce other cereals such as wheat or maize (Bouman and Tuong 2001; Cantrell and Hettel 2005). The increasing water scarcity for agriculture points to an urgent need to improve crop water productivity.

Aerobic rice is one of the water-saving systems proposed to replace the traditional lowland rice system that is now under threat (Cantrell and Hettel 2005). In aerobic rice, seed is sown directly into dry soil and irrigation is applied to keep the soil sufficiently moist for good plant growth, but the soil is never saturated. Though aerobic rice, just like 'upland rice', is grown under aerobic conditions, it is different in water management from traditional upland rice, which is completely dependent on rainfall. Changing the establishment system from transplanting to direct seeding and soil hydrological conditions from flooded to aerobic conditions will definitely bring more severe weed problems. Successful aerobic rice production therefore requires effective weed management. For this reason the feasibility of breeding for weed competitiveness in rice was explored.

Comparing two contrasting cultivars

Differences in competitive ability between two contrasting rice cultivars (Mahsuri and IR8), grown in well-fertilized irrigated conditions, were analysed by means of a mechanistic simulation model for crop–weed interaction (Bastiaans et al. 1997). Mahsuri is a native cultivar that originates from Malaysia. It is a tall-growing, highly competitive cultivar, with fast growth during the early growth stages. It belongs to the more traditional leafy cultivars with a droopy plant type and a low harvest index. IR8 is the higher-yielding, but less competitive rice cultivar. This first IRRI-bred recommended cultivar has low stature relative to Mahsuri, a more vertical leaf orientation, and a harvest index of around 0.50. In the experiment, both cultivars were grown in pure stand and in the presence of purple rice, which was added as a model weed. In all situations, IR8 gave the highest grain yield, but obviously the yield of IR8 was more affected by the presence of the weed than was the yield of Mahsuri.

Based on regular periodic samplings and non-destructive observations in the pure stand plots, INTERCOM (Kropff and Van Laar 1993), a model for interspecific competition, was parameterized. Simulation of dry-matter production and grain yield of IR8 and Mahsuri in competition with purple rice resulted in a good agreement with observed data, implying that the differences in phenological, physiological and morphological attributes of IR8 and Mahsuri were able to explain the observed differences in their competitive ability. The validated competition model was then used for a sensitivity analysis to identify which traits were responsible for the differences in competitive ability. One by one, model input parameters were increased by 10% and the consequences for simulated weed shoot biomass determined.

The result was expressed as relative sensitivity: the ratio of percentage change in simulated weed shoot biomass and percentage change in the value of the specific input parameter. The model clearly pointed at the importance of early growth characteristics (Figure 3). Increased rates of early leaf area development (EGR-leaf area) and early height growth rate (EGR-height) both gave considerable reductions in simulated weed biomass, indicating their importance for weed-suppressive ability. Maximum plant height (Max-height), which determines the vertical position of leaf area in the mixed canopy, was also found important. Increases in crop growth rate (CGR), the light extinction coefficient (K-dif) and specific leaf area (SLA) only resulted in marginal reductions in simulated weed biomass, indicating that these factors are not major determinants of weed-suppressive ability. These results exemplify the role of mechanistic simulation models in guiding the plant-breeding process: the models enable a quantitative estimation of the potential contribution of various traits to an increased competitive ability.

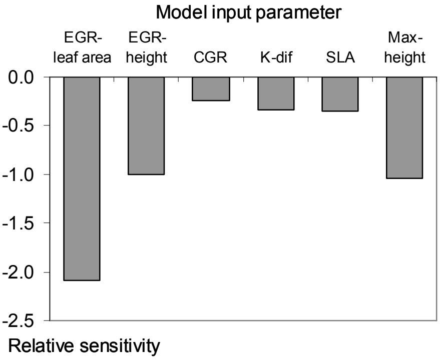

Figure 3. *Relative sensitivity, calculated as the ratio of percentage change in simulated weed biomass and percentage change in model input parameter obtained for various model input parameters (EGR=early growth rate; CGR=crop growth rate; K-dif=light extinction coefficient; SLA=specific leaf area; Max height=maximum plant height)*

Weed competitiveness and yielding ability of aerobic rice genotypes

In collaboration with the International Rice Research Institute (Los Baños, Philippines), the feasibility of breeding for weed-suppressive high-yielding aerobic rice was further investigated (Zhao 2006). In the experiments, conducted from 2001 to 2004, 40 aerobic/upland genotypes, including *indica*, *japonica*, *aus* and mixed types within *Oryza sativa* were used. Growing these genotypes in the presence of weeds revealed a large variability in weed-suppressive ability (WSA) among genotypes (Zhao et al. 2006a). Among the different germplasm groups, *indica* and *aus* germplasm appeared to be more weed-suppressive than tropical *japonica* germplasm (Figure 4). The *indica* group combined weed-suppressive ability with a strong yielding ability. Both under weed-free and weedy conditions the average grain yield was significantly higher than that of the other groups. The *aus* group showed the lowest yield reduction, which apart from its strong WSA might hint at a high level of weed tolerance. These findings indicate that *indica* and *aus* are likely to be the most suitable gene donors for improvement of WSA in aerobic rice in tropical regions.

Weedy yield and weed biomass, the two target traits in breeding for weed competitiveness, were both found to be moderately heritable, indicating that reasonable gains from selection can be expected. On top of that, early crop vigour and yield under weed-free conditions were found to have high estimated indirect selection efficiency for both weedy yield and weed biomass. This implies that selection for high-yielding, weed-competitive genotypes can be conducted in the absence of weeds. This has many practical advantages and saves breeding costs of seed, field and labour because of the smaller plot size and seed amount that are required, and the simplified selection process.

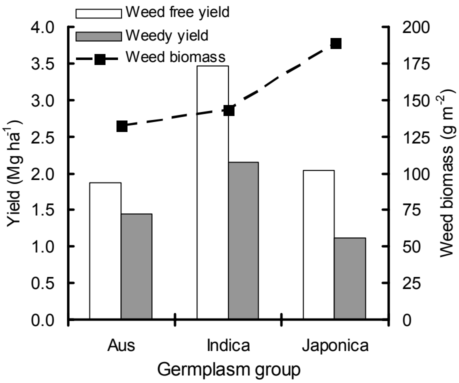

Figure 4. *Weed-free yield, weedy yield and weed biomass for three germplasm groups evaluated over three wet seasons of 2001–2003 at IRRI, Los Baños, Philippines (after Zhao 2006)*

Controversial conclusions have been drawn on the compatibility of yield potential and WSA. The current study showed that yielding ability and WSA were not only compatible, but also closely associated in aerobic/upland rice. One attribute of modern varieties is the vertical orientation of their leaves, creating a more even distribution of light over the canopy, resulting in a higher radiation use efficiency and a more productive crop. It is often assumed that vertical leaves are at the cost of the ability to suppress weeds. The results of the current study do not support this hypothesis, and even a negative association between droopy plant type and WSA was observed. One explanation for this is that WSA is determined by many different factors, such as growth rate, LAI, tillering, tiller angle, plant height and leaf erectness. Therefore, the contribution of droopy leaves to WSA might be very limited and cancelled out by the other factors. The association between plant type and WSA within the current germplasm population might also simply result from the fact that all the cultivars belonging to *indica* and *aus* germplasm groups were erect and had fast early growth. These kind of confounding factors hinder a clear analysis. What remains, however, is that nearly all studies addressed the importance of fast early growth in determining strong WSA (e.g., Johnson et al. 1998; Gibson and Fischer 2001; Zhao et al. 2006b).

One other objective of the study was to find out whether the use of more competitive cultivars can be combined with other cultural measures that strengthen the ability of the crop to suppress weeds. For this purpose three cultivars differing in competitive ability (APO, IR60080-46A and IRAT 216) were selected and sown at seeding rates of 100, 300 and 500 viable seeds m^{-2}. All weedy plots were hand-weeded once at either 3 weeks after sowing (WAS) (2003) or at 2 WAS (2004), and weeds were allowed to grow thereafter. In both years, and for all three cultivars, the weed biomass (WB; g m^{-2}) in dependence of crop plant density (N_c; plant m^{-2}) could be accurately described by a rectangular hyperbola, according to Spitters (1983):

$$WB = \frac{N_w}{b_{w0} + b_{ww}N_w + b_{wc}N_c}. \tag{1}$$

In this function, N_{w} (plant m^{-2}) represents the number of weed plants, b_{w0} (plant g^{-1}) represents the reciprocal of the average weight per weed plant in the absence of competition, b_{ww} (m^2 g^{-1}) is the intraspecific-competition coefficient for the weed plants. The effect of interspecific competition of a rice cultivar is expressed as the product of an interspecific-competition coefficient (b_{wc}; m^2 g^{-1}) and crop plant density. In both years the competition coefficient of cultivar APO was about twice as high as that of the other two cultivars. This implies that the other two cultivars should be sown at a twice higher density to obtain the same weed-suppressive effect as APO. Time of weeding also had a clear effect. With weeding at 3 WAS (2003) crop plants were more competitive than with weeding at 2 WAS (2004). This illustrates that weed suppression is strongly determined by genotype × management interaction.

INTERCROPPING

Breeding for more competitive genotypes does not provide a solution for solving weed problems in all cropping systems. Particularly in production of vegetables there are some relatively slow-growing crops such as onion, carrot and leek that will never be able to suppress weeds sufficiently. In these situations, intercropping, in which two or more crops are simultaneously grown in the same field, is an alternative option for attaining improved weed management (Liebman and Dyck 1993; Teasdale 1998). Ideally, crops whose resource use characteristics are physiologically, temporally or morphologically complementary are combined. In this way, the crops are prevented from fully competing with one another (Vandermeer 1989). At the same time, these intercrops may use a greater share of available resources and, therefore, provide opportunities for suppressing weeds through niche pre-emption or resource competition. In the concept of Vandermeer (1989) component crops in an intercrop interfere with one another by affecting one another's growing environment. A distinction is made between *competition*, when one crop creates a less favourable environment for the other, and *facilitation*, when an improved growing environment is created. With regard to weed management, facilitation, or the creation of a weed-free environment, is created through competition. Perhaps the best known example of this type of weed suppression is the use of cover crops, which are solid-grown crops grown primarily to protect and cover the soil between crop rows. One of the main challenges of this approach is that the crop that is introduced for its weed-suppressing function should provide a sufficient level of weed control without putting too much competitive pressure on the main crop. Whether this is realized depends on the main crop, on the added crop, but particularly on the combination of both.

Suitability of the main crop

Options for utilization of intercropping systems for weed control first of all depend on the main crop. In perennial cropping systems, such as vineyards, annual cover crops are often successfully introduced. Here the main crop is well established and competition can be avoided by selecting a low-growing, shallow-rooting cover crop that is able to produce a closed cover. A quick cover-crop establishment than avoids the settlement of deeper-rooting, tall-growing weeds that compete with the grape plants or hinder the harvesting operations. With annual main crops it is far more difficult to avoid competition between main and cover crop. Suitability of the main crop is then largely determined by the ability of the main crop to tolerate a certain level of interspecific competition. In a joint experimental approach, intercropping of Brussels sprouts with barley was investigated. Entomologists observed that populations of several herbivore species (e.g., *Brevicoryne brassicae, Myzus persicae*) were reduced by intercropping Brussels sprouts with barley (Bukovinszky 2004). For weed management the results were disappointing. Introduction of barley in between the rows of Brussels sprout did not prevent the establishment of weeds such as *Chenopodium album*, whereas it precluded the use of mechanical weed control options such as hoeing. Most importantly, Brussels sprout suffered quite extensively from the competitive pressure that barley posed on this crop. Apart from a lower dry-matter production, the harvest index was dramatically reduced (Figure 5). De Wit et al. (1979) already pointed at differences in the response of crops to competition and distinguished between crops where, at higher levels of competition, individual plant size is affected but harvest index remains unaffected (e.g., small cereal grains) and crops where a reduction in plant size is complemented with a reduction in harvest index (e.g., maize, Brussels sprouts). The additional sensitivity of the last category to competition makes those species far less suitable for use in an intercrop.

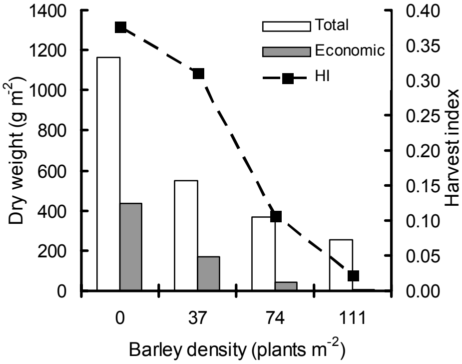

Figure 5. *Total yield, economic yield and harvest index (HI) of Brussels sprouts grown in pure stand and in mixture with different plant densities of barley. Results represent averages obtained with Brussels sprouts grown at 4.4 and 6.7 plants m*$^{-2}$

Determining the suitability of clover as a cover crop

Except for the main crop, the selection of a proper cover crop is important. It is obvious that cover-crop species that combine weed suppression with other functions are favoured. Clover species, e.g., as members of the Leguminosae, are, able to fix nitrogen. Furthermore, it is well established that clover species are able to reduce pest and disease pressure in a number of crops (Theunissen 1994). Both these characteristics are particularly favourable in organic agricultural systems. In addition, some clovers possess good potential as weed suppressor as they are able to produce a dense layer of biomass. The main constraint for using clover as an undersown cover crop is yield depression because of competition with the main crop. Attempts to reduce this competition include the screening for less competitive cover-crop species (Nicholson and Wien 1983). Recently, such a screening was carried out among a group of eight different clover species (Den Hollander and Bastiaans 2004).

In this comparison, Persian clover (*Trifolium resupinatum*) was among the fastest developing species. It produced a rapid soil cover and grew relatively tall. Subterranean clover (*T. subterraneum*) showed the slowest soil cover and remained relatively small. The differences between those two clover species were reflected in clear differences in the ability to suppress weeds. Persian clover gave a satisfactory suppression as, compared to the bare-soil control plot, the number of established weeds was reduced by 80% and those weeds that managed to survive remained relatively small. In plots with subterranean clover the number of weeds was reduced by only 25%. Regardless of clover species, the competition from clover led to unacceptable yield reductions of the main crop. When leek transplants were introduced in well-established clover canopies, individual leek plant dry weight was reduced by 75% in case of Persian clover, and still by 61% when introduced in subterranean clover. These findings indicate that species selection on its own is not sufficient to obtain an acceptable equilibrium between weed suppression and yield reduction of the crop when using clover as undersown cover crop. Obviously, additional control measures remain necessary to restrain the negative effects on the crop, a conclusion in line with findings of Lotz et al. (1997). Mechanical suppression of cover-crop growth through, e.g., mowing or root cutting (Brandsæter et al. 1998), and improved timing of establishment of the cover crop relative to that of the main crop (Müller-Schärer and Potter 1991), are examples of such management.

Competitive suppression of weeds in a leek–celery intercropping system

One alternative for minimizing the negative consequences of the competitive effect of the cover crop on the main crop is the introduction of a second cash crop. In collaboration with the Swiss Agricultural Research Station, Agroscope FAW Wädenswil a leek–celery intercropping system was studied and optimized with regard to crop yield, plant quality and weed-suppressive ability (Baumann 2001). In this case the leafy and competitive celery was introduced to improve the weed suppression of the vertically growing and weakly competitive leek (Baumann et al.

2000). Competition between leek and celery is to some extent acceptable, as long as leek is able to reach its minimum marketable plant size, simply because the resources captured by celery also result in a marketable product.

Field experiments were carried out to study the weed-suppressive ability and the intra- and interspecific competition of a leek–celery intercrop with and without additional weed competition (Baumann et al. 2001). Results showed that intercropping of leek and celery in a row-by-row replacement design provided a much better weed suppression than the leek pure stand, even though the intercrop was not able to suppress early-germinating weeds completely. Consequently, the critical period for weed control of intercropped leek lasted about two weeks shorter than that of leek pure stand. In an experiment in which *Senecio vulgaris* was planted, it was shown that the flower production and the offspring of mature weed plants was considerably reduced under intercropped leek compared with the pure stand of leek. This indicates that increasing the ability of the crop canopy to compete for light can reduce not only the biomass, but also the reproductive potential of weeds. The advantages of the intercrop relative to the pure stand with respect to weed management are schematically presented in Figure 6.

Next to weed management, crop productivity is an important element for justification of an intercrop. The experiment showed that the relative yield of the intercrop exceeded that of the pure stands by 10%, probably as a result of an optimized exploitation of resources. The percentage of marketable leek plants (pseudostem diameter ≥ 20 mm) was, however, reduced by 20%. For this reason, the focus was put on optimization of the total plant density and the mixing ratio of the intercrop, using simulation modelling. An adapted version of the eco-physiological competition model INTERCOM was used to simulate interplant competition between leek, celery and *S. vulgaris* (Baumann et al. 2002a).

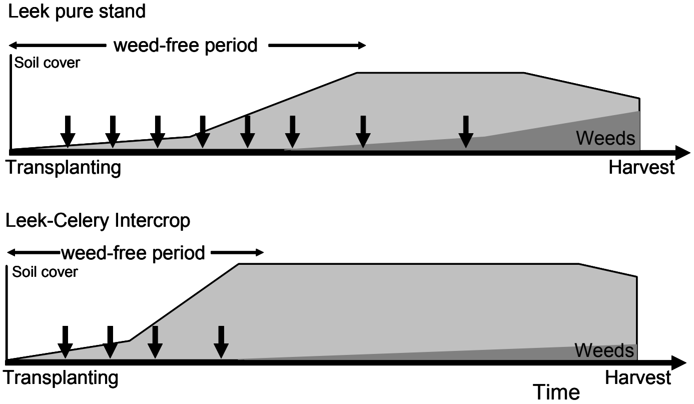

Figure 6. *Schematic representation of the implications for weed management when leek is grown in a mixture with celery, rather than in pure stand. Arrows indicate weed control interventions. The weed-free period refers to the period during which weeds should be removed in order to avoid yield reduction. In the leek pure stand an additional weed control operation is conducted after the weed-free period to avoid weed seed production*

After parameterization, based on the pure stands of the two crops and the weed, and validation, the model was used to simulate intercrop performance for various conditions and a wide range of crop densities, using different relative times of weed emergence (Baumann et al. 2002b). The results of these simulations were summarized using a descriptive hyperbolic yield density model (Spitters 1983). Based on the parameter estimates of this simple model it was found that the competitive ability of celery was about three times higher than the competitive ability of leek. Increasing the proportion of celery in the mixture will thus result in an improved weed-suppressive ability of the intercrop, but at the same time might cause severe reductions in the quality of leek. Optimization confirmed that the size of individual leek plants was the main limiting factor of this system. An intercropping system consisting of 19 leek and 9.4 celery plants m^{-2} was found optimal. The revenues of this system were 7% higher than that of the highest-yielding pure stand of leek and 9% higher than the revenues of the highest-yielding celery pure stand. Compared to leek pure stand, this mixed cropping system also gave a considerable reduction in reproductive potential of *S. vulgaris*. In conclusion, it was shown that intercropping of two main crops improved the sustainability of the system by reducing the need for labour and cost-intensive weed control measures, whereas the profitability of the system was maintained.

SEQUENTIAL COVER CROPPING

Use of cover crops in the crop-free period

Another strategy for using cover crops for weed management is to grow them during the period when the main crop is absent. Competition between the main and the cover crop is then no longer a pressing issue and problems with mechanization of the main crop are avoided. Inclusion of cover crops in crop rotations introduces two important mechanisms through which the development of weed populations might be hampered. In late summer and autumn the successful introduction of cover crops prevents growth, development and, most importantly, seed production of weeds that remain in the stubble. Cover crops fill gaps in cropping systems that would otherwise be occupied by weeds (Liebman and Staver 2001). As a result of this type of niche pre-emption, weed soil cover is substantially reduced.

In late winter and spring, cover-crop residues, used as surface mulches, suppress or retard weed emergence and growth due to both allelopathic and physical effects (e.g., Liebman and Davis 2000). Crop residues on the soil surface can also reduce weed densities by physically impeding weed seedling emergence and intercepting light that cues weed germination. Many plant species produce and release chemicals that are toxic to other plants, a phenomenon referred to as allelopathy. Allelochemicals may also be produced by microbes that transform plant products during residue decomposition. Living crops can have direct allelopathic effects on weeds, but the most important application involves the use of crop residue to suppress weed germination, establishment and growth. A number of classes of chemicals have been identified as allelopathic agents. Those found frequently include alkaloids, coumarins, cyanogenic glucosides, flavonoids, phenolic acids, polyacetylenes, quinines and terpenoids (Einhellig and Leather 1988; Worsham 1989; Rice 1995).

In Dutch agriculture, cover crops have always played a modest role and the motives for using them have changed over time. Originally, these crops were mainly used as green manure or fodder crops, and this is how they still can be found in the Dutch List of Varieties of Field Crops (PRI 2005). On arable farms, cover crops were mainly used after the main crop, for increasing the organic-matter content of the soil. On mixed farms *Brassica* spp. were grown in the same period and used as additional feed for cattle. More strict regulations on emission of nutrients have given cover crops an additional role as catch crop, meant to avoid leaching in the crop-free winter period. Furthermore, Chinese radish (*Raphanus sativa*) is being used as hatch or trap crop for *Heterodera* spp., cyst nematodes that are pathogenic to sugar beet. For this purpose, the best results are obtained if the crops are sown in spring. In organic farming systems leguminous crops are used to supply nitrogen to the soil (e.g., Liebman and Davis 2000). The weed-suppressing function of these cover crops has so far received little attention.

Optimization of the weed-suppressing function

Recently, a research programme was started with the aim of exploring the potential of cover crops to contribute to the ecological management of weed populations in organic farming systems (Kruidhof and Bastiaans 2005). The aim of this project is to explore options for enhancing cover-crop performance by optimizing both the autumn (competition) and spring (allelopathic inhibition) weed-suppressing functions (Figure 7). From each of the families Brassicaceae, Poaceae and Fabaceae a frost-sensitive and a winter-hard species were selected. Weed suppression in autumn is studied and related to morpho-physiological characteristics of the cover crops. Particular attention is given to early growth, as a fast establishment and canopy closure of the cover crop is a prerequisite for sufficient weed suppression. Furthermore, it is investigated how the concentration of allelochemicals in the cover crop can be maximized. Plant density, nutrient level and mechanical damage are factors whose effects are investigated.

Incorporation of the cover-crop residues in the soil is another important aspect, as it mediates the effect of the residues on the target plants. Pre-treatment of residues before incorporation is one element. The crops can simply be mown, but cutting the residues in pieces of different sizes and crushing are other options. All of these treatments may affect the release pattern of the allelochemicals from the cover-crop residue material. Residue incorporation strategies will also be studied. Cover-crop residues might be left on the soil surface, mixed through the upper part of the soil (e.g., 5, 10 or 20 cm), or ploughed under at a specific depth. In field experiments different equipment is tested for pre-treatment and residue incorporation. Both distribution of residues in the soil and the undesired regrowth of the cover crops are evaluated.

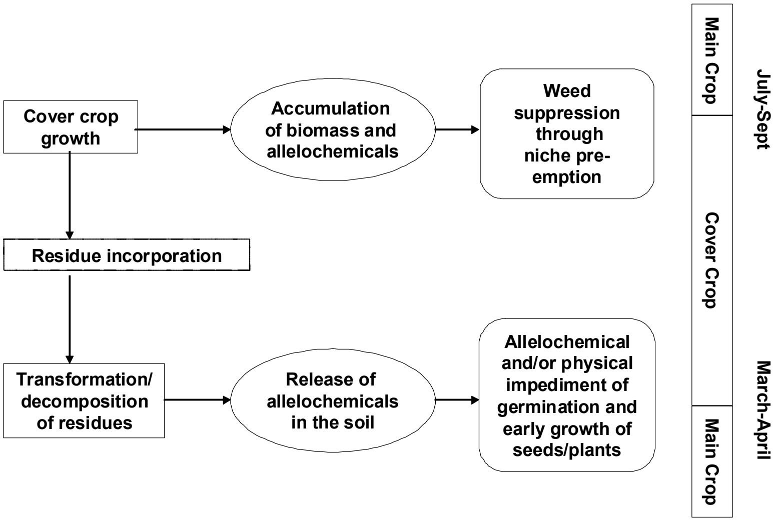

Figure 7. *Framework showing the autumn and spring weed-suppressive mechanisms through which rotational cover cropping contributes to weed management in a crop rotation (after Kruidhof and Bastiaans 2005)*

Often, allelopathic effects of plant extracts on germination of seeds in Petri dishes have been reported, whereas effects under field conditions are absent. For this reason, chemical analysis, laboratory bioassays, ring experiments and field experiments are conducted. In most experiments lettuce (*Lactuca sativa* L.) is used as test species, as seeds of this species are known to be sensitive to allelopathic compounds. In other experiments, seeds of a range of plant species are used to determine whether selective inhibition of seeds occurs. Seed size has often been reported an important mechanism of selectivity. Small-seeded species appear to be more susceptible to allelochemicals, whereas large-seeded species appear to be relatively insensitive (Putnam and Defrank 1983). As seeds of crop species are often larger than seeds of weed species this might be an important mechanism of selectivity for application of this strategy in practice.

CONCLUDING REMARKS

Especially after the introduction of herbicides, curative weed control has become the dominant strategy for dealing with weeds. Attention has shifted away from cultural control measures that largely try to avoid or reduce the potential negative consequences of weeds that are present in agro-ecosystems. The current problems related to the profuse use of herbicides have reinitiated an increased interest in alternative weed management options. A reduced recruitment of weeds from the soil seed bank, an increased competitive ability of the crop relative to that of the weed and a reduction of the weed soil seed bank all represent principles through which weed problems in agro-ecosystems can be reduced. In a number of projects research was undertaken to investigate whether diversity could be employed for the utilization of these alternative principles. The use of weed-competitive cultivars, intercropping and rotational cover cropping all contribute significantly to the management of weed populations through at least two of the fore-mentioned principles. Apart from an improved competitiveness of the crop (weed-competitive cultivars; intercropping) or a reduced recruitment (rotational cover cropping) each method generates a positive contribution to the control of the size of the weed seed bank. It is also obvious that none of the proposed methods is able to replace curative control completely. Rather, the proposed measures allow curative control measures to be applied in a less intensive, and probably less frequent, manner.

For all methods the ultimate weed-suppressive effect is determined by a combination of genotype and management. For the competitive cultivars the weed-suppressive effect can be quantitatively characterized as the product of the seeding rate of the crop and an interspecific competition index. This interspecific competition index expresses the competitive ability of a single crop plant relative to that of a weed plant. Apart from the genetic component this competition index was shown to be influenced by the timing of weed control. Later removal of weeds gave the crop a clear competitive advantage, as mainly the weeds that emerge after the weeding operation put a long-lasting competitive pressure on the crop. Postponing the weeding control measure too much might, however, reduce the efficacy of the control operation. With intercropping, the selection of the main and the undersown cover crop is an important first step. Combined with relative planting time, overall planting density and the mixing ratio of the component crops they determine the weed-suppressive ability of the intercrop. If the cover crop becomes too dominant, additional management is required to restore the desired competitive balance between the component crops. In case of rotational cover cropping the choice of the cover crop should be based on the competitive ability in autumn and the allelopathic potential in spring. Important management aspects are mainly related to incorporation of the cover-crop residue material in spring. Not only does this residue handling determine the impediment of weed seed germination, it also determines the risk of undesired regrowth of the cover crop.

Both intercropping and the use of competitive cultivars are largely based on providing a more competitive environment for the weeds. In both situations an important aspect is whether the improved competitive ability of the crop is at the cost of yielding ability. In the current research, improved weed-suppressive ability of aerobic rice cultivars was closely related to early vigour, and this trait correlated well with yielding ability. Consequently, in this case, yielding ability and weed-suppressive ability can easily be combined. For intercropping systems a different situation was found. In this case the weed-suppressive ability is mainly determined by the cover crop, whereas the yield of the main crop is most important. Improved weed suppression is closely associated with a stronger competitive pressure on the main crop and consequently there is a clear tension between weed suppression and crop yield. Introduction of a competitive second cash crop is one option to minimize the financial consequences of yield reduction in the main crop. Another option is to use the cover crop in a rotational context and avoid the competition between main and cover crop. Competition models showed to be useful tools for improved understanding and systems optimization. With breeding for more competitive cultivars they allow the quantitative assessment of the importance of various traits, whereas with intercropping they allowed the determination of the optimum mixture composition with regard to crop yield, plant quality and weed suppression.

Opportunities for implementation of the proposed strategies are quite different. Intercropping, despite its many advantages, is generally not considered a feasible system in high-input horticulture and agriculture in Western Europe. One of the obstacles is the risk of obtaining a lower yield compared to systems consisting of pure-stand crops. Furthermore, the difficulties with mechanization and hence the high labour requirement reduce the attractiveness of this system. Mechanization, however, does not necessarily have to be a major obstacle. In case of the leek–celery intercropping system, planting, tillage operations and harvesting could all be carried out using commercially available machinery. Growing cover crops in between two main crops is already common practice for many farmers. This practice is often used for many different reasons. The current research project focuses on optimization of the weed-suppressive effect of rotational cover cropping. Outcomes of this research might give directions for cover-crop selection and handling and incorporation of cover-crop residue material. For farmers that already use rotational cover cropping this might imply some simple adjustments to their current practices. For farmers who prefer to have their land fallow in between two main crops, implementation of this strategy is less likely. Competitive cultivars mainly require a serious breeding effort. For aerobic rice, indirect selection indices were developed, meaning that selection can be conducted in weed-free conditions and only requires few additional observations. Once the more competitive cultivars are available no major obstacles have to be overcome.

ACKNOWLEDGEMENTS

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OUTLOOK AND DIALOGUE

CHAPTER 22

WHEN CAN INTELLIGENT DESIGN OF CROPS BY HUMANS OUTPERFORM NATURAL SELECTION?

R.F. DENISON

University of Minnesota, Ecology, Evolution and Behavior, 100 Ecology Building,
1987 Upper Buford Circle, Saint Paul, MN 55108, USA.
E-mail: denis036@umn.edu

Abstract. Natural selection operated on the wild ancestors of crop plants for millions of years. Many seemingly intelligent design changes that we could make to enzyme structure or gene expression would duplicate (at least in phenotypic effect) variants already rejected by past natural selection. These variants died out because they decreased individual plant survival or reproduction under preagricultural conditions. Many of the variants rejected by past natural selection would also reduce crop yield or quality today, so it would be a waste of time to duplicate them using molecular methods. For example, most changes to rubisco will decrease photosynthesis (and crop yield) under current conditions, just as they would have decreased photosynthesis (and individual plant fitness) under preagricultural conditions.

A few of natural selection's 'rejects', however, would be genuine improvements by human criteria. Can we identify these promising rejects? Opportunities for crop genetic improvement that were missed by past natural selection are likely to fall into three major categories. First, and most important, conflicts of interest among competing plants, or between plants and their microbial symbionts, can cause trade-offs between individual plant fitness (favoured by past natural selection) and the collective performance of the crop community. Therefore, we can sometimes increase yield by reversing the effects of past natural selection for individual competitiveness. Second, changes in climate, soil fertility and pest populations mean that some variants that were less fit in the past will be more fit today. In this case, crop genetic improvement may accelerate changes that are already favoured by ongoing natural selection in an agricultural context. Third, eventually molecular methods may produce genotypes so different from anything that existed in the past that we cannot assume they were tested and rejected by natural selection. C_4 photosynthesis has evolved repeatedly, however, so a proposed innovation would have to be more radical than C_4 photosynthesis before we can assume it was missed by past natural selection.

The relative importance of these three kinds of opportunity is likely to change over the next few decades. Some trade-offs between individual competitiveness and the yield of the crop community have already been exploited, as in dwarf wheat and rice, but other opportunities may remain. Our ability to design radical new enzymes from scratch, or to predict the consequences of major changes in gene expression patterns, may improve over coming decades. Even after most significant opportunities to improve yield potential (yield in the absence of pests and diseases) have been fully exploited, ongoing evolution of pests and pathogens will create a continual need for 'Red Queen Breeding', generating a stream of new cultivars to keep up with the latest biotic threats.

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> "Natural Selection … is as immeasurably superior to man's feeble efforts, as the works of Nature are to those of Art." – Darwin (1859).

> "There is nothing in the process of evolution that has any aspect of community behaviour as a goal" – C.T. De Wit (1978).

INTRODUCTION

For 30 years, some biotechnology enthusiasts have been predicting that major increases in crop yields will come from improving fundamental physiological processes, such as photosynthesis or N_2 fixation (Shanmugam and Valentine 1975). For example, Zelitch (1975), claiming to have mutant plants with lower photorespiration and 40% greater net photosynthesis, suggested that "large increases in yields should be obtainable". Yet there is no crop grown commercially today whose higher yield results from genetic engineering of photosynthesis, N_2 fixation or similar processes. In fact, there has been little or no improvement in yield potential (i.e., yield without abiotic stress, diseases or pests) of major crops over the last 20 years (Cassman 1999). This lack of progress, together with evolutionary arguments presented here and previously (Denison et al. 2003a; 2003b) suggest that ongoing emphasis on improving physiological efficiency (e.g., Long et al. 2006) is misplaced. We need a new theoretical framework to guide future crop improvement.

We should begin by recognizing that natural selection has already optimized much of the genome of our crops, mostly prior to domestication. Attempts to improve most genes are therefore likely to be futile. Fortunately for crop geneticists, however, the genotypes that were favoured by natural selection are not always best by human criteria. Conflicts between past natural selection and present human goals represent the best opportunities for significant genetic improvement of crops.

How can we identify these opportunities, 'missed' by natural selection? First, do not assume that the application of human intelligence over a few years is intrinsically superior to natural selection over millennia. Engineers are increasingly recognizing that processes analogous to natural selection, such as genetic algorithms (Cogan 2001) and simulated annealing (Kirkpatrick et al. 1983) can often solve baffling optimization problems. The development of an enzyme made from DNA (Breaker and Joyce 1995) is a biochemical example of using a process analogous to natural selection to solve an otherwise intractable design problem. Human ingenuity was required to create the conditions that selected for successive improvements in enzyme activity, but the actual process of selection was independent of human judgement. What these methods have in common with natural selection is non-random selection of each new generation from a randomly varying population. Given a large population of random variants from which to select each new generation, and given enough generations – each generation typically represents only a small improvement – natural selection generates solutions so effective that they can give the superficial appearance of having been designed by an individual (Behe 1996), or perhaps a team, of superhuman intelligence.

So, for most genes, improving on natural selection will be difficult. This is especially true for many of the genes that are most important to crop yield. Enzymes involved in photosynthesis, synthesis of seed storage proteins, etc., are critical to crop yield. But, because they were also critical to the reproductive success of the wild ancestors of crops, they will already have been subject to strong selection for efficiency over millions of generations. Similarly, over-expression of a 'key enzyme' will almost always represent an option that was previously rejected by natural selection, so it is unlikely to increase crop productivity today. Crop yields may increase when photosynthesis increases with N fertilization or CO_2 enrichment (Long et al. 2006), but that is hardly evidence for the existence of trade-off-free opportunities for genetic improvement of photosynthesis that will increase yield. Similar conclusions have been drawn previously from more detailed analyses based on evolutionary theory (Denison et al. 2003b) and whole-plant physiology (Sinclair et al. 2004).

Nonetheless, it is clear that domestication and subsequent breeding of crops have resulted in enormous improvement in crop productivity under agricultural conditions, in addition to improvements in their suitability for our uses. Many opportunities for further improvements doubtless remain. The purpose of this chapter is to help those involved in crop improvement to identify areas where the chances for further progress are greatest, i.e., to identify opportunities for improvement (by human criteria) missed by past natural selection.

These opportunities can be grouped into three broad categories:

- Opportunities linked to conflicts of interest among organisms;
- New human goals or new crop environments; and
- Radical innovations not previously tested by natural selection.

The first two categories, which collectively account for most crop genetic improvement to date, will each be divided into two subcategories.

OPPORTUNITIES LINKED TO CONFLICTS OF INTEREST

Most past increases in crop yield potential have involved reversing the effects of past natural selection, in cases where conflicts of interest among individual plants have limited the collective performance of plant communities. Molecular methods have created new ways of implementing this approach.

Natural selection favours the spread of genes whose phenotypic effect favours their own spread (Dawkins 1976), whatever the consequences for a species as a whole, or for the plant communities and ecosystems where a species lives. Conflicts of interest even within an individual genome can sometimes be detected, as in cytoplasmic genes for male sterility, which favour their own spread by redirecting plant resources to seed production at the expense of pollen production (Dominguez 1995). The focus here, however, will be on conflicts of interest between individual plants and the plant community, and on conflicts between plants and their microbial symbionts. Both types of conflicts have limited the power of natural selection to optimize the collective performance of plant communities. Therefore, ameliorating the effects of such conflicts can provide opportunities for crop genetic improvement.

Trading individual competitiveness for community performance

It might seem that natural selection, operating on the wild ancestors of plants that reproduce mainly by producing seed, would already have maximized ‘seed yield’, at least in environments that resemble those where the ancestral plant evolved. But ‘seed yield’ can have at least three different meanings, even if expressed on a dry-weight basis: g plant^{-1}, g m^{-2} or g seed^{-1}. Natural selection will tend to increase some combination of g plant^{-1} and g seed^{-1}, subject to physiological and environmental constraints. But increases in g m^{-2} – the collective seed production of a group of plants – will be favoured by natural selection only as a side-effect of selection for individual seed production. Such side-effect benefits are probably common, as any increase in the inherent efficiency of an enzyme, for example, would tend to increase the productivity of individual plants. It would therefore be favoured by individual-based natural selection, while also increasing the collective productivity of groups of plants. This would leave little room for further genetic improvement by humans, except possibly through innovations so radical they have never arisen in the past, as discussed below.

There are, however, many cases in which past natural selection has favoured traits that reduce the productivity or efficiency of plant communities, despite their beneficial effects on individual productivity in a competitive environment. Taller rice plants out-compete their neighbours, but the collective seed production of the whole community is reduced by excessive investment in stems. Shorter, ‘Green Revolution’ varieties are much more productive (g m^{-2}) when grown together, but less competitive against taller genotypes (Jennings and De Jesus 1968). Dwarf fruit trees are less competitive than neighbours that keep growing (vertically or horizontally), but limited growth reduces pruning costs and allocates more resources to fruit production. Similarly, traits such as erect leaves and reduced branching reduce competitiveness while increasing yield potential (Donald 1968).

In all of these cases, changes in crop management may be required to achieve this potential. Kokubun (1988) showed that a population of single-stem soybean plants had higher seed yield (g m^{-2}) than branched soybeans, but only at high seeding rates (seeds m^{-2}). Similarly, a short wheat cultivar with erect leaves had the highest yield of all cultivars tested when weeds were controlled, but the lowest yield rank when forced to compete with weeds (Tanner et al. 1966).

Trade-offs between individual competitiveness and community-level traits will not always result in a negative relationship between weed-free yield and competitiveness with weeds (Gibson et al. 2003). A given genotype may have some defect, such as poor local adaptation, which affects both competitiveness and productivity. Major increases in yield potential may often require sacrificing some competitiveness, but that does not mean that all non-competitive genotypes have higher yield potential.

Community-level traits other than yield may also have been undermined by past natural selection for individual competitiveness. Consider water use efficiency. Natural selection will tend to favour high water use efficiency at the leaf level, increasing the ratio of CO_2 uptake per g H_2O transpired. But past natural selection may have rejected some strategies that could have increased community-level water use efficiency (Zhang et al. 1999). For example, conserving water in the soil for later use will not be favoured by natural selection if the conserved water is then used by a competing plant.

General strategies by which plant breeders might exploit individual versus community trade-offs have been discussed previously (Denison et al. 2003b), but a more recent example illustrates how molecular tools can facilitate this approach. Many plants respond to the presence of neighbours (using light cues detected by phytochrome) with an increase in stem elongation. Transplant experiments have shown that elongated plants are more fit under crowded conditions and less fit under less-crowded conditions (Dudley and Schmitt 1996).

Boccalandro et al. (2003) reasoned that, although elongation increases the fraction of total available light intercepted by taller individuals, it does not increase total light interception (and hence photosynthesis) of the plant community. The elongation response, therefore, represents a waste of resources in excessive allocation to stems. So they used genetic modification of phytochrome to make potato plants less responsive to crowding. The transgenic plants had higher tuber yield (both g plant^{-1} and g m^{-2}, as neighbouring plants all had the same genotype), although a decrease in g tuber^{-1} could affect the commercial value of the transgenic genotype. There were also effects on flowering time and stomatal conductance, so yield increases may not be due to effects on elongation alone, but this approach seems promising enough to merit further research in various crops.

Manipulation of genes related to crowding responses below-ground could also be worthwhile. Zhang et al. (1999) modelled root allocation in a water-limited environment and suggested that natural selection would favour excessive allocation to roots because of competitive interactions. Experiments by Gersani et al. (2001) confirmed this prediction, showing that interactions between roots of different soybean plants lead to excessive root proliferation at the expense of seed production.

Increasing benefits from symbiosis

Natural selection may also have failed to optimize below-ground interactions between plants and their microbial symbionts. This discussion will emphasize rhizobium symbiosis, so it is directly relevant only to legume crops and forages. Similar considerations may apply to mycorrhizas (Kiers et al. 2002), however, and perhaps also to disease-suppressing rhizosphere bacteria (Denison et al. 2003a).

Genes for nodulating plants persist in rhizobium populations because rhizobia reproduce inside nodules and are released into the soil in greater numbers than if they stayed in the soil. A legume species or cultivar that allows a given rhizobium strain to nodulate increases the soil abundance of that strain after nodule senescence, relative to rhizobia limited to soil and rhizosphere. This has been shown in the field both for soybean nodules (Kuykendall 1989), in which the differentiated, N_2-fixing bacteroid form of rhizobia retains the ability to reproduce (citations in Denison 2000) and for pea nodules (Kucey and Hynes 1989), from which only undifferentiated rhizobia escape and reproduce in soil.

After reproducing manyfold inside a nodule, why bother to fix N_2? Fixing N_2 consumes resources that rhizobia might otherwise use for their own current or future reproduction. For example, rhizobia can fix more N_2 if they use all available C as an energy source than if they accumulate some C as polyhydroxybutyrate (PHB, Cevallos et al. 1996). Rhizobium cells that hoarded PHB should have greater survival and reproduction inside senescing nodules or subsequently in the soil, however. (Experiments underway in my laboratory support this hypothesis.)

In species where N_2-fixing bacteroids will have no direct descendants in the soil, hoarding of PHB by bacteroids has no fitness benefit for rhizobia. It is therefore not surprising that only undifferentiated rhizobia, and not bacteroids, accumulate PHB in those species (Denison and Kiers 2004). Terminally differentiated bacteroids could still enhance their inclusive fitness at the expense of N_2 fixation, however, by diverting C to the production of rhizopines for consumption by undifferentiated rhizobia, presumably clonemates inside the same nodule (Denison 2000).

It might seem that rhizobia and plant have a shared interest in increasing the photosynthate supply they share. If rhizobia fix more N_2, they can increase host-plant photosynthesis (Bethlenfalvay et al. 1978) and growth, potentially increasing total photosynthate supply to nodules. The problem is that each individual plant is typically infected by several different rhizobium strains (Hagen and Hamrick 1996). These rhizobia compete for host resources, at least in the sense that a given sugar molecule cannot be shared by two different nodules. After rhizobia escape into the soil during nodule senescence, strains from the same plant are each others' most likely competitors, for the next host plant and possibly for soil resources as well. By investing in N_2 fixation, therefore, rhizobia in a nodule may benefit their worst competitors, with negative consequences for their own fitness (survival and reproduction) and that of their descendants in the soil. Thus, multiple strains per plant create a 'tragedy of the commons'. Genes that enhance rhizobium reproduction at the expense of N_2 fixation should therefore completely displace more mutualistic genes that enhance N_2 fixation, over the course of evolution. Why has this not happened?

I have suggested that the most likely explanation for the persistence of more-mutualistic genes in rhizobium populations is that legumes monitor the actual symbiotic behaviour of rhizobia in nodules – N_2 fixation, not easily-mimicked 'recognition signals' – and then impose 'sanctions' that reduce the fitness of less-mutualistic strains (Denison 2000). A mathematical model confirmed that less-mutualistic strains would spread in the absence of host sanctions (West et al. 2002). The existence of host sanctions has since been confirmed experimentally, by manipulating the N_2 concentration around individual soybean nodules. Rhizobia allowed to fix only trace amounts of N_2 reproduced at only half the rate of genetically identical rhizobia fixing N_2 normally (Kiers et al. 2003).

Sanctions reverse our earlier theoretical prediction and raise a new question. If, because of host sanctions, rhizobia that fix N_2 have twice the fitness of rhizobia that do not, then why are strains that fix little or no N_2 with local crops common in some soils worldwide? In some cases, rhizobium strains that are poor N_2 fixers on the locally dominant crop species might do better (fix more N_2 and avoid sanctions) on another species (Bala and Giller 2001), which may once have been common locally and which may persist as a weed. Unless fields are very weedy, however, release of rhizobia from nodules of this alternative host should be swamped by release from crop legume nodules. Therefore, if a sanctions-imposing crop is the dominant legume species, then the subset of locally adapted rhizobia that is most mutualistic on that crop should come to dominate the soil. Instead, less-mutualistic strains often dominate (Erdman 1950; Labandera and Vincent 1975; Denton et al. 2000).

Why are less-mutualistic strains sometimes common, if the dominant host species imposes sanctions on poorly performing nodules? If mixed nodules are common in the field, as may be the case for soybean (Moawad and Schmidt 1987), then less-mutualistic rhizobia sharing a nodule with a more-mutualistic strain might escape nodule-level sanctions. If so, then developing crops that are better at limiting the number of founding rhizobia to one per nodule could solve this problem. Alternatively, sanctions imposed by existing cultivars may not be stringent enough to prevent the spread of marginally effective rhizobia. If even mediocre rhizobia still provide a net benefit to an individual legume plant, then natural selection among legumes would have limited the imposition of sanctions, except against rhizobia that fix essentially no N_2 (Denison 2000). Improvements in host sanctions may therefore represent an opportunity for genetic improvement of legume crops and forages. A crop that killed all rhizobia inside nodules with mediocre fixation rate, while directing abundant resources to the best nodules, would tend to enrich the soil with the best local strains of rhizobia, released from its best-performing nodules.

Plants with this positive effect on rhizobium communities in the soil might be identified using a relatively simple screen. First, grow a genetically diverse population of plants, with each plant in a pot with soil containing rhizobia differing in mutualism, and save seed. Then grow a genetically uniform test cultivar in the same pots. Select seed from the first generation based on growth of the test cultivar. Plant genotypes with a beneficial effect on other soil microbes could be identified similarly, if such genotypes exist. For example, genotypes that enrich the soil with mycorrhizae more beneficial to the next crop, which may be the reverse of the current situation (Johnson et al. 1992), might be identified.

Another interesting approach was developed by Rosas et al. (1998), who designed an innovative method for identifying plant genotypes that nodulate preferentially with a specific inoculum strain. If local rhizobia are all ineffective, it would be easy to screen for green plants nodulated by the inoculum strain. But local rhizobia are often mediocre, making it difficult to identify plants nodulated mainly by the more mutualistic inoculum strain. So they made a non-fixing mutant of the inoculum strain, and screened for yellow plants. The genotypes selected were subsequently shown to admit selectively the N_2-fixing version of the inoculum strain, despite the abundance of mediocre rhizobia in the soil.

This approach, improved control of the initial infection process, has potential advantages, relative to attempting to improve post-infection sanctions. Rhizobia reproduce many-fold inside the nodule before starting to fix N_2, so unless sanctions were very effective at killing rhizobia inside nodules, there might still be many bad rhizobia released into the soil. Total exclusion from nodulation would be better. On the other hand, mediocre rhizobia might acquire the recognition signals of the inoculum strain fairly quickly, especially given the possibility of horizontal gene transfer among rhizobia (Sullivan et al. 1995). It is not clear whether an exclusion system based on recognition signals would last long enough in the field to justify the effort to develop such selective cultivars.

NEW OBJECTIVES AND NEW ENVIRONMENTS

'Collective performance' is itself a new objective, as indicated by the quotation from Professor C.T. de Wit (of Wageningen) at the beginning of this essay. Although we can sometimes predict how the traits of individual plants will affect community-level performance (Donald 1968), actual success can only be measured at the community level, that is, using field plots rather than individual plants. This section, in contrast, discusses traits that can be effectively evaluated in individual plants.

Many new objectives will still require reversal of past natural selection, although the trade-offs are between the individual competitiveness of plants and a variety of human goals, rather than collective performance of the plant community. On the other hand, improved adaptation to new environments, including new pests and pathogens, will not always require significant reversal of past evolutionary trends. For these traits, human ingenuity is used to accelerate genetic changes that are also favoured by natural selection (to the extent that it is allowed to operate) in the new environment.

New goals

Trade-offs between seed production in g plant^{-1} versus g m^{-2} were discussed in the previous section. At the level of the individual plant, there is also a trade-off between seeds plant^{-1} and g seed^{-1}. Larger seeds may be able to use seed energy reserves to out-compete neighbouring seedlings during the critical days after germination (Darwin 1859). On the other hand, smaller seeds may be dispersed farther by wind, and a plant can produce more of them per g of C and N available. Natural selection has often favoured seed sizes that are less than optimal by human criteria, although there are exceptions (e.g., coconut). Breeding for larger seed size is often an appropriate and readily achievable goal.

Major changes in seed composition have also been achieved through selection (Dudley and Lambert 1969). There are some significant biochemical constraints on this process. A higher-protein seed requires more nitrogen, of course, but also more photosynthate (g C per g seed), because of the greater energetic requirements of protein relative to starch (Sinclair and De Wit 1975). Similar constraints limit the yield of crops whose seeds have a high lipid content (Penning de Vries et al. 1974).

Plant breeders have a long history of success in improving the flavour of fruits and vegetables, sometimes by reducing levels of plant secondary compounds involved in defence against herbivores or pathogens (Ames 1983). More radical changes to plant biochemical composition are becoming easier to achieve using molecular methods, although the impact of these developments may be less than has been claimed (Schnapp and Schiermeier 2001). Production of pharmaceuticals should be limited to non-food crops, such as tobacco or guayule, to reduce the risk of contaminating food supplies (Daniell and Gepts 2004).

It is easy to imagine novel breeding objectives that would be useful in particular situations. For example, in compacted soils, roots sometimes reach deeper into the soil by following channels left by roots that grew and decayed in previous years. In one study, 41% of corn roots grew in channels left by a previous alfalfa crop (Rasse and Smucker 1998). The ideal root, from the standpoint of a subsequent crop, might consist of a coarse-weave ‘basket’ made of materials resistant to microbial degradation (e.g., lignin), surrounding a core of readily degradable materials (e.g., cellulose and protein). Once the core degraded, the basket could hold the channel open for smaller roots. Natural selection would favour such a root design only to the extent that it preferentially benefits the offspring of the plant leaving the channel, relative to competitors of the same species. Someday, humans might understand root developmental anatomy well enough to design rotation crops with such roots, although such a design might conflict with other objectives related to water and nutrient transport in roots. Or could we develop a practical screening method to select mutants that approach this root design by successive approximation? If each plant were grown in a long vertical tube containing compacted soil, differences in residual root channels after growth of each genotype might be detected simply by moistening the soil to field capacity and then looking for differences in the rate at which water added to the top of the tube drains out of the bottom.

New physical environments

A new environment may be a new location (e.g., the introduction of potatoes to Europe) or a change in biotic or abiotic conditions over time. For example, changes in the photoperiod response of flowering in soybean have been essential to the success of this crop over a wide range of latitudes in North America.

Some widespread trends in abiotic conditions include increases in atmospheric CO_2 or soil nitrogen and (with irrigation) increased predictability of soil water status (Denison et al. 2003b). The density of stomata per cm^2 leaf and the nitrogen content of leaves both appear to have decreased over time, based on comparisons of herbarium specimens collected over the past 200 years (Woodward 1987). This may represent a combination of long-term evolutionary changes of species and short-term acclimation by individual plants. Higher atmospheric CO_2 means that fewer stomata are needed to achieve a given CO_2 content in the leaf interior. A decrease in stomatal density may decrease the risk of dehydration due to excessive transpiration. The lower leaf N content presumably reflects a decrease in the concentration of the photosynthetic enzyme, rubisco. With higher CO_2 concentration, fewer rubisco molecules are needed to fix a given amount of CO_2.

To varying extents, natural selection can still occur in plant populations that are also subject to selective breeding, so stomatal density and leaf nitrogen of crop plants will continue to evolve even without deliberate selection by humans. However, evolutionary responses to changes in atmospheric CO_2 will always lag behind current conditions. Furthermore, we can predict the future, including further increases in CO_2, in ways that the 'blind watchmaker' (Dawkins 1985) of natural selection cannot. Therefore, breeding for lower stomatal density and leaf N may increase crop yield and/or water use efficiency under some conditions. However, under hot conditions, the higher stomatal conductance of modern Pima cotton cultivars is associated with higher yields, apparently because of lower leaf temperature (Lu and Zeiger 1994). Similarly, rubisco serves as an important store of nitrogen in leaves, in addition to its photosynthetic function (Stitt and Schulze 1994). Therefore, whether a decrease in stomatal density or leaf nitrogen is an improvement may depend on climate, irrigation frequency, soil fertility and the ability of crops to take up soil nitrogen late in the growing season (Denison et al. 2003b). These complications are in addition to the technical difficulty of achieving the proposed change.

The need to consider, and perhaps modify, how the crop is grown may seem burdensome, but future improvements in crop production may be just as dependent on interactions between genetics, environment and management as past improvements have been. For example, dwarf wheat and rice have higher yield potential than taller traditional cultivars, but they require better weed control, as they are less able to compete with tall weeds for light.

New pest and pathogen genotypes

'New environments' may also include newly arrived or newly evolved pests and pathogens. Conventional breeders have a long history of success in developing crops resistant to or tolerant of biotic threats. More recently, molecular methods have been used with some success. Given the theme of this essay, it is useful to divide molecular approaches to disease and pest resistance into two categories: those that probably duplicate phenotypes previously rejected by natural selection, and those that are sufficiently novel that they may not have been tested by past natural selection. This is analogous to the difference between tinkering with an existing design and engineering a new design.

Disease-related traits that we can assume were previously rejected by natural selection include increased (or more constitutive) expression of genes already present in a crop. Before using this approach to crop genetic improvement, we should at least ask why mutants with higher expression of the target gene failed to out-compete those with 'normal' levels. Assuming that the increased expression did indeed increase disease resistance, did it also impose some cost that, on average, reduced seed production?

There are many reasons why constitutive expression might reduce yield, relative to inducible expression of the same defence. For example, inducible chemical defences against insect herbivores reduce synthesis costs, avoid autotoxicity, create spatial patterns that make herbivores more evident to birds (by increasing movement from one leaf to another), limit chemical deterrence of pollinators, and provide various other benefits, relative to constitutive defences (Agrawal and Karban 1999). The ubiquity of inducible defences against pathogens suggests that inducible pathogen defence was also beneficial, at least under pre-agricultural conditions. Has this changed? In some crop fields, plant species diversity is less today than it was when these defences evolved, but this may not be true for some important crops, whose wild ancestors also grew in monospecific stands (Wood and Lenné 2001). Either way, are constitutive defences now the best solution, or should we consider increasing crop diversity in space or time (Denison et al. 2003b)?

Expression of the *NPR1* gene increases twofold in response to infection by a pathogen, and Cao et al. (1998) found that overexpression of *NPR1* in *Arabidopsis thaliana* increased resistance to two different pathogens. However, overexpressing the same gene in rice increases susceptibility to leaf lesions, especially under low light (Fitzgerald et al. 2004). This problem may explain why genotypes with higher constitutive expression of *NPR1* were rejected by past natural selection.

More innovative approaches to disease- and pest-resistance are also being developed. It might seem that an evolutionary perspective, which predicts that increased expression of existing genes will usually fail to increase yield, has little predictive power when it comes to truly novel genotypes. But how novel is novel?

Because the *Bt* toxin is not closely related to any plant toxin, insect herbivores may not evolve resistance to it as quickly as they would to new variants of toxins to which they are already resistant. This did not prevent evolution of *Bt* resistance in field populations of diamondback moth, however. An analysis of this case led Tabashnik et al. (1997) to predict that evolution of resistance in some insect pests may be 'faster than previously expected'. Subsequent evolution of *Bt* resistance in pink bollworm in the US has been slower than expected, however, apparently because of resistance management regulations requiring *Bt*-free insect refuges (Tabashnik et al. 2005). Reliable comparisons of evolution of *Bt* resistance in countries differing in resistance management would be of interest.

Using a vertebrate antibody to detect a plant pathogen and trigger chemical defences is certainly a clever idea (Bohlmann 2004). But it is probably safe to predict that ongoing pathogen evolution will overcome this new defence, sooner or later, either through mutations that prevent the antibody from recognizing the pathogen, or through mutations that reduce susceptibility to the plant's induced chemical defences. What makes the vertebrate immune system a major innovation is, among other things, its ability to generate millions of different antibodies. We will not be able to endow plants with that level of sophistication anytime soon. Meanwhile, developing crops resistant to evolving pathogens and pests "takes all the running you can do, to keep in the same place" (Carroll 1872), which led us to coin the term 'Red Queen Breeding' (Denison et al. 2003b).

RADICAL ALTERNATIVES NOT TESTED BY NATURAL SELECTION

The distinction between tinkering and engineering, applied to pest resistance in the previous section, can also be applied to physiological improvement of crop yield potential, i.e., yield per unit area, under non-limiting abiotic and biotic conditions (Evans and Fischer 1999). Simply increasing the expression of existing genes related to photosynthesis, nitrogen assimilation, drought tolerance, etc., is unlikely to increase yields reliably, except perhaps to the extent that crops are grown in (man-made) environments very different from where their ancestors evolved. Similarly, tinkering with the active site of key enzymes is unlikely to result in further increases in efficiency, beyond what millions of years of natural selection have already achieved. On the other hand, innovations so novel that they never arose over the evolutionary history of the crop and its wild ancestors might, in theory, result in substantial increases in yield potential or in their utility for humans.

Again, how novel is novel? C_4 photosynthesis, which can enhance both photosynthesis and water use efficiency, has evolved repeatedly (Kellogg 1999), so evidently natural selection has no difficulty with changes as 'minor' as the conversion of C_3 plants to C_4 (Berry 1975; Denison et al. 2003b). Therefore, if there is a superior photosynthetic system not yet found by natural selection, it is presumably even more different from C_3. A red algal rubisco with superior specificity for CO_2, relative to O_2 (Uemura et al. 1997) was hailed as a major step towards higher photosynthesis in crops (Mann 1999), but that may have been overoptimistic. The maximum turnover rate for CO_2, which is considered slow in terrestrial-plant rubisco, was even slower in the algal enzyme (Table 1 in Uemura et al. 1997).

Other novel genotypes that may someday be developed include N_2-fixing wheat or rice, a goal that is perhaps more remote today than it was thought to be more than 30 years ago (Shanmugam and Valentine 1975). One problem is the simultaneous requirement for high O_2 flux for respiration, to meet the energy requirements of N_2 fixation, together with low O_2 concentration, to protect nitrogenase from inactivation. Legumes solve this problem with adaptations including a variable gas diffusion barrier and leghemoglobin (Jacobsen et al. 1998), which would probably require tens or hundreds of genes in a new N_2-fixing crop. The alternative approach of modifying cereals to host N_2-fixing bacteria in nodule-like structures (Christiansen-Weniger 1998) has this same problem as well as the conflict of interest between host and rhizobia, discussed above.

Crops that leave more persistent root channels in soil might someday be achieved through intelligent design of root-related genes, rather than by the selection procedure outlined above. Similarly, perhaps a cover crop could be designed to shade out weeds until an overseeded crop has germinated and emerged, but then drop its leaves and die, maybe in response to a photoperiod achievable only with supplemental light. Perhaps crops could be designed to interfere with pollination in nearby weeds, by producing pollen that mimics the early steps of fertilization.

Like all new technologies, these suggestions could have unanticipated side-effects, but they illustrate the sorts of things we may eventually be able to do, once we can design a better crop 'from scratch'. However, we are unlikely to achieve this capability soon enough to help alleviate the competing demands of feeding a growing world population sustainably, while preserving enough natural ecosystems to prevent unacceptable losses of biodiversity.

THE FUTURE OF CROP GENETIC IMPROVEMENT

Any physiological improvements in yield potential are likely to be more durable than comparable improvements in resistance to pests and pathogens. The effects of climate change are essentially random with respect to their interactions with physiological adaptations. Change in the physical environment may decrease the benefit from a new genotype – increasing CO_2 will eventually decrease the comparative advantage of C_4 rice (Sage 2000) – but they may also increase that benefit. Evolution of pests and pathogens, however, is not random with respect to their ability to infect or feed on crops. This is good news for crop geneticists, in terms of job security.

The most promising route to increasing yield potential over the next two decades is to continue exploiting trade-offs between the collective performance of communities of plants (and their symbiotic partners) and the competitiveness of individual plants, sacrificing the latter to improve the former. Accelerating adaptation to the changing physical environment will probably also contribute to higher yield potential. The contributions from radical physiological innovations, not previously tested by natural selection, are unlikely to be significant over the next decade or two, but could be very important in the longer term. Meanwhile, Red Queen Breeding will be an important activity for as long as the human food chain relies on plants.

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CHAPTER 23

INTEGRATED ASSESSMENT OF AGRICULTURAL SYSTEMS AT MULTIPLE SCALES

M.K. VAN ITTERSUM[#] AND J. WERY[##]

[#] Plant Production Systems Group, Plant Sciences, Wageningen University,
P.O. Box 430, 6700 AK Wageningen, The Netherlands.
[##] UMR System (Agro.M-Cirad-Inra), 2 Place Viala, 34060 Montpellier Cedex,
France.
E-mail: martin.vanittersum@wur.nl

Abstract. Agricultural policies are increasingly replaced by agro-environmental and rural-development polices. The rationale behind this evolution is that the policies seek to enhance the sustainability of agricultural systems and contributions from these systems to sustainable development at large. The same can be argued for agricultural innovations; they are increasingly aimed at serving a range of sustainability objectives, rather than only improving productivity and quality. As a result, resource use issues related to agriculture must be analysed and addressed from an integrated and multi-scale perspective. Both the introduction of alternative agricultural resource use options and agro-environmental policies would benefit from their *ex ante* assessment. Contributions from agronomy to such integrated assessment have strong implications for its research agenda. This chapter presents an extensive example of a multi-scale assessment methodology (SEAMLESS) in which agronomy plays a significant but partial role. The methods allow the investigation of different kinds of policies and innovations and their effects on economic, environmental and social objectives of stakeholders and decision makers at farm, regional and sector level.

INTRODUCTION

Globalization, liberalization of markets, novel agro-technologies, economic development, changing societal demands and climate change drive a continuous evolution of agricultural systems around the globe. Agricultural and societal stakeholders try to influence the evolution such that sustainability of agricultural systems themselves and contributions of agricultural systems to sustainable development at large are promoted. In this context and paper, sustainable development stands for meeting the needs of present generations without jeopardizing the needs of future generations – a better quality of life for everyone, now and for generations to come, both in terms of economic, environmental and

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social issues (World Commission on Environment and Development 1987). Sustainable development, in this paper, is interpreted as a broader concept than sustainability of agriculture. The latter may imply developments within the agricultural sectors (or for specific types of farms) that are not positively contributing to sustainable development of society at large.

The factors that can be varied to achieve the objectives associated to sustainable development are merely the adoption of novel agro-technologies, the (re-)design of agricultural systems, and introduction of agricultural, environmental and rural-development policies implemented at various hierarchical levels. Institutional changes are simultaneously required to create incentives and consistency between the multi-scale and multi-objective changes (Spangenberg et al. 2002). Despite the obvious trend of liberalization, there is consensus that policies are needed to support achievement of sustainability objectives, and that these must be cost-effective and efficient (EC 2002). These policies, however, increasingly have an integrated nature: they are not solely targeted at agricultural issues, but try to achieve multiple objectives (e.g., 'cross-compliance' in the reform of the Common Agricultural Policy of the European Union). Agricultural policies are increasingly replaced by rural-development policies seeking to enhance the sustainability of agricultural systems and contributions from these systems to sustainable development of societies (Brouwer and Lowe 2000).

Sustainability and sustainable development are relative notions that are scale-dependent, i.e., what is good for the environment or economy at farm level may not be advantageous for the national or global environment or economy, or what is beneficial for the agricultural sector in general may not be desirable for the individual farmer. This implies the need for both multi-scale and integrated analysis that captures the effects of specific developments at field, farm, regional and even global level, and the effects in terms of economy, environment and social factors (Dalgaard et al. 2003; López-Ridaura et al. 2005; Verburg et al. 2006). Usually such analyses make use of indicators that characterize the pressure on systems or characterize the attributes of sustainable development (Gallopin 1997).

Both the introduction of new agro-technologies, the lay-out and design of agricultural fields, farms and sectors, and the design of agricultural, environmental and rural development policies would benefit from *ex ante* assessments to estimate their (relative) contributions to sustainability and sustainable development. Assessing the strengths and weaknesses of new technologies, systems or policies prior to their introduction would greatly facilitate transparency and consistency in decision making at the various scales. The European Commission, for instance, has introduced Impact Assessment of its policies as an essential step in the development and introduction of new policies since 2003 (EC 2005). It explicitly calls for assessment of the economic, environmental and social impacts of policies *and* consultation with stakeholders. This implies in many cases establishment of a so-called Inter-service steering group (across various Directorates General, e.g., Agriculture, Environment, Economics and Finances) that is responsible for the Impact Assessment. Impact Assessment is anticipated to contribute to a more coherent implementation of the European strategy for sustainable development (EC 2005).

Contributions from agricultural research to integrated assessment (cf. Harris 2002, here integrated and impact assessment are used as synonyms) have distinct consequences for the agronomic research agenda, i.e., how to summarize and integrate knowledge on crop growth and management and its interaction with the environment and economy. By nature it is a contribution to interdisciplinary research in which agricultural research plays only a partial role, jointly with many other disciplines such as economics, geo-informatics, information technology and sociology. The aim of this chapter is to discuss the role of agronomic research in multi-scale assessment studies, and then to present the conceptual and methodological approach of a large research project (SEAMLESS) to provide a frame in which research on crops and cropping systems can be integrated and used to the benefit of *ex ante* integrated assessments of agro-environmental policies and innovation in European agriculture. In this chapter we will discriminate between agronomic research focusing on plant and crop science (the core theme of this book) and agricultural research that is much broader and includes, e.g., agricultural economics and rural sociology.

AGRONOMIC AND AGRICULTURAL RESEARCH FOR INTEGRATED ASSESSMENT

Agronomic research and integrated assessment of agricultural systems

Today's questions regarding agricultural systems, their sustainability and their contribution to sustainable development at large can only be addressed from a systems perspective. Agro-ecosystems are the interplay of ecosystems and human societies, and their behaviour is determined by interactions with the natural and human-resource base (see Figure 1). This unavoidably leads to the conclusion that by definition the role of agronomy can only be partial in analysing and solving problems of agricultural systems at farm, regional and continental scale. Answers to agronomic questions provide only limited insight into behaviour of agricultural systems and are only part of the problem-solving package for most systems around the globe. This is clearly demonstrated for many cases in Africa (e.g., Ojiem et al. in press), but it is not difficult to find equally illustrative examples from other continents. Well-known agronomic principles are not adopted because of socio-economic factors or only play a small role in the complex problems that farming communities face. At the same time, using agronomic knowledge in integrated assessment tools is indispensable: many future studies on natural-resource use, agricultural systems and their industries reduce the agro-ecological relationships to a mere econometric function, production function or, in general, statistical relationship between some set of inputs and output(s) (Lehtonen et al. 2006; Guan et al. 2006). This hinders process-based analysis, explanation of systems' behaviour, interactions with the environment and identification of future alternatives that outperform current activities in terms of productivity and realization of positive or negative externalities. To assess performance of agricultural systems and their contributions to sustainable development and to identify promising alternative pathways, process-based knowledge of agro-ecological relationships is essential, but only to a certain degree of detail and tailored to integration with other factors and systems. This constitutes the challenge for agricultural research and its role in contributing to sustainable-development studies (Bland 1999). To what extent can we synthesize agronomic knowledge to the appropriate degree of detail for integration in interdisciplinary and multi-scale analysis of agricultural systems and their interactions with ecosystems and societies?

Methods to deliver agronomic knowledge into studies of an integrated nature are generally model-based and amongst the methods available two can often be found in literature: dynamic crop or cropping-systems simulation models with different levels of detail (Keating et al. 2003; Van Ittersum et al. 2003) and approaches generating and using so-called input–output coefficients of agricultural activities (Van Ittersum and Rabbinge 1997). These coefficients are in turn often generated using dynamic cropping-systems models complemented with other sources of agronomic information, and then used as an input in bio-economic models studying farming systems or regional land use systems (e.g., Roetter et al. 2005).

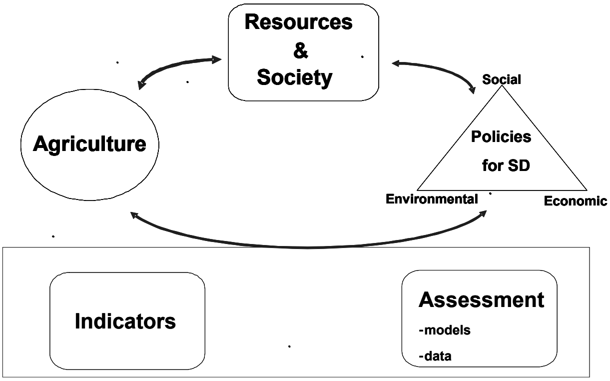

Figure 1. *The conceptual basis of integrated assessment of agricultural systems and associated policies for sustainable development (SD)*

Tools for integrated assessment of agricultural systems

Although precise documentation is scarce, it seems evident that today research tools for integrated assessment of policies and technological innovation in agriculture are still rarely used in practice (cf. McIntosh et al. 2006). Also, most of the approaches developed by research that are being used, or can be used potentially, are still largely disciplinary and focused on specific issues and/or hierarchical levels (e.g., EuroCARE 2002; EC 2003). Hence their use to assess policies and innovations, which by definition impact on different hierarchical levels (e.g., the globe, developing countries, EU25, administrative region in a country, specific farms and fields) and across economic, environmental and social domains, is restricted. They may lead at best to partial conclusions as to the behaviour of the agricultural systems. The gap between analysis at micro-level (farms) and macro-level (region or market) is still largely unresolved. Most research models are targeted at specific scales of analysis, e.g., farm (Kruseman et al. 1995; Veysset et al. 2005), watershed (Barbier and Bergeron 1999), region (Lu and Van Ittersum 2004; Bouman et al. 1999), national or continental (Deybe 1998; Lehtonen et al. 2006) or global scale (Van Tongeren et al. 2001). Also, they have been developed for specific purposes, such as evaluation of new technologies (Barbier and Bergeron 1999), macro-economic policies (Lehtonen et al. 2006; Jansen et al. 2005), nutrient policies (Wolf et al. 2005) and climate change (IMAGE team 2001). As a result possibilities for re-use for different issues are limited, whereas political agendas can evolve rapidly. Few methods were designed to deal with multi-scale assessments (Bouman et al. 1999; Jansen et al. 2005; Laborte et al. in press) and such that they can be used for a broad range of issues, e.g., breeding strategies, technological innovation, market policies, environmental policies, climate change and rural-development issues.

Another typical feature of agricultural research models is their *ad hoc* solutions in terms of software architecture and implementation. Some examples exist of cropping-systems models with significant investments in software design (e.g., Keating et al. 2003; Stöckle et al. 2003), but to our knowledge no such models have been designed to be (re-)usable in integrated frameworks. Generally, possibilities for integration, re-usability and easy maintenance of models for agricultural systems are restricted; software solutions being often targeted at a particular model, study and application. Rizzoli et al. (1998) and Van der Wal et al. (2005) argue about the advantage of modelling frameworks allowing easy maintenance and re-use of models in integrated assessment systems.

Research agenda for agronomic and agricultural research

From the previous section a research agenda for *agronomic* research aimed at contribution to integrated assessment can be derived. We think that the most important features of such an agenda are:

- Methods to enable a synthesis and summary of agronomic knowledge such that it can be used in integrated studies of a bio-economic nature. Processes and systems need to be modelled (either statically or dynamically) at the proper level of detail for specific purposes;
- The need for generic agronomic methods capable of contributing to assessments at different hierarchical levels and related to different issues;
- Software designs and implementations of agronomic models, which allow re-usability, linkage to other models and easy maintenance.

For *agricultural* research supporting integrated assessment in general we arrive at the following key features:

- Methods capable of assessing, at the proper level of detail, the economic, environmental and social issues at stake;
- Multi-scale capabilities of research methods: the methods should allow investigation of interrelationships between scales of analysis;

- Robust and open software architecture and implementation that allow linkage, re-use and maintenance of models.

In our view these features are best served by a computerized framework for integrated assessment, using individual models that can be linked, re-used and maintained through a software infrastructure using state-of-the-art developments from information technology. The individual models and some of the linkage procedures can be derived from existing studies as listed in the previous paragraph but must be amended such that they can be used in an integrated framework. The SEAMLESS project aims at developing such a framework and it will be presented in the next section. At the end of that section we return to the role of agronomic components in such a framework.

A FRAMEWORK FOR INTEGRATED ASSESSMENT OF AGRICULTURAL AND ENVIRONMENTAL POLICIES AND INNOVATION

Introduction and methodology

The European Union Integrated Project, SEAMLESS (System for Environmental and Agricultural Modelling; Linking European Science and Society, 2005–2008, Van Ittersum et al. 2006, www.seamless-ip.org) aims at developing a computerized, integrated and user-friendly framework (SEAMLESS-IF) to assess and compare, *ex ante*, alternative agricultural and environmental policy options and technological innovations. Following an analysis of requirements, the framework must allow:

- Analysis at the full range of scales (farm to EU and global), whilst focusing on the most important issues emerging at each scale;
- Analysis of the environmental, economic and social contributions of a multifunctional agriculture towards sustainable rural development and rural viability;
- Analysis of a broad range of issues, such as climate change, environmental policies, food production and costs, rural-development options, effects of an enlarging EU, international competition and effects on developing countries.

SEAMLESS-IF will have the following specific features and capabilities:

- A multi-perspective set of economic, social and environmental indicators of the sustainability and multifunctionality of systems, policies and innovations in agriculture and agroforestry;
- Quantitative models, tools and databases for integrated evaluation of agricultural systems at multiple scales and for varying time horizons;
- A software architecture, SeamFrame, that allows reusability of models, data and other knowledge, also ensuring transparency of models, their linkages and integration with other procedures.

In summary, SEAMLESS-IF aims to facilitate translation of policy questions into alternative scenarios that can be assessed through a set of indicators that capture the key economic, environmental, social and institutional issues of those questions. The indicators are assessed using an intelligent linkage of quantitative models. These models have been designed to simulate aspects of agricultural systems at specific organizational levels, i.e., point or field level, farm, region, EU and world. SEAMLESS aims at integrated use of partly existing and partly newly designed models of agricultural systems. These models use pan-European databases for environmental, economic and social issues. Some indicators, particularly social and institutional ones, will be assessed directly from data or through a post-model analysis with specific procedures going beyond the extrapolation of present trends. Smooth linkage of models designed for different scales and from biophysical and economic domains requires software architecture, and a design and technical implementation of models that allows this. The software backbone of the project, SeamFrame (Van der Wal et al. 2005; Van Ittersum et al. 2006), serves that purpose. It is also developed to facilitate re-use, maintenance and documentation of the models.

Prototype 1 of SEAMLESS-IF, including agronomic models

The first working prototype of SEAMLESS-IF, which was completed in 2006, includes an indicator calculator that draws information from the model chain provided in Figure 2 to compute selected indicators. Examples of such indicators are: farm income (for the different farm types in a region and for the EU25), nitrate leaching and contribution to global warming. The model chain comprises the agricultural sector model, CAPRI (Common Agricultural Policy Regionalised Impact), which simulates supply–demand relationships in the EU25 for agricultural commodities (Heckelei and Britz 2001). CAPRI is a comparative static-equilibrium model, solved by iterating supply and market modules. CAPRI has a supply module that consists of supply models at different scales, from farm to the European level. These are non-linear programming models allowing direct implementation of most policy measures with highly differentiated sets of agricultural activities. Allocation

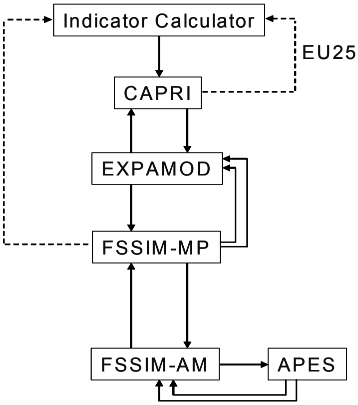

Figure 2. *Models and model chain in Prototype 1 of SEAMLESS-IF*

is based on profit-maximizing behaviour and estimated multi-product cost functions. CAPRI also estimates nutrient balances and gas emissions with global-warming potential using a matrix of coefficients linked with the levels of the activities.

In SEAMLESS-IF, CAPRI derives information on price–supply relationships from a farm model, FSSIM (Farm System SIMulator, Deybe and Flichman 1991). A restricted number of simulations of supply responses to prices with FSSIM are extrapolated through an econometric up-scaling procedure (EXPAMOD) that estimates price-supply elasticities. FSSIM is a bio-economic farm model developed to quantify the integrated agricultural, environmental and socio-economic aspects of farming systems. FSSIM includes an agricultural management module (FSSIM-AM), which computes the input–output coefficients for agricultural activities and a mathematical programming part to capture resource endowments, policy constraints and farmers' objectives (FSSIM-MP). Applied at farm (micro) level, FSSIM seeks to represent the actual farmer's behaviour using the knowledge of technical and socio-economic constraints, agro-environmental policies, the relation between production factors, the amount of output obtained and the costs of each agricultural activity (= growth of a crop rotation or livestock system) and future market prices (simulated by CAPRI). The principal characteristic of this type of model is the application of production functions, i.e., relationships between agricultural inputs (water, nitrogen, labour, etc.) and outputs (yields or emissions), partly derived from mechanistic simulation models (APES) capturing agro-ecological processes. FSSIM also uses information from surveys and expert knowledge for assessment of activities currently practiced by farmers.

FSSIM assesses both currently practiced agricultural activities and alternative ones. These alternative activities can either be on-the-shelf activities, i.e., those available but currently not practised by farmers, or in-the-pipeline activities, which may become available to farmers within the time frame of the study.

The Agricultural Production and Externalities Simulator (APES) is a modular simulation model estimating the biophysical processes of agricultural production systems, at point level, in response to weather, soil and different options of agro-technical management (cf. Van Ittersum and Donatelli 2003). APES computes the yields, as well as several inputs and externalities of crop rotations; both averages and variability across years can be generated. The processes are simulated in APES with deterministic approaches mostly based on mechanistic representations of biophysical processes. The criteria to select modelling approaches is based on the need of: (1) accounting for specific processes to simulate soil–land use interactions; (2) input data to run simulations; and (3) simulation of agricultural production activities and their management of interest.

Farm and agro-environmental typologies play an essential role in linking the models (e.g., FSSIM and CAPRI), for up- and down-scaling and for the calculation of many indicators.

Further prototypes of SEAMLESS-IF will introduce a broader diversity of agricultural activities, e.g., tropical and perennial crops in APES, animal production in FSSIM, landscape models, rural employment models and a linkage with the global trade model GTAP (Van Tongeren et al. 2001).

Examples of possible SEAMLESS-IF application

Evaluation through applications to realistic questions is an essential step in the process of development of each SEAMLESS tool (indicators, databases, typologies, models, software architecture, qualitative tools and participatory methods) and of SEAMLESS-IF as a whole. This evaluation is based on two 'Test Cases' representing the major types of questions that SEAMLESS-IF is designed to address. In each Test Case we analyse how the agricultural systems and their contribution to sustainable development will be affected by EU policies and global developments. Test Case 1 focuses on the impacts of economic policies at the EU/World level, and Test Case 2 on the impact of environmental policies and agro-ecological changes at the farm level. Analyses will be conducted both at EU level and, with more details allowed by data availability and stakeholder interactions, for typical regions of the EU representing a territorial entity with respect to environment and rural development. Examples of these typical regions are (1) the 'Neste region' in southwestern France, which represents an agricultural region where water availability and quality is a key issue; (2) the 'Pyrzyce region' of Poland, which is a typical case of an intensive cereal-based region where agriculture is still a major driver of the local economy but which is confronted with specific circumstances related to EU accession and water quality issues; and (3) the 'Massif Central region' in France, which is a mountainous area with high recreational value where agriculture is dominated by dairy production, playing a major role for landscape, grassland biodiversity, and water quality. Significant changes in the CAP related to the milk market will most likely affect this region considerably, but cheese with Certified Origin and regional policies may mitigate its effects.

To demonstrate the applicability of SEAMLESS-IF to least developed countries, two contrasting regions of Mali (Sikasso and Koutiala) have also been selected, where EU policies and trade liberalization (especially on cotton and meat) may have a significant impact on farming systems and rural development.

Test Case 1 is driven by economic-policy changes, analysing the impact of further trade liberalization as currently discussed in World Trade Organization negotiations. For this purpose, the behaviour of EU and global markets and farms in the test case regions will be compared between a baseline scenario under currently agreed policies until 2012 and a policy scenario based on a likely outcome of trade liberalization in the DOHA round of the WTO. The policy is applied at EU level through the CAPRI model and the FSSIM model. The CAPRI model simulates prices, whereas the FSSIM models for the major farm types simulate supply and externalities given certain prices. Economic, environmental and some social indicators are assessed at relevant scales using output from FSSIM and CAPRI models.

Test Case 2 analyses what would happen if the EU countries, regions and farmers would effectively apply the EU directives on water, pesticides and biodiversity. The impacts will be assessed with the economic, social and environmental indicators at the various levels represented in SEAMLESS-IF. Specific attention will be paid to the interactions between these policies and the various agro-ecological technologies (such as integrated or organic farming, conservation agriculture and agro-forestry) under different scenarios with respect to existence and degree of specific policy incentives to use these technologies. The bio-economic approach (APES-FSSIM-farm typology chain) is designed to reproduce the major factors that determine farmers' selection of alternative production systems and it will be used to identify whether or not agro-ecological technologies will be favoured by the implementation of environmental directives.

Analysing the interactions between EU environmental policies and agro-technical innovations implies the definition of complex scenarios and of a wide range of alternative agricultural activities. European agriculture and rural development are already constrained by a large and complex set of environmental directives, among which we have selected those affecting water quality and quantity (water, nitrate and pesticide directives) and biodiversity (Belhouchette et al. 2006). Deriving from these directives a set of variables and constraints that can be applied to a farm model like FSSIM is a complex task, because each country and most often each region has the freedom to define the actual application of the environmental directives. It is essential to capture this diversity because it reflects the EU strategy based on the assumption that a more ecological agriculture must be tailored to the environmental and social characteristics of each agricultural region. For the purpose of testing/improving SEAMLESS-IF and because of lack of data, the scenarios applied to EU level are simplified but the specific regions have been selected to work with national and regional decision makers and stakeholders to collect sufficient information to capture the complex constraints and incentives actually faced by the farmers. This information will be used to define realistic scenarios based on simultaneous implementation of the nitrate, water framework and bird habitat directives, but also cross-compliance rules from the CAP reform and specific regional agro-environmental schemes.

Model-based assessment of agro-ecological innovations

Integrated policy assessment tools should be able to represent the fact that new techniques become available or feasible to farms within the time horizon of the study, such as introduction of genetically modified crops (e.g., herbicide-resistant maize), a new cropping technique promoted in the region, and a new market for certified products with ecological techniques. Will such agro-ecological innovations be selected by the farmer as a response to EU environmental directives or other policies? What will be the impact on water quality, on water use by agriculture, or on biodiversity in the regions where these techniques are adopted? What effects will they have on competitiveness of EU agricultural products in the world market?

Following the approach of Rapidel et al. (2006) the cropping system is considered here as a combination of a biophysical subsystem (a plants–soil–weeds–pests combination) for each field of the farm, and a technical system (a coherent combination of management options applied on each field and allocated within a farm). As shown by Wery and Ahlawat (in press) for an example with grain legumes in Europe and in India, this approach can be used for the integrated assessment of agro-technical changes on farming-systems' sustainability, but it requires specific models to represent the biophysical and the technical subsystems. For this purpose, agro-technical innovations can be clustered:

1. Changes in the management of inputs of the biophysical system, e.g., shifting from predetermined applications of water, pesticides and nutrients to split applications based on the actual status of the biophysical system;
2. Changes in the structure of the biophysical system, i.e., shifting from pure stands to mixtures of varieties, species or crops in the same field, including intercropping and agro-forestry;
3. Diversification of the biophysical and technical systems, through inclusion of more and other crops in the crop rotation or production enterprise;
4. Institutional changes, including specific markets providing technical support and economic value to technical systems targeted at the protection of the farm environment in a specific region. The certification of origin is a typical example but it is still mainly targeted at quality of the product with limited incentives to protect the environment; and
5. Combination of the previous clusters, where the institutional environment of the farm is organized to promote agro-ecological innovations and their recognition and economic valuation by the society. Despite its limitations, organic farming is still the best example of a form of agriculture forcing farmers to adopt diversified crop rotations, crop associations, soil and nutrient management and providing recognition of these efforts and risks in a specific market.

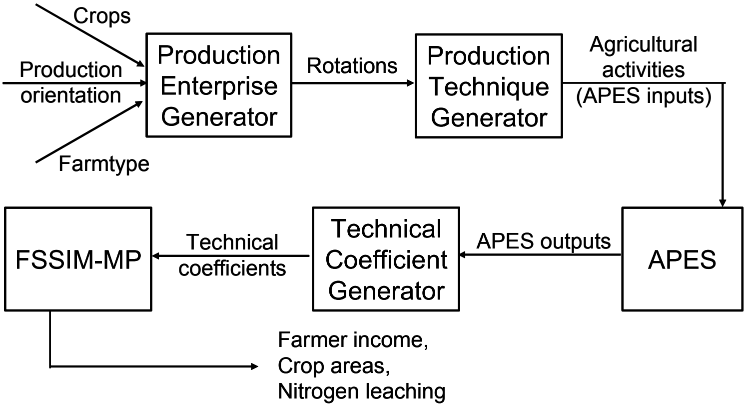

Figure 3. *Methods and tools to capture agronomic knowledge in SEAMLESS-IF. For an explanation, see the text*

Following Figure 3, a broad range of such agro-technical innovations can be generated using the so-called Production Enterprise Generator, which can generate crop rotations from a list of crops and pre-defined filters (cf. Dogliotti et al. 2003) and a Production Technique Generator, which adds production techniques to the production enterprises as combined variants of general, nutrient, water, pest and disease, and conservation management (Janssen et al. 2006). Such generated enterprises and production techniques (i.e., agro-technical innovations) can then be assessed in terms of input–output coefficients through the use of APES, which is capable of simulating defined cropping systems and production techniques, complemented with formalized expert knowledge on, e.g., labour, pest and disease management and machinery in a Technical Coefficient Generator. The derived input–output coefficients are then used in a bio-economic farm model (FSSIM-MP) to simulate allocation of current and/or alternative activities to a farm, given a set of constraints and farm objectives. That model then provides income and other indicators for the farm level.

THE ROLE OF AGRONOMY IN INTEGRATED AND MULTI-SCALE ANALYSIS

The SEAMLESS methodology has been presented as an example of a method for integrated assessment of agro-environmental policies and new technologies in agriculture. We believe it meets some key aims associated with research for integrated assessment identified in the first part of this chapter (Bland 1999). The example also illustrates both the essential and the partial role of agronomic research on plants, crops and cropping systems in integrated analysis. Too often the agronomic part is replaced by statistical relationships derived from surveys or census data, hiding or ignoring any causal relationships based on insight in agro-ecological processes, and hence rendering it impossible to forecast future developments and technological innovation. This is often the case in analyses dominated from social or economic science or carried out from a non-agricultural perspective (e.g., a nature-conservation or environmental viewpoint). On the other hand, the use of expert knowledge to assess agro-technical innovations (e.g., from farmers or farm advisors) is generally biased by the partial information they derive from their experiments (mainly production and economic aspects) and their strong dependency on the local pedo-climatic conditions. At the same time, the example illustrates that agronomic knowledge must be integrated with information on, e.g., resource endowments, variation in farm households, farmers' objectives, agricultural markets, and a variety of market and agro-environmental policies.

Agronomic principles and processes must be summarized to the proper level of abstraction, such that only the essential information is included in the analysis. This is far from trivial and depends much on the questions at stake and the scaling methods adopted (Ewert et al. 2006). Hence, it is neither easy to prescribe general procedures for this, nor to develop generic tools. In the SEAMLESS Integrated Framework it is attempted to develop stand-alone components for each hierarchical level and a flexible modelling framework to assemble the model-typology-indicator chains required to assess complex scenarios. For point and field scales, agro-ecological knowledge is captured in mechanistic simulation models. In the bio-economic farm models agronomic knowledge is summarized in input–output coefficients of discrete agricultural activities. Finally, at agricultural-sector level (EU25) agronomic knowledge is further summarized from multiple runs of the farm models, resulting in price–supply relationships or so-called elasticities.

An important question, not addressed in this paper, is the uncertainty associated with summarizing agronomic knowledge and how this affects (accumulates) in a modelling chain underlying integrated assessment. Although individual model components at field and farm level can be evaluated fairly well, this is far more complicated in a series of linked models, used for forecasting purposes. This will constitute an important research challenge.

Obviously, the SEAMLESS example only provides one of the multiple ways of dealing with the integration of agronomic knowledge in multi-scale assessment studies. There are many fundamental questions underlying this integration, which are much related to problems of up- and down-scaling and interdisciplinarity (Ewert et al. 2006; Dalgaard et al. 2003). We anticipate that agronomy must play an increasing role in pushing the envelope of such fundamental scientific questions, if it wants to play a key role in a changing research and policy agenda in which agriculture is no longer a separate activity but increasingly part of integrated economies, resource use problems and policies.

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CHAPTER 24

A DIALOGUE ON INTERDISCIPLINARY COLLABORATION TO BRIDGE THE GAP BETWEEN PLANT GENOMICS AND CROP SCIENCES

P.C. STRUIK[#], K.G. CASSMAN[##] AND M. KOORNNEEF[###]

[#] *Crop and Weed Ecology Group, Plant Sciences, Wageningen University,*
P.O. Box 430, 6700 AK Wageningen, The Netherlands.
E-mail: paul.struik@wur.nl
[##] *Department of Agronomy and Horticulture, University of Nebraska,*
P.O. Box 830724, 68583-0724 Lincoln, NE, USA.
E-mail: kcassman1@unl.edu
[###] *Max Planck Institute for Plant Breeding Research,*
Carl von Linnéweg 10, 50829 Köln, Germany.
E-mail: koornnee@mpiz-koeln.mpg.de

Abstract. In the future, more food needs to be produced with increasingly scarce natural resources. Genomics can play a key role in accelerating yield gains because it helps to improve our understanding of genetic traits and assists in breeding for better crop performance. The scientific muscle of genomics attracted tremendous research investments, but the efficiency with which these investments are paying off is still low. How can we accelerate the application of molecular genetics to our understanding of crop physiology and subsequently to crop improvement? The missing link is a more detailed understanding of the effects of gene function on crop performance at field level under agronomically relevant conditions captured in robust, physiology-based mechanistic models. With such models the most sensitive processes and mechanisms at whole-crop level that contribute to improved crop performance can be identified. To achieve the detailed understanding necessary to build and feed these models, more research on whole-plant physiology and crop ecology is required, with a focus on the complexity of scaling up knowledge from the molecular level to the farmers' fields and production systems. Such studies assess how the plant is able to integrate the information at different levels of organization into the functioning of the whole plant and predicting the phenotype of transgenic plants engineered for improvement of a complex trait.

More investment is needed in linking whole-plant physiology, crop ecology and crop simulation with molecular biology and genomics. Moreover, long-term progress can be enhanced by the formation of multidisciplinary teams that operate through networks of excellence in developing quantitative tools that integrate complex information and different levels of organization and by the exchange of young scientists between research groups working at different hierarchical levels. On the short term improvement of the characterization of experimental environments (preferably through commonly shared protocols) and of the characterization of parents for creating mapping populations is needed. In addition,

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joined multi-location trials and advanced physiological and statistical approaches for determining what aspects of the environment are most influential on the genotype × environment interactions are required.

INTRODUCTION

The need to accelerate yield gains

Our human population continues to increase and will reach 8.5 to 9 billion within the next 40 years. Future food security for this growing population will depend on *acceleration* of yield gains per unit of land and per unit of input for the major food crops at rates *well above* the historical trend of the past 50 years. The challenge is to produce more food on limited land resources and with less water because the availability of these natural resources for agriculture is decreasing rapidly as a result of economic development, which diverts these resources for non-agricultural uses. These trends are strongest in developing and emerging countries, where nearly all of future population growth will occur. It has been claimed that (functional) genomics can play a key role in the necessary acceleration of yield gains.

The potential of genomics

Genomics provides a powerful tool for identifying genes of agronomic importance. Genomics implies the study of all genes and their gene products in an organism with respect to their function and their control by environmental and developmental factors. It is suggested that the knowledge arising from genomics not only helps to improve our understanding of complex crop traits (such as yield and yield stability), but will also assist us in breeding for better crop performance and in designing better cultural practices.

For genomics, tools have been developed that allow the detection of the genes (genome sequencing), the study of the expression of these genes (micro-array, gene-chip analysis) and of their ultimate gene products (transcriptomics, proteomics, metabolomics, expression of traits). Moreover, methods have been developed to study the function of genes. The latter methods make use of genetic variation from within the available germplasm of a species, and also from induced mutants and transgenic plants that over- or under-express a specific gene.

Genomics is not paying off yet

The scientific muscle of this relatively new approach has attracted a tremendous research investment in both the private and public sectors. As a result, entire genomes of several crop species have been or will soon be sequenced, and there has been an explosion of new knowledge about genome structure and function. At issue is the efficiency with which this huge investment is paying off in terms of leveraging this genetic knowledge to meet the challenge of global food security.

Despite the remarkable recent advance in basic knowledge of plant genes and gene networks, there has been relatively little impact on crop improvement from the application of genomics and recombinant-DNA technology. Insect-resistant (*Bt*) and

Roundup-Ready® herbicide-resistant crops are the exceptions, but these technologies were developed on the basis of studies of single genes a decade before the birth of genomics. Progress in harnessing the power of genomics is still limited, despite all the promises and claims. It is not even clear yet what will be the impact of genomics on the rate of crop improvement by plant breeding.

Results are not yet visible. This certainly can be explained in part by the juvenile stage of the '-omic' technologies. More time is needed. For example, at this moment rice is the only major staple crop for which a complete genome sequence – an important tool in genomics – has been published.

The view that progress is limited may also be obscured by a focus on the use of transgenics, the easy but certainly not the only way to apply '-omics'.

Finally, '-omic' technologies are mainly applied by private research laboratories carrying out their work in secrecy and evaluating physiological processes responsible for genotype × environment interactions regarding complex traits according to their own standards. This research is not dictated by an agenda aimed at solving important scientific issues for the public good, and much of it does not undergo peer review or publication in scientific journals.

Scientific limits to genomics

There are substantial advances in understanding the function of single genes that control agronomic traits (such as pest resistance and grain quality) and several examples of traits under control of *linear* gene cascades or small gene networks (such as flowering response). Even with these impressive advances, the use of this knowledge for the improvement of our major food crops has been relatively slow. In addition, there has been much less progress in elucidating the genetic control of traits for which the genetic variation accessible to breeding is under complex genetic control involving many genes and strong genotype × environment interactions. Fecundity, effective drought resistance and nitrogen use efficiency are examples of such complex traits that are influenced by numerous compensatory feedback mechanisms and for which plant evolution has worked millions of years to perfect. The latter, by the way, could also imply that the genetic variation is limited.

There are also scientific challenges in the application of genomics research. First, in genomics priorities have to be set, as resources are limited, with regard to genetic variation: not all genes involved in traits of interest show relevant genetic variation, not all variation can be identified, for example, by QTL analysis, and costs of generating desirable variation may be too high. Second, genes do not function on their own, and knowing the molecular characteristics (biochemical function, expression regulation, etc.) often does not elucidate the controls on a complex trait such as yield. Third, molecular biology is progressing much faster than the theoretical and experimental framework connecting genes, plants and crops.

Given this situation, what is needed to accelerate the application of molecular genetics to our understanding of crop physiology and subsequently to crop improvement, especially for traits under complex genetic control? The answer to this question is, in our view, a detailed physiological analysis of the genetic variation and of the controls of the expression of genes in an agronomically relevant environment. The plea for such research at crop level is the main issue of this dialogue, because progress in this field is still limited.

THE MISSING LINK

We believe the critical missing link is a more detailed understanding of the effects of gene function on crop performance at the field level under a relevant range of environmental conditions, and capturing this knowledge in robust simulation models. Such models would facilitate identification of the most sensitive processes and mechanisms at the whole-plant and plant-community levels that contribute to improved crop performance. They would also allow prediction of phenotype from genotype in transgenic plants. To achieve this capability, a greater public investment in plant physiology and crop ecology, and a much closer collaboration between scientists in these disciplines and those in basic and applied plant genetics will be required. Lack of such collaboration has resulted in a number of spurious reports published in influential scientific journals that claim progress on improving complex traits such as crop yield potential based on molecular genetic approaches, but still await confirmation under agronomically relevant conditions. The issues and questions given above indicate that these claims are to date either unjustified or at least not supported by published results. Public research in whole-plant physiology and crop ecology must be strengthened to realize the potential of publicly funded functional genomics. Privately funded research may soon yield significant results, but these need to be embedded in scientific theory and require independent verification, confirmation and testing.

Crop physiology and ecology at whole-plant and plant-community levels are needed for the following reasons:

1. The complexity of scaling up knowledge from the molecular level to the field ecosystem level will require powerful new quantitative tools and approaches, including modularized multi-scale models, proper interfaces between hierarchical levels, specific software allowing up- and downscaling, and mathematical solutions for integration of steps differing in scale but belonging to the same process. Genes that control developmental processes and rates need to be identified with priority, and their effects will be among the first candidates to be included in whole-plant and crop models.
2. Gene function tested on the basis of comparing genetic variants (either transgenic or classical) should not only be measured in artificial growth systems (e.g., small pots in greenhouses or growth chambers) as this may not be relevant in the real world of production agriculture at the field level. It is, therefore, crucial to understand better how to test genotypes in relevant environments that can predict performance in the field.
3. A plant can only adapt successfully to changing conditions when it is able to integrate the information at different levels of organization into the functioning of the whole plant. Therefore, it must have a finely tuned coordinated control of all individual genes that contribute to the desired phenotype. Recent research on

the regulation of flowering (e.g., in wheat) provides an exciting example of how such a coordinated control system might work for a specific process. For other traits under more complex genetic control, knowledge of the coordinating control function is still lacking.

4. Achieving finely tuned coordination of introgressed genetic variation including transgenes in a gene cascade or network is a difficult task because of a number of factors that affect gene expression, including transgene copy number, RNA silencing, transgene insertion site and the employment of certain regulatory sequences to drive transgene expression. Therefore, predicting phenotype of a transgenic plant on the basis of whether transgenes are present is a major challenge and a costly undertaking. Overcoming this limitation by screening large numbers of transgenic plants becomes less efficient as the number of genes controlling the trait increases. Greater efficiency in achieving the desired level of transgene expression will be critical to improving prediction of the phenotype of transgenic plants engineered for improvement of a complex trait. Even when successful, these predictions can only be based on the expression of genes for which genetic variants differ.

TOP-DOWN OR BOTTUM-UP?

The lack of collaboration between scientists in the fields of genomics and biotechnology on the one hand and scientists in whole-plant physiology and crop ecology on the other hand is probably best illustrated by the debate on how to make use of the wealth of new information obtained by molecular biologists in computational systems analysis. Basically there are two approaches: the top-down approach and the bottom-up approach. Both approaches are facing fundamental problems.

Ecophysiological modelling is a *top-down* approach that predicts crop function based on generic relationships that describe the fundamental processes governing plant growth in relation to environmental conditions. Photosynthesis, respiration, assimilates partitioning to organs, and ontogenic development are key drivers of such models. Individual genotypes can then be represented by a set of response parameters that are valid under a wide range of conditions. The phenotype and its response to environmental conditions are broken down into simpler processes that explicitly take into account actual environmental conditions and behaviour. Such models do not have the detail necessary to simulate expression of single genes or gene networks although such capabilities could be included if the function of single genes or gene networks is known and their coordinated expression can be quantified in relation to environmental conditions.

In contrast, *the bottom-up* approach integrates knowledge at the molecular and cellular level, and a new scientific discipline – systems biology – has been developed for such research and successfully applied in single-cell organisms or relatively simple processes in plants. Examples of the latter are the explanation of phyllotaxis on the basis of gene-regulated accumulation of auxins and the explanation of the progress to flowering based on the knowledge of the expression level of a set of genes with known function. However, to extend this approach to more complex traits in higher plants and plant communities, we need greater knowledge of how to scale up prediction of gene function at the field level under a range of environmental conditions using information from quantitative estimates of gene expression preferentially obtained under these conditions.

Given these fundamental problems in both approaches we need to re-think the way green plants are organized. The organization of green plants arises as a sequence of developmental processes that allow the plant to behave as an integrated system with multiple feedback controls and cascades to coordinate the growth process. This coordinated integration is achieved by a communication system based on various types of signals and messengers. The plant as a whole also perceives changes in its abiotic and biotic environment, which then evoke responses based on signals. These signals must function across levels of organization, from the genome, cell, tissue and organ levels to the plant and plant community levels. These levels of organization or functional control systems have different principles but yet interact. Response to drought stress provides an example. Drought will induce changes in gene expression, electron transport pathways in photosystems, tissue turgor, specific leaf area, root:shoot ratio and plant-to-plant interaction. But changes in plant-to-plant interaction will affect root:shoot ratio, specific leaf area, photosynthesis of the individual leaf, etc. More insight into the functional interaction between the different levels of organization is needed – something which cannot be easily achieved by a top-down or a bottom-up approach. Understanding gene expression under agronomic conditions is virtually impossible.

THE MIDDLE-OUT APPROACH

We, therefore, need more research that starts from the different levels of biological organization for which we have detailed existing data and understanding, and then use this information to reach up and down to other levels. In human physiology this has been called *the middle-out approach*. Such integral, quantitative studies, on the one hand, integrate knowledge and understanding at the lower level of organization, and, on the other hand, are optimally embedded as an essential component in plant systems at the next-higher hierarchical level. In this way, a knowledge chain can be created that will integrate plant processes in a coherent way, supported by a chain of models or modules that can communicate with each other across levels of organization. A first example may be the modelling of fruit quality, which has been based on modules for daily changes in the available assimilate, hourly changes in water relations and daily partitioning of carbon into different types of sugars. Model parameters have also been linked to genetic variation (QTLs, mutants, transgenics).

In this middle-out approach the proper choice of level of detail is essential. Fine detail might not be required in all cases, robustness (especially across environments) might be more important. This can even be true when this would mean that the models will be rather coarse-grained. Keeping it as simple as possible is a must. How simple relations are, may be best assessed at the middle level. We need crop physiologists well trained in molecular physiology and systems analysis to assess the proper level of detail. Only they can judge the trade-off between resolution and robustness, between detail and rigour.

New physiology-based mechanistic models will be needed to integrate and quantify functional relationships across levels of organization. These models should allow us to discard obsolete details at each level of organization. They should also be developed in such a way that higher systems-level models can impose the type of lower-level information needed to improve the inputs from low to high, while the lower-level models serve to inspire the higher-level models to seek to stick to the true way plants regulate themselves.

THE CHALLENGES IN SCALING UP

Gene expression studies performed under controlled conditions can create knowledge that is less affected by environment than crop performance data, which is essential for a basic understanding of crop physiology. However, the bottom-up approach in systems biology requires a proper upscaling, linking and interfacing of the following steps: DNA – RNA expression – RNA stability – protein – protein modification – protein stability – protein functioning – metabolites. From the metabolites to traits under variable environmental conditions is then the next, giant step. Given the complexity of this chain of knowledge, it will be difficult to make use of '-omics' (based on large scale gene expression, proteomics, etc.) to improve our understanding of crop physiology; the more so as all processes can be tissue-specific and metabolites move around through the plant and interact with each other. A focus on the single-cell level, as is the case in systems biology, is already a tremendous challenge. In this respect, some of the work published in top international journals, in which claims were made of unravelling simple traits strongly associated with yield potential, should be considered with proper caution and questioned by crop physiologists. As argued before, more progress is needed before crop physiologists can make use of the '-omics' potential. Other technological breakthroughs, such as hybrid breeding in maize, also took a long time before they were widely accepted and utilized in crop improvement. Nevertheless, the proportion of the available resources allocated to crop physiology and ecophysiology is worrying to many crop scientists as the total amount of funding for plant sciences will most likely not increase in the foreseeable future.

Trying to understand the entire organism at all levels of aggregation might also be the wrong approach. Understanding the specific effects of environmental changes based on molecular information is easier to achieve. Even easier is to try to understand the molecular-physiological basis of genetic differences in such specific effects. The latter is currently the most important as this is amenable to crop improvement through breeding. Of course one can also select for high values of end traits (for example yield) without knowing how yield formation works through the brute force of mass selection in relevant target environments.

Scientists active in the field of systems analysis sometimes argue that for scaling up one does not need all the details from the lowest or intermediate levels of aggregation. In general, scaling up across several levels of aggregation simply results in the loss of impact of mechanisms or relationships at the lower levels, because they are diminished by the most influential mechanisms that operate at higher levels of aggregation. For example, to understand the effects of the *Rht* dwarf genes in wheat on yield, it is sufficient to compare the alleles of these genes in isogenic background, which does not require knowledge about the molecular function of the gene. It is sufficient to carry out well-designed experiments to unravel the crop physiological behaviour of various, well-defined genetic materials (isogenic lines).

Moreover, despite the large increase in detailed knowledge, we do not necessarily need models of increasing complexity. Scaling is about summarizing important knowledge that captures what needs to be taken to the next scale. An important question then is: How much detail is needed to get from gene or molecule to phenotype? In some cases, one can model processes at the crop level based on the information of the effect of the genetic variant and simply build relationships that circumvent the intermediate levels, thus, ignoring the consequences of lower-level traits at intermediate levels (such as circumventing the cell). This approach has been successfully applied for QTL-based modelling of flowering in barley and leaf expansion rate in maize using data from populations of recombinant inbred lines. But for more complex traits, such as grain yield, this modelling approach was not successful. Mutants and transgenic plants, which are even better near-isogenic lines, can also be used, and this is how at least breeding or biotech companies move from '-omics' to crop production.

THE CHALLENGES IN SCALING DOWN

A top-down approach with a keen focus on the 'bottom' to allow further understanding seems most feasible, provided we clearly understand the complexity of the traits involved and have detailed insight about the processes that operate at lower scales. We may wish to start with the identification of genes that are critical (rate limiting) for basic, well-studied processes (such as flower induction, cell elongation), to initiate the links between crop physiology and basic sciences. But that might only work well for developmental processes such as flowering or simple growth processes such as leaf expansion, which are easy to quantify precisely and for which the effects of environmental factors are well known and described. However, even for simple traits, top-down approaches may not always be successful. Although there is no reason for gloomy pessimism on the longer term, at this point this top-down approach seems too ambitious for complex traits such as yield. Some small successes have been reported: research on rice has shown fairly simple inheritance and relatively large effects of QTLs for important yield components, such as seed number and seed size, but the relationships between these traits and seed yield are complex, influenced by feedback mechanisms and dependent on genotype, environment and management.

The immediate challenge is, therefore, to assess the level of detail needed to bridge the gap between physiological approaches (from the crop level) and molecular approaches (to the molecule or gene) depending on the research objectives. An international effort to elaborate in one case study how this can be achieved using the input of a transdisciplinary team is advocated. Such an effort would be the best way to establish the required working relationships and mutual understanding of problems that is required of both crop physiologists and molecular geneticists, and also to demonstrate the value of this approach. A case study related to genotype × environment interactions is most suitable to achieve these goals.

ACTIONS REQUIRED

Some new approaches are needed, and we urge molecular, plant and crop scientists to collaborate more strongly. We recommend the following general, long-term actions:

1. Establishing private–public partnerships to enhance the role of genomics and its application by applying '-omics' to genetic diversity tested in field conditions.
2. More investment in research on whole-plant physiology, crop ecology and crop simulation to allow efficient integration of knowledge on molecular biology. An interesting complication is that application of genomics can best be tested with transgenics but tests are hardly allowed and/or very expensive and risky for public institutions, at least in Europe.
3. The formation of multidisciplinary teams that operate through networks of excellence in developing quantitative tools that integrate complex information at different levels of organization.
4. The exchange of young scientists between research groups that work at different hierarchical levels to develop a wider set of (T-shaped) skills to deal with complexity and levels of organization in crop science.

In order to be able to identify QTLs and candidate genes that drive complex traits so that they can be included in simulation models, we propose the following short-term actions:

1. Improving the level of detail in characterizing experimental environments, preferably through commonly shared protocols. This will allow modellers to analyse the genotype × environment interaction in a more consistent and precise way.
2. Improving the characterization of parents used for creating mapping populations. For example, careful characterization is needed in terms of the genes involved in developmental requirement (e.g., vernalization (*Vrn*) and photoperiod (*Ppd*) requirement). This will allow the design of populations with no significant genotype × environment interaction for phenology, thus, avoiding this strongly confounding effect in cases where this is desired. The use of near-isogenic materials for the study of the effect of major QTL and genes (mutants or transgenic lines) is highly recommended in specific cases. In other cases, the genotype × environment interactions in phenology might be of particular interest and can then be quantified using QTL-based crop models.
3. International collaboration to carry out a number of multi-location trials with well-designed and characterized populations and with proper characterization of

experimental environments to analyse genotype × environment interactions for other plant characteristics, not related to phenology.

4. Advanced physiological and statistical approaches for determining what aspects of the environment are most influential on the genotype × environment interaction that affect the trait in question, and the stages of crop development at which these interactions are most important.
5. A search for funding to finance international, transdisciplinary teams which will carry out a case study in which scaling across several levels of organization is achieved to identify which level of detail is needed to bridge the gap between molecular approaches and crop physiological approaches and between the genotype and the phenotype.

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LIST OF REVIEWERS

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- Bas Bouman, IRRI, The Philippines
- Ken Cassman, University of Nebraska, USA
- Scott Chapman, CSIRO Plant Industry, Australia
- Karine Chenu, INRA-INAPG, France
- Fred van Eeuwijk, Wageningen University, The Netherlands
- Jochem Evers, Wageningen University, The Netherlands
- Ken Giller, Wageningen University, The Netherlands
- Graeme Hammer, University of Queensland, Australia
- Martin van Ittersum, Wageningen University, The Netherlands
- Maarten Koornneef, Max Planck Institute, Germany
- Edith Lammerts van Bueren, Wageningen University, The Netherlands
- Bert Lotz, Plant Research International, The Netherlands
- Shaobing Peng, IRRI, The Philippines
- Tommie Ponsioen, Wageningen University, The Netherlands
- Matthieu Reymond, Max Planck Institute, Germany
- Matthew Reynolds, CIMMYT, Mexico
- Ad Schapendonk, Plant Dynamics, The Netherlands
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