Chittaranjan Kole (Ed.)

Genome Mapping and Molecular Breeding in Plants

Cereals and Millets

Springer

Genome Mapping and Molecular Breeding in Plants
Volume 1

Series Editor: Chittaranjan Kole

Volumes of the Series
Genome Mapping and Molecular Breeding in Plants

Volume 1
Cereals and Millets

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Forest Trees

Chittaranjan Kole (Ed.)

Cereals and Millets

With 25 Illustrations, 3 in Color

Springer

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Preface to the Series

Genome science has emerged unequivocally as the leading discipline of this new millennium. Progress in molecular biology during the last century has provided critical inputs for building a solid foundation for this discipline. However, it has gained fast momentum particularly in the last two decades with the advent of genetic linkage mapping with RFLP markers in humans in 1980. Since then it has been flourishing at a stupendous pace with the development of newly emerging tools and techniques. All these events are due to the concerted global efforts directed at the delineation of genomes and their improvement.

Genetic linkage maps based on molecular markers are now available for almost all plants of significant academic and economic interest, and the list of plants is growing regularly. A large number of economic genes have been mapped, tagged, cloned, sequenced, or characterized for expression and are being used for genetic tailoring of plants through molecular breeding. An array of markers in the arsenal from RFLP to SNP; tools such as BAC, YAC, ESTs, and microarrays; local physical maps of target genomic regions; and the employment of bioinformatics contributing to all the "-omics" disciplines are making the journey more and more enriching. Most naturally, the plants we commonly grow on our farms, forests, orchards, plantations, and labs have attracted emphatic attention, and deservedly so. The two-way shuttling from phenotype to genotype (or gene) and genotypte (gene) to phenotype has made the canvas much vaster. One could have easily compiled the vital information on genome mapping in economic plants within some 50 pages in the 1980s or within 500 pages in the 1990s. In the middle of the first decade of this century, even 5,000 pages would not suffice! Clearly genome mapping is no longer a mere "promising" branch of the life science; it has emerged as a full-fledged subject in its own right with promising branches of its own. Sequencing of the *Arabidopsis* genome was complete in 2000. The early 21st century witnessed the complete genome sequence of rice. Many more plant genomes are waiting in the wings of the national and international genome initiatives on individual plants or families.

The huge volume of information generated on genome analysis and improvement is dispersed mainly throughout the pages of periodicals in the form of review papers or scientific articles. There is a need for a ready reference for students and scientists alike that could provide more than just a glimpse of the present status of genome analysis and its use for genetic improvement. I personally felt the gap sorely when I failed to suggest any reference works to students and colleagues interested in the subject. This is the primary reason I conceived of a series on genome mapping and molecular breeding in plants.

There is not a single organism on earth that has no economic worth or concern for humanity. Information on genomes of lower organisms is abundant and highly useful from academic and applied points of view. Information on higher animals including humans is vast and useful. However, we first thought to concentrate only on the plants relevant to our daily lives, the agronomic, horticultural and technical crops, and forest trees, in the present series. We will come up soon with commentaries on food and fiber animals, wildlife and companion animals, laboratory animals, fishes and aquatic animals, beneficial and harmful insects,

plant- and animal-associated microbes, and primates including humans in our next "genome series" dedicated to animals and microbes. In this series, 82 chapters devoted to plants or their groups have been included. We tried to include most of the plants in which significant progress has been made. We have also included preliminary works on some so-called minor and orphan crops in this series. We would be happy to include reviews on more such crops that deserve immediate national and international attention and support. The extent of coverage in terms of the number of pages, however, has nothing to do with the relative importance of a plant or plant group. Nor does the sequence of the chapters have any correlation to the importance of the plants discussed in the volumes. A simple rule of convenience has been followed.

I feel myself fortunate to have received highly positive responses from nearly 300 scientists of some 30-plus countries who contributed the chapters for this series. Scientists actively involved in analyzing and improving particular genomes contributed each and every chapter. I thank them all profoundly. I made a conscientious effort to assemble the best possible team of authors for certain chapters devoted to the important plants. In general, the lead authors of most chapters organized their teams. I extend my gratitude to them all.

The number of plants of economic relevance is enormous. They are classified from various angles. I have presented them using the most conventional approach. The volumes thus include cereals and millets (Volume I), oilseeds (Volume II), pulse, sugar and tuber crops (Volume III), fruits and nuts (Volume IV), vegetables (Volume V), technical crops including fiber and forage crops, ornamentals, plantation crops, and medicinal and aromatic plants (Volume VI), and forest trees (Volume VII).

A significant amount of information might be duplicated across the closely related species or genera, particularly where results of comparative mapping have been discussed. However, some readers would have liked to have had a chapter on a particular plant or plant group complete in itself. I ask all the readers to bear with me for such redundancy.

Obviously the contents and coverage of different chapters will vary depending on the effort expended and progress achieved. Some plants have received more attention for advanced works. We have included only introductory reviews on fundamental aspects on them since reviews in these areas are available elsewhere. On other plants, including the "orphan" crop plants, a substantial amount of information has been included on the basic aspects. This approach will be reflected in the illustrations as well.

It is mainly my research students and professional colleagues who sparked my interest in conceptualizing and pursuing this series. If this series serves its purpose, then the major credit goes to them. I would never have ventured to take up this huge task of editing without their constant support. Working and interacting with many people, particularly at the Laboratory of Molecular Biology and Biotechnology of the Orissa University of Agriculture and Technology, Bhubaneswar, India as its founder principal investigator; the Indo-Russian Center for Biotechnology, Allahabad, India as its first project coordinator; the then-USSR Academy of Sciences in Moscow; the University of Wisconsin at Madison; and The Pennsylvania State University, among institutions, and at EMBO, EUCARPIA, and Plant and Animal Genome meetings among the scientific gatherings have also inspired me and instilled confidence in my ability to accomplish this job.

I feel very fortunate for the inspiration and encouragement I have received from many dignified scientists from around the world, particularly Prof. Arthur

Kornberg, Prof. Franklin W. Stahl, Dr. Norman E. Borlaug, Dr. David V. Goeddel, Prof. Phillip A. Sharp, Prof. Gunter Blobel, and Prof. Lee Hartwell, who kindly opined on the utility of the series for students, academicians, and industry scientists of this and later generations. I express my deep regards and gratitude to them all for providing inspiration and extending generous comments.

I have been especially blessed by God with an affectionate student community and very cordial research students throughout my teaching career. I am thankful to all of them for their regards and feelings for me. I am grateful to all my teachers and colleagues for the blessings, assistance, and affection they showered on me throughout my career at various levels and places. I am equally indebted to the few critics who helped me to become professionally sounder and morally stronger.

My wife Phullara and our two children Sourav and Devleena have been of great help to me, as always, while I was engaged in editing this series. Phullara has taken pains ("pleasure" she would say) all along to assume most of my domestic responsibilities and to allow me to devote maximum possible time to my professional activities, including editing this series. Sourav and Devleena have always shown maturity and patience in allowing me to remain glued to my PC or "printed papers" ("P3" as they would say). For this series, they assisted me with Internet searches, maintenance of all hard and soft copies, and various timely inputs.

Some figures included by the authors in their chapters were published elsewhere previously. The authors have obtained permission from the concerned publishers or authors to use them again for their chapters and expressed due acknowledgement. However, as an editor I record my acknowledgements to all such publishers and authors for their generosity and good will.

I look forward to your valuable criticisms and feedback for further improvement of the series.

Publishing a book series like this requires diligence, patience, and understanding on the part of the publisher, and I am grateful to the people at Springer for having all these qualities in abundance and for their dedication to seeing this series through to completion. Their professionalism and attention to detail throughout the entire process of bringing this series to the reader made them a genuine pleasure to work with. Any enjoyment the reader may derive from this books is due in no small measure to their efforts.

University Park,   Chittaranjan Kole
Pennsylvania,
10 January 2006

Preface to the Volume

Cereals and millets form the leading group of economic plant species. Many of them have a glorious past with records of their domestication in the earliest civilizations and are mentioned in ancient writings the world over. They are the staple food for most of the earth's population and have attracted most of the attention of plant scientists. Some of them have contributed immensely to the discovery or reinforcement of many concepts in genetics and strategies of breeding. For example, maize was a model plant in genetics and breeding in the last century and could be called the "plant Drosophila". It led to concepts such as linkage mapping, cytoplasmic inheritance, somatic crossover, mutation, chromosomal aberration, heterosis, and, most importantly, transposable elements. In the modern era also, maize and rice were among the first plant species to have molecular genetic linkage maps. The so-called grass family was elegantly used to elucidate genome homology and synteny, with rice at the center of the circle. Rice, with its small, simple genome, is a model plant today and has pride of place in being the first crop plant to have its complete genome mapped. Thanks to the Rockefeller Foundation, enormous progress has been made in rice genome research, particularly in the developing nations of Asia and Africa, which in fact laid the foundation for biotechnology research in these countries and facilitated infrastructure and human resource development. The literature on the major cereal crops such as rice, wheat, maize, and barley is voluminous. Other cereals like oat, rye, sorghum, and the millets stand in sharp contrast. We have miles to go to depict their genomes and improve upon them considering the preference and potential of these crops in some edaphoclimates.

Deliberations on genome mapping and molecular breeding in some cereals, particularly rice, wheat, and maize, would require at least a complete volume for each crop. It is widely recognized that a hundred pages or so on each of these cereals cannot do them justice. The authors have rightly devoted more pages to the advanced studies on these crops; reviews on the basic studies on them are widely available. By contrast, road maps for genome research on other cereals and millets have been thoroughly delineated by the authors.

The authors of this volume's chapters have made tremendous contributions to the study of the crop to which their respective chapters are devoted, as well as to the study of related crops. This made the deliberations not just comprehensive but philosophical as well. The long, even lifetime, experience of most of them made the experiments and examples cited especially pertinent. I was glued, I must confess, to the manuscripts during the many long days (and nights) spent as I mentally prepared myself to edit the chapters of these esteemed scientists. Even during the review of these reviews, I was a student first, an editor later, and a reader last! I am very grateful to them for giving me this unique opportunity.

We are all aware of the fast pace at which the ideas, strategies, and databases on cereals and millets are being generated. The authors took pains (and pleasure!) to update their chapters at least two or three times. Finally we had the finalized chapters in hand, knowing full well that, regardless of when the volume was published, it would be missing "the very latest". And so it goes for all the sciences; otherwise they would not be called science.

Editing this volume has gone very smoothly thanks to all the kindhearted and highly professional authors. Many of the contributing authors in this volume are my personal friends as well. My own works on molecular mapping started with cereal crops, and I retain a nostalgic love for them. Many of my research students worked with me on rice, and some of them are pursuing their own research on this crop. Editing this volume was therefore highly enriching and enjoyable for me thanks to the interactions and intellectual exchanges from the authors, old colleagues, and research students.

I look forward to receiving suggestions from you, the reader, for an updated second edition of this volume.

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Abbreviations

ABA	Abscisic Acid
ABL	Advanced Backcross Line
ABQA	Advanced Backcross QTL Analysis
AC	Amylose Content
AFLP	Amplified Fragment Length Polymorphism
ALP	Amplicon Length Polymorphism
AltSB	Aluminum Tolerant *Sorghum Bicolor*
ARC	Assam Rice Culture
ASA	Allele Specific Amplicon
BAC	Bacterial Artificial Chromosome
BARC	Beltsville Agricultural Research Center
BB	Bacterial Blight
BES	BAC End Sequence
BIL	Backcross Inbred Line
BLB	Bacterial Leaf Blight
BPH	Brown Plant Hopper
BSA	Bulked Segregant Analysis
BT	*Bacillus thuringiensis*
BVP	Basic Vegetative Phase
BYDV	Barley Yellow Dwarf Virus
CaMV	Cauliflower Mosaic Virus
CAPS	Cleaved Amplified Polymorphic Sequence
CDD	Conserved Domain Database
CHS	Chalcone Synthase
cM	centi-Morgan
CMS	Cytoplasmic Male Sterility/Cell-membrane Stability
CSSL	Chromosome Segment Substitution Line
DAP	Days After Pollination
DAPI	4′,6-Diamidino-2-Phenylindole
DH	Doubled Haploid
DHL	Doubled Haploid Line
DLA	Diseased Leaf Area
EM	Egg Mortality
eQTLs	expressed QTLs
EST	Expressed Sequence Tag
FAO	Food and Agricultural Organization
FHB	Fusarium Head Blight
FISH	Fluorescence *In Situ* Hybridization
FT	Flowering Time
GB	Greenbug Biotypes
GBSS	Granule Bound Starch Synthase
GC	Gel Consistency
GISH	Genomic *In Situ* Hybridization
GLH	Green Leaf Hopper
GT	Gelatinization Temperature

GWL	Grade of Watery Lesion
HAPPY	HAPloid genome PolYmerase chain reaction mapping
IBPGR	International Bureau of Plant Genetic Resources
ICRISAT	International Crop Research Institute for Semi-Arid Tropics (Hyderabad, India)
InDel	Insertion-Deletion
INRA	L'Institut National de la Recherche Agronomique (= National Institute for Agricultural Research) Cedex, France
IPK	Institut für Pflanzengenetik und Kulturpflanzenforschung (= Institute of Plant Genetics and Crop Plant Research), Gatersleben, Germany
IRAP	Inter Retrotransposon Amplified Polymorphism
IRGSP	International Rice Genomic Sequence Project
IRRI	International Rice Research Institute (Manila, Philippines)
IS	Insertion Sequence
ISH	*In Situ* Hybridization
ISSR	Inter Simple Sequence Repeat
ITS	Internal Transcribed Spacer
JIC	John Innes Center (Norwich, UK)
LD	Linkage Disequilibrium
LG	Linkage Group
LMA	Late Maturity α-Amylase
LOD	Logarithm Of Odds
LRL	Lateral Root Length
LRR	Leucine Rich Repeats
LWPO	Leaf Water Potential
MAR	Matrix Attachment Regions
MAS	Marker-Assisted Selection
MBC	Map-Based Cloning
MITEs	Miniature Inverted-Repeats Transposable Elements
MPSS	Massively Parallel Signature Sequencing
MT	Metric Tons
NADPH	Nicotinamide Adenine Dinucleotide Phosphate, reduced form (by addition of a 'H' atom)
NARS	National Agricultural Research Systems
NBS	Nucleotide Binding Site
NIL	Near Isogenic Lines
NOR	Nucleolus Organizing Region
NPT	New Plant Type
NSF	National Science Foundation (USA)
OA	Osmotic Adjustment
PAC	PI-Derived Artificial Chromosome
PCR	Polymerase Chain Reaction
PFGE	Pulse Field Gel Electrophoresis
PGMS	Photoperiod Sensitive Genetic Male Sterility
PHO	P-Deficiency Tolerance
PHS	Preharvest Sprouting
PHYA	Phytochrome A
PPR	Pentatrico Peptide Repeat
PS	Phenotypic Selection
PVE	Phenotypic Variance Explained

PWL	Percentage of Watery Lesion
QTL	Quantitative Trait Loci
RAPD	Random Amplified Polymorphic DNA
REMAP	Retrotransposon Microsatellite Amplified Polymorphism
RFLP	Restriction Fragment Length Polymorphism
RGA	Resistance Gene Analogue
RH	Radiation Hybrid
RIL	Recombinant Inbred Line
RIP	Recombinant Inbred Population
RLGS	Restriction Landmark Genomic Scanning
RRL	Relative Root Length
RSL	Recombinant Substitution Line
RT-PCR	Reverse Transcription PCR
RTSV	Rice Tungro Spherical Virus
RWA	Russian Wheat Aphid
RWC	Relative Water Content
RYMV	Rice Yellow Mottle Virus
SAGE	Serial Analysis of Gene Expression
SAMPL	Selective Amplification of Microsatellite Polymorphic Loci
SAP	Specific Amplicon Polymorphism
SAT	Semi Arid Tropics
SCAR	Sequence Characterized Amplified Region
SNP	Single Nucleotide Polymorphism
SRL	Seminal Root Length
SSAP	Sequence Specific Amplified Polymorphism
SSCP	Single Strand Conformational Polymorphism
SSR	Simple Sequence Repeats
STMS	Sequence Tagged Microsatellite Site
STS	Sequence Tag Site
TAGI	The Arabidopsis Genome Initiative
TCs	Tentative Consensi
TGMS	Temperature Sensitive Genetic Male Sterility
USDA	United States Department of Agriculture
VPM	Ventricosa into Persicum into Marne
WA	Wild Abortive
WBPH	White Backed Plant Hopper
WCV	Wide Compatibility Varieties
WMC	Wheat Microsatellite Consortium
YAC	Yeast Artificial Chromosome

1 Rice

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1.1
Introduction

Rice is the world's single most important crop and a primary food source for half of the world's population. Rice, wheat, and maize provide 49% of the calories consumed by the human population. Of those 23% are provided by rice, 17% by wheat and 9% by maize. Thus almost one fourth of the calories consumed by the entire world population come from rice. More than 90% of the world's rice is grown and consumed in Asia, where 60% of the earth's people live. Rice is planted to about 154 million hectares annually, or on 11% of the world's cultivated land. World rice production was 600 million tons in 2000. India has the largest area under rice (45 million hectares), and China is the largest producer of rice (190 million tons). Other major rice-producing countries are Indonesia, Bangladesh, Vietnam, Thailand, Myanmar, Japan, and the Philippines (Table 1). The importance of rice in the diet varies among countries. It accounts for over 70% of the daily calories intake in countries such as Bangladesh, Cambodia, Laos, Myanmar, and Vietnam but drops to about 40% in countries such as China and India, whose northern areas consume primarily wheat.

1.1.1
Taxonomy and Origin of Cultivated Rice

Of the two cultivated species, Asian cultivated rice, *Oryza sativa* , is grown worldwide. *Oryza glaberrima*, the African cultivated rice, is grown on a limited scale in West Africa. Like other cereals such as wheat, maize, barley, sorghum, oats, and rye, which feed the world, rice belongs to the grass family Gramineae. The genus *Oryza*, to which cultivated rice belongs, probably originated at least 130 million years ago and spread as a wild grass in Gondwanaland, the super continent that eventually broke up and drifted apart to become Asia, Africa, Australia, and Antarctica (Chang 1976). This explains the distribution of *Oryza* species on all of these continents except Antarctica (Table 2).

There are 22 wild species of genus *Oryza*. Nine of the wild species are tetraploid. The remaining wild species and the two cultivated species are diploid. Ten different genomes (Table 2) have been assigned to the different species based on chromosome pairing in interspecific hybrids or based on total DNA hybridization and molecular divergence.

The common rice, *Oryza sativa,* and the African rice, *Oryza glaberrima,* are thought to be examples of parallel evolution in crop plants. The wild progenitor of *O. sativa* is the Asian common wild rice, *O. rufipogon*, which shows a range of variation from perennial to annual types. Annual types, also given the specific name of *O. nivara*, were domesticated to become *O. sativa* (Khush 1997). In a parallel evolutionary path, *O. glaberrima* was domesticated from annual *O. breviligulata*, which in turn evolved from perennial *O. longistaminata* (Fig. 1).

Domestication of wild rices probably started about 9,000 years ago. Development of annuals at different elevations in East India, northern Southeast Asia, and western China was enhanced by alternating periods of drought and variations in temperature during the Neothermal Age about 10,000 to 15,000 years ago (Whyte 1972). Domestication in Asia could have occurred independently and concurrently at several sites within or bordering a broad belt that extends from the plains below the eastern foothills of the Himalayas in India through upper Myanmar, northern Thailand, Laos, and Vietnam to southwestern or southern China (Roschevitz 1931; Chang 1976). The earliest and most convincing archeological evidence for domestication of rice in Southeast Asia

Table 1. Total area planted, coverage of high-yielding varieties, and increase in rice production in selected countries in Asia

Country	Total area planted (million ha)		Area planted with HYVs	Production (million tons)		Increase in production
	1966	2000	(%)	1966	2000	(%)
Bangladesh	9.1	10.7	65	14.3	35.8	150
China	31.3	30.5	100	98.5	190.1	93
India	35.2	44.6	73	45.6	134.1	194
Indonesia	7.7	11.5	77	13.6	51.0	275
Myanmar	4.5	6.3	72	6.6	21.3	222
Pakistan	1.4	2.3	42	2.0	7.0	250
Philippines	3.1	4.0	89	4.1	12.4	202
Sri Lanka	0.5	0.8	91	1.0	2.8	180
Thailand	7.3	10.0	68	13.5	23.4	73
Vietnam	4.7	7.6	80	8.5	32.5	282

was discovered by Welhelm G. Solheim II in 1966 (Solheim 1972). Pottery sherds bearing the imprints of grain and husks of *O. sativa* were discovered at Non Nok Tha in the Korat area of Thailand. The remains were dated to about 4000 BC. The oldest carbonized grains found in India date to about 6750 BC (Sharma and Nanda 1980). The oldest remains of cultivated rice in China date to five centuries before Christ. Carbonized rice grains from Tongxieng County of Zejiang province were identified as being 7,040 years old.

The African cultivar *O. glaberrima* originated in the Niger River delta. The primary center of diversity for *O. glaberrima* is the swampy basin of the upper Niger River and two secondary centers to the southwest near the Guinean coast. The primary center was probably formed around 1500 BC, while the secondary centers were formed 500 years later (Porteres 1956).

1.1.2
Dispersal of Cultivated Rice

From the Himalayan foothills rice spread to western and northern India, to Afghanistan and Iran and south to Sri Lanka. The date of 2500 BC has been established for Mohenjodaro in Pakistan, while in Sri Lanka rice was a major crop as early as 1000 BC. The rice crop may well have been introduced to Greece and neighboring countries of Mediterranean by returning members of Alexander the Great's expedition to India in 324 BC. However, in all probability rice did not become an established crop in Europe until much later,

perhaps in the 15th century. Rice was introduced from India to Madagascar, to East Africa, and then to countries of West Africa. Indica rices also spread eastward to Southeast Asia and north to China.

The japonica rice was most likely domesticated somewhere in northern parts of Southeast Asia or southern China. It moved north to become a temperate japonica. From China temperate japonicas were introduced into Korea and from Korea to Japan around the beginning of the first century. In the hilly areas of Southeast Asia, japonica rices were grown under upland culture as a component of shifting cultivation before the upland tribes moved into lowlands and introduced the japonicas into lowland culture. From mainland Southeast Asia, both indica and japonica rices were introduced into Malaysia, the Philippines, and Indonesia and from the Philippines to Taiwan. Migrating Malays from Indonesia introduced tropical japonicas to Madagascar in the 5th or 6th century. Portuguese priests introduced the tropical japonicas from Indonesia to Guinea Bissau, and from there they spread to other West African countries. Thus most of the upland rice varieties grown in West Africa are tropical japonicas. The Portuguese also introduced tropical japonicas and lowland indicas to Brazil, and Spanish-speaking people brought them to other Latin American countries. Thus in Brazil today most of the upland varieties are tropical japonicas and the lowland varieties are indicas (Khush et al. 2003). The first record of rice in the U.S. dates from 1685, and it was probably introduced from Madagascar with the slave trade.

Table 2. Chromosome number , genomic composition , and geographical distribution of *Oryza* species

Species	2*n*	Genome	Distribution
O. sativa complex			
O. sativa L.	24	AA	Worldwide
O. nivara Sharma et Shastry	24	AA	Tropical and subtropical Asia
O. rufipogon Griff.	24	AA	Tropical and subtropical Asia, tropical Australia
O. breviligulata A. Chev. et Roehr.	24	AA	Africa
O. glaberrima Steud.	24	AA	West Africa
O. longistaminata A. Chev. et Roehr.	24	AA	Africa
O. meridionalis Ng	24	AA	Tropical Australia
O. glumaepatula Steud.	24	AA	South and Central America
O. officinalis complex			
O. punctata Kotschy ex Steud.	24, 48	BB, BBCC	Africa
O. minuta J.S. Pesl. ex C.B. Presl.	48	BBCC	Philippines and Papua New Guinea
O. officinalis Wall ex Watt	24	CC	Tropical and subtropical Asia, tropical Australia
O. rhizomatis Vaughan	24	CC	Sri Lanka
O. eichingeri A. Peter	24	CC	South Asia and East Africa
O. latifolia Desv.	48	CCDD	South and Central America
O. alta Swallen	48	CCDD	South and Central America
O. grandiglumis (Doell) Prod.	48	CCDD	South and Central America
O. australiensis Domin.	24	EE	Tropical Australia
O. meyeriana complex			
O. granulata Nees et Arn. ex Watt	24	GG	South and Southeast Asia
O. meyeriana (Zoll. et (Mor. ex Steud.) Baill)	24	GG	Southeast Asia
O. ridleyi complex			
O. longiglumis Jansen	48	HHJJ	Irian Jaya, Indonesia, and Papua New Guinea
O. ridleyi Hook. F.	48	HHJJ	South Asia
Unclassified			
O. brachyantha A. Chev. et Roehr.	24	FF	Africa
O. schlechteri Pilger	48	HHKK	Papua New Guinea

1.1.3
Varietal Diversity of Rice

From its subtropical origin rice is now cultivated between 55° N in China and 36° S in Chile. Cultivation and farmer selection for centuries under varied growing conditions have resulted in a myriad of rice varieties. An estimated 120,000 distinct rice varieties exist in the world. Approximately 80,000 are preserved in the Gene Bank of the International Rice Research Institute (IRRI) in the Philippines. China has about 40,000 and India about 25,000 in their gene banks. Other countries have smaller selections.

Rice varieties differ in numerous morphological and physiological traits and have been selected for adaptation to different growing conditions. Some mature in less than 80 d from sowing. Others, like Rayada rices of Bangladesh, have a growth cycle of about 280 d. These are photoperiod-sensitive deepwater rices and are planted with the onset of rains in March and harvested in December. Rice varieties also differ in endosperm traits, which determine their acceptability to various consumer groups. While the vast majority of rice varieties are nonglutinous, glutinous varieties form the everyday diet of the people of Laos and northeast Thailand. Most of the major rice-growing countries have a few aromatic varieties that are prized on the market. Varieties differ in the level of cold tolerance and tolerance to other abiotic stresses such as drought, submergence, and salinity. There are

Fig. 1. Evolutionary pathways for two cultivated species of rice
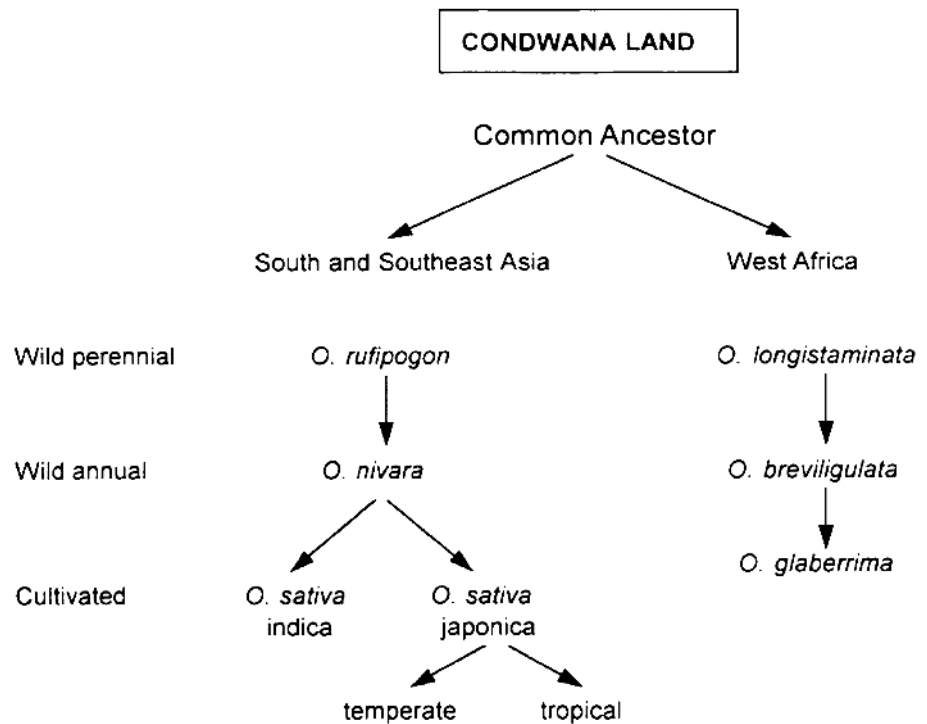
differences in resistance to diseases and insects. In some countries, varieties are classified according to the season in which they are grown. For example, in Bangladesh, where rice is grown throughout the year, varieties have been selected for adaptation to following seasons (Khush 1997).

1. Boro: Winter rice, transplanted, cold tolerant, grown December to May
2. Aus: Summer rice, broadcast, sown, drought tolerant, short life cycle, grown April to July
3. Transplanted Aman: Autumn sown, transplanted, photoperiod sensitive, grown July to December
4. Broadcast Aman: Deepwater, photoperiod sensitive, grown March to December
5. Ryadas: Deepwater, photoperiod sensitive, very long duration, grown March to December
6. Ashina: Deepwater aus, broadcast sown, grown April to August
7. Hill Rice: Grown on upland fields, usually on sloping hillsides, direct seeded, grown June to September

Similar varietal differentiations exist in southern India and Sri Lanka, where rice is grown throughout the year.

1.1.4
Rice Varietal Improvement

Since its domestication about 10,000 years ago, rice has undergone tremendous modifications so much as a result of human selection for improved traits that domesticated rice varieties can no longer survive in the wild state. The simple acts of reaping and sowing are selective. Our ancestors may not have known it, but they started the first rice-breeding programs when they began to grow rice plants for their use. Most farmers have a keen eye and a sensitive feeling for plants. Millions of farmers have applied this keen insight and sensitivity for thousands of years to select diverse varieties. Selection was first practiced on the variable and heterogeneous wild and semiwild populations, which must have narrowed the genetic variability. However, several mechanisms in primitive agriculture, such as the introduction of varieties from one region to another and occasional natural crosses, enhanced variability for further selection. Natural crosses between domesticated crop and the weed complexes were another source of variability. The third source of variability was varietal mixtures that primitive agriculturists grew as a protection against disease epidemics. Occasional intercrosses between component varieties generated variability. This

conscious and unconscious selection by humans led to the development of over 120,000 rice varieties grown around the world.

Thus farmers themselves were responsible for most rice improvement from the time of its domestication to about 1900. The best known examples are the "rono" varieties such as "Shinriki" that Japanese farmers selected in the 1890s. The rono varieties are shorter and therefore responded to nutrient inputs with higher yields. Rice-breeding stations were established in China, India, and Japan in the early 20th century. Rice breeders' initial activities were the purification of existing varieties (landraces) through pure line selection. This resulted in pure line varieties. Up to the 1960s rice farmers in tropical and subtropical Asia grew thousands of landraces or pure line varieties, and few had been touched by modern agricultural science. These varieties were tall and weak stemmed and late maturing. When nitrogenous fertilizer was applied at rates exceeding 40 kg/ha, traditional varieties tillered profusely, grew excessively tall, lodged early, and yielded less than they would have with lower fertilizer inputs.

The International Rice Research Institute (IRRI) was established in 1960 in the Philippines to address the problems of stagnant yields. A major breakthrough in raising the yield potential of tropical rice came with the development of IR8 at IRRI in 1966, which resulted in a doubling of the yield potential of rice. IR8 has a short stature and a combination of several other agronomic traits such as sturdy stems for lodging resistance, dark green and erect leaves, and high tillering capacity. Because of lodging resistance it is highly responsive to fertilizer. Since the development of IR8 a series of improved rice varieties have been developed at IRRI and by the National Agricultural Research Systems (NARS). These varieties have been improved in many other traits such as grain quality, disease and insect resistance, growth duration, and tolerance to abiotic stresses. More than 300 varieties have been selected from the breeding materials developed at IRRI (Khush and Virk 2002). These and others developed by NARS are now planted on 80% of the world's rice land. Because of widescale adoption of these varieties and associated technology, world rice production increased 135% in a 35-year period from 257 million tons in 1966 to 600 million tons in 2000, and, during the same period, average rice yield increased from 2.1 t/ha to 3.9 t/ha. Most of the major rice-growing countries achieved self-sufficiency in rice.

During this intensive breeding effort rice varieties have been developed that have genes from various ecotypes of rice. Even the genes from wild species have been introduced into modern varieties. Thus the ecotypic differentiation present in the landraces of rices no longer exists in the improved varieties. Genes from numerous landraces have been incorporated into new varieties. For example, widely grown IR64 has 20 landraces in its ancestry (Khush 1987).

1.1.5
Rice-Breeding Challenges in the 21st Century

World population continues to increase by 75 million people a year, an annual growth rate of 1.3%, with 90% of this increase occurring in the developing countries of Asia, Africa, and Latin America. Providing for population growth now requires an expansion in world grain production of 26 million tons per year. Moreover, owing to rising living standards, food habits are changing in many countries, particularly in Asia, and people are eating more high-value foods such as meat, eggs, and milk. This is driving the demand for grain at a rapid rate. A kilogram of beef produced in the feedlot requires 7 kg of grain, a kilogram of pork needs 4 kg, and a kilogram of poultry needs just over 2 kg (Brown 1997).

More than a billion people in developing countries live below the poverty line and have poor access to food. As poverty-alleviation programs in developing countries make an impact, the purchasing power of poor people will increase, as will the demand for food grains. Based on population projections and improved consumption patterns in developing countries, it is estimated that rice production must increase by 40% during the next 20 to 25 years or at the rate of about 1.1% a year. This increase will have to be achieved from less land, with less water, less labor, and fewer chemicals.

To feed 5 billion rice consumers in 2025, we have to develop rice varieties with higher yield potential and greater yield stability. Crop cultivars with higher yield potential are the key to increased productivity. Conventional hybridization and selection procedures will continue to be employed, but breakthroughs in cellular and molecular biology will be increasingly used in rice improvement. Transformation techniques allow us to introduce novel genes from unrelated sources to accomplish breeding objectives not possible through conventional breeding approaches. For example, none of the rice varieties or related wild species has beta

carotene, a precursor of vitamin A, and rice varieties with vitamin A could not be developed. Ye et al. (2000) introduced three genes, two from the daffodil (*Narcissus pseudonarcissus*) and one from the bacterium *Erwinia uredovora* into rice variety Taipei 309. This led to the establishment of a biosynthetic pathway for the production of beta carotene in rice endosperm. This so-called "golden rice" will have a great impact in alleviating vitamin A deficiency among poor rice consumers.

1.2
Construction of Molecular Linkage Maps in Rice

Genetic mapping means the identification of the location of polymorphism between parental lines that generate progenies used for statistical analysis of recombination frequency. The polymorphisms used are observed both in appearance and nucleotide sequence in genomic DNA. It is well known that Gregor Mendel succeeded in establishing the law of inheritance because he used nearly genetically pure common pea lines for target traits such as plant height or roundness of seed in his experiments. For genomewide mapping using polymorphisms in the nucleotide sequence, the parental lines must be genetically pure or homogeneous as well in terms of the target loci. In the case of rice, homogeneity in the genetic background of the parental lines can be achieved by repeated self-pollination for 5 to 6 generations. In non-self-pollinating plant species, genetic analysis can be performed by a pseudotest cross-analysis method using F_1 siblings. The basic idea in generating genetic maps of both self-pollinating and non-self-pollinating plants is to detect recombination between markers of phenotype or DNA. This chapter focuses on the genetic analysis of rice, which is a purely self-pollinating plant.

Historically, mapping of rice was tried first by linkage analysis of appearance, or phenotype (Nagao and Takahashi 1963). Several phenotypes that could be easily identified and evaluated, such as waxy, dwarfism, chlorosis, or disease resistance, were chosen for genetic mapping, which led to the development of the 12 linkage groups of rice. After this remarkable work, improvement of the linkage map was achieved using isozymes such as esterase instead of phenotypes (Nakagahra 1977). The use of isozyme was the first step in innovating the linkage map by molecular tools.

The correspondence of linkage groups and chromosomes was achieved by using trisomic rice plants with representative phenotype (Iwata and Omura 1984; Khush et al. 1984). The current numbering of chromosomes and linkage groups was unified in 1990 at the 2nd International Rice Genetics Conference (Khush 1990).

In 1986, the utility of polymorphism in genomic nucleotide sequences was first shown to be effective in tagging the human inheritable disease Huntington's disease (Botstein et al. 1980). This linkage analysis of phenotype with DNA markers led to the success in identification of the gene controlling the corresponding phenotype. Subsequently, several efforts focused on detecting polymorphisms of nucleotide sequence to generate many DNA markers distributed all over the genome of a target species. The most reliable polymorphism is restriction fragment length polymorphism (RFLP) because it can be detected as a codominant trait in Southern hybridization. Other conventional polymorphisms such as random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP), which are less time consuming but costly and less reliable as compared to RFLP, are more widely used in linkage analysis. In the case of rice, the first molecular linkage map with 135 loci defined by RFLP markers was published in 1988 using 50 progenies derived between a cross of japonica and indica cultivars of *O. sativa* (McCouch et al. 1988). This pioneered the possibility of molecular genetic analysis of the rice genome further promising the gene identification corresponding to phenotype.

After this first endeavor, several groups in Japan continued the effort to increase the number of RFLP markers for a more detailed and accurate genetic anatomy of the rice genome. In Japan, one of the countries where rice is a main staple, a pilot project of development of genetic maps with genomic RFLP markers (Saito et al. 1991) was immediately followed in 1991 by a large-scale and systematic construction of a map with high-density DNA markers mainly of RFLP. The analyzed population was 186 F_2 plants obtained by a cross between japonica cultivar Nipponbare and indica cultivar Kasalath , and the first map constructed carried a total of 883 markers (Kurata et al. 1994). The markers were mainly derived from rice cDNAs randomly selected from several libraries and partially sequenced from both ends. These markers, which correspond to expressed genes from the rice genome, are more advantageous than random genomic DNAs because their mapped positions will

1S

position	Locus				
0.0	R687				
0.3	C161				
0.6	C602				
5.3	S1442				
5.6	C970	S1543			
7.0	S10623	C1679			
8.4	R87				
8.7	C749	V32	G107	L451	
9.5	S13048				
10.9	R753	C90			
12.3	S14064	M225A	C936	C30	
	S14080	R106	C146	R1841	C1127A
12.8	V31				
13.1	C804				
16.1	G5001	R1613	T26		
16.9	W598				
19.9	C470				
20.2	R480B				
22.7	S1778				
23.8	W168B	T96			
24.1	R1944	R2747			
25.5	G359	S1651	C668	C101	
26.9	R3192				
27.4	L612				
29.0	S4325S	**C885**	S10057B		
29.8	C319				
30.9	S14085				
32.7	G1184C				
37.1	R665				
37.6	R457				
39.0	**C955**	V3A	T98		
41.4	S11941A				
42.6	V92				
43.4	S10245	W115	C399	G89A	
	G317	R618	R549B		
45.6	S11214				
46.5	R844				
47.4	S11122				
49.2	C113				
49.7	R2151S				
50.5	C1338				
52.1	C1211				
52.4	S10086				
53.9	S10591	S1959			
54.3	C916A				
55.4	R210				
58.1	R1712	R108			
58.4	R2167		G1184D		
60.3	V138				
60.6	S2139				
63.9	L429				
65.0	S1501				
65.5	S13927				
65.9	R2280				
66.3	C52903S	R2329S			
66.6	C1905		R1902A	P164	
69.1	Y5714L		P70		
69.4	S11337				
71.3	C1733S	R1903	C904		
	S1715	P67		G165B	
71.8	R2501A				
72.9	C45				
73.2	S14028	R2880	R596	R2630	C369	P145
73.5	C178	L1087	V178	S10626	S960
	Y6855RB	R1547	R2504	C585	

CEN

position	Locus				
87.6	S13849	S765			
87.9	R2159	V139			
90.9	P102				
91.4	**R1928**				
92.2	Y3154R	G270			
93.6	**S13994**	Y2820R			
94.7	S1945				
95.0	Y2820L				
96.3	W394	R3072			
96.9	M163A	M201		S10634B	P23
97.8	R2374B				
99.0	S13559	L588			
100.9	**R2635**				
102.0	G3001				
102.3	V67	S2457			
102.8	C1456	S12831			
103.6	C409	G302			
103.9	R1485	C981			
104.2	R2374				
104.5	G6003				
106.7	C808	R2816			
108.1	L666				
108.6	P77				
109.7	B2G7				
110.6	R886				
113.4	C10728S				
113.7	C122				
114.5	V176	V119	C667	S1457	
116.9	S2717				
119.4	R1012	C2690			
121.0	C922A				
121.5	R178				
122.0	R559				
122.5	S11702	S14096			
123.0	S10045				
123.5	C1162	C1271	C1370	C2782D	
	W116	F30	R290	S14051	
123.8	L417	S1870			
124.3	S10634A				
125.1	C137				
126.5	G1372	F446	C1459	R530	L172B
126.8	C911	C854	L884		C3057
127.6	G393	B194A	R1416	C9A	S1776
129.0	L268				
129.3	S10712	W156			
129.6	C1282	B370	L685		
	C1335	R2417	T36		
130.4	L694				
132.4	C250	R2882	R37		
134.3	R3347				
134.6	R758				
135.1	S2245	S10271	R655		
136.2	C836	C555			
136.5	W578		G1087B		
137.0	R2347A				
137.3	**C813**	L669			
137.6	R1623S				
138.4	P42	C2340			
139.6	C3125				
139.9	C1979				
140.5	**R494**	**S13781**	V2	R2648	L825
140.8	G370	S721			
141.9	C191A				
142.7	**C1257**	T22	R2125	R836	
143.2	S10925	S10346			
143.5	**C1127B**	M225B	C1000		
144.0	C283	C713	R578		
	S10846	**R3279**	R3265		
144.5	C434				
145.3	S12805				
145.6	C86	**C727A**			
146.7	**S2596**	**S13471**			
	C86	L610	B270	R1764	
147.2	W78		P22		
147.5	C1439				
	S2523				
151.4	L819				
155.0	R2625	L543	G1133		
155.3	S13312				
155.6	C3120				
158.0	G2200	R2657A	L1082	R2018	
	R2414	W170A	S1008	C346	G6001
158.3	V137				
158.6	S10526				
158.9	R1545	G7003	G7002	G7001	
159.4	S11062	C547	V1		
159.7	T91				
160.0	R3203				
161.1	B183				
161.9	S1046	S877A	R2833		
162.2	G54				
163.8	C742				
164.1	S2672				
164.4	C1482				
166.0	P26				
167.2	C225				
167.5	W858	R503			
169.7	S10581				
170.3	L726				
176.5	T44	R117	G5005		
180.8	S13931				
181.1	C112	R2727			
181.7	P61				

1L

Fig. 2. Rice molecular genetic map with 2,275 markers (Harushima et al. 1998) developed using 186 F$_2$ population from the cross Nipponbare × Kasalath

2S

position	Locus			
0.0	C1357			
2.5	L653	R2510		
3.3	V70			
3.6	W8A			
4.7	R2710	R1373	C673	
6.6	T89			
7.7	S910			
8.4	R2460			
11.5	S10889			
14.5	R1738B			
15.0	S14004	R810	G365	G1064
17.0	R2242S			
17.5	R459	R2403S		
17.8	G1003			
18.3	C1796			
18.6	C915			
18.9	C421			
20.8	C994	S1511		
21.9	G1327			
23.5	T83			
24.3	T82			
26.8	R2344			
27.9	C1246			
30.1	C132		C709A	
30.6	C149			
31.1	R2164	S10038B	R3086	
31.6	C365			
32.7	R394	C1654		
33.0	W113			
33.5	C630			
36.2	G227	V140		
36.7	C2168	T93	G357	
37.0	S10927S	V5B		
37.6	V5A			
39.4	S14115			
41.9	Y2724R	**R712**		
42.2	C980			
44.3	P17			
46.4	S2525			
46.7	B103	R1589		
48.6	P6	M130	G1340	R3228 P86
48.9	C626	R3191	S2577	
49.7	Y2757R	S12891		
50.5	G243	Y2757L		
52.7	S10576	C990	G1184A	C2184B
53.0	S1917			P141 P117
53.8	S2328			
54.1	R1989			
54.6	C196			
54.9	R1736			
55.4	R2975			
55.7	R1843	C106		
56.0	S14043	V141	C898	
56.5	S10847A			M162B
57.3	C777	P167		
60.3	G132			

CEN

2L

position	Locus			
77.0	S2068	S1412	P165	
77.9	G1314B			C796A
78.7	S10814	G1456		
79.0	C499	R447		
82.9	C1566			
83.7	R3041			
85.1	C621	R26	S1716	
85.4	L22			
86.8	G45	S1072		
88.4	R1424			
88.7	C953A	S12507	S10886B	
89.8	B159			
90.1	R1870B			C1061
90.4	C37	R1870A	G3004	
91.2	C436			
91.5	S11120			
92.0	S14078	R1826		
92.3	S1785			
93.4	S10641	M162A	S10582	
	R1906	S10301B		
94.2	C41			
95.0	S10844			
96.4	C645			
98.0	S2287			
98.3	C920			
99.4	R1381			
99.7	R2792	R2284		
100.2	C424	S14054	R1737	
100.7	S13984			
103.4	R2370A			
104.2	R480A			
106.1	R63	C1769	F27	
107.4	F19	S908		
107.7	G39	R427	C747	
108.0	L737			
109.4	C1236	P49		
110.8	L107			
114.9	G57	G1185	R3393	R2216
117.4	W142A			
119.6	R418	**C892**		
120.8	R3128			
122.3	C1419	T54	C520	
122.6	R2643			
123.1	C92			
125.0	S11127	B252	C601	C1408
127.7	S1911			
128.5	L629			
131.2	R685			
132.2	C370			
132.8	B241			
134.2	C932			
134.7	V4	C679	W2D	M242B
135.5	R2609			
136.1	C560			
139.3	S10030			
139.8	C253			
140.1	R759			
140.4	C1119			
141.5	R3324	G275		
143.4	C348			
143.7	R2734			
144.0	S1730	L181		
144.3	C1221			
147.3	C1137A	S11867A	L1011	R3309
	C978	R3014A	S824	
148.1	C1137B			
148.4	C440	R1560	R1521	
149.5	G7009	G1234	C379	G7015
150.0	G7011	G7017	G7014	
	G7025	R2821		
150.3	R2511	G7024	C1445	G7012
150.6	G7026			G7020
150.9	S1916	G7010	G7030	
	G7021	R208		
152.3	S10020B	G7018	G7027	
	G7022	**R1792**	G7013	
153.1	G7016	G7019	V68	C2782B
	R1496	R2702B	C1901	
154.7	C1470			

Fig. 2. (continued)

3S

position	Locus			
0.0	V80			
1.1	S10760	R1468A	M83	
	V10B	S1792		
1.6	S1714			
1.9	C515			
2.2	C567	C1173		
2.5	V10A	R1468B		
6.0	C721			
6.3	C1153	S2520		
7.4	W164	W151		
7.9	Y1065R	V134	V37A	S1554
9.3	R1713			
10.9	C1279			
11.2	C814B			
11.5	**R2247**			
12.0	S1473			
12.5	L837			
13.3	C725			
14.4	R3131			
14.8	R1811	C238		
15.2	S2514			
16.8	S13946	M168B	S13095	R265A
17.9	C25			
18.4	V131A	R518	T52	
20.3	S2769			
21.1	V53B			
21.4	C1051			
21.7	S2264			
24.2	S2344	S879		
24.5	R3212			
24.8	R461	R3361		
27.2	M61B			
27.5	R176			
27.8	C316	S12158		
28.2	R2479			
30.0	S1513			
30.5	C245	R549A		
30.8	C169	C563		
33.0	S10968			
34.4	G55	R2584		
35.8	R2015	C875	R2778	C326
36.6	S1845	R3130	S2680	
37.4	C706	R944	R2185	
38.8	R2293			
39.6	S10251			
40.1	C63	R1868		
41.7	L761	R867		
42.2	S2072	C161B	W104	
42.5	S13802			
43.3	C68			
43.6	S1524			
43.9	S1650	C614	R134	
44.2	C12534S			
46.4	C1488	C2045	R2849	
46.7	C606	R1690	C2184A	
47.2	S804			
47.5	R216	R1158	R411B	S941
48.3	G62	S1828		
48.6	R1638	P57	S14073	
	B246	R143		
49.1	R1862			
50.6	R3156			
53.0	B212			
55.5	R3126			
56.3	S11433	M127A	C269A	
57.2	R2906S			
59.6	C1135			
61.5	C198	C1452		
62.0	C582			
63.4	C730			
65.0	C603	R2387	R2170	
65.5	C12845S	C361	R1420	
	R44	R2877S		
66.3	C1186			
67.4	Y3870L			
68.8	S11493			
69.3	Y3870R	V9B		
69.8	G144			
70.1	R2982			
73.5	C1677	R663	C2942	
76.8	S1764			
80.2	S14055	V142		
83.2	C524			
84.4	T84			
86.2	C80	B196	T34	T86
87.0	R606A	S2274	V143	
	L708	R3235	R2816B	V127
87.5	C269B	S2470	M127B	
	R3239	R1908		
87.8	V8			
88.1	C816			
88.4	G332			
88.7	S10558	S10301A	M116	M148A
	G1316	C549	M163B	
89.7	C1122	W100A	R440	
90.5	L481			
91.3	C2394	S1466		
92.1	P60	P65		
95.9	S11539			
97.7	S13580			
98.2	R19			
100.1	S10742			
100.6	M183	R250		

CEN

3L

position	Locus			
111.2	V6B			
116.9	C136			
121.0	S10057A			
121.4	C746			
121.7	R2752A			
123.6	Y3335L	R654		
124.1	R2847	V30C	S10209	S10283A
	S2606	G4002	C1936	S1803
124.9	C1351			
127.6	C944			
128.1	V120	R1770	R1538	W1871A
128.4	M10	R321		
128.7	R2060			
129.0	S10087	S738	S10281	
129.4	R2690			
130.2	W821			
132.0	C1468	C5	S11669	
136.4	R689			
137.1	**C595**	**R2462**	W819	
137.8	G249			
138.9	S770			
139.2	**C1401**			
140.0	R1618	S1912	R2632	
141.1	L154	R2311	**R2628**	L119
141.4	**R2404**	R2856	**C2540**	
142.8	G1015			
143.6	S2516			
144.4	C217			
145.8	**C1329**	V7	R2443	
146.9	S13014	G232		
147.4	S13428	S11941B	R3226	
	cDbTUB30	M10D		
147.7	M143	S10656		
148.1	S759			
148.5	V6A			
149.0	C831			
150.1	S851			
150.4	R2224			
152.8	C1442	C393A	G3003	R707
153.0	C1141			
153.6	C1484	R2966	R3020	R1927
154.7	G164			
155.0	S906			
156.1	B240	R273		
156.6	S10594	C393B	R3385	
157.1	C1219			
157.6	S1866	S13262	C1164	
159.0	S11301	S1509		
159.5	W580	R1925	C1354	S1469	B203B
160.3	G1318			
161.4	V71			
165.2	S13122	T90	S1571	M40
167.2	L375			

Fig. 2. (continued)

4S

Position	Locus				
0.0	R2373				
2.8	P34				
3.1	V39	R2502			
4.8	R1854	P85			
6.6	C9B	B194B	C56	R2218	L363E
6.9	Y1065L	G1184B			
7.7	C1156	C953B			
9.4	P66	P30			
9.9	Y3635R				
10.4	C946	V28			
10.7	S2713				
11.5	G3006				
12.2	P16				
15.4	C708				
17.4	C820				
18.4	T2				
18.7	R288	C954	C10501SA		
	C1794	S2106	R2949B	P46	G124B
19.0	C933	V144	B90	S11182	V11B
	M203	G5002	S2242		
21.9	L363D				
22.6	V145	P74			
22.9	V85				
23.4	S1621	V162			
23.7	P28				
28.2	P158	G3015D			
30.4	S10983	C734	P150		
39.1	P161				
48.7	S10644				
52.8	P155				
53.1	C975	P160			
56.1	C891	C459A			
58.1	C140				
58.4	V12				
58.7	L1051				
59.1	G271				
59.5	S10628				
60.6	C2043				
62.2	R93				
62.7	R2406				
65.2	R2921				
65.5	B74				
66.6	C1087	W388	C558		
67.4	S12568	R2547	R1721	C513	
67.7	C2807				
68.0	S12448				
69.1	V135				
70.1	R2343				
70.4	C1047	W390			
71.2	L353	S1408	C76B	G7007A	
72.0	G7008				
72.3	G7004	G7006	C559	R278	V11C
72.6	G7005	S13322			
73.1	C619				
74.5	C377	S10585			
74.8	R896				
76.1	R1849	C1399			
76.7	C335				
77.0	C523A	M105	C793	R577	
	W102	T33	R2226		
78.4	M132	C1238B	W641		
81.9	R374	R3351			
82.7	L1091				
83.2	R2783	G282			
83.7	R2370B				
84.0	R3262				
85.4	L1082B	S11922			
87.3	V128	R1783	R3261	S733	
87.6	V15				
94.6	R738				
96.2	R2737				
97.6	C79	G3015C			
97.9	C1100	S13536			
100.9	S2486	R2785			
102.0	G102				
102.3	R514				
102.6	S12119				
102.9	C810	R1408B	L102		
104.4	V65				
106.7	V125				
107.6	B387	Y5212R			
108.1	G264				
108.4	G235	Y5212L	S1544	C600	
	V61	Ky4		Kyy03	
108.7	V17	V60	Kyu08	Ph	
109.0	V16				
110.1	L449	R78			
111.2	G267				
111.5	L116	B151	G177	R2376	C55
112.6	V38				
113.4	R740				
114.5	S14001	R99	C1016	T46	R634
115.9	Y3451R				
123.3	R1427				
124.2	R2231				
128.6	V121	M134			
128.9	C107	S2008			
129.2	S1890		R2184S		
129.5	C445	S1623	R3074		

4L

5S

Position	Locus				
0.0	TEL1				
3.0	C597	C263	L865	C2526	
4.6	V40	C568			
4.9	V30A	C919			
5.4	S14158	V72			
6.5	S12936	S782			
6.8	S2649	W937			
8.1	C2067				
8.4	S1548	C10501SB			
9.5	V82	S2208	S2309		
12.0	R830				
12.5	G396				
14.3	S2136				
16.3	F5002				
18.8	R3166				
19.9	R2213S				
20.4	R2693S	R1674			
20.9	R476B	R3332			
21.2	S13147				
21.5	R708				
24.5	G71				
24.8	V122				
27.5	C119	R1838	P25	R2752B	
28.9	S12447				
30.5	C859	C259B			
31.3	R2232	C1255			
36.3	W168A	S10593	Y1060L	Y1049L	
37.1	Y1060R				
38.1	P21				
40.4	C249				
40.9	V146				
43.9	C1004	R569			
44.4	S1873				
44.9	S12928				
46.0	C282A				
47.4	S14177				
48.5	S10265A	R566	R3238		
49.4	S1850B	S10091			
51.8	V64				
52.3	C309			S11168	P9
52.6	S2351	P169		P37	W39
53.4	R413	R2558		P8	
53.7	C1388	R448		P71	
	C288A	R3069	M231		
	R2059	G260	W100B		
57.5	V147				
59.7	G5004				
60.0	M141				
63.0	S2467				
63.6	R3313				
64.4	S1780				
64.7	G1458				
65.0	R372				
66.2	T20				
68.0	R1436				
70.2	G3010				
70.5	C1239				
70.8	P83	P105			
71.1	V163				
71.4	R188				
71.7	R2289				
72.5	S2692	C2269S	S811	W170B	
73.3	W653				
74.9	C1268	G366			
75.7	R2724				
77.9	R1553	C1991	R3103		
78.2	R1320	S10569			
83.2	C308				
84.8	C624	R594			
88.7	L1013				
89.5	P68	C43			
92.0	C128	R3182	C903		
92.8	W754				
92.8	C2161				
93.3	S964				
93.6	C536	C1479			
93.9	R2117A				
95.8	C1402				
97.8	C191C				
98.5	C62				
99.0	C67B	R3149S	R2249	C419	
99.9	B2H12				
100.3	C1018	C455	G81		
100.6	C336				
101.1	R317S				
101.4	L176				
101.9	S11241	R2023			
102.2	W162				
104.9	S2678	C466	L172A		
105.2	S869				
106.0	S2581	B344	S1762	S1831	
106.5	C2782A				
107.9	C246	S10693			
108.2	R3085				
108.5	S11116	C1629			
108.8	G1103				
109.1	C686				
109.4	Y2744R	R2953			
109.9	R1607	S985	S974		
110.5	R1714				
112.9	R521				
113.2	S14121				
113.7	R3134	R2754			
114.0	R1939				
114.3	S12419				
115.4	C999B	G188	C1264		
	C1447	L755	C444		
118.1	S11036				
118.4	V41	M146			
119.5	C1230				

5L

CEN

Fig. 2. (continued)

6S

Position	Locus			
0.0	V83			
0.6	V63			
0.9	V91	W160	Y617R	C1003B
1.6	Y4621L	S924		
3.0	R2869			
3.5	S10068	S2330	S10372	V81
	Y617L	C474	R560	G3012
	S1515	P140		
4.5	V84			
5.0	S2107	C952		
5.9	R3139			
7.2	C425A	C916B		
7.9	C76A	wx	S1084	
	T3	C402		
9.3	C1496	C1084	T81	
9.6	C688			
9.9	Y3637R	R1952		
10.2	V42	V30B	S10283B	
	R2967	R2291	C1368	
	R2750	R1962	R2749	
11.0	C764			
11.8	B174	C425B		
12.3	G30	R2634	C1032	
12.6	B67			
12.9	C226A	C191B	Y4622LA	
	S2069	R845		
13.2	S1434			
14.6	G165A			
14.9	B2F7	L533	C498	
15.2	S1520	G7007D		
17.4	W490	R1966	R1014B	
18.0	L1092			
18.5	R1954			
32.1	L688	R2349		
32.7	M160			
33.5	Y2587L	G200	Y4073L	R2147
35.3	S1553			
36.7	alk			
37.2	V73			
37.7	C1478			
39.6	P126	P124		
49.7	V19B			
50.4	V19A	P108	S11149	
	R2171	S913		
50.7	P123			
52.9	R1679	Y4836L		
53.2	P129	P137		Y2668LB
53.5	C235	P138	S2539	V11D
	Y4836R	P125		
54.0	P130			
54.3	Y2145L			
59.9	P127	P5		
62.2	R2123			
63.3	L136			
63.6	C999A	V74		
63.9	V96	R538	P135	P136 T85
	S881B	P3		
64.7	R688	S2570	S12672	C282B
	C574	S12926	C991	C58 C709C
	R1960	S771	R2777	R111 W475C
	C629	C294	G3009	P128 P81
	C288B	P121	P7	P69 P56
65.8	Y2686R			
66.1	S1809	S1810		
66.4	P139			
66.7	C81	R3188	R2140S	
67.5	C133A			
68.6	V148	R2654		
69.1	P31			
69.6	R32			
71.5	C60213			
71.8	G5003	L414	S793	P78
72.1	L246			
72.4	G3013			
72.7	L1047			
73.0	R437			
74.9	C214	G2028	T27	
77.4	C829	S881A		
78.8	S10555			
79.1	G122	R1519	P59	
79.9	L131			
80.2	R2955			
81.0	G1369			
81.3	R674			
83.9	S12715			
85.9	S10324A	S1437	G1314A	P72
86.7	R2266B			
88.1	W475A	T59		
88.9	R1559			
90.3	R2549	P41		
91.1	P12			
97.6	L263			
98.6	G12	S2652	C358	Y2686L C692
98.9	V86	C554	R959	
99.4	S1994	C882		
101.6	P276			
103.8	S1306S			
106.0	B284	G1091	G329	
	W129	C556	R2403	
106.3	L655			
109.3	C259C			
111.8	C962			
112.1	C1424	R2071	C2851	S947 R2395
112.4	L124			
114.3	S14023	R11	R2091	
114.6	C61277S			
115.7	C69			
117.9	R1782			
118.2	R1028			
118.5	S2760			
118.8	R1479			
120.4	R1888	R2614		
121.5	R1608	R2117B	W1077	R1394B
122.3	G342			
122.6	V20			
123.1	V43	B204B	S11239	
	C607	R1167		
124.3	P73	P144		
125.0	P98			

CEN

6L

7S

Position	Locus			
0.0	S2329			
0.8	S14082S	G50	G3011	R1744
1.9	S14164			
2.2	V108			
2.5	W112			
3.8	C261			
5.1	C615			
7.0	V44			
7.9	M61			
8.8	C2523S			
11.0	C1023B	C1057	S14083	R1561
16.1	T94			
24.1	R565	P24		
24.7	S12540			
25.0	R2829			
25.3	B2G4	S11633		
25.9	S10012			
30.8	L538T7			
35.5	V177	R2401		
36.6	M166			
41.6	R1488	R3113	P13	
42.7	R1807			
44.3	C67A	R277	V100	Rc
45.1	G338			
45.4	L627	G2009	C1023A	
46.4	C636			
46.9	L538T3			
47.4	R1582			
47.7	R46			
49.0	Y2707L	C383		
49.3	C226B	C1006	V126	G1068 P132 G3007
	C479	R610	C39	Y2707R
	R1382	C145	R568	R742B P35 C796B
49.6	S10038A			T55 Y1055L
50.1	C492	C1464		
50.4	R1788			
50.7	P120			
52.1	C735	R643A	C1226	
52.9	Y4622LB	R1233		
55.3	R430			
55.6	R1440	R606B		
56.9	R646			
60.2	G20	S1723	W8B	
61.3	R3089	V27	V103	S954
61.8	S11250	C285	L1073	
66.0	S11177	S1001		
68.2	S14048	S12288		
69.0	S10794			
69.8	C909			
70.6	C451			
72.2	C1467			
73.0	R2286	R972		
74.6	C1008	R1477A	W1	S846 S1082
74.9	S13940	C529		
75.2	C1237	T21		
75.7	C1521			
77.9	R1357	R1477B		
78.2	V93			
79.2	P82			
79.5	L585	R2394		
80.0	R1422	R1988	R2561	S2279
80.3	S10051	C437	S980	V102 C1485
80.8	S1563			
82.2	R2677	V101		
83.0	R1245	S2055		
83.8	B2F2			
88.0	S1848			
88.4	W146			
88.8	S13453			
90.7	C847			
91.0	C1412			
93.9	S2089	M228		
94.5	R3349			
95.8	V104			
97.7	R2658S	R1789	C507	
98.0	S14165	R2358	T101	
98.3	S10832			
100.2	S13161			
100.6	C1340	T87	T32	G379
101.0	T79			
104.2	C596			
113.7	C844			
114.0	R2576S	R2577S		
114.5	S14029			
115.0	S11279			
115.8	L831	C924		
116.1	C586			
116.4	S2267			
116.7	R411	V46	T31	
117.0	S1979	C213	C728	

CEN

7L

Fig. 2. (continued)

8S

Position	Locus				
0.0	S2104				
0.3	S2518				
0.6	T92				
1.4	B119				
1.7	C400	P53	R1943		
2.8	C1017		V47	S11506	
3.4	T77	T78			
12.6	G278		R3003		
13.2	C390	V48			
13.7	C770	R2007	C277		
16.2	C181	C1009			
16.5	C905		C922B		
16.8	R1010				
19.9	R2367				
24.7	R2285				
26.2	C166				
27.0	R2723	P39			
28.9	V112				
33.3	V116				
34.1	V115				
35.2	R2976				
36.3	R902	S2108	T1		
36.6	S1850A				
38.0	S1633A	S1461			
38.3	S2092				
39.4	C1121				
39.7	R2736	R544			
42.4	W154				
43.0	T19	S10038C			
44.2	G1010				
44.7	R1813				
45.1	V89A	V97			
47.0	S1633B				
47.5	C483				
48.3	S779	V106			
48.8	S10588				
49.3	C1107				
49.6	G104	R80			
52.6	R2201	C411			
53.1	B317	V150	V75	R2381	P99
	R2466	C1115	C1374		
54.2	S10543	V123	C1356		
			P63		
56.6	L457				
57.2	C347	C623			
59.4	S14109				

CEN

8L

64.7	R1394A				
65.0	P18	S13495	L411	G2132	
65.5	R727				
65.8	V114				
66.1	S10324B				
68.5	L363F				
69.9	S2014				
70.4	L128				
73.8	S815	G1073			
74.9	C929	W1871B			
76.4	W128				
76.7	S10631				
78.5	S10622				
78.8	S1609	S11114			
82.6	S2524				
82.9	P27				
84.8	L677				
86.7	G187	S11102			
88.0	M235				
88.6	R2676				
89.4	M242C	W2A			
90.2	R2382				
94.6	R202	R1985	L133		
100.4	S14074				
102.4	S10655				
103.2	C10122S				
103.6	R2118				
105.4	V130	C502			
105.9	G56				
106.2	G1149				
107.0	V133	S14003	W152		
108.9	R2027				
110.3	V129	S2314	R2662		
116.3	C153	R1880	P15		
117.0	S1570				
117.3	V49	R662			
117.6	C83	S1088	T95		
118.1	R1963	R404	C222		
118.9	S11322	S1433	V99		

Fig. 2. (continued)

9S

CEN

Position	Locus						
0.0	C152	Y2769L				W167	S831
	W470	V89B				R470	S877B
0.5	S13769						
0.8	G36	P107	V52	V151			
	G95	C711	C313	C1232			R265B
	C438	R3104	R2920	S1535			
	R2807	T35	L710	S781			
1.6	T50						
2.0	L776	S2662	G1047				
3.2	S10709						
4.6	R1164						
25.3	R1687	P104	P33				
33.5	C1454						
36.5	P162						
37.4	S2660						
38.0	G103	M148B					
38.3	C1176						
39.1	G6002	S13922	R1016				
	C2985	R79					
43.2	C397						
43.8	C873						
44.1	R41						
45.0	S752						
46.8	R742A						
48.4	S1824	S14067					
52.5	R1751						
53.9	C472						
57.4	S2074						
58.5	S2778	G385					
59.1	S1974S						
61.5	S10275	C2070	P52				
65.1	S1801	C52178S	W574	C1227			
65.4	R2272	G123					
66.2	W2B	M242A					
66.7	P88						
67.9	R2638	R2790					
69.1	G1087A	P134	V111				
70.8	R1562	C846					
71.7	W601						
72.1	C53053S	C670SA					
74.7	S1057	S14195	R3312				
77.3	C609	C2194	G2065	T58			
77.6	C662	C1720					
79.2	V109	V132					
81.1	C1263	S2655					
81.4	L442	C442					
81.7	G1085	R3330					
84.7	C985	C482					
85.0	S10578						
85.8	B163	S955					
86.9	C570						
87.2	C47						
93.0	C506						
93.3	S2275	C632					
94.1	R1683						
94.4	S10879	Y4926L	T102	L984	T4		
94.7	G293	G1445					
95.0	V50	V51					
96.1	S11302	C35S	S1456				

9L

10S

CEN

Position	Locus						
0.0	L769						
1.2	L169	S10620	S14152				
2.0	S10906	C701					
2.3	R2965						
3.1	R2946						
4.4	R2309	G7007E	G89B				
5.1	C709B						
6.4	C404						
6.9	B10	S2083	S10265B				
7.7	R1933						
9.6	S2348	R2657C					
9.9	P148						
11.0	C913A						
11.5	C1757S						
12.5	C288C						
13.5	S10020A	C1123	R2174	B46	T56		
15.1	S11777			P87			
15.4	G1125	R3014B	T29		G1128C		
15.9	C489	R2052	R2194	C8			
16.5	R1261						
17.5	C148						
18.3	L682						
18.6	G1084						
20.8	P156	P133					
21.9	S1837						
22.2	V152						
22.7	S1856	R1629	P14				
23.8	V53A	G4101					
24.3	S1786B						
25.4	P89	P44					
25.7	C961	V23					
29.8	C1166	G1082	R1738A				
	R2447	S11069					
30.3	M168A						
31.4	S10886A						
31.7	C1633						
34.6	Y1053R						
36.9	V153	Y1053L	R3320				
38.5	R3285	R3217					
40.7	C677	T53	R2084				
41.0	R2604	R2252					
41.8	C1195	C1286					
42.8	G7007C						
43.1	S10008	C814A					
43.4	C234						
43.7	C1369	T38					
44.0	R2825						
44.8	G37						
47.1	S11014						
50.5	S11148						
52.8	R1877						
53.1	G291	R2303					
53.4	S12564						
54.2	C1361						
54.5	S10019						
54.8	V76						
55.1	G4003						
57.0	V77	S10602					
58.1	G2155	S14155					
58.4	C488						
59.6	C121						
68.4	R716						
70.9	V37B						
71.2	C16	S11131					
72.3	C809						
72.6	C797						
82.9	C239	L704	C223	C371			
83.4	F15						
83.7	C405	G127					

10L

Fig. 2. (continued)

11S

Position	Locus			
0.0	TEL2B	C562	R77	
	S10264	B2E11	S1409	
0.3	G24A	V56	V57A	
	R2984	WUBA		
1.4	S1756			
1.7	C389	M18		
2.2	R2253A	C732A		
2.7	S790C			
3.5	C362B	B42		
4.0	C104B			
4.3	T28			
4.7	W120A	R1466		
5.4	SINE1_r6			
5.9	S10792	P45		
6.4	R328B	S10723	S1615	R2954
7.2	V59			
7.5	C1116B	R2104		
8.0	C294	R769A	R3202	C718
8.5	R642A	G189A	R3023	S2668
8.8	S10637B			
9.1	R1938			
10.2	C83B	R2918		
16.6	C794A			
16.9	S14061			
18.8	S10318	W118	M136B	L254
19.6	C1350	S1559		
20.1	S10616A			
25.1	V79	M136A		
28.0	C410			
28.4	C477			
30.3	C496	M234	R682	
	T40	T43		
31.3	M148C			
32.0	R2316			
32.6	L428			
33.3	S2712			
34.7	G320	F5003		
45.0	S2137			
45.5	T13			
48.6	C535	S10207		
53.9	G44	C996B	W1206	
55.5	V154	R2347B	S1915	
	R391	V24	P131	
55.8	S790A			
56.1	C3			
56.4	G7007F	L1067	P97	
56.9	R1891	V95	V155	R120
	S10616B	T14	S1736	S10563
57.2	C754			
58.0	G202			
63.8	R728	P54		
65.2	C459B			
65.9	T57			
68.0	G257			
71.6	V117			
77.1	M244			
77.4	S16073	V98		
79.6	M251	R3341	S11988	
79.9	C1172			
80.2	W1871C	V105		
83.9	P64			
85.4	P79	C1003A	V78	C189
	P101	S723		
88.7	S10928			
89.8	R2458	C50	P11	V9C
90.3	C6			
91.1	G4001			
98.4	G1465			
99.5	T45			
101.1	G376			
103.6	P76			
104.1	L833			
106.0	L468	G389	C82	
109.3	C950			
110.7	R2968	S13316S		
111.2	G181			
111.5	Y2668LA			
112.0	L1044	R251		
113.3	Y6854L	V36		
115.2	R1506			
116.1	S12886	C10150S	S1612	Y6855RA C481S
	P119	L363B	P143	
116.4	L190	S1872	G3002	
	C10295S	C105		
116.7	R543			
117.0	S10559	S10003	V55	
	V90	F12		
118.6	TEL3			

11L

12S

Position	Locus			
0.0	TEL2A			
5.5	G24B			
5.8	V57B			
6.3	R3266	F8		
7.1	G193			
7.4	R2253B			
7.9	C732B			
9.5	S790B	R3025S		
9.8	C362A	C104A	G1112	
	R328A	W120B		
10.1	R1957			
10.4	C1116A			
10.9	R769B			
11.6	S2556	G189B	C3029S	
12.3	R642B			
13.4	S10637A			
25.9	C1336			
26.5	S2572	S13913		
27.0	R1534			
30.0	R367	S2423	V9A	
	G124A	R2672	T80	
39.7	R3375	V110	G1225	P36
40.2	S10520	L363C		
40.5	G1391			
43.0	P4			
46.4	R617	V26	R2219	
46.9	R1869			
47.2	V175	P96		
47.5	S10904	L491	W161	W483
48.6	S10704	B15	W305	
	G1128B	P80		
50.8	C443	V156	V94	
	V11A	R887	C625 V3B	
51.1	S14025	Y6854R		
53.9	L1087B	P38	L363A	
56.0	S13752			
57.4	S1436	G1128A		
61.1	S826	G1184F		
63.7	G2140			
64.8	S894			
66.0	C2			
70.7	V124	S11679	M10C	T5 S1786A
71.2	C1060	G3014		
71.7	G402			
72.5	S2545	C751	C449	
72.9	S10363			
73.8	C185			
89.0	C2808			
89.9	R2708	R643B		
91.8	R1709			
95.1	C460	R2961		
95.6	C87			
95.9	S11447	G148		
97.8	C1069	C454		
100.2	S861			
101.4	W326	R2672B	L405	
103.6	L714	C996A	G1406	
104.7	S10074			
107.2	R1684		R2501B	
108.0	R496	R1759	S1830	R1552
108.8	G1106	R2292	C930	S13561 L405B
109.3	S11076			
109.8	Y2824R		S1016	
110.1	C901	V58	B203A	R2706A

12L

CEN

Fig. 2. (continued)

indicate the relative position of the gene with respect to other genes in the genome. The second genetic map constructed by the Japanese group (Harushima et al. 1998) is shown in Figs. 2 and 3. The information derived from these maps have become indispensable in gene cloning by providing clues for the tagging of target phenotypes. As in the case of cloning a gene, *Xa21*, for resistance against bacterial blight disease (Song et al. 1995) many mapped cDNAs provided a pivotal point for hitting the target gene. The latest high-density genetic map constructed by the Japanese team consists of 3,267 DNA markers including RFLP, simple sequence repeat (SSR), single-strand conformational polymorphism (SSCP), and cleaved amplified polymorphic sequence (CAPS) markers (http://rgp.dna.affrc.go.jp/publicdata/geneticmap2000/index.html). About 70% of these markers were derived from rice and the remaining from other cereals such as barley, wheat, and maize.

Other genetic maps constructed for rice consist mainly of RFLP markers (Causse et al. 1994; Xiong et al. 1997; Cho et al. 1998). Among them, the map constructed by a team at Cornell University is unique in aiming at the development of universal DNA markers applicable to important cereal crops, such as maize, barley, and oat, other than rice (Causse et al. 1994). This idea was brought about by the fact that there exists colinearity of gene order among these cereals, which belong to the grass family Poaceae. These species diverged from a common ancestor about 60 to 70 million years ago, and current descendants still share common ancestral characteristics in their genomes (Kellog 2001). This collinearity in genetic traits, called synteny among grasses, has been fundamental in rationalizing rice as a model or reference plant among the grass species. In addition, it has the smallest genome size and is one of the most well and deeply studied cereal crops. The rice genetic map by Cornell University carries such DNA markers as to show a distinct hybridization pattern with other major cereals.

The latest genetic map uses markers easily reproduced by polymerase chain reaction (PCR). This characteristic is prerequisite, not only for basic research, but also for the practical application of markers to breeding, such as easy selection of siblings with preferable traits once identified by DNA markers or by the gene itself. RFLP markers are the most accurate because of their codominancy, but it is very tedious and costly to perform Southern hybridization to detect

Fig. 3. Rice varieties (*left*: Nipponbare, *right*: Kasalath)

RFLP. On the other hand, PCR is relatively cheaper and easy to use once specific primers for amplification are developed. For satisfying this demand, the best marker, the simple sequence repeat (SSR) marker, has been generated since the beginning of molecular genetic analysis. However, the discovery of Class I SSR, which is less than 20 nucleotides long and shows polymorphism among rice cultivars, requires several laborious steps. Very recently, high-quality genome sequences of japonica cultivar Nipponbare (Feng et al. 2002; Sasaki et al. 2002b; Rice Chromosome 10 sequencing Consortium 2003), a draft sequence of indica cultivar 93-11 (Yu et al. 2002a), and BAC end sequences of indica cultivar Kasalath (Katagiri et al. 2004) have become publicly available. This information offers the opportunity to survey SSRs all over the rice genome and to discover effective SSRs closely linked to target phenotypes. The most extensive and detailed genetic map using SSRs as markers was published in 2002 (McCouch et al. 2002). This map should be useful for map-based cloning and marker-based breeding in the near future.

The molecular genetic map is a required tool for changing the strategy of genetic analysis and breeding of important crops, especially self-pollinating ones.

In the case of corn, which is also a very important cereal crop with a wide range of uses in both industry and agriculture, genetic mapping has been pursued by several private companies. However, these maps have been mainly for in-house use, and the public sector has been rendered to undertake redundant efforts to construct publicly available genetic maps. The University of Missouri-Columbia coordinates the efforts of the public sector and publishes a map with 1,736 loci, including 1,156 loci probed by cDNAs (Davis et al. 1999). Genetic maps have also been constructed for other cereal crops such as wheat (Qi et al. 2004a), barley (Kleinhofs 2004), sorghum (Bowers et al. 2003), and pearl millet (Qi et al. 2004b) by utilizing previously established DNA markers from rice and other crops. For example, wheat and barley are closely related species and DNA markers of both could be shared for mapping. A similar situation is found in the case of mapping of sorghum by using maize DNA markers as a common tool. The detailed genetic map information with images of polymorphisms is now available through Internet databases such as GrainGenes (http://wheat.pw.usda.gov) or Gramene (http://www.gramene.org).

Sharing DNA markers derived from cDNAs of each target cereal species could reveal the existence of significant remnants of ancestral genome structure (Ahn and Tanksley 1993; Moore et al. 1993). The family Poaceae is thought to have diverged about 60 to 70 million years ago from an ancestor common to many current grass species (Kellog 2001). During this long period, each species evolved to adapt to each habitat under natural and, in the case of cultivated species, artificial selection pressures. Molecular genetic analysis using expressed genes as tools for mapping could prove the existence of their common ancestry, although they have undergone different evolutionary pathways. The existence of colinearity among the grass species could be very useful in clarifying the existence of one gene in other species with a syntenous genome structure. For example, the orthologous genes of a waxy gene on rice chromosome 6 are found on chromosome 9 of maize and chromosome 7 of wheat, which are proved to be a syntenic part of these genomes (Devos and Gale 1997). This was first shown in 1993 based on genetic mapping or recombination events. Subsequently, many researchers have sought to apply synteny to tag the phenotype or to isolate the gene in wheat, barley, or maize corresponding to a similar phenotype in rice. The success or failure of this strategy is highly dependent on the existence

of true synteny at the target genomic region and the extent of saturation of DNA markers in the genetic map used for analysis. Recent progress in genome sequencing of the whole rice genome and partial maize genome enables detailed evaluation of synteny between them (Lai et al. 2004). Also, genome sequences of a limited genomic area of sorghum (Draye et al. 2001), barley (Dubcovsky et al. 2001; Caldwell et al. 2004), and wheat (Feuillet and Keller 1999) were used for this evaluation. As a result, microlevel synteny based on sequence comparison is in most cases not valid because of rearrangement, insertion by transposable element, or translocation to other chromosomes (Bennetzsen and Ma 2003). However, synteny observed by mapping of orthologous genes is still very important for interpreting the evolution of Poaceae and to understand microlevel synteny as a clue for research.

1.3
Molecular Mapping of Simple and Complex Traits in Rice

In comparison to the classical morphological markers and isozymes, DNA markers are now becoming an essential tool for genetic investigations because of ability to generate and track an unlimited number of loci that can be linked to any trait of interest. Aside from RFLP, a variety of DNA markers such as RAPD (Williams et al. 1990), SSR (Litt and Lutty 1989), sequence tagged sites (STS) (Olson et al. 1989), sequence characterized amplified region (SCAR) (Martin et al. 1991), CAPS (Koniecyzn and Asubel 1993), and AFLP (Vos et al. 1995) have been developed. Unlike RFLP, most of these recently developed markers are PCR-based with simplified protocols and require minute quantities of DNA. However, the dominant nature of some PCR markers like RAPD and AFLP makes distinguishing homozygotes from heterozygotes difficult. Currently, SSR markers are the most preferred class of markers for marker-assisted selection (MAS) because of its codominant nature, simpler protocols, abundance, and higher level of polymorphism. Due to the availability of considerable amounts of sequence data, single nucleotide polymorphism (SNP) (Brookes 1999) is gaining momentum as an excellent tool to navigate the genome due to its simplicity, abundance, and amenability for automation. A number of softwares such as Mapmaker/QTL (Lincoln et al. 1992), Qgene (Nelson 1997), QTL map-

per (Wang et al. 1999a), QTL Cartographer (Basten et al. 2001), PLABQTL (Utz and Melchinger 1996), and MQTL (Tinker and Mather 1995) have been developed to detect quantitative trait loci (QTL). DNA markers and their usefulness in crop improvement have been widely reviewed (Paterson et al. 1991; Burrow and Blake 1998; Brar 2002; Subudhi and Nguyen 2004). In this section, we will provide an update on molecular marker utilization to investigate both simple and complex traits in rice.

1.3.1
Disease Resistance

The advent of molecular markers greatly facilitated genetic analysis of disease resistance genes in rice. In the case of rice blast (*Magnaporthe grisea*) and bacterial leaf blight (BLB) (*Xanthomonas oryzae* pv. *oryzae*), a large number of major genes had been earlier identified by classical genetic studies and thus targeted for mapping investigations using a variety of marker systems and approaches. Besides blast and bacterial leaf blight, sheath blight caused by *Rhizoctonia solani* Kühn also limits rice productivity significantly. Few reports are available on the mapping of genes responsible for resistance to sheath blight, rice yellow mottle virus, stem rot, bacterial leaf streak, rice stripe disease, and rice tungro virus.

In most of these studies, either near isogenic lines (NIL) or bulked segregant approach (BSA) (Michelmore et al. 1991) was extensively preferred for identifying markers linked to the resistant genes. Additionally, segregating populations were developed from crosses involving resistant and susceptible cultivars to develop closely linked markers for MAS. Most of the BLB resistance genes are major genes, and though a similar trend was followed for some time to analyze blast resistance, more emphasis is currently given to identify QTL for partial resistance that, in combination with major genes, can improve the durability of resistance. The candidate-gene approach is also demonstrated as an efficient way of mapping resistance genes or resistance QTL in rice (Wang Z et al. 2001).

Bacterial Leaf Blight
To date, more than 20 resistance genes against various strains of *Xanthomonas oryzae* pv. *Oryzae* (*Xoo*) have been identified. Many of those genes have been assigned to rice chromosomes using molecular mark-

ers (Table 3). Mapping of these genes facilitated pyramiding of a number of genes through MAS providing a higher degree of resistance in rice-breeding programs. Physical mapping, cloning, and characterization of resistant genes were also possible in many cases. Despite the clustering of several bacterial blight resistance genes on chromosome 11 along with some blast resistance genes, clear identity of those genes has been demonstrated by their unique location on the rice chromosomes. Other BLB resistance genes were mapped on rice chromosomes 4, 5, 6, and 8. To date, three BLB resistance genes, *Xa-1*, *Xa-21*, and *Xa-26*, have been isolated by map-based cloning (Song et al. 1995; Yoshimura et al. 1998; Sun et al. 2004). High-resolution genetic maps of the *Xa-7-* and *Xa-27(t)-* carrying genomic regions have been constructed to expedite cloning of these genes (Porter et al. 2003; Gu et al. 2004). Considerable progress has been made in the physical mapping of BLB resistance genes: *Xa-4* (Wang W et al. 2001; Sun et al. 2003), *xa-5* (Yang et al. 1998; Blair et al. 2003), *xa-13* (Sanchez et al. 1999), and *Xa-22 (t)* (Wang et al. 2003a). BAC clones carrying these resistant genes have been identified or candidate genes have been identified from sequence information obtained from the target BAC clone.

Though most of the genes discussed above involve qualitative resistance, quantitative component of resistance was also investigated (Li et al. 1999, 2001c). Using NIL sets for four BLB genes, *Xa-4*, *xa-5*, *xa-13*, and *Xa-21*, Li et al. (2001a) demonstrated that a qualitative component of the resistance genes is reflected by their large effects against corresponding avirulent *Xoo* races and the quantitative component is their residual effect against corresponding virulent races and their epistatic effects. Another study by Li et al. (1999a) involving a recombinant inbred line (RIL) population from a cross Teqing × Lemont and three strains of *Xoo*, CR4, CR6, CXO 8, revealed several QTL for resistance, and interestingly a major gene, *Xa-4*, was mapped onto chromosome 11. Lemont was susceptible to all three strains while Teqing was resistant to CR4 and CX8 but susceptible to CR6. Teqing allele at the *Xa-4* locus behaved like a dominant resistance gene against CR4 and CXO8.

Blast
More than 40 major blast resistance genes have been identified and deployed in rice-breeding programs. Despite considerable progress in the mapping and identification of a number of blast resistance genes

Table 3. Molecular marker facilitated tagging of disease resistance genes in rice

Gene	Source of resistance	Linked markers	Chromo-some	Reference
Bacterial blight				
Xa-1	Kogyoku	XNpb 235	4	Yoshimura et al. 1996
Xa-3	Chugoku45	XNpb 181	11	Yoshimura et al. 1995
Xa-4	IR20	XNpb 181	11	Yoshimura et al. 1995
xa-5	IR1545-339	RG 556	5	Yoshimura et al. 1995
Xa-7	IRBB7	M5	6	Porter et al. 2003
Xa-10	IRBB10	CDO365	11	Yoshimura et al. 1995
xa-13	IR66699-5-5-4-2	RG 136	8	Zhang et al. 1996a
Xa-21	*O. longistaminata*	RG 103	11	Ronald et al. 1992
Xa-22(t)	Zhachanglong	R1506	11	Lin et al. 1996b
Xa-26(t)	Minghui 63	R1506, M224	11	Yang et al. 2003
Xa-27(t)	*O. minuta*	M631/M1230	6	Gu et al. 2004
Blast				
Pi-1	LAC23	RZ536	11	Yu et al. 1996
Pi-2	5173	RG64	6	Yu et al. 1991
Pi-4	Tetep	RG869	12	Yu et al. 1991
Pi-5	Moroberekan	RG498	4	Wang et al. 1994
Pi-7	Moroberekan	RG16, G103A	11	Wang et al. 1994
Pi-10	Tongil	RRF6, RRH18	5	Naqvi et al. 1995
Pi-11 (Pi-zh)	Zhai-Ye-Qing 8	BP127	8	Zhu et al. 1993
Pi-12	Hong-jiao-zhan	RG869	12	Zheng et al. 1996
Pi-18	Suweon 365	RZ536	11	Ahn et al. 2000
Pi-20	IR24	XNpb88	12	Imbe et al. 1997
Pi-21	Owarihatamochi	G271/G317	4	Fukuoka and Okuno 2001
Pi-24(t)	Azucena	K5	1	Sallaud et al. 2003
Pi-25(t)	IR64	RG520	2	Sallaud et al. 2003
Pi-26(t)	Azucena	RG313	5	Sallaud et al. 2003
Pi-27(t)	IR64	Est-2	6	Sallaud et al. 2003
Pi-28(t)	Azucena	RZ617/RGA-IR86	8	Sallaud et al. 2003
Pi-29(t)	IR64	RZ500	10	Sallaud et al. 2003
Pi-30(t)	IR64	OpZ11-f RGA-IR14	11	Sallaud et al. 2003
Pi-31(t)	IR64	O10-800	12	Sallaud et al. 2003
Pi-32(t)	IR64	AF6	12	Sallaud et al. 2003
Pi-33	IR64, Bala	R1813	8	Berruyer et al. 2003
Pi-44	RIL276 from Co 39 × Moroberekan	CDO520	11	Chen et al. 1999
Pi-b	Tjahaja	RZ213	2	Miyamoto et al. 1996
Pi-ta, Pi-ta^2	Tadukan	XNpb088	12	Rybka et al. 1997
Pi-k^m	Tsuyuake	G181	11	Kaji and Ogawa 1996
Pi-ar	SC09	OPK17	–	deAraújo et al. 2004
Pi-g(t)	Guangchangzhan	RM166, RM208	2	Zhou et al. 2004
Leaf and neck blast				
Pi-24(t)	Zhong 156	RGA3$_{620}$	12	Zhuang et al. 2002
Pi-25(t)	Gumei 2	RGA7$_{470}$	6	Zhuang et al. 2002
Panicle blast				
Pb-1	–	CDO 226	11	Fuji et al. 1995
Rice Stripe disease				
Stv-b(i)	Modan	ST10, XNpb257	11	Hayano-Saito et al. 1998

Table 3. (continued)

Gene	Source of resistance	Linked markers	Chromosome	Reference
Rice Tungro Spherical virus				
RTSV	ARC11554	RZ262	4	Sebastian et al. 1996
Yellow mottle virus				
RYMV	Azucena	RG341/RG869	12	Ghesquiere et al. 1997

(Table 3), only two such resistance genes, *Pi-b* and *Pi-ta*, have been isolated and characterized (Wang et al. 1999b; Bryan et al. 2000). Due to the mapping of several blast resistance genes to the same rice genomic region, there is confusion regarding the identity of those genes. For example, the allelic relationship between *Pi-4* and *Pi-ta* is not clear (Inukai et al. 1994). Rybka et al. (1997) could not separate the genes *Pi-ta* and *Pi-ta2* even in a large mapping population. Physical mapping is now revealing information on the allelic nature of these genes. A physical mapping study by Jeon et al. (2003) suggested that *Pi-3(t)* and *Pi-5(t)* are the same resistance gene. Similarly, a detailed physical map of the *Pi-2(t)* (Jiang and Wang 2002; Liu et al. 2002a) revealed that *Pi-9(t)* and *Pi-2(t)* are either allelic or tightly linked.

The first comprehensive QTL mapping study by Wang GL et al. (1994) elucidated the number and nature of blast resistance genes. Ten QTLs conferring partial resistance based on the number of lesions, lesion size, or the diseased leaf area (DLA) were mapped in an RIL population developed from a cross Moroberekan (with durable resistance) × Co 39 (susceptible). Two dominant blast resistance genes, *Pi-5(t)* and *Pi-7(t)*, were associated with these QTLs for partial resistance. The researchers' data suggest that stable resistance in Moroberekan is due to the combination of genes conferring both partial and complete resistance, and some QTL for partial resistance may be alleles of the major resistant loci. Since then, QTLs for field resistance to blast have been identified using several different mapping populations (Fukuoka and Okuno 2001; Sirithunya et al. 2002; Tabien et al. 2002; Sallaud et al. 2003; Talukder et al. 2004; Wu et al. 2004). Sallaud et al. (2003) mapped nine unlinked loci [*Pi-24(t)* to *Pi-32(t)*] in a double haploid (DH) population of the cross IR64 × Azucena. A major gene conferring partial resistance against leaf blast has been demonstrated (Zenbayashi et al. 2002).

Several conclusions can be drawn from these mapping studies involving blast and bacterial blight resistance. (1) Although breeding blast-resistant cultivars through deployment of these major resistance genes is simple, the major limitation is the lack of durability, which can be improved by pyramiding of multiple resistance genes. Both QTL and major genes are required for durable resistance. (2) Since many of these QTL were localized in the vicinity of many major resistance genes, it reinforces the hypothesis that QTL and major genes are probably different alleles of the same loci (Robertson 1985). (3) Partial resistance genes might be defeated major genes with residual effectiveness and race specificity.

Sheath Blight

The major obstacle in breeding rice cultivars resistant to sheath blight disease is lack of resistance sources. Quantitatively inherited resistance can be helpful in protecting rice crop from this disease in field conditions. The first QTL study was conducted by Li et al. (1995a) in an F_4 bulked population from a cross between the susceptible variety "Lemont" and the resistant variety "Teqing". Six QTLs contributing to resistance were located on six chromosomes and collectively explained approximately 47% of the phenotypic variation. Except for one QTL (*QSbr4a*), which accounted for 6% of the genotypic variation, the other five putative resistance loci (*QSbr2a, QSbr3a, QSbr8a, QSbr9a*, and *QSbr12a*) colocalized with QTLs for morphological traits.

Zou et al. (2000) used an F_2 clonal population of another cross Jasmine 85 × Lemont and, based on field disease evaluations for 2 years, six QTLs, *qSB-2, qSB-3, qSB-7, qSB-9-1, qSB-9-2*, and *qSB-11*, were located on chromosomes 2, 3, 7, 9, and 11, respectively. The QTLs *qSB-2, qSB-3, qSB-7*, and *qSB-9-2* from Jasmine 85 explained 21.2%, 26.5%, 22.2%, and 10.1% of the total

phenotypic variation, respectively; while *qSB-9-1* and *qSB-11* from Lemont were responsible for 9.8% and 31.2% of the total phenotypic variation. Contrary to the observation of Li et al. (1995a), this study did not demonstrate any linkage of detected resistance loci to the loci for heading date or plant height. A dominant sheath blight resistant gene *Rsb1,* carried by a transgenic cultivar "4011," was mapped recently on rice chromosome 5 near RM39 (Che et al. 2003).

Rice Yellow Mottle Virus , Stem Rot , Bacterial Leaf Streak , and Rice Stripe Disease

Ghesquiere et al. (1997) identified a QTL for rice yellow mottle virus (RYMV) resistance on chromosome 12 in two DH rice populations developed from crosses IR64 × Azucena and IRAT177 x Apura, and it corresponded to regions known to harbor major blast resistance genes. In the former DH population, Albar et al. (1998) detected 15 QTLs for RYMV resistance on seven chromosomes, and most of the resistant QTL alleles were from the resistant parent "Azucena". Resistance was correlated to plant morphology. There was one QTL of resistance on chromosome 12 independent of plant morphology that interacted with a QTL on chromosome 7 to control the virus content (Pressoir et al. 1998).

A selective genotyping approach was used to map two loci for resistance to stem rot (*Sclerotium oryzae*) in populations developed from the crosses between an *O. rufipogon* derived resistant line and susceptible line (Ni et al. 2001). These two loci on chromosome 2 (AFLP marker TAA/GTA167 and near RZ166 and RG139) and chromosome 3 (near RM232) jointly explained 50% of the phenotypic variation.

Using both F_2 and RIL population tested over 2 years, Tang et al. (2000) mapped 11 QTLs conferring resistance to bacterial leaf streak on six chromosomes. Six of the QTLs were detected in both seasons. Five QTLs with the largest effects were significant in both seasons. The detected QTLs explained 85% of the genetic variation in 1997. Bulked segregant analysis of the extremes of the F_2 population identified three QTLs of large effect.

Graphical genotyping and linkage analyses with molecular markers were used by Hayano-Saito et al. (1998), who determined the chromosomal location of the rice stripe disease resistance gene *Stvb(i)* from indica rice cv "Modan" on chromosome 11 between XNpb220 and XNpb257/XNpb254. A tightly linked marker, ST10, was developed on the basis of the results of RAPD analysis for MAS. Hayano-Saito et al. (2000) also physically mapped *Stvb(i)* in an approximately 286-kb region covering two overlapping BAC clones.

1.3.2
Insect Resistance

Major insect pests of rice include gall midge, stem borer, brown plant hopper, and green leafhopper. While many of these insects damage rice crop by feeding others, particularly leafhoppers, act as vectors of many viruses, spreading viral diseases in rice crop. Host plant resistance is an ideal and environmentally friendly approach to lessening the damage to rice crop, and a large number of germplasms with resistance to various insect species have been identified accordingly, and inheritance of insect resistance has been elucidated (Khush and Brar 1991).

Progress has been made in breeding for resistance to gall midge and different plant and leafhoppers (Table 4). For stem borer, a damaging pest in most rice-growing areas of the world, a resistance source has been rare in available germplasms. Selvi et al. (2002), however, identified RAPD markers $K6_{695}$ and $AH5_{660}$ linked to yellow stem borer resistance at distances of 12.8 cM and 14.9 cM, respectively, using BSA.

Tan et al. (2004) mapped two white-backed plant hopper (WBPH) resistant genes, which are the same as *Qbp1* and *Qbp2* genes for brown plant hopper resistance in an RIL population from B5 × Minghui 63. Of the two WBPH resistance genes, one designated as *Wbph7(t)* was located within a 1.1-cM region between R1925 and G1318 on chromosome 3, and the other designated as *Wbph8(t)* was within a 0.3 cM region flanked by R288 and S11182 on chromosome 4. Yamasaki et al. (1999) used ovicidal response as a criterion for resistance against WBPH and mapped the traits, percentage of watery lesions (PWL), and WBPH egg mortality (EM) in an RIL population developed from a cross of japonica cultivar Asominori and indica cultivar IR24. Out of a total of 10 QTLs for ovicidal response, QTL on chromosome 6 (R1954-L688) was most significantly associated with the ovicidal response and accounted for 69.9% of phenotypic variance for PWL and 46% of phenotypic variance for EM.

The first green leafhopper mapping study by Sebastian et al. (1996) revealed a dominant gene conferring resistance to GLH and RTSV located within 5.5 cM of RFLP marker RZ 262 on rice chromosome 4.

Table 4. Molecular-marker-facilitated tagging of insect resistance genes in rice

Resistance Gene	Source	Linked markers	Chromosome	Reference
Gall midge				
Gm2	Phalguna, ARC6650	RG329, RG476	4	Mohan et al. 1994
Gm4(t)	Abhaya	R1813	8	Mohan et al. 1997b
Gm6(t)	Duokong #1	RG214	4	Katiyar et al. 2001a
Brown plant hopper				
Bph1	Mudgo	XNpb248, em5814N, em2802N	12L	Hirabayashi and Ogawa 1995; Sharma et al. 2003
Bph?	IR64	Sdh-1, RG463	12	Huang et al. 1997b
Bph10	*O. australiensis*	RG457	12	Ishii et al. 1994
Bph (t)	Sanguizhan	RZ404	9	Mei et al. 1996
bph2	IR1154-243	KAM4	12L	Murata et al. 1998; Murai et al. 2001
Bph9	Pokkali	OPR04	12L	Murata et al. 2000
White backed plant hopper				
Wbph-1	–	RG146B	7	McCouch et al. 1991
Wbph?	Asominori	R1954, L668	6	Yamasaki et al. 1999
Wbph?	IR64	RG103, RG167	11	Kadirvel et al. 1999
Wbph7(t)	B5	R1925, G1318	3	Tan et al. 2004
Wbph8(t)	B5	R288, S11182	4	Tan et al. 2004
Green leaf hopper and Green rice leaf hopper				
GLH	ARC11554	RZ262	4	Sebastian et al. 1996
Glh$_{Ib1}$	Ptb8	OPA19$_{320}$	–	Padmavathi et al. 2001
Grlp3/Grh4	DV85 Norin PL-6	XNpb144	3	Yasui and Yoshimura 1999 Fukuta et al. 1998
Grlp11/Grh2	DV85 Norin PL-6	G1465	11	Yasui and Yoshimura 1999 Fukuta et al. 1998
Grh1	IR24 Norin PL2	C309 R566	5 5	Yasui and Yoshimura 1999 Tamura et al. 1999

Padmavathi et al. (2001) mapped a gene *glh$_{Ib1}$* of Ptb8 conferring resistance to the Indian biotype of green leafhopper using RAPD markers. The resistance locus was closely linked with three QTLs controlling total tiller number, effective tiller number, and 100-grain weight. Wang et al. (2004) recently conducted a QTL mapping study of antibiosis to green leafhopper in an RIL population of rice developed from the cross Taichung65 (susceptible) × ARC10313 (resistant) and identified four QTLs on chromosomes 3, 5, 11, and 12. Two major QTLs on chromosomes 3 and 11 explained 25.3% and 56.8% of phenotypic variance, respectively, and were localized close to two green rice leafhopper (*Nephotettix cincticeps*) resistance genes,

Grh4 and *Grh2*, mapped earlier onto the same position using different sources (Fukuta et al. 1998; Yasui and Yoshimura 1999).

Thirteen biotypes of gall midge (*Orseolia oryzae* Wood-Mason) and seven gall midge resistance genes are reported in the literature (Sardesai et al. 2001). Despite the emergence of new biotypes, the development of resistant varieties has been possible due to the involvement of a single dominant gene in most cases and the identification of resistance sources among available germplasms. Several *Gm* genes have been tagged with molecular markers (Table 4), and MAS is practiced in some cases (Sardesai et al. 2001). *Gm2* is the first example of a mapped gall midge resistant

gene from "Phalguna" on chromosome 4 that confers resistance to biotypes 1, 2, and 5 (Mohan et al. 1994). Another gall midge resistant gene *Gm4(t)* from the source "Abhaya" was mapped on chromosome 8 (Mohan et al. 1997b). Katiyar et al. (2001a) mapped *Gm6(t)* from Chinese rice cultivar Duokang # 1, which confers resistance against four biotypes of Asian rice gall midge in China on chromosome 4, and demonstrated that it is nonallelic to *Gm2* located in its vicinity (Mohan et al. 1994). *Gm6(t)* was later pyramided with *Gm2* by traditional breeding (Katiyar et al. 2001b). YAC and BAC clones encompassing the genes have been identified as being able to clone *Gm2* and *Gm6(t)* (Rajyashri et al. 1998; Katiyar et al. 2001a).

At least 12 major brown plant hopper (BPH) (*Nilaparvata lugens* Stål) resistance genes have been identified in indica rice cultivars and two wild species of rice, *O. australiensis* and *O. officinalis*. *Bph10* from *O. australiensis* was mapped onto chromosome 12 (Ishii et al. 1994). Jena et al. (2003) and Renganayaki et al. (2002) mapped two BPH genes resistant to an Indian biotype and biotype 4 onto chromosomes 11 and 3, respectively, using lines with resistance genes introgressed from *O. officinalis*. In both cases, BSA was used in conjunction with RAPD markers. One of these genes was designated *Bph13 (t)* (Renganayaki et al. 2002). *Bph1* locus was mapped onto chromosome 12 at a distance of 10.7 cM from XNpb248 (Hirabayashi and Ogawa 1995) and mapped near *bph2* (Murata et al. 1998). A high-resolution map of this region (Murai et al. 2001) demonstrated that *Bph1* and *bph2* were nonallelic, and an AFLP marker KAM4 completely cosegregated with *bph2*. Four additional genes, *bph4, bph9, bph11(t)*, and *bph12(t)*, have been mapped onto chromosomes 6, 12, 3, and 4, respectively (Hirabayashi et al. 1998, 1999; Murata et al. 2000; Kawaguchi et al. 2001).

The quantitative nature of resistance to BPH has been demonstrated in a number of studies (Alam and Cohen 1998; Yamasaki et al. 2000; Huang et al. 2001; Xu XF et al. 2002). Alam and Cohen (1998) first reported the mapping of seven QTLs associated with resistance to two Philippine BPH populations in a DH population developed from the cross IR64 × Azucena. These QTLs are located on chromosomes 1, 2, 3, 4, 6, and 8 and individually accounted for 5.1 to 16.6% of the phenotypic variance. Most of these QTLs were derived from IR64 and conferred a relatively durable resistance under field conditions. Yamasaki et al. (2000) used an RIL population derived from a cross between a japonica variety Asominori with ovicidal response

and an indica variety IR24 without ovicidal response and detected two QTLs each on 1L and 6S for both grades of watery lesions (GWL) and egg mortality (EM). The 6S QTL explained 72.1% and 85.1% of the phenotypic variance for GWL and EM, respectively. The QTL on 1L explained 19.8% and 17.8% of the phenotypic variations for GWL and EM, respectively. Both alleles from Asominori increased GWL and EM. The Asominori allele at the 6S QTL was essential for the ovicidal response to BPH, and the Asominori allele at the 1L QTL could increase the EM of BPH in the presence of the Asominori allele at the 6S QTL. Two RFLP loci, R1954 linked to 6S QTL and C112 linked to 1L QTL, can be used for MAS. Using F_3 families from a B5 × Minghui 63 cross, Huang et al. (2001) identified two QTLs on chromosome 3 (*Qbp1*) and chromosome 4 (*Qbp2*) for BPH resistance that explained 26.4% and 14.3% of the phenotypic variation and are different from at least nine of the ten previously identified BPH resistance genes. Xu et al. (2002b) mapped seven main-effect QTLs and many epistatic QTL pairs onto 12 rice chromosomes in an RIL population from the Lemont × Teqing cross. The main-effect and epistatic QTLs together accounted for more than 70% of the total phenotypic variation in damage scores. Teqing contributed the resistance allele at four main-effect QTLs, and the Lemont allele resulted in resistance at the other three. The Teqing allele controlling leaf and stem pubescence was associated with resistance, while the Lemont allele for glabrous stem and leaves was associated with susceptibility, indicating that this gene might have contributed to resistance through antixenosis. These studies revealed that there are many major genes and QTLs on the rice genome that are conferring BPH resistance and should be pyramided to provide durable resistance to this pest.

1.3.3
Traits Relevant for Hybrid Rice Breeding

Development of semidwarf rice varieties beginning in the early 1960s was a significant accomplishment in improving rice productivity in all rice-growing areas. With growing demand for more food for the increasing world population, new strategies need to be developed to further elevate the stagnant rice productivity plateau. Exploitation of heterosis is one such strategy that has been demonstrated well in China to shift the yield ceiling beyond the level of current semidwarf rice cultivars (Yuan et al. 1994). Although yield advantage of hybrids is about 20% over the inbred va-

rieties, the high cost of hybrid seeds is reducing the profit margins of rice farmers. Many challenges and opportunities still exist to reap the benefits of this technology in rice. A number of genetic tools, such as cytoplasmic genetic male sterility, environment-sensitive genic male sterility, and wide compatibility, are being employed and refined to facilitate hybrid rice breeding. Significant progress has been made on molecular marker utilization to accelerate hybrid rice breeding (Table 5).

CMS-Fertility Restoration

Cytoplasmic genetic male sterility (CMS) is the most effective male sterility system to produce hybrid seeds in rice (Virmani 1996). Despite the discovery of numerous CMS systems, the wild-abortive (WA) CMS is still commonly used in commercial rice hybrids because it gives stable CMS lines for which fertility restorers are available in abundance (Virmani 1999). The usefulness of other CMS systems (CMS-TN, CMS-MS577, CMS-*O. perrenis*, CMS-O. *glumaepetula*) has been limited because of the nonavailability of restorer lines in developing rice hybrids. Reports regarding the number, chromosome location, and effects of fertility restorer genes are conflicting. Bharaj et al. (1995), from a trisomic analysis, reported the involvement of two *Rf* loci on chromosomes 7 and 10. Using a population from the cross of two isogenic lines, Zhang et al. (1997) mapped one locus *Rf3* on chromosome 1. Another study by Yao et al. (1997) using an F_2 population of a cross between Zhengshan 97A and Minghui 63 identified two loci on chromosomes 1 and 10, and the locus on chromosome 1 was the same as that reported by Zhang et al. (1997). Since then, Ichikawa et al. (1997) mapped a restorer locus for BT-CMS system in a region near by, but further investigation is needed to clarify whether it is same as or different from that of Yao et al. (1997). Tan et al. (1998) used QTL strategy to map two restorer loci on chromosome 10, of which one QTL linked to marker C1361 explained 71.5% of the phenotypic variance, and the second QTL located between RFLP makers R2309 and RG257 explained 27.3% of the phenotypic variance. As many as four loci on chromosomes 2, 3, 4, and 5 were associated with fertility-restoring ability to WA cytoplasm (Zhu et al. 1996). Liu et al. (2004b) studied a novel type of gametophytic CMS system, called Honglian CMS (CMS-HL) used in hybrid rice production in China, and mapped two fertility restorer loci, *Rf5* and *Rf6(t),* on chromosome 10. A rice nuclear gene *Rf-1* was re-

cently fine mapped and has been subsequently cloned (Komori et al. 2003, 2004).

Environmental Genetic Male Sterility

Commercial production of hybrid seeds in CMS systems is cumbersome due to the involvement of three different lines, A (male sterile), B (maintainer), and R (fertility restorer) lines. Moreover, application of a CMS system is limited in germplasm in which maintainer and restorers are scarce. Due to the discovery of nuclear sterility factors that are regulated by environmental factors, viz., temperature and/or photoperiod, simplification of hybrid seed production is now possible. Several temperature-sensitive genetic male sterile (TGMS) lines have been developed through irradiation (Virmani 1996). These mutants are male sterile under high temperature but revert to partial to full fertility under low-temperature conditions. In all TGMS lines, male sterility is controlled by a single recessive gene (Virmani 1999). So far, seven TGMS genes have been mapped on rice chromosomes 8, 7, 6, 2, 9 (Wang et al. 1995; Subudhi et al. 1997; Yamaguchi et al. 1997; Koh et al. 1999; Dong et al. 2000; Reddy et al. 2000; Wang et al. 2003b) (Table 5). A reverse TGMS gene in line J207S was mapped using the AFLP technique combined with BSA (Jia et al. 2001). This *rtms1* gene was mapped between RM239 and RG257 with a genetic distance of 3.6 cM and 4.0 cM, respectively. The reverse-TGMS rice exhibits sterility at lower temperature and will have applications in a much larger area. AFLP and RAPD markers were utilized in this study to tag the genes, and then the linked markers were mapped onto specific rice chromosomes using a reference mapping population.

Male fertility in photoperiod-sensitive genetic male sterile (PGMS) rice is regulated by photoperiod length. The first PGMS rice was a spontaneous mutant in japonica rice cultivar Nongken58. PGMS rice can be multiplied under short-day conditions but is to be planted under long-day conditions to produce hybrid seeds. Zhang et al. (1994a) used bulked DNA from the extreme fertile and extreme sterile individuals of a large F_2 mapping population developed from a cross 32001S (PGMS line) × Minghui 63 and mapped two loci, *pms1* and *pms2,* on chromosome 7 and 3, respectively. The effect of *pms1* was two to three times larger than that of *pms2,* and the dominance was almost complete at both loci. The PGMS line "32001S" was developed by transferring the PGMS trait from the original Nongken58S. Later, Mei et al. (1999a,b) used

Table 5. Molecular-marker-facilitated tagging of genes for hybrid breeding in rice

Genes	Source	Linked markers	Chromosome	References
TGMS				
tms1	5460S	RZ562	8	Wang et al. 1995
tms2	Norin PL12	R643A, R2440	7	Yamaguchi et al. 1997
tms3	IR32364TGMS	RZ144	6	Subudhi et al. 1997
tms4	TGMS-VN1	RM27	2	Dong et al. 2000
tms5	AnnongS-1	C365-1	2	Wang YG et al. 2003
tgms	SA2	RM257	9	Reddy et al. 2000
Reverse TGMS				
rtms1	J207S	RM239	10	Jia et al. 2001
PGMS				
pms-1	32001S	RG477	7	Zhang et al. 1994a
pms-2	32001S	RG191	3	Zhang et al. 1994a
pms-3	Nongken58S	C751, RZ261	12	Mei et al. 1999a,b
Male sterility				
ms-h(t)	Hwacheong ms-h	RG451, RZ404	9	Koh et al. 1999
Fertility restorer (BT)				
Rf-1	MTC10R	OSRRF	10	Akagi et al. 1996
Rf2	IR64	CDO686	2	Yang et al. 1997
Fertility restorer (WA)				
Rf-3	IR24	RG532	1	Zhang et al. 1997
Rf?	IR24	C1361	10	Tan et al. 1998
Rf5	T24	RG374, RG394	1	Shen et al. 1998
Rfu	Minghui 63	C4003	10	Yao et al. 1997
Fertility restorer (HL)				
Rf5	MY23	RM3150	10	Liu et al. 2004b
Rf6(t)	93-11	RM5373	10	Liu et al. 2004b
Wide compatibility				
S − 5	02428	R2349	6	Liu et al. 1997
S − 5	Nekken 2	RG213	6	Yanagihara et al. 1995
Hybrid breakdown				
Hwd1	Siborunauli, Col.No.15	C701, R2309	10	Fukuoka et al. 1998
Hwd1	Siborunauli, Col.No.15	C796B, R1382, C492, C145	7	Fukuoka et al. 1998

the PGMS line Nongken58S in two crosses and identified two PGMS loci. One locus was *pms1*, identified previously by Zhang et al. (1994b), and the second locus designated as *pms3* was on chromosome 12, which was later fine mapped by Li et al. (2001a). Both had a strong effect on fertility and behaved like a pair of duplicated genes in controlling sterility. A comparison of the *pms3* region between Nongken58S and 32001S indicated that there was no transfer of this region from Nongken58S. This implies that transfer of a complete set of PGMS genes is not necessary for the development of PGMS lines. On the contrary, Wang

et al. (1997) showed that a mutation on the *pms1* locus did not result in the sterility of the PGMS line Nongken58S. Another study by He et al. (1999b) revealed that both stability of sterility and reversibility of fertility are the joint effects of the additive effects of the QTL and additive-by-additive components of two-locus interactions.

Wide-Compatibility Genes

To enhance the level of heterosis for yield in rice, intersubspecific crosses (e.g., indica/japonica) were

proposed. But hybrid sterility observed in those crosses was a major deterrent in the utilization of heterosis at the subspecific level. The discovery of wide-compatibility varieties (WCV) (Ikehasi and Araki 1984) offered the possibility of realizing high-yield heterosis through the production of fertile hybrids in many indica/japonica crosses. The wide-compatibility locus was named S_5, and a three-allele system (S_5^i, S_5^j, and S_5^n to represent indica, japonica, and neutral alleles at the S_5 locus, respectively) was proposed to explain the partial hybrid sterility in those crosses (Ikehasi and Araki 1986). Hybrid sterility is observed in the S_5^i–S_5^j combination but not in the S_5^n–S_5^i or S_5^n–S_5^j combination. S_5 locus was closely linked to marker genes C and *wx* (Ikehasi and Araki 1987) and to isozymes *Amp3* and *Est2* (Malik and Khush 1996) on chromosome 6. The map location was also confirmed using RFLP markers (Liu et al. 1992; Yanagihara et al. 1995). A genomewide mapping by Liu et al. (1997) revealed a tightly linked marker R2349 for the S_5 locus and two more additional minor loci on chromosome 2 and 12, whose combined effect could lead to partial sterility even in the presence of the wide-compatibility gene.

The complex genetic basis of wide compatibility in different WCVs is evident from several studies (Li HB et al. 1997a; Wang et al. 1998). In a QTL study involving the rice cultivar "Dular" with a high level of wide compatibility, Wang et al. (1998) identified five loci on chromosomes 1, 3, 5, 6, and 8 with significant effect on fertility segregation. These loci jointly explained 55.5% of the phenotypic variation, and the location of locus on chromosome 6 was the same as the earlier mapped S_5; the locus with largest effect was on chromosome 5. Two complex interactions between two loci and three loci were proposed to explain the level of hybrid fertility. Interactions between loci have also been detected to play a role in the expression of hybrid sterility in indica/japonica crosses (Wu et al. 1996; Li HB et al. 1997a). Zhuang et al. (2002) mapped a locus *S–c* closely linked to an RFLP marker RG227 on chromosome 3 and suggested that the "one-locus sporogametophytic" model could explain F_1 hybrid pollen sterility in cultivated rice.

Understanding Heterosis

A fundamental assumption for hybrid breeding is the advantage of heterozygotes. Although two major hypotheses, the dominance hypothesis and the overdominance hypothesis, were proposed earlier to explain the genetic basis of heterosis, no consensus has been reached to date. With the help of molecular marker technology and high-density molecular linkage maps, it is now possible to critically evaluate those hypotheses in rice (Xiao et al. 1995; Yu et al. 1997; Li et al. 2001b; Luo et al. 2001; Hua et al. 2002, 2003). Recently, Hua et al. (2003) investigated the genetic basis of heterosis by using an "immortalized F_2" population developed by randomly permutated intermating of 240 recombinant inbred lines derived from the cross between the parents of the elite hybrid Shanyou 63. Using a 231 marker linkage map in conjunction with the data gathered from a field trial of the hybrids and parental recombinant lines over 2 years, 33 heterotic loci were detected for four traits–yield, tillers per plant, grains per panicle, and thousand grain weight. Because of little overlapping of the QTLs for the traits with the heterotic loci, the involvement of a different group of factors for heterosis and trait performance is expected. It was concluded that all kinds of genetic effects, including partial, full, and overdominance at single-locus level and all three forms of digenic interactions (additive by additive, additive by dominance, and dominance by dominance) contributed to heterosis and that these genetic components were not mutually exclusive as explanation of the manifestation of heterosis. Heterosis in Shanyou 63 could be explained by heterotic effects at the single-locus level combined with the marginal advantages of double heterozygotes caused by dominance-by-dominance interaction at the two-locus level.

Using the same immortalized F_2 population Hua et al. (2002) concluded that heterozygotes were not necessarily advantageous for trait performance even among genotypes derived from the above highly heterotic hybrid. Earlier in an intersubspecific cross of rice, Xiao et al. (1995) suggested that dominance was the genetic basis of heterosis in rice and both dominance and overdominance hypotheses may be based on a single-locus theory. On the contrary, epistasis and overdominance are primarily responsible for explaining inbreeding depression and heterosis (Li et al. 2001b; Luo et al. 2001). Yu et al. (1997) investigated an $F_{2:3}$ population derived from a highly heterotic rice cross combination and detected a high level of digenic interactions involving loci that are distributed all over the rice genome in expression of heterosis. In most studies (Xiao et al. 1995; Li et al. 2001b; Luo et al. 2001) backcrossed recombinant inbred lines were used, but such populations do not provide estimates

for some genetic components at both single- and multilocus levels to study the genetic basis of heterosis.

Predicting Heterotic Crosses

The selection of parental lines that would result in improved performance of rice hybrids is a challenging task for hybrid rice breeders. Hybrid rice breeding would be accelerated phenomenally if a reliable, simple, and efficient method could replace large-scale crossing and field evaluation to predict heterotic cross combinations. A number of criteria commonly used to breed heterotic rice hybrids are *per se* performance, combining ability, and genetic diversity. During the last two decades, with the advent of molecular markers, genetic diversity is now estimated more efficiently compared with morphological variation. Several investigations in rice have been conducted to define the correlation between a hybrid performance and the molecular divergence in the parental lines. Two different measures of F_1 heterozygosity based on molecular data are general heterozygosity and specific heterozygosity. General heterozygosity is based on all molecular markers used in the study, whereas specific heterozygosity involves only those markers that affect a trait in a significant way. The results from the studies undertaken so far are conflicting. For example, Zhang et al. (1994b, 1995b) evaluated a diallel set of 28 indica × indica hybrids and detected high correlation between specific heterozygosity and heterosis for yield and its component traits. But Xiao et al. (1996b) found genetic distance measures useful for predicting yield and heterosis of intrasubspecific hybrids but not of intersubspecific hybrids. Similar conclusions were drawn in two other studies involving a wide assembly of germplasms (Zhang et al. 1996b; Zhao et al. 1999). These studies make clear that the correlation between genetic distance and heterosis is not of universal occurrence and the degree of correlation is variable because of germplasm diversity and the complex genetic basis of heterosis.

Since a moderate level of genotypic divergence between parents of intersubspecfic hybrids plays an important role in heterosis (Li et al. 1998), Liu and Wu (1998) suggested optimal accumulation of favorable alleles and removal of unfavorable alleles in parental lines using MAS rather than broadening the genetic diversity or heterozygosity in indica/japonica hybrid breeding programs. The discovery of favorable alleles and unfavorable alleles may be useful for hybrid rice breeding (Liu et al. 2002b).

1.3.4
Grain Quality

The preference for cooking, processing, and eating quality rice differs greatly around the world. To meet the consumer demand for rice of a specific quality, breeders' objective to improve grain quality changes accordingly. Rice grain quality can be defined in many ways. The major components of rice grain quality include appearance, milling, cooking, eating, and nutritional qualities; they are determined by physical and chemical characteristics. Most grain quality mapping studies have involved the *O. sativa* germplasm (He et al. 1999a; Tan et al. 1999, 2000, 2001a; Li Z et al. 2003b; Zhou et al. 2003b). Three recent reports concerned crosses involving *O. rufipogon* (Septiningsih et al. 2003a) and African rice *O. glaberrima* (Aluko et al. 2004; Li et al. 2004). Although most *O. rufipogon* alleles are inferior, information about the inferior grain quality QTLs can be useful in reducing the linkage drag while introgressing yield-improving QTLs from wild species. Similarly, the new QTL from *O. glaberrima* and a high level of transgressive variation in *O. sativa* × African rice *O. glaberrima* should provide further opportunity to improve grain quality.

Major grain quality genes mapped in rice include aroma, cooked kernel elongation, and waxy gene. Using an NIL developed by introgression of the scent gene (*fgr*) from Della in Lemont background, Ahn et al. (1992) identified a marker, RG28, linked to this gene on chromosome 8 at a distance of 4.5 cM. Later this gene was found to be linked to cooked kernel elongation QTL located in the proximity of RZ323 in a line, B8462T3-710, derived from Basmati 370 (Ahn et al. 1993). A similar gene for scented kernel (*sk-2*) was mapped on chromosome 8 near markers RG28 and XNpb369 (Yano et al. 1991). The major component of rice aroma is a compound 2-acetyl-1-pyrolline (AcPy) (Buttery et al. 1983), and the gene regulating this compound was also mapped near RG28 on chromosome 8 in a DH population derived from IR64 x Azucena (Lorieux et al. 1996).

The grain quality QTL studies are listed in Table 6. Rice milling quality is judged by three main factors: brown rice percentage, milled rice percentage, and head-milled rice. Grain length, grain width, width-length ratio, grain shape, and degree of chalkiness determine the quality of appearance of rice. Red pericarp in rice was studied in both classical mutants (Kinoshita 1998) and QTL studies (Bres-Patry et al. 2001; Septiningsih et al. 2003a), and major genes/QTL

were localized on chromosomes 1 and 7 and possibly a modifier on chromosome 12. The protein content and the fat content in rice grains were also mapped to facilitate improvement of the nutritional quality (Tan et al. 2001b; Hu et al. 2004).

Amylose content (AC) is one of the most important determinants of rice cooking and eating quality and is known to be controlled by a major locus *waxy* (*wx*) on chromosome 6 (Wang et al. 1992; He et al. 1999a; Tan et al. 1999). Sano et al. (1986) identified two different alleles of the *wx* locus corresponding to the indica and japonica subspecies using RFLP markers. In a study involving 89 nonglutinous rice cultivars, Ayres et al. (1997) identified eight different alleles of waxy genes that accounted for more than 85% of the variation in amylose content. But involvement of some minor genes in modifying this major locus has also been reported (McKenzie and Rutger 1983). AC of rice grain affects the gelatinization temperature (GT) and gel consistency (GC). A QTL study in both F_2 and RIL populations derived from a cross Zhenshan 97 x Minghui 63 indicated that AC, GC, and GT are controlled by the *wx* locus or surrounding region on chromosome 6 (Tan et al. 1999). Improvement of four quality traits, such as AC, GC, GT, and chalky endosperm in Zhenshan 97, an elite parent of hybrid rice, by introgressing the waxy region from Minghui 63 through molecular marker MAS, further testifies to the importance of this chromosome 6 region (Zhou et al. 2003b). A recent study by Larkin et al. (2003) indicated that the waxy gene encoding granule bound starch synthase affects viscosity characteristics significantly, whereas a tight-linked starch synthase locus has a lesser effect. By contrast, Han et al. (2004) reported the contribution of starch branching enzymes to viscosity characteristics. A single QTL study involving jasmine rice KDML 105 indicated involvement of several QTLs for controlling AC, GC, and GT (Lanceras et al. 2000).

1.3.5
Abiotic Stress Tolerance

Drought
Drought imposes serious limitations on rice productivity in rainfed ecosystems. In rainfed rice-growing areas, yield is greatly determined by the amount and distribution of rainfall. There is a tremendous amount of genetic variation for drought tolerance among world rice germplasms because some geno-

types perform remarkably better than others under drought conditions (Price et al. 2002a). Some of these lines use drought escape mechanisms, while others have an inherent ability to fight drought. Both shoot- and root-related traits contributing toward drought tolerance have been recently reviewed (Pathan et al. 2004). Shoot-related traits that are important in the context of drought tolerance are osmotic adjustment (OA), leaf water potential (LWP), cell-membrane stability (CMS), osmolytes, leaf rolling, leaf drying, and relative water content. A number of root morphological attributes to improve drought tolerance are root thickness, root weight, root length, root number (penetrated and total), and root penetration index. Most studies use F_2, DHL, or RIL as mapping populations to study root and shoot traits related to drought tolerance (Table 7).

Osmotic adjustment (OA) allows plants to maintain higher turgor to sustain normal physiological functions. The indica cultivars are known to have high OA capacity compared to japonica cultivars. There has been no attempt to exploit the existing genetic variation in breeding programs because the methods of OA measurement are both time consuming and labor intensive and also there is no distinct relationship between OA and rice productivity under drought. In the first report, Lilley et al. (1996) mapped a major QTL for OA onto chromosome 8 between markers RG978 and RG1. Of five QTLs for dehydration tolerance on chromosomes 1, 3, 7, and 8, two QTLs on chromosomes 3 and 7 overlapped with QTL for leaf rolling (Champoux et al. 1995) and total root number (Ray et al. 1996). Subsequently, Zhang et al. (2001) and Robin et al. (2003) used DH and advanced backcross populations to map several QTLs for OA. A comparison of these results revealed that there is some consistency in QTL locations (Pathan et al. 2004). For example, the QTL for OA on chromosome 8 (Lilley et al. 1996) was mapped in the same chromosomal region by both Zhang et al. (2001) and Robin et al. (2003). Similarly, another QTL on chromosome 1 (RG140-ME2_12) was consistent in both reports. On chromosome 3, one QTL for OA was detected between markers RZ313 and RG224 in two rice populations (Zhang et al. 2001; Robin et al. 2003). Tripathy et al. (2000) detected nine QTLs for cell membrane stability (CMS) using the DH population developed from the cross CT9993 × IR62266.

On comparing the location of QTL (Champoux et al. 1995; Courtois et al. 2000; Price et al. 2002b) involving drought-avoidance traits like leaf rolling,

Table 6. Grain quality QTL studies in rice

Reference	Populations	Number of lines and population type	No. and types of markers used	Methods/software used	Traits	Number of QTL	Chromosomal location of QTL	Variance % explained
He et al. 1999a	ZYQ 8 × JX 17	132, DHL	243 RFLP and SSR	Mapmaker/QTL	Amylose content	2	5,6	11.8–91.1
					Gel consistency	2	2,7	14.2–20.2
					Alkali Spreading score	2	Both on 6	24.6–82.4
					Percentage of grain with white core	2	8,12	10.0–21.9
					Square of white core	1	3	8.8
Lanceras et al. 2000	CT9993 × KDML105	141, RIL	191 RFLP, SSR, and AFLP	MQTL	Amylose content	4	3,4,6,7	9.2–58.7
					Gel consistency	3	6,6,7	10.5–53.1
					Gelatinization temperature	3	2,6,6	8.6–60.3
Bao et al. 2000	ZYQ 8 × JX 17	132, DHL	243 RFLP and SSR	Mapmaker/QTL	Paste viscosity (Rapid Visco Analyzer profile)	20	1,2,5,6,7,12	10.0–63.7
Tan et al. 2000	Zhenshan 97 x Minghui 63	241, F_2	150 RFLP, and SSR	Mapmaker/QTL and QTL Cartographer	Grain length	3	2,3,7	6.5–63.8
					Grain width	3	1,5,6	10.4–55.2
					Length-width ratio	2	3,5	36.4–37.8
	Zhenshan 97 × Minghui 63	238, RIL	171 RFLP, and SSR	Mapmaker/QTL and QTL Cartographer	Grain length	2	3,11	7.2–57.6
					Grain width	2	5,6	4.6–44.0
					Length-width ratio	2	3,5	25.4–33.3
Tan et al. 2001a	Zhenshan 97 × Minghui 63	238, RIL	162 RFLP and SSR	Mapmaker/QTL and QTL Cartographer	Brown rice (%)	1	5	10.0
					Milled rice (%)	2	3,5	4.8–7.0
					Head rice (%)	1	3	10.1
					Protein (%)	2	6,7	6.0–13.0
					Flour color L*	9	5,6,8,4,7,1,3,6,8	4.3–25.4
Yoshida et al. 2002	Reiho × Yamadanishiki	91, DHL	145 RAPD, AFLP, and SSR	QGENE	Grain length	4	1,4,6,11	11.9–23.4
					Grain width	4	3,4,5,6	8.7–27.1
					Grain thickness	3	2,4,5	9.7–19.6
					White core (%)	2	12, unknown	14.2–17.3
					White belly (%)	3	All three on 5	8.7–20.7
					Cracked grains (%)	4	1,3,11,12	9.7–14.3
					Amylose content	1	8	9.9
					Protein content (brown rice)	4	2,3,11,12	11.0–15.1
					Protein content (polished rice)	5	1,3,4,11,12	9.9–18.2

Table 6. (continued)

Reference	Populations	Number of lines and pop-ulation type	No. and types of markers used	Methods/software used	Traits	Number of QTL	Chromosomal location of QTL	Variance % explained
Septiningsih et al. 2003b	IR64 × O. rufipogon (IRGC 105491)	285 BC$_2$F$_2$ families	165 SSR and RFLP	QGENE and QTL Cartographer	Filled/total rough rice	1	8	5.1
					Grain density	2	4,8	10.1–11.2
					Dehusked rice grain	1	10	6.5
					Green grain	2	7,12	5.1–5.3
					Damaged-yellow rice grain	3	7,9,10	4.9–28.3
					Red grain	2	7,12	5.5–43.0
					Milled rice grain	1	10	5.8
					Head rice grain	3	1,2,5	5.2–5.5
					Broken rice grain	4	1,1,2,5	5.4–6.4
					Crushed rice grain	2	2,3	4.9–5.2
					Amylose content	1	6	21.9
					Gel consistency	1	6	6.6
Aluko et al. 2004	Caiapo × O. glaberrima (IRGC 103544)	312, DHL from a BC$_3$F$_1$ population	100 SSR	Mapmaker/QTL and QTL Cartographer	Brown rice (%)	3	1,7,8	2.8–4.9
					Head rice (%)	5	1,3,6,8,11	7.6–54.1
					Rice bran (%)	4	2,4,7,10	4.0–39.7
					Milled rice (%)	2	5,7	5.3–6.1
					Amylose (%)	3	3,6,8	10.9–73.7
					Alkali spread score	2	both on 6	44.0–50.1
					Protein (%)	4	1,2,6,11	4.5–15.0
					Grain length	2	3,6	4.7–12.5
					Grain l/w ratio	2	1,6	4.0–14.0
Hu et al. 2004	Gui 630 × 02428	81, DHL	232 RFLP	Mapmaker/QTL	Rice protein content	5	1,4,5,6,7	6.9–35.0
					Rice fat content	3	1,2,5	7.7–25.5
Li et al. 2004	V20A × O. glaberrima (IRGC 103544)	308 BC$_3$F$_1$	110 RFLP and 20 SSR	QGENE and QTL Cartographer	Amylose content	3	2,6,12	7–8
					Kernel elongation	1	3	6
					Grain length	3	1,3,10	5–27
					Grain width	3	1,11,12	12–33
					Length:width ratio	3	3,4,12	13–14
					Brown rice yield	2	7,12	5–22
					Crude protein content	1	8	10
					Gel consistency	2	2,7	69–70

Table 7. Quantitative trait loci studies for drought stress tolerance and associated traits in rice

Reference	Parents and cross	Mapping population	No. and types of markers used	Methods/software used	Traits scored	Number of QTL	Chromosomal location of QTL	Variance % explained
Champoux et al. 1995	Co 39 × Moroberekan	203 RILs	127 RFLP	Mapmaker/QTL	Root thickness	18	1,2,3,4,6,7,8,9,10,11,12	13–33
					Root shoot ratio	16	1,2,3,4,6,7,8,9,10,11,12	9–22
					Root dry weight/tiller	14	1,2,3,4,6,7,8,9,12	11–18
					Deep root weight	8	2,3,4,7,8,9,,12	6–17
Ray et al. 1996	Co 39 × Moroberekan	203 RILs	125 RFLP	Mapmaker/QTL	Total root number	19	1,2,3,4,5,6,7,8,9,11,12	8–19
					Root penetration index	6	2,4,5,6,11	7–13
					Tiller number	10	1,2,4,6,8,11,12	7–14
					Penetrated root number	4	1,3,6,12	6–8
Lilley et al. 1996	Co 39 × Moroberekan	52 RILs	127 RFLP	ANOVA and Mapmaker/QTL	Osmotic adjustment	1	8	32
					Dehydration tolerance	5	1,3,7,8	27–36
Quarrie et al. 1997	IR20 × 63-83	123 F$_2$	228 RFLP, AFLP	ANOVA and QTL Cartograoher	Leaf weight	7	2,4,8	Total 54%
					ABA accumulation	10	2,3,4,6,7,9	Total 55%
Price and Tomos 1997	Bala × Azucena	178 F$_2$	71 RFLP	Mapmaker/QTL	Root length	17	1,2,3,5,6,10,11	5–38
					Root cell length	1	–	10
					Root thickness	3	2,3,5	7–21
					Root volume	3	1,8,12	6–10
Yadav et al. 1997	IR64 × Azucena	125 DHLs	175 RFLP, RAPD, isozyme	ANOVA	Total root weight	6	1,5,6,7,9	5–11
					Deep root weight	5	1,6,7,9	4–15
					Deep root weight to shoot ratio	9	1,2,5,6,7,8,9	4–22
					Deep root weight per tiller	6	1,2,6,7,8,9	4–20
					Maximum root length	8	1,2,6,7,8,9	4–21
					Root thickness	5	1,2,5,8	4–10
Courtois et al. 2000	IR64 × Azucena	135 DHLs	175 RFLP, AFLP, Isozyme	QTL Cartographer	Leaf rolling	11	1,3,4,5,7,9	5–23
					Leaf drying	10	1,4,5,6,7,10,11,12	5–19
					Relative water content (RWC)	11	1,3,5,6,9,12	6–19
					Relative growth rate under stress	10	1,2,3,5,6,7,9	8–17
Hemamalini et al. 2000	IR64 × Azucena	56 DHLs	175 RFLP, AFLP, Isozyme	Mapmaker/QTL	Morphological and physiological traits	36	1,2,3,4,5,6,7,8,9,10,12	11–29
Tripathy et al. 2000	CT9993 × IR62266	154 DHLs	315 RFLP, AFLP, SSR	PLABQTL	Cell-membrane stability	9	1,3,7,8,9,11,12	13–42

Table 7. (continued)

Reference	Parents and cross	Mapping population	No. and types of markers used	Methods/software used	Traits scored	Number of QTL	Chromosomal location of QTL	Variance % explained
Price et al. 2000	Bala × Azucena	205 RILs	135 RFLP, AFLP	QTL Cartographer	Total root number	3	1,10	5–10
					Penetrated root number	7	2,3,5,10,11	5–17
					Penetration ratio	7	2,3,5,10,11	7–18
					Tiller number	1	1	12
Zheng et al. 2000	IR64 × Azucena	109 DHLs	175 RFLP, RAPD, isozyme	Mapmaker/QTL	Root penetration index	4	2,3,7,8	9–14
					Penetrated root number	2	2,7	8–9
					Total root number	2	1,7	9–14
					Penetrated root thickness	4	1,3,4,9	10–16
Ali et al. 2000	IR58821x IR52561	166 RILs	399 RFLP, AFLP	Mapmaker/QTL	Total root number	2	3,7	9–12
					Penetrated root number	7	1,2,3	7–27
					Root penetration index	6	2,3,10	8–26
					Penetrated root thickness	8	1,2,4,6,7,10	6–14
					Penetrated root length	5	1,2,3,7,11	6–13
Zhang et al. 2001	CT9993 × IR62266	154 DHLs	315 RFLP, AFLP, SSR	Mapmaker/QTL	Osmotic adjustment	5	1,2,3,8,9	8–13
					Root penetration index	4	3,4,12	8–11
					Basal root thickness	6	2,3,4,8,9,12	9–38
					Penetrated root thickness	11	1,2,4,6,7,9,12	9–31
					Penetrated root length	1	11	17
					Total root dry weight	5	1,2,4,6,10	9–20
					Penetrated root dry wt.	3	4,9,12	11–17
					Root pulling force	6	2,3,4,5,11	9–20
Kamoshita et al. 2002a	CT9993 × IR62266	154 DHLs	315 RFLP, AFLP, SSR	QTLMapper	Shoot biomass	7	2,6,11,12	9–57
					Deep root mass	7	1,2,3,7,11	9–36
					Deep root ratio	6	1,2,5,11	8–52
					Deep root per tiller	6	1,2,4,5,11	5–40
					Rooting depth	9	1,2,5,7,9,11	5–17
					Root thickness 0–10cm	6	1,2,4,8,11	7–36
					Root thickness 20–25cm	3	2,4	15–22

Table 7. (continued)

Reference	Parents and cross	Mapping population	No. and types of markers used	Methods/software used	Traits scored	Number of QTL	Chromosomal location of QTL	Variance % explained
Kamoshita et al. 2002b	IR58821 × IR52561	184 RILs	399 RFLP, AFLP	QTLMapper	Shoot biomass	2	4,8	13–14
					Deep root mass	5	2,3,4,9,11	7–21
					Deep root ratio	5	2,3,4,9,11	6–27
					Deep root per tiller	6	2,4,6,7	9–22
					Rooting depth	5	1,4	6–30
					Root thickness 0–10 cm	6	1,3,8,9	6–15
					Root thickness 20–25 cm	2	4	12–23
Price et al. 2002b	Bala × Azucena	205 RILs	135 RFLP, AFLP	QTL Cartographer	Leaf rolling	5	1,3,5,6,7	5–20
					Leaf drying	11	1,2,3,4,5,7,8,11,12	6–18
					RWC	8	1,3,4,5,6,8,9,10	9–26
Courtois et al. 2003	IAC165 × Co39	125 RIL	182 RFLP, SSR	QTL Cartographer	Maximum root length	3	1,9,12	7–14
					Root thickness	4	1,4,7	6–24
					Root weight (0–30 cm)	5	1,6,7,11,12	8–22
					Root weight (30–60 cm)	2	4,8	9–11
					Root weight (60–90 cm)	3	4,10,11	7–10
					Total root weight	3	1,3,11	10–12
					Deep root weight	3	4,7,8	11–12
Zheng et al. 2003	IR1552 × Azucena	96 RILs	239 RFLP, AFLP, SSR	QGENE	Seminal root length	4	1,2,7,9	11–14
					Adventious root number	8	1,2,3,4,9	11–20
					Lateral root length	5	1,3,5,6	12–14
					Lateral root number	3	3,4,6	12–13

leaf drying, and relative water content, it is evident that there is consistency of several QTLs across genotypes, screening environments, and years (Pathan et al. 2004). Of the 18 QTLs for drought avoidance at seedling, early vegetative, and late vegetative stages in the field (Champoux et al. 1995), five were consistently identified during three different growth stages and four across at least two growth stages. Courtois et al. (2000) detected 11 QTLs for leaf rolling, 10 for leaf drying, and 11 for RWC. Many of these QTLs were detected across different trials. For example, of 11 QTLs for leaf rolling, three QTLs, one each on chromosomes 1, 5, and 9, were common in three trials. Using an RIL population developed from the cross Bala × Azucena, Price et al. (2002b) detected 17 QTLs for leaf rolling, leaf drying, and relative water content in two different years and in two different locations. When QTLs of all drought-avoidance traits were examined, QTLs for leaf rolling and RWC on chromosome 1 (RG331-RZ14) were consistent in both Co 39 x Moroberekan and IR64 × Azucena populations. The QTLs for OA and root traits were also mapped in this region across different genetic backgrounds. The region of chromosome 3 between RZ519 and CDO795 carried QTLs for leaf rolling, leaf drying, and RWC in all three populations. A similar overlapping of QTLs for many of these shoot- and root-related traits were also evident (Pathan et al. 2004).

Root morphology is fundamentally important for improving drought tolerance in rice. A large number of QTL studies involving root-related traits such as thickness, weight, length, number (penetrated and total), and root penetration index are available (Table 7). Most japonica cultivars have well-developed root systems compared to indica cultivars. Root morphology and drought avoidance in rice under both field and greenhouse conditions were first investigated in an RIL population derived from the cross Co 39 x Moroberekan (Champoux et al. 1995). They showed that 12 of 14 QTLs were associated with field drought tolerance and overlapped with QTLs for root morphology (root thickness, root/shoot ratio, and root dry weight). Later, Ray et al. (1996) used the same RILs to locate QTLs associated with root penetration ability in rice. Additionally, five more populations have been used for QTL mapping of root traits (Table 7). Despite different experimental conditions, several QTLs for root traits were consistent across different mapping populations (Pathan et al. 2004). The most recent study of Courtois et al. (2003) involved an RIL population developed from the cross IAC165 x Co 39 in

which root traits (maximum length, thickness, and dry weight in various layers) were measured in greenhouse. For each trait, one to four QTLs were detected and each QTL on chromosomes 1, 4, 9, 11, and 12 explained 5.5 to 24.8% of the phenotypic variation. Most QTLs in this population overlapped with one or more root traits in earlier studied populations (Courtois et al. 2003). It is thus necessary to test the utility of this QTL information under natural field situations.

The above populations were used in a number of studies to establish a relationship between an individual drought tolerance trait and yield under drought stress. Babu et al. (2003) used a DH population of 154 rice lines from the cross CT9993-5-10-1-M × IR62266-42-6-2 in three field experiments at two locations and identified 47 QTLs, individually explaining 5 to 59% of the phenotype variation for various plant water stress indicators, phenology, and production traits. A region on chromosome 4 (RG939-RG476-RG214) with root-related traits was observed to have a pleiotropic effect on yield traits under stress. Venuprasad et al. (2002) used a similar strategy employing an IR64 × Azucena DH population and found a positive correlation between maximum root length and grain yield under stress but a negative correlation under unstressed conditions. They further reported that QTLs responsible for grain yield and component traits were not pleiotropic with loci for desirable root morphology under low-moisture stress at the vegetative stage, and so it may be possible to combine higher grain yield and desirable root morphological traits to improve rice productivity in rainfed ecosystems.

Zheng et al. (2003) used an RIL population derived from a cross between the lowland rice variety IR1552 and the upland rice variety Azucena and compared the QTL results with earlier reports. In all these studies Azucena provided positive alleles for root elongation. The researchers screened several candidate genes from expressed sequence tags (EST) and cDNA-AFLP and mapped two genes for cell expansion, four cDNA-AFLP clones from root tissues of Azucena in the QTL region for seminal root length (SRL), and lateral root length (LRL) under upland conditions, respectively. Nguyen TT et al. (2004) used differential display to identify candidate genes and mapped several of these adjacent to the QTLs for root thickness and OA capacity in a CT9993 × IR62266 population.

Submergence

Rice varieties tolerant to flooding or submergence are needed to improve productivity in rainfed lowland and flood-prone areas of South and Southeast Asia. An Indian cultivar FR13A is the most widely used source of flooding tolerance. Although considered earlier as a polygenic trait, a number of molecular marker analyses (Table 8 A) indicated that a major locus, *Sub1*, located on chromosome 9 and controlling 69% of the phenotypic variation, is responsible for this trait (Xu and Mackill 1996). This major locus was confirmed along with the discovery of four additional QTLs using an RIL population developed from the cross IR74 x FR13A (Nandi et al. 1997). A high-resolution map of this major locus was constructed (Xu et al. 2000). Sripongpangkul et al. (2000) used another submergence-tolerant traditional Indian cultivar Jalamagna and mapped a QTL for submergence tolerance with large effect onto the same position on chromosome 9. Among six more genomic regions for leaf and stem elongation, the most important QTL *QIne1* located near *sd1* on chromosome 1 had a large effect on internode elongation and contributed significantly to the submergence tolerance under flooding. From this study it is evident that genes for submergence tolerance are different from genes for elongation ability. Using three different mapping populations the same group Toojinda et al. (2003) identified the same major QTL on chromosome 9 consistently in different years and different genetic backgrounds. Several other QTLs specifically expressed under certain environments or genetic backgrounds were also mapped on chromosomes 1, 2, 5, 7, 10, and 11.

Salinity

Salinity is a major constraint on rice productivity, affecting 20% of irrigated land worldwide. There has been rapid growth in understanding of the component traits for salt tolerance and mechanism in other plant species, such as production of compatible solutes, salt compartmentation, sodium uptake, and preference for potassium to sodium. However, this knowledge has not been translated into improvements in salt tolerance in cereal crops including rice (Flowers and Yeo 1995). The major bottleneck is the complex genetic and physiological mechanism along with high environmental influence associated with salinity tolerance. Because of the involvement of large number of genes for this complex trait and its asso-

ciated components, a QTL approach may be ideal to dissect the component traits to enhance salinity tolerance.

A number of QTL studies in rice to dissect various component traits of salinity are listed in Table 8 B. There is hardly any agreement in those reports regarding the map position of the identified loci for component traits of salt tolerance. Koyama et al. (2001) described QTLs responsible for sodium uptake, potassium uptake, and regulation of the $Na^+:K^+$ ratio that are independent of vegetative growth. Because of localization of QTLs for Na^+: and K^+: uptake on different chromosomes, the uptake pathways are independent like the $Na^+::K^+$ ratio. The independence of QTL location for Na^+ and K^+ transport was also reported by Lin et al. (2004), who mapped eight QTLs of which two major QTLs, *qSNC-7* and *qSKC-1,* explained 48.5% and 40.1% of the total phenotypic variation, respectively. Zhang et al. (1995a) detected one gene for salt tolerance on chromosome 7 near RG4. Flowers et al. (2000) opined that transferability of markers linked to physiological traits like ion transport and selectivity across populations is not possible, and thus novel protocols to identify the differentially expressed genes would be necessary.

Few reports are available on genetic control of aluminum (Al) tolerance (Wu et al. 2000; Nguyen et al. 2001, 2002, 2003) and ferrous iron toxicity tolerance (Wu et al. 1997; Wan et al. 2003) in rice (Table 8 C). One locus for tolerance against ferrous iron toxicity on chromosome 1 appeared to be identical in both reports, with a large effect. The molecular mechanism of aluminum tolerance is little understood and the aforementioned QTL reports indicated a complex genetic basis for this trait. Wu et al. (2000) identified several QTLs conferring Al tolerance in an RIL population developed from the cross IR1552 (sensitive) × Azucena (tolerant). Their results showed that an additive effect is important for Al tolerance in younger seedlings but an epistatic effect is important in older seedlings. Nguyen et al. (2001) identified five QTLs for Al tolerance scattered over five chromosomes with a major QTL located on chromosome 1, whereas in another study involving a DH population from the cross CT9993 × IR62266, ten QTLs were localized on nine chromosomes. The Al tolerance QTL on chromosome 1 was found conserved across three genetic backgrounds. Ma et al. (2002) used a population of backcross inbred lines (BIL) derived from the cross between a japonica variety, Koshihikari, and an indica variety, Kasalath, in which Koshihikari showed

Table 8. Quantitative trait loci studies for tolerance to submergence, salinity, aluminum, cold and ferrous iron toxicity in rice

Reference	Parents and cross	Mapping population	No. and types of markers used	Methods/software used	Traits scored	Number of QTL	Chromosomal location of QTL	Variance % explained
A. Submergence tolerance								
Xu and Mackill 1996	IR40931-26 × PI543851	169 F_2	RFLP and RAPD	Mapmaker/QTL	Submergence tolerance	One major	9	69
Nandi et al. 1997	IR74 × FR13A	74 RILs	202 AFLP	Mapmaker/QTL	Submergence tolerance	Five	6,7,9 (major),11,12	19–27
Sripongpangkul et al. 2000	IR74 × Jalamagna	165 RILs	144 RFLP, AFLP and Isozyme	QTL Mapper	Submergence tolerance	13	1,3,4,5,7,8,9,10,11,12	11–36
					Leaf elongation	3	4,6,7	9–14
					Internodes elongation	3	1,2,4	9–37
Toojinda et al. 2003	IR49830 × CR6241	65 DHLs	105 RFLP, SSR, AFLP, RAPD	MQTL	% plant survival	3	1,5,9	16–48
					Total shoot elongation	3	2,5,9	24–74
					Tolerance score	2	5,9	28–72
					Leaf senescence	2	5,9	30–72
	FR13A × CT6241	172 RILs	183 RFLP, SSR, AFLP, and others	MQTL	% plant survival	4	7,9,10	5–77
					Total shoot elongation	4	9,10	10–52
					Relative shoot elongation	2	9	10–12
					Tolerance score	9	7,9,10	8–63
					Leaf senescence	4	5,7,9,10	10–53
	Jao-Hom-Nin × KDML105	188 F_2	99 SSR, STS and others	MQTL	% plant survival	3	10,11	3–15
					Total shoot elongation	2	7,9	2–3
					Relative shoot elongation	5	1,8,10,11,12	2–5
					Leaf senescence	6	1,2,4,9	3–19
B. Salt tolerance								
Zhang et al. 1995a	M-20 × 77-170	85 F_2	130 RFLP	ANOVA	Salt tolerance	One	7	Major gene
Koyama et al. 2001	IR4630 × IR15324	118 RILs	RFLP, AFLP, SSR QTL Cafe	GENSTAT and	Ion Conc, ratios, ion uptake, and dry mass	11	1,4,6,9	6–20%
Lin et al. 2004	Nona Bokra × Koshihikari	133 F_2	154 RFLP	Mapmaker/QTL	Na^+ and K^+ uptake of the roots and shoots	11	1,4,6,7,9	12–40

Table 8. (continued)

Reference	Parents and cross	Mapping population	No. and types of markers used	Methods/software used	Traits scored	Number of QTL	Chromosomal location of QTL	Variance % explained
C. Aluminum tolerance								
Wu et al. 2000	IR1552 × Azucena	150 RILs	207 RFLP, AFLP	QGENE	Al tolerance after 2 weeks of stress	3	1,3,12	9–19
					Al tolerance after 4 weeks of stress	3	1,9,12	9–20
Nguyen VT et al. 2001	Chiembau × Omon269-65	188 F_3 families	164 RFLP	Mapmaker/QTL	Aluminum tolerance	9	1,2,3,5,6,10,11,12	6–51
Nguyen VT et al. 2002	CT9993 × IR62266	146 DHLs	280 RFLP, SSR, AFLP	Mapmaker/QTL	Aluminum tolerance	19	1,2,3,4,6,7,8,9, 10,12	9–29
Ma et al. 2002	Koshihikari × Kasalth	183 BILs	162 RFLP	PLABQTL	Aluminum tolerance	3	1,2,6	27 (total)
Nguyen BD et al. 2003	IR64 × *O. rufipogon* (Acc 106424)	171 F_6 RILs	151 RFLP, SSR	Mapmaker/QTL	Aluminum tolerance	7	1,2,3,7,8,9,12	9–26
D. Cold tolerance								
Takeuchi et al. 2001	Akihikari × Koshihikari	212 DHLs	135 RFLP and 34 RAPD	–	cold tolerance at booting stage	3	1,7, 11	5–22
Andaya and Mackill 2003a	M-202 × IR50	191 RILs	175 SSR	PLABQTL	Cold tolerance at the booting stage	8	1,2,3,5,6,7,9,12	11–17
Andaya and Mackill 2003b	M-202 × IR50	191 RILs	175 SSR	PLABQTL	Cold tolerance at the vegetative stage	15	1,3,4,6,8,10,11,12	9–41
Fujino et al. 2004	Italica Livorno × Hayamasari	122 BILs	186 RFLP, SSR	QTL Cartographer	Low temperature germinability	3	3,4	6–35
E. Ferrous iron toxicity tolerance								
Wu et al. 1997	IR64 × Azucena	123 DHLs	175 RFLP, RAPD, isozymes	Mapmaker/QTL	Ferrous iron toxicity tolerance	3	1,8	11–33
Wan et al. 2003	Nipponbare/Kasalth //Nipponbare	96 BC_1F_9 backcross inbred lines	245 RFLP	Mapmaker/QTL	Ferrous iron toxicity tolerance	4	1,3	21–48

higher tolerance at various Al concentrations than Kasalath, probably because of exclusion mechanisms rather than internal detoxification. Three chromosomal regions on chromosomes 1, 2, and 6 controlling Al tolerance explained about 27% of the phenotypic variation and were confirmed using substitution lines. Kasalath contributed positive alleles at the QTL on chromosome 6 but were unfavorable for loci on chromosomes 1 and 2. Nguyen et al. (2003) used IR 64 × *O. rufipogon* RIL and identified nine QTLs, of which QTLs for relative root length (RRL) located on chromosomes 1 and 9 were consistent across different genetic backgrounds. A major QTL for RRL on chromosome 3 was also reported to be conserved across many cereals.

Cold Tolerance

Low-temperature stress reduces rice growth and yield because of poor germination, poor seedling growth, delayed heading, and spikelet sterility in most temperate regions and high-elevation areas in tropics. A list of QTL mapping studies on cold tolerance in rice is given in Table 8 D. Andaya and Mackill (2003a,b) employed a QTL mapping strategy to investigate cold tolerance during both the vegetative and booting stages of rice. In an RIL population (temperate japonica M-202 × tropical indica, IR50), they identified a single major QTL, *qCTS12a,* on chromosome 12 that accounted for 41% of the variation and several minor QTLs distributed over eight rice chromosomes. In another study they reported eight QTLs for cold tolerance at the booting stage on chromosomes 1, 2, 3, 5, 6, 7, 9, and 12 with a contribution of 11 to 17% to the total phenotypic variation. Two major QTLs, *qCTB2a* and *qCTB3* from the tolerant parent M-202, explained approximately 17% of the phenotypic variance, and IR50 alleles in two QTLs contributed to cold tolerance. But for the same trait only three QTLs on chromosomes 1, 7, and 11 explained 5 to 22% of the phenotypic variation in a DH population from the cross Akihikari (moderately low-temperature susceptible) × Koshihikari (low-temperature tolerant) (Takeuchi et al. 2001). Saito et al. (2003) introgressed fragments of cold-tolerant variety Silewah into Norin-PL8 and a cold-sensitive variety Kirara 397 background and identified three QTLs on chromosomes 3 and 4 for cold tolerance at the booting stage.

Fujino et al. (2004) mapped three QTLs for low-temperature germinability on chromosomes 3 and 4 in a population of 122 backcross inbred lines (BIL)

derived from a cross between temperate japonica varieties, Italica Livorno, and Hayamasari. A major QTL, *qLTG-3-1,* on chromosome 3 accounted for 35.0% of the total phenotypic variation and two additional QTLs, *qLTG-3-2* on chromosome 3 and *qLTG-4* on chromosome 4, explained 17.4% and 5.5% of the total phenotypic variation, respectively. The Italica Livorno alleles contributed toward improvement in germinability at low temperatures. Miura et al. (2001) studied this trait in a BIL population from the cross Nipponbare × Kasalath and identified five putative QTLs on chromosome 2, 4, 5, and 11 explaining 40.7% of the total phenotypic variation.

Nutrient Deficiency and Toxicity
Few studies have been directed toward mapping of QTL for nutrient deficiency and toxicity (Table 8 E). The tolerance against phosphorous (P) deficiency was investigated by a number of researchers using different mapping populations (Ni et al. 1998; Wissuwa et al. 1998; Ming et al. 2001; Hu et al. 2001). Four QTLs for P-deficiency tolerance were identified on chromosomes 2, 6, 10, and 12 in a BIL population from the cross Nipponbare × Kasalath (Wissuwa et al. 1998). The major QTL for P uptake (*Pup1*) on chromosome 12 that explained 28% of the phenotypic variability was confirmed and fine mapped by employing both NIL and substitution mapping strategy (Wissuwa et al. 2002). The locus *Pup1* cosegregated with the marker S13126. Ni et al. (1998) used the extreme RILs (sensitive and tolerant) from a cross IR20 × IR55178-3B-9-3 (sensitive to P deficiency) in conjunction with AFLP markers and mapped a major QTL for P-deficiency tolerance (*PHO*) on chromosome 12 along with several minor QTLs on chromosomes 1, 6, and 9. Additionally, QTL studies targeted to locate genes for tolerance to ferrous iron (Fe^{2+}) toxicity, low potassium stress, and manganese toxicity were available (Wu et al. 1998a,b; Wang et al. 2002).

1.3.6
Important Agronomic Traits

Rice breeders usually target yield and yield-attributing traits that have a high impact on improving productivity. Some of the agronomic traits that have been thoroughly investigated in a wide array of populations under different environmental situations are plant height, heading date, yield, and its component traits. The genetic basis of many of these

traits is understood to some degree by determining the number and location of genes/QTLs on the map, gene effects, and interaction with other QTLs and also environment. It is evident from the progress made so far that molecular markers and their application has revolutionized the concept of quantitative traits and breeding strategy to facilitate further genetic gain in rice productivity. Since this aspect has been reviewed earlier (Yano and Sasaki 1997; Zhang and Yu 2000), our discussion will be limited to recently reported QTL mapping studies. For many of these so-called quantitative traits, such as heading date, plant height etc., loci with major effect have been identified and a few of them have been cloned.

Plant Height

The reduction of plant height through the use of a semidwarfing (*sd*) gene was instrumental in boosting rice productivity. A total of 13 genes responsible for semidwarfism have been plotted onto the molecular linkage map of rice (Huang et al. 1996). The semidwarfing genes located on different rice chromosomes 1 are *d-10*, *sd-1*, and *d-18* (chromosome 1), *d-5*, *d-30*, and *d-32* (chromosome 2), *d-56* (chromosome 3), *d-31* and *d-11* (chromosome 4), *sdg* (chromosome 5), *d-9* (chromosome 6), *d27* (chromosome 11), and *d-33* (chromosome 12) (Zhang and Yu 2000). Besides these qualitative genetic loci, a large number of studies have investigated plant height using a QTL approach and mapped several QTLs distributed over all 12 chromosomes (Table 9). Huang et al. (1996) compared the QTL-mapping results across five populations to analyze the correspondence between the qualitative genes and the QTLs for plant height and found a very strong correspondence between the map positions of QTLs and the major dwarfing genes.

The genetic basis of QTL effects and their interaction with environments for plant height and heading date was investigated by evaluating the DH population of IR64 × Azucena in nine environments in Asia (Li et al. 2003c). Thirty-seven main-effect QTLs and 29 epistatic QTLs were identified, and many of them were detected in multiple environments with consistency in direction but of variable magnitude. Response of some QTLs was different in different environments. Therefore, information regarding the magnitude of QTL x Environment interaction would be essential even for highly heritable traits for effective MAS.

Heading Date and Photoperiod Sensitivity

Manipulation of the heading date in rice is an important objective in all rice-breeding programs. The vegetative growth duration and photoperiod sensitivity both determine the time of flowering. Although a number of major genes controlling photoperiod sensitivity have been known, few of these genes have been assigned to rice chromosomes: *Se-1, Se3, Se-5* on chromosome 6, *E1* on chromosome 7, and *E-3* on chromosome 3 (Kinoshita 1998). Since the tagging of the first major photosensitive gene *Se-1* with a molecular marker (Mackill et al. 1993), a large number of QTLs with both major and minor effects have been mapped onto rice chromosomes (Table 10) (Li et al. 1995b; Xiao et al. 1995, 1996c; Lin et al. 1996a, 1998; Yano et al. 1997). QTL mapping by Yano et al. (1997) further confirmed that *Se-1* locus was the same as *Hd1*, which explained 67% of the phenotypic variation. Of four additional QT loci, *Hd-2* and *Hd-4* were mapped on chromosome 7 and *Hd-3* and *Hd-5* on chromosomes 6 and 8, respectively. Three additional QTLs were identified using a BIL population of the same cross Nipponbare x Kasalath (Lin et al. 1998). A fine mapping study using an advanced backcross progeny revealed *Hd1, Hd2,* and *Hd3* loci as Mendelian factors (Yamamoto et al. 1998). Further characterization of these QTLs and their interaction were done by developing the QTL-NILs through MAS (Yamamoto et al. 2000; Lin et al. 2000, 2002, 2003; Monna et al. 2002b) ultimately leading to cloning and isolation of some of these QTLs (Yano et al. 2000; Kojima et al. 2002; Takahashi et al. 2001).

Yield and Yield Components

A few reviews summarizing the progress of QTL mapping of complex agronomic traits in rice are available (Yano and Sasaki 1997; Zhang and Yu 2000). A large number of QTL studies have been directed toward mapping the genes for yield and yield-contributing factors (Table 9). Populations used in those studies were either F_2, or RIL or DHL or advanced backcross lines (ABL) derived from either intersubspecific crosses or interspecfic crosses involving wild relatives. The varying number of QTLs identified in different experiments and the differential QTL effects and their contribution to the total phenotypic variation of a specific trait might be due to variable population size or variable statistical threshold to declare the QTL, linkage density, and genotypes. From a practical point of view, comparison of QTLs and their

Table 9. Quantitative trait loci studies for yield and yield components involving cultivated rice

Reference	Parents and cross*	Mapping population	No. and types of markers used	Methods/software used	Traits scored	Number of QTL	Chromosomal location of QTL	Variance % explained
Lin HX et al. 1996a	Tenasai (I) × CB (I)	480 F$_2$	91 RFLP	Mapmaker/QTL	Grain weight/plant	3	1,2,4	9–11
					No. of panicles/plant	2	2,4	9–26
					Grain number/panicle	2	1,2	9–17
					Spikelet no./panicle	1	8	16
					Spikelet fertility	1	1	12
					1000-grain weight	3	1,4,5	9–15
					Spikelet density	1	8	11
					No. of first branches/main panicle	1	8	21
	Waiyin 2 (I) × CB (I)	480 F$_2$	101 RFLP	Mapmaker/QTL	Grain weight/plant	3	1,4,5	10–22
					No. of panicles/plant	2	5,6	10–12
					Spikelet no./panicle	2	6,11	13
					Spikelet fertility	1	1	9
					1000-grain weight	2	2,10	15–23
					Spikelet density	2	2,6	12–13
Xiao et al. 1996	9024 (I) × LH 422 (J)	194 RILs	141 RFLP	ANOVA and Mapmaker/QTL	Plant height	6	1,2,5,6,7,8	8–12
					Days to heading	3	3, 8,11,	7–51
					Days to maturity	2	8,11	7–74
					Panicle length	2	7,9	6–10
					Panicles/plant	1	4	7
					Spikelets/panicle	4	3,4,8	5–19
					Spikelet setting density	3	3,4,10	7–21
					Grains/panicle	3	3,4,5	14–22
					Percentage seed set	1	5	12
					1000-grain weight	3	3,4,5	10–15
					Spikelets/plant	4	3,4,9,11	5–11
					Grains/plant	3	3,4,5	15–16
					Grain yield	2	8,12	6–9

Table 9. (continued)

Reference	Parents and cross*	Mapping population	No. and types of markers used	Methods/software used	Traits scored	Number of QTL	Chromosomal location of QTL	Variance % explained
Wu et al. 1996	Palawan (JV) × IR42 (I)	231 F_2	104 RFLP	Mapmaker/QTL	Plant height	4	1,2	11–17
					Tiller number	2	4,12	4–7
					Panicle number	1	4	5
					Panicle length/plant	2	2,6	5–6
					Primary branch no./panicle/plant	1	4	7
					1000 Grain weight	1	1	10
Lu et al. 1997	ZYQ8 (I) × JX17 (J)	132 DHLs	137 RFLP	Mapmaker/QTL	Heading date	4	1,8,10	9–35
					Plant height	5	3,4,7,8,10	9–24
					1000-grain weight	6	1,2,3,5,6,8	8–19
					Spikelets/panicle	2	4,6	13–25
					Grains/panicle	2	4,6	13–28
					Seed set %	3	4,5,7	10–23
Redona and Mackill 1998	Labelle (TJ) × Black Gora (I)	195 F_2	116 RFLP	PLABQTL	Panicle size	2	3,6	8–16
					Spikelet fertility	4	1,3,5	7–11
					Grain length	7	2,3,4,7,10	8–21
					Grain breadth	4	2,3,7,8	3–11
					Grain shape	3	3,4,7	11–26
					Grain weight	2	4,8	8–10
He et al. 2001	ZYQ8 (I) × JX17 (J)	107 RIL	154 RFLP,AFLP, RAPD, SSR	Mapmaker/QTL	Days to heading	2	8,12	11–35
					Plant height	2	1,4	12–32
					No. of spikelets per panicle	2	4,6	10–15
					No. of grains per panicle	1	4	13
Yamagishi et al. 2002	Akihikari (Tmp. J) × Koshihikari (Tmp. J)	212 DHLs	169 RFLP, RAPD	QGENE	No. of primary branches per panicle	5	3,5,7,8,11	6–19
					No. of secondary branches per panicle	3	3,6,7	5–26
					No. of spikelets on secondary branch	1	6	20

Table 9. (continued)

Reference	Parents and cross*	Mapping population	No. and types of markers used	Methods/software used	Traits scored	Number of QTL	Chromosomal location of QTL	Variance % explained
Hittalmani et al. 2003	IR 64 (I) × Azucena (J)	135 DHLs	175 RFLP, isozyme, RAPD, cloned genes	Mapmaker/QTL	Biomass per plant	6	1,3,4,7	9–16
					Fertility %	1	4	8–13
					Heading date	7	1,3,4,7,8,9	8–32
					Harvest index	11	1,3,4,7,8	9–26
					No. of panicles per plant	6	1,3,4,12	8–28
					No. of spikelets per panicle	6	1,4,7	8–41
					Panicle exsertion	3	1,4,8	9–23
					Plant height	4	1,3,7	8–63
					Panicle length	8	1,3,4,6,7,10	8–37
					1000 grain weight	10	1,3,6,10	8–30
					Grain yield per plant	3	4,9,10	8–15
Mei et al. 2003	Lemont (J) × Teqing (I)	254 RILs and two test cross populations	182 RFLP, morphological markers	QTLMapper	Heading date	6	3,4,7,8,11,12	5–17
					Plant height	5	3,4,6,8	6–16
					Flag leaf length	2	2,3	12–19
					Flag leaf width	5	1,4,6,8,12	6–25
					Panicle length	3	2,8,10	9–16
					Spikelet No. per panicle	4	1,3,6,11	5–16
					Spikelet fertility	7	3,5,6,7,8,10,11	7–13
Kobayashi et al. 2003	M23 (I) × AK (J)	191 RILs	182 RFLP	QGENE	Culm length	31	1,2,3,4,5,6,9,10,11,12	5–19
					Panicle length	59	1,2,3,4,5,6,7,8,9,10,11,12	5–23
					Panicle number	38	1,2,3,4,5,6,7,8,11,12	5–39
					Tiller number	14	1,2,3,4,6,9,11	5–20
Teng et al. 2004	ZYQ 8 (I) × JX17 (I)	127 DHL	108 RFLP, isozyme	Mapmaker/QTL	Net Photosynthetic rate	2	4,6	15–17
					Chlorophyll content	3	1,3,8	11–14
					Stomatal resistance	1	4	11
					Transpiration rate	2	4,7	12–14

*I: Indica; J: Japonica; TJ: Tropical Japonica; Tmp. J: Temperate Japonica; JV: Javanica

Table 10. Quantitative trait loci analysis of yield and yield components in crosses involving wild species

Reference	Parents and cross*	Mapping population	No. and types of markers used	Methods/software used	Traits scored	Number of QTL	Chromosomal location of QTL	Variance explained per QTL (%)
Xiao et al. 1998	V20A/O.rufipogon (IRGC105491) //V20B//V20B ///Ce64	300 BC$_2$ testcross	102 RFLP, 20 SSR	ANOVA	Days to heading	7	1,3,5,6,7,8,12	3–15
					Days to maturity	8	1,3,5,6,7,8,12	3–11
					Plant height	6	1,4,8,9,12	8–45
					Panicle length	7	1,2,4,8,9,12	4–14
					Panicles/plant	2	1,2	3–4
					Spikelets/panicle	4	1,6,9	4–8
					Spikelets/plant	1	1	4
					Grains/panicle	5	1,4,8,12	4–13
					Grains/plant	6	1,2,4,5,8	2–7
					Percentage seed set	7	2,3,4,5,7,8	3–15
					1000-grain weight	8	2,3,4,5,8,9,11,12	3–10
					Grain yield	7	1,2,4,5,8,12	3–5
Xiong et al. 1999	Aijiao Nante (I) × O. rufipogon (Acc. P16)	172 F$_2$	348 RFLP, SSR, AFLP	Mapmaker/QTL	Heading date	4	3,6,8,11	2–52
					Plant height	4	1,7,8,9	8–60
					Tillers/plant	2	1,4	9–18
					Panicle length	2	1,7	19–26
					Secondary branches/panicle	2	1,7	8–18
					Spikelets/panicle	3	1,7,8	7–8
					Spikelet density	2	1,3	9–10
Moncada et al. 2001	O.rufipogon (IRGC #105491) × Caiapo //Caiapo//Caiapo	274 BC$_2$F$_2$	125 RFLP, SSR	QGENE	Days to heading	4	2,3,7	5–13
					Plant height	6	1,2,4,5	6–22
					Panicles/plant	2	6,11	8
					Grains/plant	4	1,2,6,11	6–9
					1000-grain weight	5	1,3,11	5–22
					Yield/plant	2	1,11	7–14

Table 10. (continued)

Reference	Parents and cross*	Mapping population	No. and types of markers used	Methods/software used	Traits scored	Number of QTL	Chromosomal location of QTL	Variance explained per QTL (%)
Brondani et al. 2002	*O. glumaepatula* (Acc. RS16) × BG90-2	96 BC$_2$F$_2$	157 SSR, STS	QGENE	Days to flowering	11	2,3,4,5,7	12–26
					Plant height	7	1,3,5	12–21
					Tiller no.	6	4,5,7,8,11	12–33
					Panicle number	7	5,8,11	10–31
					Panicle length	9	4,5,7,8,11	12–19
					Spikelets/panicle	7	1,2,3,4	11–48
					% filled grains/panicle	12	1,2,3,4,5,6,7,11	11–24
					100-grain weight	20	2,3,4,5,7,8,11	12–33
					Grain yield/plant	18	1,2,3,4,5,6,7,11	12–39
					Filled grain number/panicle	16	1,2,3,4,5,7,11	12–42
					Grain yield/panicle	23	1,2,3,4,5,6,7,11	11–32
Thomson et al. 2003	*O.rufipogon* (IRGC #105491) × Jefferson (TJ)	353 BC$_2$F$_2$	153 SSR, RFLP	QGENE and QTL Cartographer	Days to heading	11	1,2,3,4,7,10	6–26
					Grains/panicle	10	1,2,3,4,8,9,11,12	5–11
					Spikelets/panicle	6	1,2,3,9,12	6–10
					Panicle length	6	1,2,4,9,10,12	6–21
					Percentage Seed Set	7	1,3,4,5,6,10	6–16
					Grain weight	8	1,2,3,5,9,10,12	6–15
					Panicles/plant	2	3,7	6–8
					Yield/plant	5	2,3,6,9	7–17
Septiningsih et al. 2003	IR64 × *O.rufipogon* (IRGC #105491)	285 BC$_2$F$_2$	165 RFLP, SSR	QGENE and QTL Cartographer	Days to heading	6	2,7,11,12	3–6
					Days to maturity	4	4,7,8	2–5
					Plant height	5	1,4,6,10,11	3–56
					Panicle length	5	1,3,9,10	2–7
					Panicles/plant	5	1,2,11	4–10
					Grains/panicle	2	2,3	5
					Spikelets/panicle	1	3	6
					Percentage Seed Set	3	1,2,11	1–8
					Grains/plant	3	1,2,12	2–5
					Grain weight	5	1,2,3,7	5–11

*I: Indica; J: Japonica; TJ: Tropical Japonica; Tmp. J: Temperate Japonica; JV: Javanica

map positions across different populations, though useful for marker-assisted improvement of quantitative traits, is inhibited by the use of different sets of DNA markers. Epistasis and QTL × E interaction further limits the use of QTL information for crop improvement. Development of a series of NILs with different combinations of QTLs will be essential to demonstrate the effect of QTL and epistatic interaction. This approach was used to identify the QTLs that could improve yield and lodging resistance in rice (Ishimaru 2003; Kashiwagi and Ishimaru 2004). From an analysis of QTL-NILs in conjunction with candidate gene strategy Ishimaru et al. (2004) identified a new gene sucrose phosphate synthase, which controls plant height.

In addition to the plant type attributes, photosynthetic rate determines the dry matter production and yield. Photosynthesis and its related physiological traits were studied using molecular markers to identify the responsible QTLs (Teng et al. 2004). Because of nonoverlapping of the QTLs for yield components with those for photosynthetic ability, it was concluded that photosynthetic ability does not influence yield (Ishimaru et al. 2001) because many factors other than photosynthetic ability influence grain yield.

1.3.7
QTL × Environment Interaction

In most QTL mapping studies, the QTL × Environment (E) interaction factor is often overlooked. Realizing the importance of epistasis and environmental influence in analyzing the genetic basis of quantitative traits, the main effects, epistatic effects, and environmental effects in rice have been characterized in several studies (Li et al. 1997b; Yan et al. 1998, 1999; Cao et al. 2001; Liao et al. 2001; Xing et al. 2002; Yu et al. 2002). The QTL × E interaction is responsible for the fluctuation in the phenotypic expression of traits in different environments, making phenotype-based selection difficult. The most common way to deduce Q × E interaction is by evaluating the segregating mapping population in different environments and then comparing with QTL mapping results. Hittalmani et al. (2003) evaluated a DH population for 11 growth- and yield-related traits in nine different environments across four countries in Asia and identified many QTLs that are stable across environments. They also evaluated the clustering of QTLs for traits like plant height, panicle number, panicle length, and

spikelet number in the same chromosomal regions. Thirty-four of 126 QTLs detected for 11 traits were common in more than one environment and were spread over 10 chromosomes. Plant height was least influenced by environment and 0 to 4 QTLs were detected per trait per location. A similar study was conducted earlier by Zhuang et al. (1997) using F_2/F_3 populations from an indica/indica cross combination Tenasai2/CB. While this type of study gives an indication about the stability of QTL expression, it does not quantify the individual Q × E interaction effects and the reasons for the instability of QTL in different environments. Evaluating the same IR64 × Azucena DH population at nine locations in Asia, Li et al. (2003c) not only identified the main effect and epistatic QTL but also quantified the Q × E effect for heading date and plant height. QTLs are either not expressed or weakly expressed in multiple environments. This inconsistency is further compounded by the epistasis and significant Q × E interaction with its direction being opposite to QTL main effects. This interaction might be either trait specific or gene specific and thus should be considered before MAS is performed to improve quantitative traits.

Seedling Vigor

Cultivars with improved seedling vigor emerge rapidly and uniformly from soil, ensuring an optimum stand establishment in temperate rice-growing areas and high-elevation areas in the tropics and subtropics. A number of quantitatively inherited traits such as long mesocotyls and coleoptiles, rapid root growth, and longer shoots determine the seedling vigor. Though considerable variation exists for these traits, improvement of seedling vigor of modern cultivars through breeding has not been satisfactory. Seedling vigor in general is higher in temperate japonica and indica rices than tropical japonicas. Redona and Mackill (1996) used an F_2 population from a cross Labelle (low-vigor japonica) × Black Gora (high-vigor indica) and mapped 13 QTLs distributed on chromosomes 1, 2, 3, 5, 6, 7, and 9, each accounting for 7 to 38% of the phenotypic variation for four seedling characteristics, i.e., shoot length, root length, coleoptile length, and mesocotyl length. Both parents contributed positive alleles to high seedling vigor. In an RIL population, Cui et al. (2002) identified four important genomic regions (RG393-C1087-RZ403 interval on chromosome 3, C246-RM26-C1447 and R830-R3166-RG360-C734b

intervals on chromosome 5, and waxy gene region on chromosome 6) that harbor QTL clusters for a number of traits associated with seedling vigor. Additionally, they mapped a number of biochemical or physiological traits such as total amylase activity, alpha-amylase activity, reducing sugar content, root activity, and seed weight, which are associated with seedling vigor.

1.3.8
Utilization of Wild Species for Mapping and Introgression of Agronomic Traits

Most wild relatives of crop species are phenotypically inferior and are often regarded unuseful for crop production. With the current innovative genomics approach, it is now possible to mine previously undiscovered genes in wild species that will have the potential to improve yield, quality, and other agronomic traits (Table 10). By using a unique mapping procedure called advanced backcross QTL analysis (Tanksley et al. 1996), it was possible to discover those masked genes (Xiao et al. 1996b). In a study involving a wild species *O. rufipogon* Acc. IRGC 105491, Xiao et al. (1998) found beneficial alleles from 35 of 68 identified QT loci, and particularly two QTLs, namely, *yld1.1* and *yld2.1* of this wild species, when added to cultivated species, improved yield by 17 to 18% without delaying maturity or increasing plant height. A number of parallel studies involving the same accession of *O. rufipogon* in combination with different rice cultivars from Brazil, USA, and Asia uncovered *O. rufipogon*-derived alleles with the potential to improve rice productivity (Moncada et al. 2001; Septiningsih et al. 2003b; Thomson et al. 2003). *O. glumapaetula*, a diploid wild relative of rice, was used in another study (Brondani et al. 2002) that demonstrated the existence of positive alleles in this species to improve tiller number and panicle number.

1.4
Molecular Characterization of Rice Germplasm

Molecular markers have become more common for analyzing germplasm resources in many field crop species including rice. The most familiar application has been the assessment of the amount of genetic variability present in germplasm collections. Additionally, molecular marker technology is helping in the identi-

fication of redundancies and gaps in germplasm collections, screening of new potential accessions, variety identification, and purity testing. Several reports document the amount of diversity present in wild species of rice (Qian et al. 2001; Sun et al. 2001; Thomas et al. 2001; Park et al. 2003; Zhou et al. 2003a), whereas in some cases the phylogenetic relationship among *Oryza* species has also been determined (Aggarwal et al.1999; Ge et al. 1999).

Since the use of isozymes in classifying the Asian rice varities (Glaszmann 1987), a number of genetic fingerprinting techniques have been developed to characterize and classify rice accessions. The techniques used involved RFLP (Sun et al. 2001), AFLP (Zhu et al. 1998), RAPD (Mackill 1995), intersimple sequence repeat (ISSR) (Blair et al. 1999; Joshi et al. 2000), STS (Yashitola et al. 2002), SSR (Ni et al. 2002), minisatellites (Zhou and Gustafson 1995), simple repetitive and hypervariable DNA sequences (Ramakrishna et al. 1994, 1995), and restriction landmark genomic scanning (RLGS) (Kawase 1994).

The genetic relationship among rice cultivars was examined using a number of different marker systems (Joshi et al. 2000). Spada et al. (2004) examined the genetic relationship among cultivated Italian rice germplasm using AFLP and SSR markers and grouped them into two main clusters: a small one comprising four exotic accessions and a larger one capable of being split into four subgroups. Song et al. (2003) compared the molecular-marker-based and pedigree-based genetic similarity among Korean rice cultivars. They showed that molecular data are more effective in identifying individual cultivars, and both pedigree data and DNA data are helpful in assessing overall patterns of genetic variation among rice germplasm. Using a set of 55 SSR markers scattered over all 12 chromosomes, Singh et al. (2004) could establish the identity of 23 aromatic rice genotypes including the Basmati types. They also fingerprinted 20 individual plants, grown from the nucleus, breeder, foundation, certified, and farmer-saved seed samples of Pusa Basmati 1 and found no variation among them.

The application of molecular markers is also emphasized for germplasm management (Jackson 1997). It is possible to predict the quantitative variation within rice germplasm using molecular markers to expedite the utilization of biodiversity available and maintained at genebanks (Virk et al. 1996). Zhu et al. (1998) analyzed the biodiversity of 57 rice germplasm

accessions using AFLP and grouped them into three groups that corresponded to isozyme groups I, II, and VI. The utility of PCR-based approaches, amplicon length polymorphism (ALP), and PCR-based RFLP, relative to that of southern-based RFLP, was demonstrated by Ghareyazie et al. (1995) to classify the Iranian rice varieties. Xu et al. (1998) successfully separated japonicas from indica varieties by ALP. To examine the pattern of diversity among 38 US rice cultivars belonging to two rice subspecies, Ni et al. (2002) used 111 SSR markers and concluded that japonica varieties are more diverse than indica cultivars on chromosomes 6 and 7 but less diverse on chromosome 2. Two subsets of around 30 SSR markers could show the same level of discriminating ability as that of 111 markers. In a recent study involving 101 SSR markers, Yu et al. (2003) grouped 193 lines from 26 countries into three major groups and nine subgroups. Group I represented the classical indica subspecies, whereas groups II and III belonged to the japonica subspecies. Most variation (93.5%) in the entire sample was caused by intrasubspecies differences, whereas indica-japonica differentiation contributed only 6.5% to the total variation. The largest number of markers on chromosomes 9 and 12 and the smallest number of markers on chromosomes 4 and 8 distinguished indicas from japonicas. This study revealed that the wide diversity among these rice germplasm was caused by selection for ecogeographical adaptation on multilocus associations.

Germplasm chacterization may be helpful for hybrid rice breeding. Subudhi et al. (1998) characterized 72 CMS lines developed at IRRI with AFLP and showed that resolution was much higher than for those based on qualitative and quantitative phenotypic traits. Genetic grouping of CMS lines based on AFLP may be useful for breeders in selecting genetically diverse CMS lines for hybrid seed production without performing a test cross of individual lines. Although molecular markers are helpful for identification, protection and parentage determination of hybrids (Wang et al. 1994a), molecular-marker-based genetic distance is not yet perfected to predict heterosis for complex traits (Kwon et al. 2002a; Xu et al. 2002a).

1.5
Progress in Marker-Assisted Breeding

Molecular marker technology has the potential to accelerate the cultivar development process in a number of ways (Tanksley et al. 1989; Mohan et al. 1997a; Subudhi and Nguyen 2004). Manipulation of most agronomic traits in crop plants is difficult because of complex polygenic inheritance. The ability to dissect and clone factors responsible for those complex traits offers a unique opportunity to improve crop productivity. Transfer of desirable genes among cultivars and precise introgression of novel genes from wild and weedy relatives into cultivars can be hastened significantly by using molecular markers. Pyramiding of genes through conventional plant-breeding approaches is difficult, laborious, and, in most cases, impossible. Durability of resistance can be enhanced if two or three genes for resistance against the same pathogen or insect can be pyramided into a single cultivar using molecular markers. Thus, molecular markers have obvious advantages for efficient selection of target traits under a variety of situations such as (1) when the trait is difficult or expensive to evaluate, (2) when several genes are to be pyramided, (3) when quick and precise transfer of genes is needed by reducing linkage drag, specifically introgression of alien genes, and (4) when selection of desirable plants is needed at early seedling stage.

With the development of high-density molecular linkage maps of rice (Harushima et al. 1998; McCouch et al. 2002), molecular tags for any trait of interest can be found anywhere on the genome and can be used to transfer useful genes from one varietal background to another. It is also possible for the breeders to conduct several cycles of selection in a year using molecular markers. Successful integration of molecular marker technology in plant-breeding programs, however, will require the development of high-throughput, rapid, reliable, and inexpensive genotyping tools that are capable of assaying large breeding populations with little DNA. Besides SSR, single nucleotide polymorphism (SNP) is gradually becoming popular as a high-throughput genotyping tool. Multiplex PCR to target multiple loci and multiple genotypes is being investigated (Fan et al. 2000; Hirschhorn et al. 2000; Buetow et al. 2001). There is progress in the development of new technology to eliminate electrophoresis (Tyagi and Kramer 1996). It is expected that DNA chips capable of expression profiling of several target genes

simultaneously in large population will be designed for use in breeding programs in the near future. This section updates the progress made in marker utilization in rice cultivar development.

1.5.1
MAS for Disease Resistance

The impact of DNA-marker-assisted selection on breeding disease-resistant rice cultivar has been impressive as reflected by a number of studies. In lieu of RFLPs, PCR-based molecular tools in the form of STS and SSR have been developed to implement genotypic selection. Two important diseases, BLB and blast, were the prime targets for which a large number of genes have been identified and mapped in a wide range of germplasms. Rice lines with improved resistance against BLB and blast are being developed through successful pyramiding of multiple disease resistance genes. A number of STS and SSR markers linked to various BLB and blast genes and their primer sequences are listed in Table 11.

Among several STS markers generated from AFLP fragments linked to the rice bacterial blight resistance gene *Xa7*, M5 was found to be cosegregating with the gene (Porter et al. 2003). Gu et al. (2004) saturated the *Xa27(t)* genomic region with markers derived from the genomic sequence of *O.sativa* cv. Nipponbare and developed markers, viz., M631, M1230, and M449, that cosegregate with the gene. SSR and STS markers linked to BB resistance genes, *xa5, xa13, Xa21,* have been identified and developed (Ronald et al. 1992; Yoshimura et al. 1995; Zhang et al. 1996a; Blair and McCouch 1997).

In backcross breeding programs, DNA-marker-based selection can hasten the incorporation of desirable genes. Chen et al. (2000, 2001) improved bacterial blight resistance of two elite restorer lines "6078" and "Minghui 63" by incorporating *Xa21* from "IRBB21" through MAS. The hybrids developed using these improved restorer lines showed improvement in yield under disease infestation.

Mapping of many blast resistance genes followed by fine mapping and development of PCR-based markers have significantly accelerated the breeding of blast-resistant cultivars in rice. Analyzing the molecular profile and blast resistance data of the RIL population of Co 39 x Moroberekan (Wang et al. 1994b), three RI lines carrying different genes for complete resistance, and two RI lines with genes for partial resistance were identified for quick development of NILs

(Inukai et al. 1996). Liu et al. (2003) demonstrated the utility of MAS by improving the resistance of Zhenshan 97 against rice blast. Selection was performed by using an SSR marker linked to *Pi1* gene on chromosome 11.

For blast resistance gene *Pi10*, Naqvi and Chattoo (1996) developed SCAR markers from linked RAPD fragments. Hittalmani et al. (1995) developed an STS marker for a tightly linked RFLP marker RG64 and detected specific amplicon polymorphism (SAP) between the resistant and the susceptible genotypes upon digestion of the PCR products with a restriction enzyme *Hae*III. Efficiency of selecting resistant plants with this STS marker was 95%, but use of flanking markers improved the selection efficiency to 100%. Pan et al. (2003) developed three RAPD markers, BAR 15486, BAR 15782, and BAR 15844, tightly flanking the *Pi15* gene with recombination frequencies of 0.35%, 0.35%, and 1.1%, respectively, for marker-aided gene pyramiding. A pair of primers that specifically amplified a susceptible *pi-ta* allele was developed to verify the absence of *Pi-ta* gene (Jia et al. 2004).

Using sequence data found in public databases and degenerate primer pairs based on the P-loop, nucleotide binding sites, and kinase domain motifs of previously cloned resistance genes, Conaway-Bormans et al. (2003) developed PCR-based markers that cosegregate with the gene *Pi-z* that confers complete resistance to five races of blast and is located on the short arm of chromosome 6. The ability to identify polymorphism in a wide range of rice germplasms offers a valuable alternative to conventional phenotypic screening for rapid introgression of genes into susceptible varieties as well as the incorporation of multiple genes into individual lines for more-durable blast resistance. Hayashi et al. (2004) surveyed SNPs and insertion-deletions (InDels) in the chromosomal region containing the blast resistance genes *Piz* and *Piz*-t and generated SNP markers to discriminate resistant and susceptible alleles.

1.5.2
MAS for Insect Resistance

Among the mapped insect resistance genes (Table 4), considerable progress has been made in the development of PCR-based markers for gall midge resistance genes. Since rice breeders evaluate their segregating population in endemic areas where the pest occurrence is severe in particular parts of the year, it is very time consuming and labor intensive to breed vari-

Table 11. Primer sequences of PCR-based markers used for marker-assisted selection in rice

Gene	Linked marker	Forward primer (5'–3')	Reverse primer (5'–3')	Enzyme	Reference
xa5	RG556	TAG CTG CTG CCG CCG TGT GC	AAT ATT TCA GTG TGC ATC TC	MaeII	Yoshimura et al. 1995
xa5	RG207	ATT GTT ACG TTT GGT GGG GG	GCC ATG GCG ACT GTC AGT CG	–	Blair and McCouch 1997
Xa7	M5	CGA TCT TAC TGG CTC TGC AAC TCT GT	GCA TGT CTG TGT CGA TTC GTC CGT ACG A	–	Porter et al. 2003
xa8	RM263	CCC AGG CTA GCT CAT GAA CC	GCT ACG TTT GAG CTA CCA CG	–	Chen et al. 1997
Xa10	RM206	CCC ATG CGT TTA ACT ATT CT	CGT TCC ATC GAT CCG TAT GG	–	Chen et al. 1997
Xa10	RM254	AGC CCC GAA TAA ATC CAC CT	CTGGAG GAG CAT TTG GTA GC	–	Chen et al. 1997
xa13	RG136	TCC CAG AAA GCT ACT ACA GC	GCA GAC TCC AGT TTG ACT TC	HinfI	Zhang et al. 1996a
xa13	RM230	GCC AGA CCG TGG ATG TTC	CAC CGC AGT CAC TTT TCA AG	–	Chen et al. 1997
Xa21	pTA248	AGA CGC GGA AGG GTG GTT CCC GGA	AGA CGC GGT AAT CGA AAG GAT GAA A	–	Chunwongse et al. 1993
Xa27(t)	M631	CTG CAT CCA TGC CGG TGG CCG	AAA CGT CAC ATG AAG ACT CCA ATT GT	NdeI	Gu et al. 2004
Xa27(t)	M1230	AGG GAT GTC GAG ATG AGA GCT TC	GGT GTC CTT CTT TAC GGG GCT CC	EcoRI	Gu et al. 2004
Pi-1	Pi-1	CTC CTT CTC CGA CCG TGC TC	AGC ATA GAA GCA CAT CAT TG	–	Cho et al. 2003
Pi2	RG64	GTT GTT TGA GCT CTC CAA TGC CTG TTC	GGA CCG GCA TGT AAC GTG ACG TC	HaeIII	Hittalmani et al. 1995
Pi-5	JJ80	TTA TGA GAT TAG GAG TGT AT	ATG TAA AGG CAA AAG CTG AT	–	Cho et al. 2003
Pi-9	pB8	CCC AAT CTC CAA TGA CCC ATA AC	CCG GAC TAA GTA CTG GCT TCG ATA	–	Liu et al. 2002a
Pi-9	pB14	TGG TGC ACT CAG AAA GAA	GCA GTG TCA TCT TGT CTC C	–	Liu et al. 2002a
Pi10	OPF6-1	GGG AAT TCG GTT TTA CAA CCA CCG	GGG AAT TCG GAT CTC CGG GGG TAG	–	Naqvi and Chattoo 1996
Pi10	OPF6-2	TTT TAC AAC CAC CGG TTT TAT GAC	ATC TCC GGG GGT AGA GCA CTG TTT	–	Naqvi and Chattoo 1996
Pi-12	P265.560	CAG CTG TTC AGT CGT TTG	CAG CTG TTC ATA CAA GAA AT	–	Zheng et al. 1995
Pi33	C483	CTT CCA CCA TAA AAC CGG AG	ACA CCG GTG ATC TTG TAG CC	–	Berruyer et al. 2003
Pi-b	Pi-b	ATC AAC TCT GCC ACA AAA TCC	CCC ATA TCA CCA CTT GTT CCC C	–	Cho et al. 2003
Pi-ta	Pi-ta440	CAA CAA TTT AAT CAT ACA CG	ATG ACA CCC TGC GAT GCA A	–	Jia et al. 2002
Pi-ta	Pi-ta1042	AGC AGG TTA TAA GCT AGG CC	CTA CCA ACA AGT TCA TCA AA	–	Jia et al. 2002
Pi-z	MRG5836	TAT AAG CCG CAG CCA AAT TC	AAA AAC CTA GAA AAT GGG AAA ATG	·	Conaway-Bormans et al. 2003
Pi-z	MRG2431	ATC CAA ATC CAA TGG TGC AG	GTG GCG AAA GGG AAC ATT CT	–	Conaway-Bormans et al. 2003
Gm2	PF8	GGG ATA TCG GGG ATG AAA TGC CAA	GGG ATA TCG GTC ATT GCA GTG GAG	–	Nair et al. 1995
Gm2	PF10	GGA AGC TTG GCT TAT AGT AAC TAG	GGA AGC TTG GAA ATG CAA GAT CTT	–	Nair et al. 1995
Gm4	E20	TTA TTG ATG AGG ACT TAG GG	TGG ATA GGT TAG CAG AGC TG	–	Nair et al. 1996
Gm6	RG214	GGT AGA CAC GCG GGC GAG GTT G	CAC GCT CAA TCC AGG TGG ACA	–	Katiyar et al. 2001a
Gm6	RG476	GAT GGC AAG CCA ATC AGA TCG	GAA GTG AGG AAG CCT ACA GTA AGC C	–	Katiyar et al. 2001a
Bph2	Bph2	TAA CTG GTG TTA GTG CGA ATG C	AAT TCA CGG CAT GTG AAG CCC TAG	–	Murai et al. 2001
Bph13	AJ09	TCG ACC TAG AAA GGC CTG TGT	CAC TGG AAA TTT GAG CGA GAA	–	Renganayaki et al. 2002
tms3	F18	AGA GAG TGA TCT ATG CCC TG	GCG GAC CGT GGA AGC TGG GG	–	Lang et al. 1999

Table 11. (continued)

Gene	Linked marker	Forward primer (5′–3′)	Reverse primer (5′–3′)	Enzyme	Reference
tms5	C365-1	ATT TTG GTT GCG CAT TAG AGG	GAA ATA TGC CAA GTA CGG AGG AT	–	Wang et al. 2003b
tms5	G227-1	ACA CAT CAG CAA TTC ATC TAC	AAC AGC ATT TCC CCC TAC TAC A	–	Wang et al. 2003b
tms (?)	TS200	CGG AAT GTA ATT CAC ATG C	CAG AGA AAC ATC AGT TGT GG	–	Reddy et al. 2000
rtms1	Rev1	CAG GTC CCT AAC CCT TAG CAA AG	CAC GAA CAA GAA GGA ATG AG	–	Jia et al. 2001
Rf1	S12564	CTA GTT AGC CGA ATA ACT GAG GTT C	TTT GTG GGT TTG TGG CAT TGA GAA AAT	*Tsp509I*	Komori et al. 2003
Rf1	C1361	AAA GCA ACC GAC TTC AGT GGC ATC ACC	CTG GAC TTC ATT TCC CTG CAG AGC	*MwoI*	Komori et al. 2003
wx	wx	CTC TCT CAC CAT TCC TTC AG	CAC AAG CAG AGA AGT GAA GC	–	Han et al. 2004
sbe1	sbe1	GAG TTG AGT TGC GTC AGA TC	AAT GAG GTT GCT TGC TGC TG	–	Han et al. 2004
sbe2	sbe2	CCG AGG GAA TGC CAG GAG TAC CAG	GAA CCA CAA CCA AGT CCA AGG CAA	–	Han et al. 2004
sbe3	sbe3	GTC TTG GAC TCA GAT GCT GGA CTC	ATG TAT AAC TGG CAG TTC GAA CGG	*SpeI*	Han et al. 2004
fgr	RG28	TGC CAA GTA TCC CCT GAT TCC	TTT GTG CCT CCT TTG CAG ATT C	–	Garland et al. 2000

eties resistant to a number of biotypes. Allele-specific PCR-based markers from RAPD fragments linked to *Gm2* and *Gm4* have been developed for MAS (Nair et al. 1995, 1996; Sardesai et al. 2001). In each case, RAPD fragments tightly linked to the genes were sequenced and primers were designed. Katiyar et al. (2001a) developed a PCR-based MAS kit containing the primer pairs based on the terminal sequences of the linked markers RG214 and RG476 for transferring the *Gm6(t)* gene into susceptible cultivars in China. Among the mapped BPH resistance genes, *bph2* has been mapped with higher resolution and an AFLP marker KAM4 showing complete cosegregation with the gene has been converted into an STS (Murai et al. 2001).

1.5.3
MAS for Grain Quality

Among the grain quality traits, amylose content is an ideal candidate for MAS because these traits can be evaluated only after the reproductive stage. Genetic basis of amylose content has mostly focused on the *Wx* gene encoding the granule bound starch synthase (GBSS). A G-T polymorphism at the 5′ splice site of the first intron of GBSS was earlier identified by Ayres et al. (1997) to determine amylose production, and this was exploited by Bormans et al. (2002) to develop a non-gel-based assay for MAS of grain quality. Larkin and Park (2003) cloned and sequenced GBSS cDNA from a number of cultivars differing in amylose content and found two SNPs in exons 6 and 10 that resulted in amino-acid substitutions, which makes changes in quality characteristics. The association of these point mutations with the functional differences between GBSS alleles could be useful in the development of varieties with superior eating, cooking, and processing characteristics. Besides *Wx* gene, starch branching enzyme 3 (*Sbe3*) played an important role for variation in amylose content. Liu et al. (2004c) developed tags for *Sbe1* and *Sbe3* by exploiting the sequence diversity for MAS of amylose content. In another study, Zhou et al. (2003b) improved eating and cooking quality traits in Zhenshan 97, an elite parent of hybrid rice by introgressing the waxy region from Minghui 63 and by using an SSR *waxy* marker and two flanking RFLP markers, C688 and C952. Garland et al. (2000) tested polymorphism in homologous regions of the marker RG28 linked to the major fragrance gene of rice (*fgr*) and detected a small mononucleotide repeat that was polymorphic

between a pair of fragrant and nonfragrant cultivars and converted into a codominant PCR-based marker. Two more SSR markers, RM223 and RM42, were also mapped in the vicinity of *fgr* to distinguish fragrant varieties from nonfragrant varieties.

1.5.4
MAS in Hybrid Rice Breeding

Attributes such as fertility restoration, PGMS, TGMS, and reverse TGMS are either difficult to evaluate or can be evaluated only in the progeny of test crosses. In hybrid rice-breeding programs, these genes are often transferred to different genetic backgrounds to develop inbred lines by successive backcrossing. Development of molecular tags for these traits would allow selection at the seedling stage, resulting in considerable savings in both time and effort.

Komori et al. (2003) fine mapped the *Rf-1* gene that restores the pollen fertility in BT-type male sterile cytoplasm by using nine PCR-based markers developed from tightly linked RFLP markers on chromosome 10. Due to the tight linkage of the *Rf-1* to the flanking markers S12564 *Tsp509I* and C1361 *MwoI*, it will now be possible to transfer the *Rf-1* gene more efficiently and precisely. For the same restorer gene, a number of PCR-based markers have been developed and utilized by many other investigators (Akagi et al. 1996; Ichikawa et al. 1997; Mishra et al. 2003). The discovery of the tight linkage of the marker R2349 with the wide-compatibility gene S_5 (Liu et al. 1997) provides an opportunity to transfer the S_5^n alleles to different varieties in intersubspecific hybrid breeding.

Introgression of the TGMS or reverse TGMS gene through conventional breeding is cumbersome because it involves identification of TGMS plants in the segregating generation followed by induction of fertility by rationing at appropriate temperatures. Lang et al. (1999) developed both dominant and codominant STS markers from the RAPD markers linked to the *tms3* gene and reported an accuracy of 85% in MAS at the vegetative stage. For *tms4(t)* on chromosome 2, Dong et al. (2000) converted an AFLP marker E5/M12-600 mapped at a distance of 3.3 cM into an STS for marker-assisted transfer of this gene to different genetic backgrounds. Wang et al. (2003b) developed one STS marker, C-365-1, and another CAPS marker, G227-2, that flanked the *tms5* gene at a distance of 1.04 and 2.08 cM, respectively. Lopez et al. (2003) reported a successful transfer of *tms2* from Norin PL12 to an aromatic Thai cultivar KDML 105 using linked

SSR markers RM2 and RM11 on chromosome 7. The accuracy of selecting sterile plants during segregating generation was more than 90%. Jia et al. (2001) sequenced and converted a closely linked AFLP marker, *rev1,* 4.2 cM from the *rtms1* gene into a SCAR marker that could facilitate MAS of the *rtms1* gene.

1.5.5
Gene Pyramiding

Because different resistance genes provide resistance to different races or isolates, gene pyramiding is often considered a viable approach to improve durability of resistance in crop cultivars. Pyramiding of both major and minor genes may lead to durable resistance. The process of stacking of genes in a single cultivar can now be achieved more efficiently by performing MAS. It expedites the variety development process by offering the opportunity to select for all desirable genes simultaneously as well as eliminating the time-consuming process of inoculation for different races or isolates at different time intervals. Additionally, it allows the identification of individuals with desirable attributes in the segregating generation at the early vegetative stage well ahead of flowering to facilitate further crossing and/or backcrossing. A number of reports have demonstrated successful pyramiding of blast or BLB resistance genes (Huang et al. 1997a; Hittalmani et al. 2000; Sanchez et al. 2000; Singh et al. 2001).

Huang et al. (1997a) pyramided four bacterial blight (BB) resistance genes, *Xa-4, xa-5, xa-13,* and *Xa-21*, in different combinations. Breeding lines with two, three, and four resistance genes were developed, and these pyramid lines showed a wider spectrum and a higher level of resistance than lines with only a single gene. Sanchez et al. (2000) later transferred three BB resistance genes, *xa5*, *xa13*, and *Xa21*, to three promising but susceptible new plant type (NPT) lines, IR65598-112, IR65600-42, and IR65600-96, by employing STS markers. The BC$_3$F$_3$ NILs having more than one BB resistance gene showed a wider resistance spectrum and manifested increased levels of resistance to the Xoo races. The accuracy of selection in identifying homozygous resistant plants for *xa5* and *xa13* in two populations was 95% and 96%, respectively. Another marker-aided pyramiding experiment involving the above three BB genes into PR106, a widely grown cultivar in India, was conducted by Singh et al. (2001). Davierwala et al. (2001) used 11 STMS and 6 STS markers to identify lines with the

resistance genes *xa5* and *Xa4* in an F$_3$ population of a cross between IR-64 and IET-14444.

Hittalmani et al. (2000) pyramided three major genes, *Pi1, Piz-5,* and *Pi-ta*, for blast resistance located on chromosomes 11, 6, and 12, respectively, using DNA markers. For *Piz-5*, a PCR-based SAP marker was used, whereas flanking markers were used for the other two. Field testing of the pyramided lines in the Philippines and India showed enhanced resistance against leaf blast in comparison with the lines with a single gene.

Effort has been made in a number of cases to combine both molecular breeding and genetic transformation to improve elite rice lines. Narayanan et al. (2002) stacked three major genes, *Pi-1*, *Piz-5,* and *Xa21,* in line Co 39 and two major genes, *Piz-5* and *Xa21,* in line IR50 by using both MAS and genetic transformation for resistance against blast and bacterial blight. In the first stage blast-resistant isolines were developed by four rounds of backcrossing in conjunction with MAS and, in the second stage, the resistant isolines were transformed with *Xa21*, which is known to confer resistance to all races of *Xanthomonas oryzae pv. oryzae.* In another study, Datta et al. (2002) reported the development of transgene-pyramided rice cv. IR72 lines using MAS that showed durable and broad-spectrum resistance against disease and insect pests by conventional crossing of two independently developed transgenic lines with different genes such as *Xa21* (for BB resistance), a *Bt* fusion gene (for yellow stem borer resistance), and chitinase gene (for tolerance of sheath blight). In this study the transgenes were used as the STS markers for rapid development of homozygous pyramided lines. Jiang et al. (2004) pyramided *Xa21* gene for resistance to BB and a fused *Bt* gene (*cry*1Ab/*cry*1Ac) conferring resistance to lepidopteran insects into a restorer line "Minghui 63." Results from the field trials indicated that hybrids of the pyramided line with the CMS lines "Zhenshan 97A" and "Maxie A" maintained similar yield levels under conditions without chemical spray. With the help of STS and SSR markers for both *Xa21* and *waxy* genes, Ramalingam et al. (2002) isolated 20 true-breeding lines with high amylose content and *Xa21* from four crosses.

1.5.6
MAS for Other Traits and QTL

Any trait that has been tagged with a molecular marker is amenable for MAS. Using a microsatellite

marker RM219 and a codominant PCR-based marker RM464A (derived from a microsatellite marker RM464) that are linked to *Sub1* by 3.4 and 0.7 cM, respectively, Xu et al. (2004) developed several NILs from the submergence tolerance source IR40931-26 in temperate japonica cultivar M-202 background. These two markers were tested in 55 diverse indica and japonica rice cultivars and breeding lines, and RM219 showed 14 different alleles, whereas none of the 55 cultivars had the same allele as the tolerant source. But RM464A showed three different alleles in the 55 cultivars. Thus, RM219 will be useful in breeding programs to select for the *Sub1* gene in a wide range of backgrounds, whereas RM464A will be helpful in selection for the *Sub1* gene in japonica rice background. Siangliw et al. (2003) successfully transferred *Sub1* from three submergence-tolerant lines to Thai Jasmine rice cultivar KDML105 by marker-assisted backcross breeding.

To improve drought tolerance, a marker-assisted backcross program was implemented to transfer the Azucena alleles at four QTLs for deeper roots (on chromosomes 1, 2, 7, and 9) from selected DH lines into IR64 (Shen et al. 2001). After evaluating 29 selected BC_3F_3 NILs in replicated experiments it was concluded that introgression of those QTLs in some of these NILs improved target root traits compared to IR64. For example, in the case of three tested NILs carrying target 1, one had significantly improved root traits over IR64. Three of the seven NILs carrying target 7 alone, as well as three of the eight NILs carrying targets 1 and 7, showed significantly improved root mass at depth. Four of the six NILs carrying target 9 had significantly improved maximum root length. But, because of likely cointrogression of linked QTLs, some NILs were taller than IR64 and all of them had a decreased tiller number.

Cho et al. (1994) established the order of DNA (RG220-RG109-RG381), morphological [anthocyanin activator (A), purple node (*Pn*), purple auricle (*Pau*)], and isozyme markers (*EstI-2*) of the semidwarf gene (*sd-1*) region on chromosome 1 and, after selfing of marker-aided selected individuals for four generations, demonstrated that three markers, *EstI-2*, RG220, and RG109, were tightly linked with *sd-1* locus and that genotypic selection for this recessive trait was effective at the seedling stage.

Anther culturability of rice is a quantitative trait controlled by nuclear-encoded genes. To increase the efficiency of green plant regeneration from microspores of 43 rice cultivars and two F_2 populations,

"MG RI036"/"Milyang 23" and "MG RI036"/"IR 36", Kwon et al. (2002b,c) used three markers, RG323, RG241, and RZ400, that are tightly linked to the QTL on chromosome 10. They reported that marker RZ400 was effective in identifying genotypes with good and poor regenerability and will be helpful in introgressing this trait into elite lines.

Ahmadi et al. (2001) introgressed the rice yellow mottle virus (RYMV)-resistant allele of two QTLs from an upland resistant japonica variety, Azucena, into a lowland susceptible indica variety IR64 by using RFLP and microsatellite markers in backcross breeding. The efficient introgression was reflected from the improved performance of the introgressed lines.

1.5.7
MAS for Introgression of Alien Genes

Wild and exotic accessions provide a useful resource for a large number of useful genes, particularly biotic and abiotic stress tolerance. Using molecular markers, introgression of such useful genes is now possible with minimum linkage drag in backcrossing programs. In rice, genes from wild species of rice have been identified in the advanced backcross progenies from crosses involving wild species, *O. australiensis* and *O. brachyantha,* using molecular markers (Ishii et al. 1994; Brar et al. 1996). Substitution lines have been developed with chromosome segments of *O. glaberrima* in *O. sativa* background using RFLP markers during the backcrossing process and should constitute useful resources for rice improvement (Doi et al. 1997).

1.6
Map-Based Cloning of Rice Genes and QTL

One of the important triumphs of molecular genetics and related genomics is the direct application of genetic map information to isolate a gene corresponding to a phenotype. The identification of a gene behind a phenotype has been a major goal in genetics since Mendel's discovery of the laws of inheritance. Many novel discoveries in the 21st century, including the principle of recombination by T.H. Morgan, the reality of inheritance by Avery et al., the structure of DNA by F.H.C. Crick and J.D. Watson, molecular cloning by P. Berg in conjunction with new technology of DNA analysis and manipulation such as nucleotide

sequencing by F. Sanger, and polymerase chain re-action by K. Mullis led to the success of identification of genes corresponding to phenotypes. The most well-known case is the competition of gene hunting for Huntington disease, a serious human-inherited disease (Allitto et al. 1991). Through this competition, tagging of phenotype by DNA markers provides an efficient strategy for gene cloning. The success of this case greatly encouraged the efforts of isolation of a gene behind a phenotype relying on molecular genetics. This idea was also applied subsequently to plants (Tanksley et al. 1995), and the efforts of generating reliable DNA markers were launched as described in previous sections.

Among plants, *Arabidopsis* was chosen as a model system for gene isolation by molecular genetics, such as map-based cloning or positional cloning (Giraudat et al. 1992; Arondel et al. 1992). Although *Arabidopsis* is a weed; it has been the target for full genome sequencing because of its small genome size (130 Mb) (Arabidopsis Genome Initiative 2000). The idea to sequence the *Arabidopsis* genome was also supported by the existence of collection of mutants to apply genomics information to identify the genes responsible for mutation. Many important genes of *Arabidopsis* have been identified by map-based cloning since 1992. Using a similar strategy of map-based cloning, though depending on the degree of preparation of infrastructure such as molecular genetic map, a genomic library with large-sized insert DNA, and genome sequence information, challenges to isolate genes have been made successful in tomato (Mao et al. 2000), wheat (Yan et al. 2003), and barley (Brueggeman et al. 2002) as well as in rice.

For performing an effective map-based or positional cloning strategy, first, the resource of the target phenotype must be genetically pure and must be established as a single Mendelian factor. This is a prerequisite to applying molecular genetic tools effectively in the accurate tagging of a phenotype. In addition, the target phenotype must be preferably distinct to obtain a clear segregation pattern. Second, the DNA markers to tag the phenotype must be codominant and must have high density in the vicinity of plausible position of the gene in the case of PCR-based markers. Third, once a candidate region is fixed within a few hundred kilobase, the region must be narrowed further by increasing the population size to reduce the number of candidate genes. To facilitate the analysis of many plant samples, pooling of five siblings as one sample to check recombination must be adapted. Fourth,

genomic libraries with large-size inserted DNA such as yeast artificial chromosome (YAC), bacterial artificial chromosome (BAC), P1-derived artificial chromosome (PAC), and cosmid libraries must be prepared and made available for screening by DNA markers tagged to the phenotype. If a positive genomic clone is identified, the sequence must be determined. And if the candidate gene region still spans more than 100 kb at this stage, genetic narrowing of the candidate region must be done using the sequence information to identify new polymorphisms such as SNP. Even if a plausible gene is found among the predicted genes within a candidate region, the target gene must be carefully confirmed because tandem duplicated similar genes are common in the rice genome. When several allelic variants exist for a common phenotype, Southern hybridization by a candidate DNA fragment should give supporting evidence if different patterns are obtained for each allelic variant. It is preferable to look for expressed genes by screening a cDNA library constructed from a specific tissue where the target phenotype is expressed.

The final step of map-based cloning is the confirmation of the biological function of the candidate gene. This is performed by transformation. The direction of transformation is either gain or loss of function depending on the characteristics of the candidate genome fragment. Transformation of rice plants by introducing *Agrobacterium tumefaciens* as an infectious tool of alien DNA has become easier and more efficient (Hiei et al. 1997). The candidate genomic region is cut out by an appropriate restriction enzyme and ligated to some eukaryotic expression vector carrying a suitable promoter (e.g., CaMV 35S). The japonica rice is considered better than indicas in their response to cell culture, although recently *Agrobacterium*-mediated transformation has been quite efficient in indica rice. Still the efficiency of transformation in the case of rice needs to be improved to obtain a large number of regenerated rice plants to obtain reliable data.

Some of the well-documented examples of map-based cloning include the isolation of disease resistance genes against viruses and bacteria. The identification of a disease resistance gene in plants was first reported in *HM1* of maize that controls the expression of the NADPH-dependent HC toxin reductase for resistance against the fungus *Cochliobolus carbonum* race 1 (Johal and Briggs 1992). However, this was performed in 1992 by transposon-induced mutagenesis, not by map-based genetics. At that time, molecular tools in maize for map-based cloning were not well

established. Then, in 1993, Tanksley's group at Cornell University published the isolation of the *Pto* gene of tomato that confers resistance to races of *Pseudomonas syringae* by map-based cloning (Martin et al. 1993). The success of this pioneering work on map-based cloning can be attributed to the well-developed molecular genetic tools for tomato such as a molecular genetic map, YAC library, cDNA library, and an efficient transformation strategy.

In 1995, the first report of success in isolation of rice disease resistance gene, *Xa21,* was published (Song et al. 1995). *Xa21* gene confers resistance to most races of *Xanthomonas oryzae* pv.*oryzae* (*Xoo*), including race 6, and carries both a leucine-rich repeat motif and a serine-threonine kinase-like domain. The tagging of *Xa21* by DNA markers on rice molecular genetic maps led to the identification of a closely cosegregating marker located on chromosome 11. This DNA marker was derived from rice genomic DNA and showed 20 to 30% identities to diverse proteins carrying leucine-rich repeat (LRR) motif. This LRR motif was identified in several disease-resistant genes from dicotyledonous plants (Staskawicz et al. 1995). The cloning of corresponding cDNA to this marker revealed its whole structure, which is commonly found in resistance (*R*) gene. Finally, the candidate was confirmed by the resistance reaction of the transformant in a susceptible japonica rice variety T-309. After this first success, several disease resistance genes of rice such as *Xa1*, which confers resistance against *Xoo* race 1 (Yoshimura et al. 1998), *Xa26,* which confers resistance against a wide range of *Xoo* races (Sun et al. 2004), *Pib*, which confers resistance against *Magnaporthe grisea* strain ARPC90-18C (Wang ZX et al. 1999), and *Pita,* which confers resistance against *M.grisea* strain O-137 (Bryan et al. 2000) were identified by map-based cloning. These isolated rice disease resistance genes carry common structural characteristics with dicotyledonous plants such as *Arabidopsis,* and this information gives us a hint for understanding the mechanism, evolution and biology of plant-microbe interaction in cereals (Ayliffe and Lagudah 2004).

Besides disease resistance genes, other agronomically and biologically important genes have been isolated from rice by map-based cloning. This includes the genes responsible for signal transduction of the plant hormone gibberellin, which controls plant growth and height. This trait is very important in rice and other cereal crops, particularly in increasing the capacity of light reception, and thereby increasing

yield efficiency. Breeding efforts in the 1960s focusing on this trait led to the so-called "Green Revolution" (Conway 1997). The genes used in this program for rice and wheat are *sd1* and *Rht1*, respectively, which have been identified by map-based cloning and revealed to be involved in signal transduction pathway of gibberellin biosynthesis. The rice *sd1* (Ashikari et al. 2002; Sasaki et al. 2002a; Monna et al. 2002a; Spielmeyer et al. 2002) and wheat *Rht1* (Peng et al. 1999) are controlled by gibberellin $(GA)_{20}$ oxidase and a nuclear transcription factor, respectively. Later, orthologs of *Rht1* were isolated from rice as *slender 1 (slr1)* (Ikeda et al. 2001). Although the amino-acid sequences of both proteins are homologous, the mutated point of each gene corresponds to a different functional motif regarding the activity under gibberellin. This causes dwarfness in the case of *Rht1* and the slender phenotype in the case of *slr1*. Other important genes involved in gibberellin signal transduction, *d*1 (Ashikari et al. 1999; Fujisawa et al. 1999) and *gid2* (Sasaki et al. 2003), were also isolated by map-based cloning. The *d*1 and *gid2* are thought to play roles at the early and late steps of signal transduction, respectively.

The above-mentioned cases of gene isolation are applicable if the corresponding phenotype is coded by a specific gene. However, many important traits used for crop production are controlled not by a single gene, but by a gene network. This network can be analyzed as a genetically defined character known as QTL. Some well-known QTLs in rice include those for grain weight, number of panicles, flowering time, and culm length. Recent development in genetic mapping with many DNA markers enables accurate identification of QTLs by an interval mapping method (Lander and Botstein 1986). Also, DNA markers make it accurate to judge the genotype of any genomic region among the siblings. Therefore, a QTL identified in an F_2 population can be separated into each locus by repeated backcrossing and genotyping of each sibling. The resultant chromosome segment substitution lines (CSSL) can be used for making a segregating population by focusing on one of the loci of the target QTL to isolate as a single Mendelian factor (Tanksley 1993). This strategy was first developed to identify genes of QTL conferring fruit size (Frary et al. 2000) and also has been used to identify QTLs of rice-flowering time (Yano et al. 2001). Crossing japonica rice variety Nipponbare and indica rice variety Kasalath, so far 15 QTLs of flowering time have been identified with a significant LOD score (Fig. 4). They were found

without clustering at a specific locus. By inspecting the response to photoperiod for each locus using corresponding CSSL, about half of the QTLs of flowering time were revealed to be photoperiod sensitive. Detection of flowering under a controlled photoperiod condition is very clear and the data obtained are reliable. This condition satisfies the prerequisite for a successful map-based cloning strategy as described above.

Among photoperiod-sensitive QTLs of rice-flowering time, six loci have been fine mapped, and four of them have been succesfully identified. The first gene identified is called *Hd1* (Yano et al. 2000) located at chromosome 6 and mainly contributing to flowering time (LOD = 44.2) of the cross of Nipponbare and Kasalath. The *Hd1* gene was revealed to be an ortholog of *CONSTANS* (*CO*) gene of *Arabidopsis* that was identified also as a gene involved in flowering time (Putterill et al. 1995) and characterized by transcription factors carrying zinc-finger domain and CCT motif. The difference in sequence between Nipponbare and Kasalath is recognized in many positions, and the most crucial factor that affects the activity of gene products is the 2-bp deletion in the second exon of the Kasalath allele (Yano et al. 2000). The other isolated genes were *Hd3a* (Kojima et al. 2002) and *Hd6* (Takahashi et al. 2001), orthologs of *Arabidopsis* flowering time (*FT*), and casein kinase 2 alpha (*CK2α*), respectively. These three rice genes have orthologs in *Arabidopsis*, but the fourth one, called *Ehd1* (Doi et al. 2004), which might be the same as *Hd14* and carries a homologous sequence with B-type response regulator, does not have any ortholog in *Arabidopsis*. The identified genes of rice QTL of flowering time must be further analyzed for their biochemical and physiological function with regard to their interactive gene network as QTL (Sasaki et al. 2003). In addition to these studies, CSSL for each locus of QTL is used in the genetic analysis of interaction of each locus by crossing each locus.

Besides flowering time, other rice QTLs such as grain size and weight (Thomson et al. 2003), seed dormancy (Takeuchi et al. 2003), durable resistance to blast (Liu et al. 2004a), resistance to UV-B (Ueda et al. 2004), and eating and cooking quality (Li et al. 2003b) have been targeted to clarify genes involved in their expression. Examples of isolated rice genes by map-based cloning are listed in Table 12. The candidate genes for some of them have already been tagged closely, and some are still being analyzed by genetic

mapping for tagging. The map-based cloning strategy must be complemented by other methods such as transposon tagging by *Tos17* (Hirochika 2001) or T-DNA insertional mutagenesis (An et al. 2003; Sallaud et al. 2004). The latter one, in principle, can identify only one gene by each tagged line, but in the case of transposon insertion affecting the phenotype relating to a target QTL, the information must be helpful to accelerate the identification by combining genetic and reverse-genetic analysis.

1.7
Advanced Works

1.7.1
Rice Physical Maps

Physical maps expedite positional cloning, whole genome sequencing, and thorough analysis of genome organization. Several cloning vectors such as yeast artificial chromosome (YAC) (Burke et al. 1987), bacterial artificial chromosome (BAC) (Shizuya et al. 1992), and P1-derived artificial chromosome (PAC) (Ioannou et al. 1994) have been developed to clone large chromosome fragments for facilitating the construction of physical maps. Umehara et al. (1995) constructed a YAC library, which was used later for developing a physical map of rice (Saji et al. 2001). Wu et al. (2002) screened this YAC library using specific primers designed from 6,731 unique expressed sequence tags (ESTs) from 19 cDNA libraries and placed 6,591 EST sites on this YAC-based physical map that covered 80.8% of the rice genome. Expressed sequence tags are partial nucleotide sequences of expressed genes and are obtained by random sequencing of many cDNA clones. ESTs provide the cheapest and fastest means to catalog all genes by comparing the homology of the DNA sequences and its inferred protein sequences with those of other organisms deposited in various databases. Uchimiya et al. (1992) reported the first set of rice ESTs. Later, the Japanese Rice Genome Program undertook extensive EST sequencing in rice (Yamamoto and Sasaki 1997). As of 16 June 2005, about 1,184,706 ESTs of rice have been deposited in GenBank (http://www.ncbi.nlm.nih.gov/dbEST/dbEST_summary.html).

BAC and PAC libraries were constructed to develop an accurate sequence ready physical map for obtaining a reliable genome sequence (Baba et al. 2000; Chen et al. 2002). Fingerprinting was applied
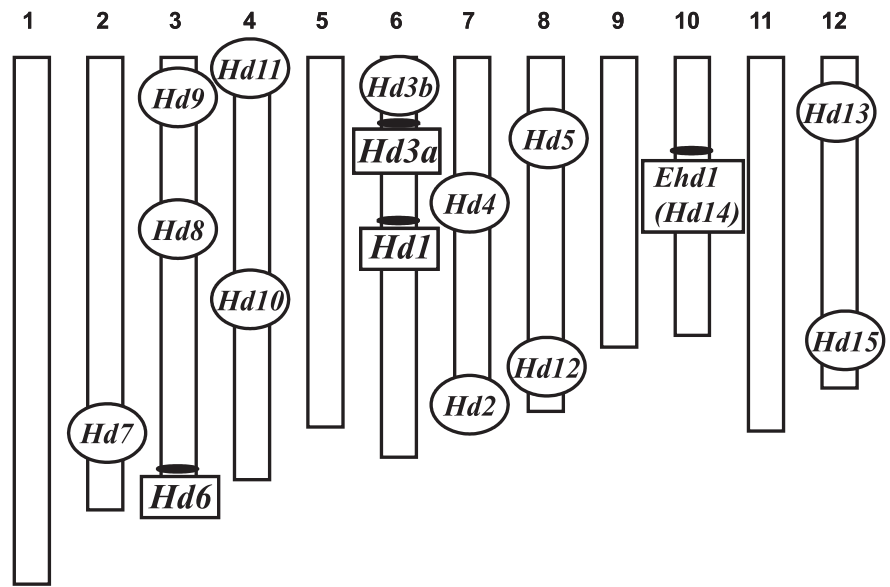
Fig. 4. Chromosomal location of each gene involved in rice flowering time QTL. The position of four genes, Hd1, Hd3a, Hd6 and Ehd1(Hd14) are shown as *bold lines* because they were identified

to determine the contiguous BAC clones and a physical map of the whole rice genome was established and integrated with the rice genetic map (Chen et al. 2002). This integrated genetic and physical map provides an essential tool for efficient and rapid isolation of agriculturally important genes in rice and comparative genome analysis among grass relatives.

1.7.2
Tools for Rice Functional Genomics

Genome mapping and sequencing are two important tools of structural genomics and have been the major focus of plant genomics for the past two decades. Both whole genome and EST sequencing in rice has generated enormous amounts of sequence data to provide the platform for functional genomics investigations. The greatest challenge now is to elucidate the function of each individual gene sequence in the growth and development of the rice plant. Quantifying the spatial and temporal expression patterns at mRNA and protein levels helps clarify the role of each annotated gene. A genomewide approach is being pursued rather than a traditional gene-by-gene approach to realize this important goal. Two major tools, DNA microarrays and insertional mutagenesis, have become essential components of rice functional genomics studies.

1.7.3
DNA Microarray

DNA microarray is a powerful tool for functional genomics studies because it allows quantification of gene expression on a global scale (Deyholos and Galbraith 2001). Application of this technology to the recently completed whole rice genome sequence data will be beneficial in terms of assigning a function to those annotated sequences. Schena et al. (1995) used this technology for the first time to compare the expression pattern of 48 EST clones between roots and leaves in *Arabidopsis*. Microarrays are available in two formats: cDNA array (Schena et al. 1995) and oligonucleotide array (Lockhart et al. 1996).

The cDNA microarrays are largely preferred by plant scientists for transcription profiling because a large number of cDNA and EST clones can be easily generated. Rice cDNA microarrays have been used to study the role of phytohormones, brassinosteroids (BRs), gibberellins (GAs), and abscisic acid (ABA) in growth and development (Yazaki et al. 2003; Yang et al. 2004), monitoring gene expression during pollination and fertilization (Lan et al. 2004), metabolic changes under phosphorous stress (Wasaki et al. 2003), and abiotic stress response (Kawasaki et al. 2001; Cooper et al. 2003; Rabbani et al. 2003).

There are only a few studies involving rice oligoarrays. Zhu et al. (2003) used a rice 21,000 gene chip microarray covering half the rice genome to inves-

Table 12. Examples of rice genes isolated mainly by map-based cloning

Gene	Phenotype	Characteristics of predicted gene product	Chromo-some	Reference
Xa1	Resistance to *X.oryzae pv. oryzae* (race 1)	NBS-LRR type of plant R-gene	4	Yoshimura et al. 1998
Xa21	Resistance ro *X.oryzae pv. oryzae* (race 21)	Receptor-LRR type of plant R-gene	11	Song et al. 1995
Xa26	Resistance to *X.oryzae pv. oryzae*	NBS-LRR type of plant R-gene	11	Sun et al. 2004
Pib	Resistance to *M.grisea* (race 003)	NBS-LRR type of pant R-gene	2	Wang et al. 1999b
Pita	Resistance to *M.grisea*	NBS-LRR type of plant R-gene	12	Bryan et al. 2000
Spl7	Spotted leaf (lesion-mimic)	Heat stress transcription factor	5	Yamanouchi et al. 2002
Spl11	Spotted leaf (lesion-mimic)	U-box/armadillo repeat protein	12	Zeng et al. 2004
*d*1	Dwarf (Daikoku)	Alpha subunit of heterotrimeric GTP-binding protein	5	Spielmeyer et al. 2002; Peng et al. 1999
ebisu dwarf (d2)	Dwarf	Cytochrome P450(CYP90D2)	1	Hong et al. 2003
gid2	Dwarf	F-box protein	2	Peng et al. 1999
sd1	Semi-dwarf (Dee-geo-woo-gen, IR8)	GA20 oxidase (GA20ox-2)	1	Ashikari et al. 2002; Sasaki et al. 2002a; Monna et al. 2002a; Spielmeyer et al. 2002
moc1	Abnormal tillering	GRAS family nuclear protein	6	Li XY et al. 2003
LAX	Lax panicle	Helix-loop-helix transcription factor	1	Komatsu et al. 2003
PLAST-CHRON1	Timekeeper of leaf initiation	Cytochrome P450(CYP78A11)	10	Miyoshi et al. 2004
slg	Slender glume	Ubiquitin-related modifier	7	Nakazaki et al. 2003
Rf1	Fertility restoration	Mitchodrially targeted pentatricopeptide repeat protein	10	Komori et al. 2004; Akagi et al. 2004
Hd1	QTL of flowering time	Transcription factor, *CONSTANS* family	6	Yano et al. 2000
Hd3a	QTL of flowering time	*FT* family	6	Kojima et al. 2002
Hd6	QTL of flowering time	Casein kinase *CK2?* family	3	Takahashi et al. 2001
Ehd1	QTL of flowering time	B-type response regulator	10	Doi et al. 2004

tigate nutrient portioning during rice grain filling. This study revealed that different isoforms of different enzymes of the starch biosynthesis pathway are expressed in different tissues and at different developmental stages, suggesting synchronization in the expression of coordinately regulated genes. Yazaki et al. (2004) constructed oligoarrays of 20,500 transcriptional units identified by the rice full length cDNA consortium and identified new ABA and GA responsive genes.

Recently, 28,469 full-length cDNA clones from cv. Nipponbare have been characterized (Full-Length cDNA Consortium 2003). One important conclusion from this study is that there are 19,000 to 20,500 transcriptional units in the rice genome. Seventy-six per-

cent of these clones were assigned tentative functions, and 64% of these are homologous to *Arabidopsis* proteins. Osato et al. (2004) analyzed these sequences and found large numbers of sense-antisense transcript pairs, which suggests gene regulation by the antisense transcripts.

1.7.4
Insertional Mutagenesis

The reverse genetics approach offers an efficient strategy to validate the function of most rice genes. Various physical, chemical, and biological methods can be employed to systematically disrupt the genes of rice plant, and several such mutant populations have been

generated in a number of laboratories (Hirochika et al. 2004). Biological agents such as T-DNA of *Agrobacterium* (Zambryski et al. 1980) and transposable element *Ac/Ds* of corn (McClintock 1956) have been used to generate mutant populations for gene discovery in rice (Izawa et al. 1997; Jeon et al. 2000). Since the sequences of these agents are known, it is much easier to simultaneously disrupt and tag the gene from its altered phenotypic expression. T-DNA tagged lines helped in the identification of cold responsive genes (Lee et al. 2004), genes for Mg-chelatase (Jung et al. 2003), Poly (A) binding proteins (Han and An 2003). Hirochika (1997) discovered an endogenous transposon *Tos17* as an efficient tool for insertional mutagenesis compared to *Ac/Ds* system. *Tos17* is activated by tissue culture and is widely distributed over the rice genome and preferentially integrated into low-copy-number genomic regions (Yamazaki et al. 2001). A rice homeobox gene *OSH15* responsible for dwarf phenotype due to alteration in internode architecture was identified by this method (Sato et al. 1999).

Another resource for functional genomics investigation is a collection of deletion mutants of an indica cv. IR64 developed at the International Rice Research Institute (Leung et al. 2000). These mutant populations were generated using fast neutron, gamma irradiation, and diepoxybutane. These mutant populations are being evaluated for alteration in morphology and response to biotic and abiotic stresses to develop a database. Compared with the mutants generated by the T-DNA, *Ac/Ds*, or *Tos17*, IR64 mutants are not amenable for easy identification of genes responsible for phenotypic change due to absence of a tag. But PCR screening of these mutant populations may be employed to identify gene mutations.

1.8
Future Scope of Work

The rice genome sequence has been completely and accurately decoded by the International Rice Genome Sequencing Project (IRGSP) (http://rgp.dna.affrc.go.jp/IRGSP/). This map-based precise sequence information of the japonica rice variety Nipponbare will have a significant impact on any further rice research. About 90% of the cultivated and consumed rice belongs to the indica type, not japonica. Aside from some morphological and physiological characteristics, no detailed information on the difference between these two subspecies has been made available so far. Although both belong to a common species, *Oryza sativa*, the progenies derived from crossing the two subspecies have low or no fertility. The Nipponbare genome sequence can be used as a reference to understand the difference between two subspecies and among varieties within each subspecies as well. In this sense, the Nipponbare genome sequence will serve as the gold standard for further genomics research based on comparison of genome sequence and structure. The subspecies japonica is mainly cultivated in Japan, Korea, Taiwan, and Italy, and their genetic diversity is narrow. On the other hand, the subspecies indica is mainly cultivated in China, India, Thailand, and Indonesia, and their genetic diversity is wide (Nakagahra et al. 1997). Comparison of these two subspecies of *O. sativa* will prove the validity of the gold standard sequence to easily and promptly attain genome information of other cereals. The most desirable tool for further work on comparative genomics is a physical map that enables genomewide comparison of the structures and gene repertoire of target species. Once such a reliable physical map is available, the sequence information at the precise corresponding genomic region could be easily obtained by routine sequencing work. Although there are about 120,000 indica varieties worldwide (Khush 1997), it is quite reasonable first to start with the variety Kasalath, which has been used as a parent of the F_2 population for construction of a molecular genetic map and, consequently, has been used to identify QTLs such as flowering time (Yano et al. 2001).

A BAC library of variety Kasalath was constructed to facilitate comparison with the variety Nipponbare. End sequencing of the clones of this library was carried out, and 78,427 high-quality BAC end sequences (BESs) were collected. At an average read length of 482 bp, a total length of 37.8 Mb sequence was obtained (Katagiri et al. 2004). After removal of BESs containing repetitive sequences and use of the Nipponbare sequence as a standard, a total of 12,170 clones with paired BESs were mapped *in silico* onto the 12 rice chromosomes. These clones consisted of 450 contigs and showed a total physical length of 308.5 Mb, indicating genome coverage of about 80%. Confirmation of the chromosomal position of the Kasalath BAC clones mapped on chromosome 1 using specific DNA markers revealed that the map accuracy was extremely high, at least 94.8%. A frequency of 0.71% for single nucleotide polymorphisms (SNPs) and 1.23 sites per kilobase for InDels (1-16bp length), respectively, were

observed between Nipponbare and Kasalath (Kata-giri et al. 2004). The Chinese indica varieties, Guangluai 4 (Feng et al. 2002) and 93-11 (Yu et al. 2002a), have been sequenced using a map-based strategy and a whole genome shotgun strategy, respectively. A detailed comparison of the 2.3-Mb genome sequence of chromosome 4 of Guangluai 4 to the corresponding chromosome of Nipponbare revealed a 0.37% frequency of SNPs (Feng et al. 2002). The genomewide frequency of SNPs and InDels between Nipponbare and 93-11 were calculated as 0.37% and 1.05 sites per kilobase, respectively (Yu et al. 2002a). Unfortunately, mapping of genomic clones of these Chinese indica varieties to Nipponbare sequences has not yet been performed. However, these results clearly showed the utility of the Nipponbare sequence as a powerful resource to perform comparative genomics of a wide range of indica subspecies. Such minute information on polymorphism is directly linked to the identification of new alleles regarding the difference of phenotype in a quantitative manner. Genetic analysis of a quantitative trait and extensive data of SNPs among many rice varieties including wild relatives of O.sativa are two main indispensable points for improvement of rice.

Cultivated rice, O. sativa, has many wild relatives (Table 2) (http://www.knowledgebank.irri.org/wildRiceTaxonomy/default.htm). Some of them are not diploid but tetraploid and some have a genome size two to three times that of O. sativa (400 Mb). Although not yet clearly demonstrated by molecular genetic data, O. sativa is said to have evolved from O. rufipogon via O. nivara aided by efforts of domestication by humans (Khush 1997). During this domestication process, some genome arrangements occurred and the rice species suitable to an existing environmental condition and with high yield have been selected to breed. On the other hand, traits without any agricultural value were not incorporated into the breeding program. However, it is well known that genes associated with tolerance to biotic and abiotic stresses still remain in wild relatives of O. sativa. In addition, recent achievement of the genome sequence of O. sativa can reveal the distinct tandem repeat of many genes and partly polyploidy nature of rice chromosomes. The genome size of each wild Oryza species is reported to be larger than O. sativa. For example, the genome size of O. glumepatula is 475 Mb (Uozu et al. 1997). So far, there is no molecular information on what happened to both japonica and indica genomes during the domestication process such as whether the deletion of a genic or intergenic region occurred or whether segmental or tandem duplication occurred before or after this genome size reduction. Molecular interpretation on such points based on a comparison of genomes is needed to understand the history of domestication and on how to improve the current cultivated species with more favorable traits.

Using DNA markers derived from expressed genes, it is clear that the rice genome carries colinearity of gene alignment with other cereals such as millet, sorghum, barley, maize, and wheat (Gale and Devos 1998). This syntenic relationship is undoubtedly the major driving force that establishes rice as the model or reference plant for cereal genomics. The *Rht1* gene of wheat and *D*8 of maize have been identified using this information (Peng et al. 1999). However, success in identifying targeted genes is so far limited. This is because of the unexpected complex structure of the cereal genomes revealed first by mapping of disease resistance genes by common markers and second by genome sequencing. Although current cereals diverged from their common ancestor about 60 million to 70 million years ago (Kellog 2001) and carry its footprint in each diverged species, each must have its specific genome structure to assert itself to survive under biotic and abiotic stresses. If it has received selection pressure by breeding, it must also alter its genome structure to accept selection. As a result, the linearity of each gene within ancestral species must be shuffled and rearranged during the evolution of each species. The remnant of the ancestral structure is observed as broken colinearity in rice, sorghum, maize, and barley, which was recently identified by detailed sequence comparison (Bennetzen and Ma 2003). This breakage was observed even for a commonly existing housekeeping gene locus like alcohol dehydrogenase I (Tikhonov et al. 1999; Tarchini et al. 2000; Bennetzen and Ramakrishna 2002).

So far, our knowledge of the validity of synteny based on sequence information is very limited. This is also true in the case of dicotyledonous plants using *Arabidopsis* as a model species with revealed genome sequence (Rossberg et al. 2001; Boivin et al. 2004). Synteny itself is a very important concept to be verified to understand the evolutionary history of each genus. Detailed sequence comparison of a specific gene locus could clarify what happened by diversification like duplication, insertion of a transposable element, or insertion of a species-specific new gene. Extensive collection of sequence information on more

gene loci and preferably on genome of related species is required for further understanding of plant. This, of course, must be carefully performed based on a close interaction of genetics and biology of the target genus.

The ultimate goal of rice genomics researchers today is to apply rice genome information to breed rice varieties with improved yield, superior quality, and resistance to biotic and abiotic stresses. This can only be achieved once we assign a function to each and every annotated sequence and discover the complex interactions among them. The recent release of the rice genome sequence coupled with technological advances in microarray technology and reverse genetics tools will expedite such activities by providing a global perspective on response of rice genes at different growth and developmental stages.

The most important benefit that has accrued from rice genomics studies is the application of MAS in rice-breeding programs. It has become routine in most rice-breeding programs all over the world because molecular markers have demonstrated their utility in making the selection process much easier and more efficient. Most of the success stories of MAS have been limited to simply inherited traits (reviewed earlier). The role of MAS in improving complex agronomic traits, however, has been minimal. This is because of the complexity resulting from pleiotropy, epistasis, and genotype × environment interaction associated with the quantitative traits (Tanksley 1993). Despite the complexity associated with many economically important traits, there has been successful cloning of QTLs controlling many useful traits. Thanks to advances in genomics, we are now in a better position than ever before to enhance our understanding of the molecular basis of these complex traits. Complex genetic traits can be studied in detail by integrating QTL mapping with microarrays. Generation and utilization of novel genetic stocks such as mutants, near-isogenic lines, substitution lines, deletion lines, and transgenic lines for gene expression studies will further facilitate the genetic analysis of complex agronomic traits. With advances in gene chip technology and our understanding of complex traits, it may be possible to select the desirable rice lines from the unique expression pattern of several genes associated with a number of agronomic traits.

To derive benefit from the accurate rice genome sequence it is imperative to catalog the allelic variation in the available germplasm for important agronomic traits at the nucleotide level. Both wild and cultivated rice germplasms are reservoirs of genes for

yield, biotic, and abiotic stresses (Tanksley and Mc-Couch 1997). Rice genome information should be exploited to unlock useful variations for rice improvement. Correlating these nucleotide sequence variations with the phenotypic variation will help select desirable alleles for incorporation by genetic engineering or MAS. Since there is no control over the number of transgenes or the sites of integration associated with transformation by *Agrobacterium* or particle bombardment, Terada et al. (2002) developed a method to insert genes in targeted sites using homologous recombination. With the knowledge of the rice genome sequence this method can further be refined for precise incorporation of useful genes for rice improvement.

References

Aggarwal RK, Brar DS, Nandi S, Huang N, Khush GS (1999) Phylogenetic relationships among *Oryza* species revealed by AFLP markers. Theor Appl Genet 98:1320–1328

Ahmadi N, Albar L, Pressoir G, Pinel A, Fargette D, Ghesquiere A (2001) Genetic basis and mapping of the resistance to rice yellow mottle virus. III. Analysis of QTL efficiency in introgressed progenies confirmed the hypothesis of complementary epistasis between two resistance QTLs. Theor Appl Genet 103:1084–1092

Ahn S, Tanksley SD (1993) Comparative linkage maps of the rice and maize genomes. Proc Natl Acad Sci USA 90:7980–7984

Ahn SN, Bollich CN, Tanksley SD (1992) RFLP tagging of a gene for aroma in rice. Theor Appl Genet 84:825–828

Ahn SN, Bollich CN, McClung AM, Tanksley SD (1993) RFLP analysis of genomic regions associated with cooked-kernel elongation in rice. Theor Appl Genet 87:27–32

Ahn SN, Kim YK, Hong HC, Han SS, Kwon SJ, Choi HC, Moon HP, McCouch SR (2000) Molecular mapping of a new gene for resistance to rice blast (*Pyricularia grisea* Sacc.). Euphytica 116:17–22

Akagi H, Yokozeki Y, Inagaki A, Nakamura A, Fujimura T (1996) A codominant DNA marker closely linked to the rice nuclear restorer gene, *Rf-1*, identified with inter-SSR fingerprinting. Genome 39:1205–1209

Akagi H, Nakamura A, Yokozeki-Misono Y, Inagaki A, Takahashi H, Mori K, Fujimura T (2004) Positional cloning of the rice *Rf-1* gene, a restorer of BT-type cytoplasmic male sterility that encodes a mitochondria-targeting PPR protein. Theor Appl Genet 108:1449–1457

Alam SN, Cohen MB (1998) Detection and analysis of QTLs for resistance to the brown planthopper, *Nilaparvata lugens*, in a doubled-haploid rice population. Theor Appl Genet 97:1370–1379

Albar L, Lorieux M, Ahmadi N, Rimbault I, Pinel A, Sy AA, Fargette D, Ghesquiere A (1998) Genetic basis and mapping

of the resistance to rice yellow mottle virus. I. QTLs identification and relationship between resistance and plant morphology. Theor Appl Genet 97:1145–1154

Ali ML, Pathan MS, Zhang J, Bai G, Sarkarung S, Nguyen HT (2000) Mapping QTLs for root traits in a recombinant inbred population from two indica ecotypes in rice. Theor Appl Genet 101:756–766

Allitto BA, MacDonald ME, Bucan M, Richards J, Romano D, Whaley WL, Falcone B, Ianazzi J, Wexler NS, Wasmuth JJ (1991) Increased recombination adjacent to the Huntington disease-linked D4S10 marker. Genomics 9:104–112

Aluko G, Martinez C, Tohme J, Castano C, Bergman C, Oard JH (2004) QTL mapping of grain quality traits from the interspecific cross *Oryza sativa* x *O. glaberrima*. Theor Appl Genet 109:630–639

An S, Park S, Jeong DH, Lee DY, Kang HG, Yu JH, Hur J et al (2003) Generation and analysis of end sequence database for T-DNA tagging lines in rice. Plant Physiol 133:2040–2047

Andaya VC, Mackill DJ (2003a) Mapping of QTLs associated with cold tolerance during the vegetative stage in rice. J Exp Bot 54:2579–2585

Andaya VC, Mackill DJ (2003b) QTLs conferring cold tolerance at the booting stage of rice using recombinant inbred lines from a japonica × indica cross. Theor Appl Genet 106:1084–1090

Arabidopsis Genome Initiative (2000) Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. Nature 408:796–815

Arondel V, Lemieux B, Hwang I, Gibson S, Goodman HM, Somerville CR (1992) Map-based cloning of a gene controlling omega-3 fatty acid desaturation in *Arabidopsis*. Science 258:1353–1355

Ashikari M, Wu J, Yano M, Sasaki T, Yoshimura A (1999) Rice gibberellin-insensitive dwarf mutant gene *Dwarf1* encodes the alpha-subunit of GTP-binding protein. Proc Natl Acad Sci USA 96:10284–10289

Ashikari M, Sasaki A, Ueguchi-Tanaka M, Itoh H, Nishimura A, Datta S, Ishiyama K, Saito T, Kobayashi M, Khush GS, Kitano H, Matsuoka M (2002) Loss-of-function of a rice gibberellin biosynthetic gene, *GA20 oxidase* (*GA20ox-2*), led to the rice 'Green Revolution'. Breed Sci 52:143–150

Ayliffe MA, Lagudah ES (2004) Molecular genetics of disease resistance in cereals. Ann Bot 94:765–773

Ayres NM, McClung AM, Larkin PD, Bligh HFJ, Jones CA, Park WD (1997) Microsatellites and a single-nucleotide polymorphism differentiate apparentamylose classes in an extended pedigree of US rice germplasm. Theor Appl Genet 94:773–781

Baba T, Katagiri S, Tanoue H, Tanaka R, Chiden Y et al (2000) Construction and characterization of rice genomic libraries: PAC library of japonica variety, Nipponbare and BAC library of indica variety, Kasalath. Bull NIAR 14:41–49

Babu RC, Nguyen BD, Chamarerk V, Shanmugasundaram P, Chezhian P, Jeyaprakash P, Ganesh SK, Palchamy A, Sada-

sivam S, Sarkarung S, Wade LJ, Nguyen HT (2003) Genetic analysis of drought resistance in rice by molecular markers: Association between secondary traits and field performance. Crop Sci 43:1457–1469

Bao JS, Zheng XW, Xia YW, He P, Shu QY, Lu X, Chen Y, Zhu LH (2000) QTL mapping for the paste viscosity characteristics in rice (*Oryza sativa* L.). Theor Appl Genet 100:280–284

Basten CJ, Weir BS, Zeng ZB (2001) QTL Cartographer version 1.15. Department of Statistics. North Carolina State University, Raleigh, NC

Bennetzen JL, Ramakrishna W (2002) Numerous small rearrangements of gene content, order and orientation differentiate grass genomes. Plant Mol Biol 48:821–827

Bennetzen JL, Ma J (2003) The genetic colinearity of rice and other cereals on the basis of genomic sequence analysis. Curr Opin Plant Biol 6:128–133

Berruyer R, Adreit H, Milazzo J, Gaillard S, Berger A, Dioh W, Lebrun MH, Tharreau D (2003) Identification and fine mapping of *Pi33*, the rice resistance gene corresponding to the *Magnaporthe grisea* avirulence gene *ACE1*. Theor Appl Genet 107:1139–1147

Bharaj TS, Virmani SS, Khush GS (1995) Chromosomal location of fertility restoring genes for wild abortive cytoplasmic male-sterility using primary trisomics in rice. Euphytica 83:169–173

Blair MW, McCouch SR (1997) Microsatellite and sequence-tagged site markers diagnostic for the rice bacterial leaf blight resistance gene *xa-5*. Theor Appl Genet 95:174–184

Blair MW, Panaud O, McCouch SR (1999) Inter-simple sequence repeat (ISSR) amplification for analysis of microsatellite motif frequency and fingerprinting in rice (*Oryza sativa* L.). Theor Appl Genet 98:780–792

Blair MW, Garris AJ, Iyer AS, Chapman B, Kresovich S, McCouch SR (2003) High resolution genetic mapping and candidate gene identification at the *xa5* locus for bacterial blight resistance in rice (*Oryza sativa* L). Theor Appl Genet 107:62–73

Boivin K, Acrakan A, Mbulu RS, Clarenz O, Schmidt R (2004) The Arabidopsis genome sequence as a tool for genome analysis in Brassicaceae. A comparison of the Arabidopsis and *Capsella rubella* genome. Plant Physiol 135:735–744

Bormans CA, Rhodes RB, Kephart DD, McClung AM, Park WD (2002) Analysis of a single nucleotide polymorphism that controls the cooking quality of rice using a non-gel based assay. Euphytica 128:261–267

Botstein D, White RL, Skolnick M, Davis RW (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet 32:314–331

Bowers JE, Abbey C, Anderson S, Chang C, Draye X, Hoppe AH, Jessup R et al (2003) A high-density genetic recombination map of sequence-tagged sites for sorghum as a framework for comparative structural and evolutionary genomics of tropical grains and grasses. Genetics 165:367–386

Brar DS (2002) Molecular marker assisted breeding. In: Jain SM, Brar DS, and Ahloowalia BS (eds) Molecular Techniques in

Crop Improvement. Kluwer Academic Publishers, Boston, pp 56–83

Brar DS, Dalmacio R, Rlloran R, Aggarwal R, Angeles R, Khush GS (1996) Gene transfer and molecular characterization of introgression from wild *Oryza* species into rice. In: Rice Genetics III. IRRI, Manila, Philippines, pp 477–486

Bres-Patry C, Lorieux M, Clement G, Bangratz M, Ghesquiere A (2001) Heredity and genetic mapping of domestication-related traits in a temperate japonica weedy rice. Theor Appl Genet 102:118–126

Brondani C, Rangel PHN, Brondani RPV, Ferreira ME (2002) QTL mapping and introgression of yield-related traits from *Oryza glumaepatula* to cultivated rice (*Oryza sativa*) using microsatellite markers. Theor Appl Genet 104:1192–1203

Brookes AJ (1999) The essence of SNPs. Gene 234:177–186

Brown LR (1997) The agricultural link: how environmental deterioration could disrupt economic progress. World Watch Paper No 136, World Watch Institute, Washington, DC

Brueggeman R, Rostoks N, Kudrana D, Killian A, Han F, Chen J, Druka A, Steffenson B, Kleinhofs A (2002) The barley stem rust-resistance gene *Rpg1* is a novel disease-resistance gene with homology to receptor kinases. Proc Natl Acad Sci USA 99:9328–9333

Bryan GT, Wu KS, Farrall L, Jia Y, Hershey HP, McAdams SA, Faulk KN, Donaldson GK, Tarchini R, Valent B (2000) A single amino acid difference distinguishes resistant and susceptible alleles of the rice blast resistance gene *Pi-ta*. Plant Cell 12:2033–2046

Buetow KH, Edmonson M, MacDonald R, Clifford R, Yip P, Kelley J, Little DP, Strausberg R, Koester H, Cantor CR, Braun A (2001) High throughput development and characterization of a genome wide collection of gene-based single nucleotide polymorphism markers by chip-based matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Proc Natl Acad Sci USA 98:581–584

Burke DT, Carle GF, Olson MV (1987) Cloning of large segments of exogenous DNA into yeast by means of artificial chromosome vectors. Science 236:806–812

Burrow MD, Blake TK (1998) Molecular tools for the study of complex traits. In: Paterson AH (ed) Molecular Dissection of Complex Traits. CRC Press, Boca Roton, FL, pp 13–29

Buttery RG, Ling LC, Juliano BO, Turnbaugh JG (1983) Cooked rice aroma and 2-acetyl-1-pyrroline. J Agr Food Chem 31:823–826

Caldwell KS, Langridge P, Powell W (2004) Comparative sequence analysis of the region harboring the hardness locus in barley and its collinear region in rice. Plant Physiol 136:3177–3190

Cao G, Zhu J, He C, Gao Y, Yan J, Wu P (2001) Impact of epistasis and QTL × Environment interaction on the developmental behavior of plant height in rice (*Oryza sativa* L.). Theor Appl Genet 103:153–160

Causse MA, Fulton TM, Cho YG, Ahn SN, Chunwongse J, Wu K, Xiao J, Yu Z, Ronald PC, Harrington SE, Second G, McCouch SR, Tanksley SD (1994) Saturated molecular map of the rice genome based on an interspecific backcross population. Genetics 138:1251–1274

Champoux MC, Wang G, Sarkarung S, Mackill DJ, O'Toole JC, Huang N, Mccouch SR (1995) Locating genes associated with root morphology and drought avoidance in rice via linkage to molecular markers. Theor Appl Genet 90:969–981

Chang TT (1976) The origin, evolution, cultivation, dissemination and diversification of Asian and African rices. Euphytica 25:435–444

Che KP, Zhan QC, Xing QH, Wang ZP, Jin DM, He DJ, Wang B (2003) Tagging and mapping of rice sheath blight resistant gene. Theor Appl Genet 106:293–297

Chen DH, dela Vina M, Inukai T, Mackill DJ, Ronald PC, Nelson RJ (1999) Molecular mapping of the blast resistance gene, *Pi44(t),* in a line derived from a durably resistant rice cultivar. Theor Appl Genet 98:1046–1053

Chen M, Presting G, Barbazuk WB, Goicoechea JL, Blackmon B, Fang G, Kim H, Frisch D, Yu Y, Sun S, Higingbottom S, Phimphilai J, Phimphilai D, Thurmond S, Gaudette B, Li P, Liu J, Hatfield J, Main D, Farrar K, Henderson C, Barnett L, Costa R, Williams B, Walser S, Atkins M, Hall C, Budiman MA, Tomkins JP, Luo M, Bancroft I, Salse J, Regad F, Mohapatra T, Singh NK, Tyagi AK, Soderlund C, Dean RA, Wing RA (2002) An integrated physical and genetic map of the rice genome. Plant Cell 14:537–545

Chen S, Lin XH, Xu CG, Zhang QF (2000) Improvement of bacterial blight resistance of 'Minghui 63', an elite restorer line of hybrid rice, by molecular marker-assisted selection. Crop Sci 40:239–244

Chen S, Xu CG, Lin XH, Zhang Q (2001) Improving bacterial blight resistance of '6078', an elite restorer line of hybrid rice, by molecular marker-assisted selection. Plant Breed 120:133–137

Chen X, Temnykh S, Xu Y, Cho YG, McCouch SR (1997) Development of a microsatellite framework map providing genome-wide coverage in rice (*Oryza sativa* L.). Theor Appl Genet 95:553–567

Cho YG, Eun MY, McCouch SR, Chae YA (1994) The semidwarf gene, *sd-1*, of rice (*Oryza-sativa* L). 2. Molecular mapping and marker-assisted selection. Theor Appl Genet 89:54–59

Cho YG, McCouch SR, Kuiper M, Kang MR, Pot J, Groenen JTM, Eun MY (1998) Integrated map of AFLP, SSLP and RFLP markers using a recombinant inbred population of rice (*Oryza sativa* L.). Theor Appl Genet 97:370–380

Cho YC, Choi IS, Baek MK, Suh JP, Hong HC, Kim YG, Koizumi S, Jena KK, Choi HC, Hwang HG (2003) Resistant genes and their effects to rice blast in isogenic lines of genetic background of Chucheongbyeo and Suweon345. Rice Genet Newslett 20:101–105

Chunwongse J, Martin GB, Tanksley SD (1993) Pre-germination genotypic screening using PCR amplification of half-seeds. Theor Appl Genet 86:694–698

Conaway-Bormans CA, Marchetti MA, Johnson CW, McClung AM, Park WD (2003) Molecular markers linked to the blast resistance gene *Pi-z* in rice for use in marker-assisted selection. Theor Appl Genet 107:1014–1020

Conway G (1997) The Doubly Green Revolution: Food for All in the 21st Century. Penguin Books, London

Cooper B, Clarke JD, Budworth P, Kreps J, Hutchinson D, Park S, Guimil S, Dunn M, Luginbuhl P, Ellero C, Goff SA, Glazebrook J (2003) A network of genes associated with stress response and seed development. Proc Natl Acad Sci USA 100:4945–4950

Courtois B, McLaren G, Sinha PK, Prasad K, Yadav R, Shen L (2000) Mapping QTLs associated with drought avoidance in upland rice. Mol Breed 6:55–66

Courtois B, Shen L, Petalcorin W, Carandang S, Mauleon R, Li Z (2003) Locating QTLs controlling constitutive root traits in the rice population IAC 165 × Co 39. Euphytica 134:335–345

Cui KH, Peng SB, Xing YZ, Xu CG, Yu SB, Zhang Q (2002) Molecular dissection of seedling-vigor and associated physiological traits in rice. Theor Appl Genet 105:745–753

Datta K, Baisakh N, Thet KM, Tu J, Datta SK (2002) Pyramiding transgenes for multiple resistance in rice against bacterial blight, yellow stem borer and sheath blight. Theor Appl Genet 106:1–8

Davierwala AP, Reddy APK, Lagu MD, Ranjekar PK, Gupta VS (2001) Marker assisted selection of bacterial blight resistance genes in rice. Biochem Genet 39:261–278

Davis GL, McMullen MD, Baysdorfer C, Musket T, Grant D, Staebell M, Xu G, Polacco M, Koster L, Melia-Hancock S, Houchins K, Chao S, Coe EH Jr (1999) A maize map standard with sequenced core markers, grass genome reference points and 932 expressed sequence tagged sites (ESTs) in a 1736-locus map. Genetics 152:1137–1172

Devos KM, Gale MD (1997) Comparative genetics in the grasses. Plant Mol Biol 35:3–15

Deyholos MK, Galbraith DW (2001) High density microarrays for gene expression analysis. Cytometry 43:229–238

Doi K, Iwata N, Yoshimura A (1997) The construction of chromosome substitution lines of African rice (*Oryza glaberrima* Steud) in the background of japonica rice (*Oryza sativa* L.). Rice Genet Newslett 14:39–41

Doi K, Izawa T, Fuse T, Yananouchi U, Kubo T, Shimatani Z, Yano M, Yoshimura A (2004) *Ehd1*, a B-type response regulator in rice, confers short-day promotion of flowering and controls *FT-like* gene expression independently of *Hd1*. Genes Develop 18:926–936

Dong NV, Subudhi PK, Luong PN, Quang VD, Quy TD, Zheng HG, Wang B, Nguyen HT (2000) Molecular mapping of a rice gene conditioning thermosensitive genic male sterility using AFLP, RFLP and SSR techniques. Theor Appl Genet 100:727–734

Draye X, Lin YR, Qian XY, Bowers JE, Burow GB, Morrell PL, Peterson DG, Presting GG, Ren SX, Wing RA, Paterson AH (2001) Toward integration of comparative genetic, physical, diversity, and cytomolecular maps for grasses and grains, using the sorghum genome as a foundation. Plant Physiol 125:1325–1341

Dubcovsky J, Ramakrishna W, SanMiguel PJ, Busso CB, Yan L, Shiloff BA, Bennetzen JL (2001) Comparative sequence analysis of collinear barley and rice bacterial artificial chromosomes. Plant Physiol 125:1342–1353

Fan JB, Chen X, Halushka MK, Berno A, Huang X, Ryder T, Lipshutz RJ, Lockhart DJ, Chakravarti A (2000) Parallel genotyping of human SNPs using generic high-density oligonucleotide tag arrays. Genome Res 10:853–860

Feng Q, Zhang Y, Hao P, Wang S, Fu G, Huang Y, Li Y et al (2002) Sequence and analysis of rice chromosome 4. Nature 420:316–320

Feuillet C, Keller B (1999) High gene density is conserved at syntenic loci of small and large grass genomes. Proc Natl Acad Sci USA 96:8265–8270

Flowers TJ, Yeo AR (1995) Breeding for salinity resistance in crop plants: Where next? Aust J Plant Physiol 22:875–884

Flowers TJ, Koyama ML, Flowers SA, Sudhakar C, Singh KP, Yeo AR (2000) QTL: their place in engineering tolerance of rice to salinity. J Exp Bot 51:99–106

Frary A, Nebbitt TC, Frary A, Grandillo S, van der Knapp E, Cong B, Liu J, Meller J, Elber R, Alpert KB, Tanksley SD (2000) *fw2.2*: A quantitative trait locus key to the evolution of tomato fruit size. Science 289:85–87

Fuji K, Hayano-Saito Y, Shumiya A, Inoue M (1995) Genetical mapping based on the RFLP analysis for the panicle blast resistance derived from a rice parental line St. No. 1. Breed Sci 45 (Suppl 1):209 (in Japanese)

Fujino K, Sekiguchi H, Sato T, Kiuchi H, Nonoue Y, Takeuchi Y, Ando T, Lin SY, Yano M (2004) Mapping of quantitative trait loci controlling low-temperature germinability in rice (*Oryza sativa* L.). Theor Appl Genet 108:794–799

Fujisawa Y, Kato T, Ohki S, Ishikawa A, Kitano H, Sasaki T, Asahi T, Iwasaki Y (1999) Suppression of the heterotrimeric G protein causes abnormal morphology including dwarfism in rice. Proc Natl Acad Sci USA 96:7575–7580

Fukuoka S, Namai H, Okuno K (1998) RFLP mapping of the genes controlling hybrid breakdown in rice (*Oryza sativa* L.). Theor Appl Genet 97:446–449

Fukuoka S, Okuno K (2001) QTL analysis and mapping of *pi21*, a recessive gene for field resistance to rice blast in Japanese upland rice. Theor Appl Genet 103:185–190

Fukuta Y, Tamura K, Hirae M, Oya S (1998) Genetic analysis of resistance to green rice leafhopper (*Nephotettix cincticeps* Uhler) in rice parental line, Norin-PL6, using RFLP markers. Breed Sci 48:243–249

Gale MD, Devos KM (1998) Plant comparative genetics after 10 years. Science 282:656–659

Garland S, Lewin L, Blakeney A, Reinke R, Henry R (2000) PCR-based molecular markers for the fragrance gene in rice (*Oryza sativa*. L.) Theor Appl Genet 101:364–371

Ge S, Sang T, Lu BR, Hong DY (1999) Phylogeny of rice genomes with emphasis on origins of allotetraploid species. Proc Natl Acad Sci USA 96:14400–14405

Ghareyazie B, Huang N, Second G, Bennett J, Khush GS (1995) Classification of rice germplasm. 1. Analysis using ALP and PCR-based RFLP. Theor Appl Genet 91:218–227

Ghesquiere A, Albar L, Lorieux M, Ahmadi N, Fargette D, Huang N, McCouch SR, Notteghem JL (1997) A major quantitative trait locus for rice yellow mottle virus resistance maps to a cluster of blast resistance genes on chromosome 12. Phytopathology 87:1243–1249

Giraudat J, Hauge BM, Valon C, Smalle J, Parcy F, Goodman HM (1992) Isolation pf the Arabidopsis *ABI3* gene by positional cloning. Plant Cell 4:1251–1261

Glaszmann JC (1987) Isozymes and classification of Asian rice varieties. Theor Appl Genet 74:21–30

Gu K, Tian D, Yang F, Wu L, Sreekala C, Wang D, Wang GL, Yin Z (2004) High-resolution genetic mapping of *Xa27(t)*, a new bacterial blight resistance gene in rice, *Oryza sativa* L. Theor Appl Genet 108:800–807

Han JJ, An G (2003) Flower-preferential poly(A) binding (PAB) protein gene from rice. Plant Sci 165:103–112

Han YP, Xu ML, Liu XY, Yan CJ, Korban SS, Chen XL, Gu MH (2004) Genes coding for starch branching enzymes are major contributors to starch viscosity characteristics in waxy rice (*Oryza sativa* L.). Plant Sci 166:357–364

Harushima Y, Yano M, Shomura A, Sato M, Shimano T, Kuboki Y, Yamamoto T, Lin SY, Antonio BA, Parco A, Kajiya H, Huang N, Yamamoto K, Nagamura Y, Kurata N, Khush GS, Sasaki T (1998) A high density rice genetic map with 2275 markers using a single F$_2$ population. Genetics 148:479–494

Hayano-Saito Y, Tsuji T, Fujii K, Saito K, Iwasaki M, Saito A (1998) Localization of the rice stripe disease resistance gene, *Stv-b(i)*, by graphical genotyping and linkage analyses with molecular markers. Theor Appl Genet 96:1044–1049

Hayano-Saito Y, Saito K, Nakamura S, Kawasaki S, Iwasaki M (2000) Fine physical mapping of the rice stripe resistance gene locus, *Stvb-i*. Theor Appl Genet 101:59–63

Hayashi K, Hashimoto N, Daigen M, Ashikawa I (2004) Development of PCR-based SNP markers for rice blast resistance genes at the *Pi-z* locus. Theor Appl Genet 108:1212–1220

He P, Li SG, Qian Q, Ma YQ, Li JZ, Wang WM, Chen Y, Zhu LH (1999a) Genetic analysis of rice grain quality. Theor Appl Genet 98:502–508

He YQ, Yang J, Xu CG, Zhang ZG, Zhang Q (1999b) Genetic bases of instability of male sterility and fertility reversibility in photoperiod-sensitive genic male-sterile rice. Theor Appl Genet 99:683–693

He P, Li JZ, Zheng XW, Shen LS, Lu CF, Chen Y, Zhu LH (2001) Comparison of molecular linkage maps and agronomic

trait loci between DH and RIL populations derived from the same rice cross. Crop Sci 41:1240–1246

Hemamalini GS, Shashidhar HE, Hittalmani S (2000) Molecular marker assisted tagging of morphological and physiological traits under two contrasting moisture regimes at peak vegetative stage in rice (*Oryza sativa* L.). Euphytica 112:69–78

Hiei Y, Komari T, Kubo T (1997) Transformation of rice mediated by *Agrobacterium tumefaciens*. Plant Mol Biol 35:205–218

Hirabayashi H, Ogawa T (1995) RFLP mapping of *Bph-1* (brown plant hopper rresistance gene) in rice. Breed Sci 45:369–371

Hirabayashi H, Angeles ER, Kaji R, Ogawa T, Brar DS, Khush GS (1998) Identification of the brown plant hopper resistance gene derived from *O. officinalis* using molecular markers in rice (abstract in Japanese). Breed Sci 48 (Suppl 1):82

Hirabayashi H, Kaji R, Angeles ER, Ogawa T, Brar DS, Khush GS (1999) RFLP analysis of a new gene for resistance to brown plant hopper derived from *O. officinalis* on rice chromosome 4 (abstract in Japanese). Breed Sci 49 (Suppl 1):48

Hirochika H (1997) Retrotransposons of rice: their regulation and use for genome analysis. Plant Mol Biol 35:231–240

Hirochika H (2001) Contribution of the *Tos17* retrotransposon to rice functional genomics. Curr Opin Plant Biol 4:18–122

Hirochika H, Guiderdoni E, An G, Hsing Y, Eun MY, Han C, Upadhyaya N, Ramachandran S, Zhang Q, Pereira A, Sundaresan V, Leung H (2004) Rice mutant resources for gene discovery. Plant Mol Biol 54 (3):325–334

Hirschhorn JN, Sklar P, Lindblad-Toh K, Lim YM, Ruiz-Gutierrez M, Bolk S, Langhorst B, Schaffner S, Winchester E, Lander ES (2000) SBE-TAGS: an array-based method for efficient single-nucleotide polymorphism genotyping. Proc Natl Acad Sci USA 97:12164–12169

Hittalmani S, Foolad MR, Mew T, Rodriguez RL, Huang N (1995) Development of a PCR-based marker to identify rice blast resistance gene, *pi-2(t)*, in a segregating population. Theor Appl Genet 91:9–14

Hittalmani S, Parco A, Mew TV, Zeigler RS, Huang N (2000) Fine mapping and DNA marker-assisted pyramiding of the three major genes for blast resistance in rice. Theor Appl Genet 100:1121–1128

Hittalmani S, Huang N, Courtois B, Venuprasad R, Shashidhar HE, Zhuang JY, Zheng KL, Liu GF, Wang GC, Sidhu JS, Srivantaneeyakul S, Singh VP, Bagali PG, Prasanna HC, McLaren G, Khush GS (2003) Identification of QTL for growth- and grain yield-related traits in rice across nine locations of Asia. Theor Appl Genet 107:679–690

Hong Z, Ueguchi-Tanaka M, Umemura K, Uozu S, Fujioka S, Takatsuto S, Yoshida S, Ashikari M, Kitano H, Matsuoka M (2003) A rice brassinosteroid-deficient mutant, *ebisu dwarf (d2)* is caused by a loss of function of a new member of cytochrome P450. Plant Cell 15:2900–2910

Hu B, Wu P, Liao CY, Zhang WP, Ni JJ (2001) QTLs and epistasis underlying activity of acid phosphatase under phosphorus

sufficient and deficient condition in rice (*Oryza sativa* L.). Plant and Soil 230:99–105

Hu ZL, Li P, Zhou MQ, Zhang ZH, Wang LX, Zhu LH, Zhu YG (2004) Mapping of quantitative trait loci (QTLs) for rice protein and fat content using doubled haploid lines. Euphytica 135:47–54

Hua JP, Xing YZ, Wu WR, Xu CG, Sun XL, Yu SB, Zhang QF (2003) Single-locus heterotic effects and dominance by dominance interactions can adequately explain the genetic basis of heterosis in an elite rice hybrid. Proc Natl Acad Sci USA 100:2574–2579

Hua JP, Xing YZ, Xu CG, Sun XL, Yu SB, Zhang QF (2002) Genetic dissection of an elite rice hybrid revealed that heterozygotes are not always advantageous for performance. Genetics 162:1885–1895

Huang N, Courtois B, Khush GS, Lin HX, Wang GL, Wu P, Zheng KL (1996) Association of quantitative trait loci for plant height with major dwarfing genes in rice. Heredity 77:130–137

Huang N, Angeles ER, Domingo J, Magpantay G, Singh S, Zhang G, Kumaravadivel N, Bennett J, Khush GS (1997a) Pyramiding of bacterial blight resistance genes in rice: marker-assisted selection using RFLP and PCR. Theor Appl Genet 95:313–320

Huang N, Parco A, Mew T, Magpantay G, McCouch S, Guiderdoni E, Xu JC, Subudhi P, Angeles ER, Khush GS (1997b) RFLP mapping of isozymes, RAPD and QTLs for grain shape, brown planthopper resistance in a doubled haploid rice population. Mol Breed 3:105–113

Huang Z, He G, Shu L, Li X, Zhang Q (2001) Identification and mapping of two brown planthopper resistance genes in rice. Theor Appl Genet 102:929–934

Ichikawa N, Kishimoto N, Inagaki A, Nakamura A, Koshino Y, Yokozeki Y, Oka M, Samoto S, Akagi H, Higo K, Shinjyo C, Fujimura T, Shimada H (1997) A rapid PCR-aided selection of a rice line containing the *Rf-1* gene which is involved in restoration of the cytoplasmic male sterility. Mol Breed 3:195–202

Ikeda A, Ueguchi-Tanaka M, Sonoda Y, Kitano H, Koshioka M, Futsuhara Y, Matsuoka M, Yamaguchi J (2001) *slender* rice, a constitutive gibberellin response mutant, is caused by a null mutation of the *SLR1* gene, an ortholog of the height-regulating gene *GAI/RGA/RHT/D8*. Plant Cell 13:999–1010

Ikehasi H, Araki H (1984) Variety screening of compatibility types revealed in F_1 fertility of distant cross in rice. Jpn J Breed 34:304–313

Ikehasi H, Araki H (1986) Genetics of F_1 sterility in remote crosses of rice. In: IRRI (ed) Rice Genetics, IRRI, Manila, Philippines, pp 119–130

Ikehasi H, Araki H (1987) Screening and genetic analysis of wide compatibility in F_1 hybrids of distant crosses in rice, *Oryza sativa*. Technical Bulletin 22 Tropical Agriculture Research Center, Japan

Imbe T, Oba S, Yanoria MJT, Tsunematsu H (1997) A new gene for blast resistance in rice cultivar IR24. Rice Genet Newslett 14:60–62

Inukai T, Nelson TJ, Zigler RS, Sarkarung S, Mackill D, Bonman JM, TAkamure, Kinoshita T (1994) Allelism of blast resistance genes in near-isogenic lines of rice. Phytopathology 84:1278–1283

Inukai T, Zeigler RS, Sarkarung S, Bronson M, Dung LV, Kinoshita T, Nelson RJ (1996) Development of pre-isogenic lines for rice blast resistance by marker aided selection from a recombinant inbred population. Theor Appl Genet 93:560–567

Ioannou PA, Amemiya CT, Garnes J, Kroisel PM, Shizuya H, Chen C, Batzer MA, Dejong PJ (1994) A new bacteriophage P1-derived vector for the propagation of large human DNA fragments. Nat Genet 6:84–89

Ishii T, Brar DS, Multani DS, Khush GS (1994) Molecular tagging of genes for brown planthopper resistance and earliness introgressed from *Oryza australiensis* into cultivated rice, *Oryza sativa*. Genome 37:217–221

Ishimaru K (2003) Identification of a locus increasing rice yield and physiological analysis of its function. Plant Physiol 133:1083–1090

Ishimaru K, Kobayashi N, Ono K, Yano M, Ohsugi R (2001) Are contents of Rubisco, soluble protein and nitrogen in flag leaves of rice controlled by the same genetics? J Exp Bot 52:1827–1833

Ishimaru K, Ono K, Kashiwagi T (2004) Identification of a new gene controlling plant height in rice using the candidate-gene strategy. Planta 218:388–395

Iwata N, Omura T (1984) Studies on the trisomics in rice plants (*Oryza sativa* L.). VI. An accomplishment of a trisomic series in japonica rice plants. Jpn J Genet 59:199–204

Izawa T, Ohnishi T, Nakano T, Ishida N, Enoki H, Hashimoto H, Itoh K, Terada R, Wu C, Miyazaki C, Endo T, Iida S, Shimamoto K (1997) Transposon tagging in rice. Plant Mol Biol 35:219–229

Jackson MT (1997) Conservation of rice genetic resources: the role of the International Rice Genebank at IRRI. Plant Mol Biol 35:61–67

Jena KK, Pasalu IC, Rao YK, Varalaxmi Y, Krishnaiah K, Khush GS, Kochert G (2003) Molecular tagging of a gene for resistance to brown planthopper in rice (*Oryza sativa* L.). Euphytica 129:81–88

Jeon J, Lee S, Jung K, Jun S, Jeong D, Lee J, Kim C, Jang S, Lee S, Yang K, Nam J, An K, Han M, Sung R, Choi H, Yu J, Choi J, Cho S, Cha S, Kim S, An G (2000) T-DNA insertional mutagenesis for functional genomics in rice. Plant J 22:561–570

Jeon JS, Chen D, Yi GH, Wang GL, Ronald PC (2003) Genetic and physical mapping of *Pi5(t),* a locus associated with broad spectrum resistance to rice blast. Mol Genet Genom 269:280–289

Jia JH, Zhang DS, Li CY, Qu XP, Wang SW, Chamarerk V, Nguyen HT, Wang B (2001) Molecular mapping of the reverse thermo-sensitive genic male-sterile gene (*rtms1*) in rice. Theor Appl Genet 103:607–612

Jia YL, Wang ZH, Singh P (2002) Development of dominant rice blast *Pi-ta* resistance gene markers. Crop Sci 42:2145–2149

Jia YL, Wang ZH, Fjellstrom RG, Moldenhauer KAK, Azam MA, Correll J, Lee FN, Xia YW, Rutger JN (2004) Rice *Pi-ta* gene confers resistance to the major pathotypes of the rice blast fungus in the United States. Phytopathology 94:296–301

Jiang J, Wang S (2002) Identification of a 118-kb DNA fragment containing the locus of blast resistance gene *Pi-2(t)* in rice. Mol Genet Genom 268:249–252

Jiang GH, Xu CG, Tu JM, Li XH, He YQ, Zhang QF (2004) Pyramiding of insect- and disease-resistance genes into an elite indica, cytoplasm male sterile restorer line of rice, ’Minghui 63’. Plant Breed 123:112–116

Johal GS, Briggs SP (1992) Reductase activity encoded by the HM1 disease resistance gene in maize. Science 258:985–987

Joshi SP, Gupta VS, Aggarwal RK, Ranjekar PK, Brar DS (2000) Genetic diversity and phylogenetic relationship as revealed by inter simple sequence repeat (ISSR) polymorphism in the genus *Oryza*. Theor Appl Genet 100:1311–1320

Jung KH, Hur J, Ryu CH, Choi Y, Chung YY, Miyao A, Hirochika H, An G (2003) Characterization of a rice chlorophyll-deficient mutant using the T-DNA gene trap system. Plant Cell Physiol 44:463–472

Kadirvel P, Maheswaran M, Gunathilagaraj K (1999) Molecular mapping of quantitative trait loci (QTLs) associated with whitebacked planthoper in rice. Intl Rice Res Notes 24(3):12–14

Kaji R, Ogawa T (1996) RFLP mapping of blast resistance gene *Pik-m* in rice. Intl Rice Res Notes 21(2–3):47

Kamoshita A, Zhang JX, Siopongco J, Sarkarung S, Nguyen HT, Wade LJ (2002a) Effects of phenotyping environment on identification of quantitative trait loci for rice root morphology under anaerobic conditions. Crop Sci 42:255–265

Kamoshita A, Wade LJ, Ali ML, Pathan MS, Zhang J, Sarkarung S, Nguyen HT (2002b) Mapping QTLs for root morphology of a rice population adapted to rainfed lowland conditions. Theor Appl Genet 104:880–893

Kashiwagi T, Ishimaru K (2004) Identification and functional analysis of a locus for improvement of lodging resistance in rice. Plant Physiol 134:676–683

Katagiri S, Wu J, Ito Y, Karasawa W, Shibata M, Kanamori H, Katayose Y, Namiki N, Matsumoto T, Sasaki T (2004) End sequencing and chromosomal *in silico* mapping of BAC clones derived from an indica rice cultivar, Kasalath. Breed Sci 54:273–279

Katiyar SK, Tan Y, Huang B, Chandel G, Xu Y, Zhang Y, Xie Z, Bennett J (2001a) Molecular mapping of gene *Gm-6(t)* which confers resistance against four biotypes of Asian rice gall midge in China. Theor Appl Genet 103:953–961

Katiyar S, Verulkar S, Chandel G, Zhang Y, Huang B, Bennett J (2001b) Genetic analysis and pyramiding of two gall midge resistance genes (*Gm-2* and *Gm-6t*) in rice (*Oryza sativa* L.). Euphytica 122:327–334

Kawaguchi M, Murata K, Ishii T, Takumi S, Mori N, Nakamura C (2001) Assignment of a brown plant hopper (*Nilaparvata lugens* Stål) resistance gene *bph4* to the rice chromosome 6. Breed Sci 51:13–18

Kawasaki S, Bochert C, Deyholos M, Wang H, Brazille S, Kawai K, Galbraith D, Bohnert H (2001) Gene expression profiles during the initial phase of salt stress in rice. Plant Cell 13:889–906

Kawase M (1994) Application of the restriction landmark genomic scanning (RLGS) method to rice cultivars as a new fingerprinting technique. Theor Appl Genet 89:861–864

Kellog EA (2001) Evolutionary history of the grasses. Plant Physiol 125:1198–1205

Khush GS (1987) Development of rice varieties suitable for double cropping. In: Tropical Agriculture Research Series No 20. Tropical Agriculture Research Center, Ministry of Agriculture, Forestry and Fisheries, Japan, pp 235–246

Khush GS (1990) Report of meetings to discuss chromosome numbering system in rice. Rice Genet Newslett 7:12–13

Khush GS (1997) Origin, dispersal, cultivation and variation of rice. Plant Mol Biol 35:25–34

Khush GS, Brar DS (1991) Genetics of resistance to insects in crop plants. Adv Agron 45:223–274

Khush GS, Virk PS (2002) Rice improvement: past, present and future. In: Kang MS (ed) Crop Improvement: Challenges in the Twenty-First Century. Food Products Press, New York, pp 13–38

Khush GS, Singh RJ, Sur SC, Librojo AL (1984) Primary trisomics of rice. Origin, morphology, cytology, and use in linkage mapping. Genetics 107:141–163

Khush GS, Brar DS, Virk PS et al (2003) Classifying rice germplasm by isozyme polymorphism and origin of cultivated rice. Discussion Paper No 46. IRRI, Manila, Philippines, p 279

Kikuchi S, Satoh K, Nagata T, Kawagashira N, Doi K, Kishimoto N, Yazaki J, Ishikawa M, Yamada H, Ooka H, Hotta I, Kojima K, Namiki T, Ohneda E, Yahagi W, Suzuki K, Li CJ, Ohtsuki K, Shishiki T, Otomo Y, Murakami K, Iida Y, Sugano S, Fujimura T, Suzuki Y, Tsunoda Y, Kurosaki T, Kodama T, Masuda H, Kobayashi M, Xie QH, Lu M, Narikawa R, Sugiyama A, Mizuno K, Yokomizo S, Niikura J, Ikeda R, Ishibiki J, Kawamata M, Yoshimura A, Miura J, Kusumegi T, Oka M, Ryu R, Ueda M, Matsubara K, Kawai J, Carninci P, Adachi J, Aizawa K, Arakawa T, Fukuda S, Hara A, Hashizume W, Hayatsu N, Imotani K, Ishii Y, Itoh M, Kagawa I, Kondo S, Konno H, Miyazaki A, Osato N, Ota Y, Saito R, Sasaki D, Sato K, Shibata K, Shinagawa A, Shiraki T, Yoshino M, Hayashizaki Y, Yasunishi A (2003) Collection, mapping, and annotation of over 28,000 cDNA clones from japonica rice. Science 301:376–379

Kinoshita T (1998) Report of committee on gene symbolization, nomenclature, and linkage groups. Rice Genet Newslett 15:13–74

Kleinhofs A (2004) Integrated molecular and morphological/physiological marker maps. (2004) Barley Genet Newslett 34:111–122

Kobayashi S, Fukuta Y, Sato T, Osaki M, Khush GS (2003) Molecular marker dissection of rice (*Oryza sativa* L.) plant architecture under temperate and tropical climates. Theor Appl Genet 107:1350–1356

Koh HJ, Son YH, Heu MH, Lee HS, McCouch SR (1999) Molecular mapping of a new genic male-sterility gene causing chalky endosperm in rice (*Oryza sativa* L.). Euphytica 106:57–62

Kojima S, Takahashi Y, Kobayashi Y, Monna L, Sasaki T, Araki T, Yano M (2002) *Hd3a*, a rice ortholog of the *Arabidopsis FT* gene, promotes transition to flowering downstream of *Hd1* under short-day conditions. Plant Cell Physiol 43:1096–1105

Komatsu K, Maekawa M, Ujiie S, Satake Y, Furutani I, Okamoto H, Shimamoto K, Kyozuka J (2003) *LAX* and *SPA*: Major regulators of shoot branching in rice. Proc Natl Acad Sci USA 100:11765–11770

Komori T, Yamamoto T, Takemori N, Kashihara M, Matsushima H, Nitta N (2003) Fine genetic mapping of the nuclear gene, *Rf-1,* that restores the BT-type cytoplasmic male sterility in rice (*Oryza sativa* L.) by PCR-based markers. Euphytica 129:241–247

Komori T, Ohta S, Murai N, Takakura Y, Kuraya Y, Suzuki S, Hiei Y, Imaseki H, Nitta N (2004) Map-based cloning of a fertility restorer gene, *Rf-1*, in rice (*Oryza sativa* L.). Plant J 37:315–325

Koniecyzn A, Asubel FM (1993) A procedure for mapping *Arabidopsis* mutations using co-dominant ecotype-specific PCR-based markers. Plant J 4:403–410

Koyama ML, Levesley A, Koebner RMD, Flowers TJ, Yeo AR (2001) Quantitative trait loci for component physiological traits determining salt tolerance in rice. Plant Physiol 125:406–422

Kurata N, Nagamura Y, Yamamoto K, Harushima Y, Sue N, Wu J, Antonio BA, Shomura A, Shimizu T, Lin SY, Inoue T, Fukuda A, Shimano T, Kuboki Y, Toyama T, Miyamoto Y, Kirihara T, Hayasaka K, Miyao A, Monna L, Zhong HS, Tamura Y, Wang ZX, Momma T, Umehara Y, Yano M, Sasaki T, Minobe Y (1994) A 300 kilobase interval genetic map of rice including 883 expesed sequences. Nat Genet 8:365–372

Kwon SJ, Ahn SN, Jeong EG, Jeon YH, Hwang HG, Choi HC, Moon HP (2002a) Relationship between genetic divergence and hybrid performance in japonica rice grown in a cold water-irrigated field. Euphytica 128:389–396

Kwon YS, Kim KM, Eun MY, Sohn JK (2002b) QTL mapping and associated marker selection for the efficacy of green plant regeneration in anther culture of rice. Plant Breed 121:10–16

Kwon YS, Kim KM, Kim DH, Eun MY, Sohn JK (2002c) Marker-assisted introgression of quantitative trait loci associated

with plant regeneration ability in anther culture of rice (*Oryza sativa* L.). Moll Cells 14:24–28

Lai J, Ma J, Swigonova Z, Ramakrishna W, Linton E, Llaca V, Tanyolac B, Park YJ, Jeong OY, Bennetzen JL, Messing J (2004) Gene loss and movement in the maize genome. Genome Res 14:1924–1931

Lan LF, Chen W, Lai Y, Suo JF, Kong ZS, Li C, Lu Y, Zhang YJ, Zhao XY, Zhang XS, Zhang YS, Han B, Cheng J, Xue YB (2004) Monitoring of gene expression profiles and isolation of candidate genes involved in pollination and fertilization in rice (*Oryza sativa* L.) with a 10K cDNA microarray. Plant Mol Biol 54: 471–487

Lanceras JC, Huang ZL, Naivikul O, Vanavichit A, Ruanjaichon V, Tragoonrung S (2000) Mapping of genes for cooking and eating qualities in Thai jasmine rice (KDML105). DNA Res 7:93–101

Lander ES, Botstein D (1986) Strategies for studying heterogeneous genetic traits in humans by using a linkage map of restriction fragment length polymorphisms. Proc Natl Acad Sci USA 83:7353–7357

Lang NT, Subudhi PK, Virmani SS, Brar DS, Khush GS, Li ZK, Huang N (1999) Development of PCR-based markers for thermosensitive genetic male sterility gene *tms3(t)* in rice (*Oryza sativa* L.). Hereditas 131:121–127

Larkin PD, McClung AM, Ayres NM, Park WD (2003) The effect of the Waxy locus (Granule Bound Starch Synthase) on pasting curve characteristics in specialty rices (*Oryza sativa* L.). Euphytica 131:243–253

Larkin PD, Park WD (2003) Association of waxy gene single nucleotide polymorphisms with starch characteristics in rice (*Oryza sativa* L.). Mol Breed 12:335–339

Lee S, Kim J, Kim S, Kim SJ, Lee K, Han SK, Choi HS, Jeong DH, An G, Kim SR (2004) Trapping and characterization of cold-responsive genes from T-DNA tagging lines in rice. Plant Sci 166:69–79

Leung H, Wu C, Baraoidan M, Bordeos A, Ramos M, Madamba S, Cabauatan P, Vera Cruz C, Portugal A, Reyes G, Bruskiewich R, McLaren G, Lafitte R, Gregorio G, Bennett, Brar D, Khush G, Schnable P, Wang G, Leach J (2000) Deletion mutants for functional genomics: progress in phenotyping, sequence assignmemts, and database development. In: Khush GS, Brar DS, Hardy B (eds) Rice Genetics IV. Science Publishers, Inc, Enfield, NH, USA, pp 239–251

Li ZK, Pinson SRM, Marchetti MA, Stansel JW, Park WD (1995a) Characterization of quantitative trait loci (QTLs) in cultivated rice contributing to field-resistance to sheath blight (*Rhizoctonia solani*). Theor Appl Genet 91:382–388

Li ZK, Pinson SRM, Stansel JW, Park WD (1995b) Identification of quantitative trait loci (QTLs) for heading date and plant height in cultivated rice (*Oryza sativa* L). Theor Appl Genet 91:374–381

Li HB, Wang J, Liu AM, Liu KD, Zhang QF, Zou JS (1997a) Genetic basis of low-temperature-sensitive sterility in *indica-japonica* hybrids of rice as determined by RFLP analysis. Theor Appl Genet 95:1092–1097

Li ZK, Pinson SRM, Park WD, Paterson AH, Stansel JW (1997b) Epistasis for three grain yield components in rice (*Oryza sativa* L). Genetics 145:453–465

Li ZK, Pinson SRM, Stansel JW, Paterson AH (1998) Genetic dissection of the source-sink relationship affecting fecundity and yield in rice (*Oryza sativa* L.). Mol Breed 4:419–426

Li ZK, Luo LJ, Mei HW, Paterson AH, Zhao XZ, Zhong DB, Wang YP, Yu XQ, Zhu L, Tabien R, Stansel JW, Ying CS (1999) A "defeated" rice resistance gene acts as a QTL against a virulent strain of *Xanthomonas oryzae* pv. *oryzae*. Mol Gen Genet 261:58–63

Li XH, Lu Q, Wang FL, Xu CG, Zhang QF (2001a) Separation of the two-locus inheritance of photoperiod sensitive genic male sterility in rice and precise mapping the *pms3* locus. Euphytica 119:343–348

Li ZK, Luo LJ, Mei HW, Wang DL, Shu QY, Tabien R, Zhong DB, Ying CS, Stansel JW, Khush GS, Paterson AH (2001b) Overdominant epistatic loci are the primary genetic basis of inbreeding depression and heterosis in rice. I. Biomass and grain yield. Genetics 158:1737–1753

Li ZK, Sanchez A, Angeles E, Singh S, Domingo J, Huang N, Khush GS (2001c) Are the dominant and recessive plant disease resistance genes similar?: A case study of rice R genes and *Xanthomonas oryzae* pv. *oryzae* races. Genetics 159:757–765

Li XY, Qian Q, Fu ZM, Wang YH, Xiong GS, Zeng DL, Wang XQ, Liu XF, Teng S, Hiroshi F, Yuan M, Luo D, Han B, Li JY (2003a) Control of tillering in rice. Nature 422 (6932):618–621

Li Z, Wan J, Xia J, Yano M (2003b) Mapping of quantitative trait loci controlling physico-chemical properties of rice grains (*Oryza sativa* L.). Breed Sci 53:209–215

Li ZK, Yu SB, Lafitte HR, Huang N, Courtois B, Hittalmani S, Vijayakumar CHM, Liu GF, Wang GC, Shashidhar HE, Zhuang JY, Zheng KL, Singh VP, Sidhu JS, Srivantaneeyakul S, Khush GS (2003c) QTL × Environment interactions in rice. I. Heading date and plant height. Theor Appl Genet 108:141–153

Li JM, Xiao JH, Grandillo S, Jiang LY, Wan YZ, Deng QY, Yuan LP, McCouch SR (2004) QTL detection for rice grain quality traits using an interspecific backcross population derived from cultivated Asian (*O. sativa* L.) and African (*O. glaberrima* S.) rice. Genome 47:697–704

Liao CY, Wu P, Hu B, Yi KK (2001) Effects of genetic background and environment on QTLs and epistasis for rice (*Oryza sativa* L.) panicle number. Theor Appl Genet 103:104–111

Lilley JM, Ludlow MM, McCouch SR, O'Toole JC (1996) Locating QTL for osmotic adjustment and dehydration tolerance in rice. J Exp Bot 47:1427–1436

Lin HX, Qian HR, Zhuang JY, Lu J, Min SK, Xiong ZM, Huang N, Zheng KL (1996a) RFLP mapping of QTLs for yield and related characters in rice (*Oryza sativa* L). Theor Appl Genet 92:920–927

Lin X, Zhang D, Xie Y, Gao H, Zhang Q (1996b) Identification and mapping a new gene for bacterial blight resistance in rice based on RFLP markers. Phytopathology 86:1156–1159

Lin SY, Sasaki T, Yano M (1998) Mapping quantitative trait loci controlling seed dormancy and heading date in rice, *Oryza sativa* L., using backcross inbred lines. Theor Appl Genet 96:997–1003

Lin HX, Yamamoto T, Sasaki T, Yano M (2000) Characterization and detection of epistatic interactions of 3 QTLs, *Hd1, Hd2,* and *Hd3* controlling heading date in rice using nearly isogenic lines. Theor Appl Genet 101:1021–1028

Lin HX, Ashikari M, Yamanouchi U, Sasaki T, Yano M (2002) Identification and characterization of a quantitative trait locus, *Hd9*, controlling heading date in rice. Breed Sci 52:35–41

Lin HX, Liang ZW, Sasaki T, Yano M (2003) Fine mapping and characterization of quantitative trait loci *Hd4* and *Hd5* controlling heading date in rice. Breed Sci 53:51–59

Lin HX, Zhu MZ, Yano M, Gao JP, Liang ZW, Su WA, Hu XH, Ren ZH, Chao DY (2004) QTLs for Na^+ and K^+ uptake of the shoots and roots controlling rice salt tolerance. Theor Appl Genet 108:253–260

Lincoln S, Daly M, Lander E (1992) Mapping genes controlling quantitative traits with MAPMAKER/QTL 1.1, 2nd ed, Whitehead Institute Technical Report, Whitehead Institute, Cambridge, MA

Litt M, Luty JA (1989) A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. Am J Hum Genet 44:397–401

Liu A, Zhang Q, Li H (1992) Location of a gene for wide-compatibility in the RFLP linkage map. Rice Genet Newslett 9:134–136

Liu KD, Wang J, Li HB, Xu CG, Liu AM, Li XH, Zhang Q (1997) A genome-wide analysis of wide compatibility in rice and the precise location of the *S5* locus in the molecular map. Theor Appl Genet 95:809–814

Liu XC, Wu JL (1998) SSR heterogenic patterns of parents for marking and predicting heterosis in rice breeding. Mol Breed 4:263–268

Liu G, Lu G, Zeng L, Wang GL (2002a) Two broad-spectrum blast resistance genes, *Pi9(t)* and *Pi2(t),* are physically linked on rice chromosome 6. Mol Genet Genom 267:472–480

Liu XC, Ishiki K, Wang WX (2002b) Identification of AFLP markers favorable to heterosis in hybrid rice. Breed Sci 52:201–206

Liu SP, Li X, Wang CY, Li XH, He YQ (2003) Improvement of resistance to rice blast in Zhenshan 97 by molecular marker-aided selection. Acta Bot Sin 45:1346–1350

Liu B, Zhang SH, Zhu XY, Yang QY, Wu SZ, Mei MT, Mauleon R, Leach J, Mew T, Leung H (2004a) Candidate defense genes as predictors of quantitative blast resistance in rice. Mol Plant-Micr Interact 17:1146–1152

Liu XQ, Xu X, Tan YP, Li SQ, Hu J, Huang JY, Yang DC, Li YS, Zhu YG (2004b) Inheritance and molecular mapping of two fertility-restoring loci for Honglian gametophytic

cytoplasmic male sterility in rice (*Oryza sativa* L.). Mol Genet Genom 271:586–594

Liu XY, Gu MH, Han YP, Ji Q, Lu JF, Gu SL, Zhang R, Li X, Chen JM, Korban SS, Xu ML (2004c) Developing gene-tagged molecular markers for functional analysis of starch-synthesizing genes in rice (*Oryza sativa* L.). Euphytica 135:345–353

Lockhart DJ, Dong H, Byrne MC, Follettie MT, Gallo MV, Chee MS, Mittmann M, Wang C, Kobayashi M, Horton H, Brown EL (1996) Expression monitoring by hybridization to high-density oligonucleotide arrays. Nat Biotechnol 14:1675–1680

Lopez MT, Toojinda T, Vanavichit A, Tragoonrung S (2003) Microsatellite markers flanking the *tms2* gene facilitated tropical TGMS rice line development. Crop Sci 43:2267–2271

Lorieux M, Petrov M, Huang N, Guiderdoni E, Ghesquiere A (1996) Aroma in rice: genetic analysis of a quantitative trait. Theor Appl Genet 93:1145–1151

Lu CF, Shen LS, Tan ZB, Xu YB, He P, Chen Y, Zhu LH (1997) Comparative mapping of QTLs for agronomic traits of rice across environments by using a doubled-haploid population. Theor Appl Genet 94:145–150

Luo LJ, Li ZK, Mei HW, Shu QY, Tabien R, Zhong DB, Ying CS, Stansel JW, Khush GS, Paterson AH (2001) Overdominant epistatic loci are the primary genetic basis of inbreeding depression and heterosis in rice. II. Grain yield components. Genetics 158:1755–1771

Ma JF, Shen RF, Zhao ZQ, Wissuwa M, Takeuchi Y, Ebitani T, Yano M (2002) Response of rice to Al stress and identification of quantitative trait loci for Al tolerance. Plant Cell Physiol 43:652–659

Mackill DJ (1995) Classifying japonica rice cultivars with RAPD markers. Crop Sci 35:889–894

Mackill DJ, Salam MA, Wang ZY, Tanksley SD (1993) A major photoperiod-sensitivity gene tagged with RFLP and isozyme markers in rice. Theor Appl Genet 85:536–540

Malik SS, Khush GS (1996) Identification of wide compatibility varieties (WCVs) and tagging of WC genes with isozyme markers. Rice Genet Newslett 13:121–123

Mao L, Begum D, Chuang HW, Budiman MA, Szymkowiak EJ, Irish EE, Wing RA (2000) *JOINTLESS* is a MADS-box gene controlling tomato flower abscission zone development. Nature 406:910–913

Martin GB, Williams JGK, Tanksley SD (1991) Rapid identification of markers linked to a *Pseudomonas* resistance gene in tomato by using random primers and near-isogenic lines. Proc Natl Acad Sci USA 88:2336–2340

Martin GB, Brommonschenkel SH, Chunwongs J, Farry A, Ganal MW, Spivey R, Wu T, Earle D, Tanksley SD (1993) Map-based cloning of a protein kinase gene conferring disease resistance in tomato. Science 262:1432–1436

McClintock B (1956) Controlling elements and the gene. Cold-Spring Harbor Symposia on Quantitat Biol 21:197–216

McCouch SR, Kochert G, Yu ZH, Wang ZY, Khush GS, Coffman WR, Tanksley SD (1988) Molecular mapping of rice chromosomes. Theor Appl Genet 76:815–829

McCouch SR, Khush GS, Tanksley SD (1991) Tagging genes for disease and insect resistance via linkage to RFLP markers. In: Rice Genetics II. IRRI, Manila, Philippines, pp 443–449

McCouch SR, Teytelman L, Xu YB, Lobos KB, Clare K, Walton M, Fu BY, Maghirang R, Li ZK, Xing YZ, Zhang QF, Kono I, Yano M, Fjellstrom R, DeClerck G, Schneider D, Cartinhour S, Ware D, Stein L (2002) Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.). DNA Res 9:199–207

McKenzie KS, Rutger JN (1983) Genetic analysis of amylose content, alkali spreading score and grain dimensions in rice. Crop Sci 23:306–313

Mei M, Zhuang C, Wan R, Wu J, Hu W, Kochert G (1996) Genetic analysis and tagging of gene for brown planthopper resistance in indica rice. In: Rice Genetics III. Proc 3rd Intl Rice Genet Symp, 16–20 Oct 1995, IRRI, Manila, Philippines, pp 590–595

Mei HW, Luo LJ, Ying CS, Wang YP, Yu XQ, Guo LB, Paterson AH, Li ZK (2003) Gene actions of QTLs affecting several agronomic traits resolved in a recombinant inbred rice population and two testcross populations. Theor Appl Genet 107:89–101

Mei MH, Chen L, Zhang ZH, Li ZY, Xu CG, Zhang QF (1999a) *pms3* is the locus causing the original photoperiod-sensitive male sterility mutation of 'Nongken 58S'. Science in China Series C-Life Sciences 42:316–322

Mei MH, Dai XK, Xu CG, Zhang QF (1999b) Mapping and genetic analysis of the genes for photoperiod-sensitive genic male sterility in rice using the original mutant Nongken 58S. Crop Sci 39:1711–1715

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

Ming F, Zheng XW, Mi GH, Zhu LH, Zhang FS (2001) Detection and verification of quantitative trait loci affecting tolerance to low phosphorus in rice. J Plant Nutr 24:1399–1408

Mishra GP, Singh RK, Mohapatra T, Singh AK, Prabhu KV, Zaman FU, Sharma RK (2003) Molecular mapping of a gene for fertility restoration of wild abortive (WA) cytoplasmic male sterility using a basmati rice restorer line. J Plant Biochem Biotechnol 12:37–42

Miura K, Lin SY, Yano M, Nagamine T (2001) Mapping quantitative trait loci controlling low temperature germinability in rice (*Oryza sativa* L.). Breed Sci 51:293–299

Miyamoto M, Ando I, Rybka K, Kadama O, Kawasaki S (1996) High resolution mapping of the indica-derived rice blast resistance genes. 1. *Pi-b*. Mol Plant-Micr Interact 9:6–13

Miyoshi K, Ahn BO, Kawakatsu T, Ito Y, Ito JI, Nsgato Y, Kurata N (2004) *PLASTCHRON1*, a timekeeper of leaf initiation in

rice, encodes cytochrome P450. Proc Natl Acad Sci USA 101:875–880

Mohan M, Nair S, Bentur JS, Prasad Rao U, Bennett J (1994) RFLP and RAPD mapping of the rice *Gm2* gene that confers resistance to biotype 1 of gall midge (*Orseolia oryzae*). Theor Appl Genet 87:782–788

Mohan M, Nair S, Bhagwat A, Krishna TG, Yano M, Bhatia CR, Sasaki T (1997a) Genome mapping, molecular markers and marker-assisted selection in crop plants. Mol Breed 3:87–103

Mohan M, Sathyanarayanan PV, Kumar A, Srivastava MN, Nair S (1997b) Molecular mapping of a resistance-specific PCR-based marker linked to a gall midge resistance gene (*Gm4t*) in rice. Theor Appl Genet 95:777–782

Moncada P, Martinez CP, Borrero J, Chatel M, Gauch H, Guimaraes E, Tohme J, McCouch SR (2001) Quantitative trait loci for yield and yield components in an *Oryza sativa* x *Oryza rufipogon* BC$_2$F$_2$ population evaluated in an upland environment. Theor Appl Genet 102:41–52

Monna L, Kitazawa N, Yoshino R, Suzuki J, Masuda H, Maehara Y, Tanji M, Sato M, Nasu S, Minobe Y (2002a) Positional cloning of rice semidwarfing gene, *sd-1*: rice "green revolution gene" encodes a mutant enzyme involved in gibberellin synthesis. DNA Res 9:11–17

Monna L, Lin HX, Kojima S, Sasaki T, Yano M (2002b) Genetic dissection of a genomic region for a quantitative trait locus, *Hd3*, into two loci, *Hd3a* and *Hd3b* controlling heading date in rice. Theor Appl Genet 104:772–778

Moore G, Gale MD, Kurata N, Flavell R (1993) Molecular analysis of small grain cereal genomes-current status and prospects. Bio/Technology 11:584–589

Murai H, Hashimoto Z, Sharma PN, Shimizu T, Murata K, Takumi S, Mori N, Kawasaki S, Nakamura C (2001) Construction of a high-resolution linkage map of a rice brown planthopper (*Nilaparvata lugens* Stål) resistance gene *bph2*. Theor Appl Genet 103:526–532

Murata K, Fujiwara M, Kaneda C, Takumi S, Mori N, Nakamura C (1998) RFLP mapping of a brown plant hopper (*Nilaparvata lugens* Stal) resistance gene *bph2* of indica rice introgressed into a japonica breeding line 'Morin-Pl4'. Genes Genet Syst 73: 359–364

Murata K, Fujiwara M, Murai H, Takumi S, Mori N, Nakamura C (2000) *Bph9*, a dominant brown planthopper resistance gene, is located on the long arm of rice chromosome 12. Rice Genet Newslett 17:84–86

Nagao S, Takahashi M (1963) Trial construction of twelve linkage groups in Japanese rice. Genetical studies on rice plant. XXVII. J Fac Agri Hokkaido Univ 53:72–130

Nair S, Rao UP, Bennett J, Mohan M (1995) Detection of a highly heterozygous locus in recombinant inbred lines of rice and its possible involvement in heterosis. Theor Appl Genet 91:978–986

Nair S, Kumar A, Srivastava MN, Mohan M (1996) PCR-based DNA markers linked to a gall midge resistance gene, *Gm4t*,

has potential for marker-aided selection in rice. Theor Appl Genet 92:660–665

Nakagahra M (1977) Genic analysis for esterase isoenzymes in rice cultivars. Jpn J Breed 27:141–148

Nakagahra M, Okuno K, Vaughan D (1997) Rice genetic resources; history, conservation, investigative characterization and use in Japan. Plant Mol Biol 35:69–77

Nakazaki T, Okumoto Y, Horibata A, Yamahira S, Teraishi M, Nishida H, Inoue H, Tanisaka T (2003) Mobilization of a transposon in the rice genome. Nature 421:170–172

Nandi S, Subudhi PK, Senadhira D, Manigbas NL, SenMandi S, Huang N (1997) Mapping QTLs for submergence tolerance in rice by AFLP analysis and selective genotyping. Mol Gen Genet 255:1–8

Naqvi NI, Bonman JM, Mackill DJ, Nelson RJ, Chattoo BB (1995) Identification of RAPD markers linked to a major blast resistance gene in rice. Mol Breed 1:341–348

Naqvi NI, Chattoo BB (1996) Development of a sequence characterized amplified region (SCAR) based indirect selection method for a dominant blast-resistance gene in rice. Genome 39:26–30

Narayanan NN, Baisakh N, Cruz CMV, Gnanamanickam SS, Datta K, Datta SK (2002) Molecular breeding for the development of blast and bacterial blight resistance in rice cv. IR50. Crop Sci 42:2072–2079

Nelson JC (1997) QGENE: Software for marker-based genomic analysis and breeding. Mol Breed 3:239–245

Nguyen VT, Burow MD, Nguyen HT, Le BT, Le TD, Paterson AH (2001) Molecular mapping of genes conferring aluminum tolerance in rice (*Oryza sativa* L.). Theor Appl Genet 102:1002–1010

Nguyen VT, Nguyen BD, Sarkarung S, Martinez C, Paterson AH, Nguyen HT (2002) Mapping of genes controlling aluminum tolerance in rice: comparison of different genetic backgrounds. Mol Genet Genom 267:772–780

Nguyen BD, Brar DS, Bui BC, Nguyen TV, Pham LN, Nguyen HT (2003) Identification and mapping of the QTL for aluminum tolerance introgressed from the new source, *Oryza rufipogon* Griff., into indica rice (*Oryza sativa* L.). Theor Appl Genet 106:583–593

Nguyen TT, Klueva N, Chamareck V, Aarti A, Magpantay G, Millena ACM, Pathan MS, Nguyen HT (2004) Saturation mapping of QTL regions and identification of putative candidate genes for drought tolerance in rice. Mol Genet Genom 272:35–46

Ni JJ, Wu P, Senadhira D, Huang N (1998) Mapping QTLs for phosphorus deficiency tolerance in rice (*Oryza sativa* L.). Theor Appl Genet 97:1361–1369

Ni J, Colowit PM, Oste, JJ, Xu K, Mackill DJ (2001) Molecular markers linked to stem rot resistance in rice. Theor Appl Genet 102:511–516

Ni J, Colowit PM, Mackill DJ (2002) Evaluation of genetic diversity in rice subspecies using microsatellitte markers. Crop Sci 42:601–607

Olson M, Hood L, Cantor C, Botstein D (1989) A common language for physical mapping of the human genome. Science 245(4925):1434–1435

Osato N, Yamada H, Satoh K, Ooka H, Yamamoto M, Suzuki K, Kawai J, Carninci P, Ohtomo Y, Murakami K, Matsubara K, Kikuchi S, Hayashizaki Y (2004) Antisense transcripts with rice full-length cDNAs. Genome Biol 5 (1): Art. No. R5

Padmavathi G, Siddiq EA, Kole C (2001) Genetics and molecular mapping of resistance to the Indian biotype of green leafhopper *Nephotettix virescens* (Distant). In: Proc 8th Natl Rice Biotechnol Network Meeting, 21–25 Oct 2001, Aurangabad, India, pp 150–153

Pan QH, Hu ZD, Takatoshi T, Wang L (2003) Fine mapping of the blast resistance gene *Pi15*, linked to *Pii*, on rice chromosome 9. Acta Bot Sin 45:871–877

Park KC, Kim NH, Cho YS, Kang KH, Lee JK, Kim NS (2003) Genetic variations of AA genome *Oryza* species measured by MITE-AFLP. Theor Appl Genet 107:203–209

Paterson AH, Tanksley SD, Sorrells ME (1991) DNA markers in plant improvement. Adv Agron 46:39–90

Pathan MS. Subudhi PK, Courtois B, Nguyen HT (2004) Molecular dissection of abiotic stress tolerance in sorghum and rice: a case study. In: Nguyen HT, Blum A (eds) Physiology and Biotechnology Integration for Plant Breeding. Marcel Dekker, Inc, New York, USA, pp 525–569

Peng J, Richards DE, Hartley NM, Murphy GP, Devos KM, Flintham J, Beagles J, Fish LJ, Worland AJ, Pelica F, Sudhakar D, Christou P, Snape JW, Gale MD, Harberd NP (1999) 'Green revolution' genes encode mutant gibberellin response modulators. Nature 400:256–261

Porter BW, Chittoor JM, Yano M, Sasaki T, White FF (2003) Development and mapping of markers linked to the rice bacterial blight resistance gene *Xa7*. Crop Sci 43:1484–1492

Porteres R (1956) Taxonomic agrobotanique de riz cultives, *O. sativa* L. et *O. glaberrima* Steudel. J Agri Trop Bot Appl 3:341–856

Pressoir G, Albar L, Ahmadi N, Rimbault I, Lorieux M, Fargette D, Ghesquiere A (1998) Genetic basis and mapping of the resistance to rice yellow mottle virus. II. Evidence of a complementary epistasis between two QTLs. Theor Appl Genet 97:1155–1161

Price AH, Tomos AD (1997) Genetic dissection of root growth in rice (*Oryza sativa* L). 2. Mapping quantitative trait loci using molecular markers. Theor Appl Genet 95:143–152

Price AH, Steele KA, Moore BJ, Barraclough PB, Clark LJ (2000) A combined RFLP and AFLP linkage map of upland rice (*Oryza sativa* L.) used to identify QTLs for root-penetration ability. Theor Appl Genet 100:49–56

Price AH, Cairns JE, Horton P, Jones HG, Griffiths H (2002a) Linking drought-resistance mechanisms to drought avoidance in upland rice using a QTL approach: progress and new opportunities to integrate stomatal and mesophyll responses. J Exp Bot 53:989–1004

Price AH, Townend J, Jones MP, Audebert A, Courtois B (2002b) Mapping QTLs associated with drought avoidance in up-

land rice grown in the Philippines and West Africa. Plant Mol Biol 48:683–695

Putterill J, Robson F, Lee K, Simon R, Coupland G (1995) The *CONSTANS* gene of Arabidopsis promotes flowering and encodes a protein showing similarities to zinc finger transcription factors. Cell 80:47–857

Qi LL, Echalier B, Chao S, Lazo GR, Butler GE, Anderson OD, Akhunov ED et al (2004a) A chromosome bin map of 16,000 expressed sequence tag loci and distribution of genes amomg the three genomes of polyploid wheat. Genetics 168:701–712

Qi X, Pittaway TS, Lindup S, Liu H, Waterman E, Padi FK, Hash CT, Zhu J, Gale MD, Devos KM (2004b) An integrated genetic map and a new set of simple sequence repeat markers for pearl millet, *Pennisetum glaucum*. Theor Appl Genet 109:1485–1493

Qian W, Ge S, Hong DY (2001) Genetic variation within and among populations of a wild rice *Oryza granulata* from China detected by RAPD and ISSR markers. Theor Appl Genet 102:440–449

Quarrie SA, Laurie DA, Zhu JH, Lebreton C, Semikhodskii A, Steed A, Witsenboer H, Calestani C (1997) QTL analysis to study the association between leaf size and abscisic acid accumulation in droughted rice leaves and comparisons across cereals. Plant Mol Biol 35:155–165

Rabbani MA, Maruyama K, Abe H, Khan MA, Katsura K, Ito Y, Yoshiwara K, Seki M, Shinozaki K, Yamaguchi-Shinozaki K (2003) Monitoring expression profiles of rice genes under cold, drought, and high-salinity stresses and abscisic acid application using cDNA microarray and RNA get-blot analyses. Plant Physiol 133:1755–1767

Rajyashri KR, Nair S, Ohmido N, Fukui K, Kurata N, Sasaki T, Mohan M (1998) Isolation and FISH mapping of yeast artificial chromosomes (YACs) encompassing an allele of the *Gm2* gene for gall midge resistance in rice. Theor Appl Genet 97:507–514

Ramakrishna W, Lagu MD, Gupta VS, Ranjekar PK (1994) DNA fingerprinting in rice using oligonucleotide probes specific for simple repetitive sequences. Theor Appl Genet 88:402–406

Ramakrishna W, Chowdari KV, Lagu MD, Gupta VS, Ranjekar PK (1995) DNA fingerprinting to detect genetic variation in rice using hypervariable DNA sequences. Theor Appl Genet 90:1000–1006

Ramalingam J, Basharat HS, Zhang G (2002) STS and microsatellite marker-assisted selection for bacterial blight resistance and waxy genes in rice, *Oryza sativa* L. Euphytica 127:255–260

Ray JD, Yu L, McCouch SR, Champoux MC, Wang G, Nguyen HT (1996) Mapping quantitative trait loci associated with root penetration ability in rice (*Oryza sativa* L). Theor Appl Genet 92:627–636

Reddy OUK, Siddiq EA, Sarma NP, Ali J, Hussain AJ, Nimmakayala P, Ramasamy P, Pammi S, Reddy AS (2000) Ge-

netic analysis of temperature-sensitive male sterilty in rice. Theor Appl Genet 100:794–801

Redona ED, Mackill DJ (1996) Molecular mapping of quantitative trait loci in japonica rice. Genome 39:395–403

Redona ED, Mackill DJ (1998) Quantitative trait locus analysis for rice panicle and grain characteristics. Theor Appl Genet 96:957–963

Renganayaki K, Fritz AK, Sadasivam S, Pammi S, Harrington SE, McCouch SR, Kumar SM, Reddy AS (2002) Mapping and progress toward map-based cloning of brown planthopper biotype-4 resistance gene introgressed from *Oryza officinalis* into cultivated rice, *O. sativa*. Crop Sci 42:2112–2117

Rice Chromosome 10 Sequencing Consortium (2003) In-depth view of structure, activity, and evolution of rice chromosome 10. Science 300:1566–1569

Robertson D (1985) A possible technique for isolating genomic DNA for quantitative traits in plants. J Theor Biol 117:1–10

Robin S, Pathan MS, Courtois B, Lafitte R, Carandang S, Lanceras S, Amante M, Nguyen HT, Li Z (2003) Mapping osmotic adjustment in an advanced back-cross inbred population of rice. Theor Appl Genet 107:1288–1296

Ronald PC, Albano B, Tabien R, Abenes L, Wu KS, McCouch S, Tanksley (1992) Genetic and physical analysis of the rice bacterial blight disease resistance locus, *Xa21*. Mol Gen Genet 236:113–120

Roschevitz RJ (1931) A contribution to the knowledge of rice. Bull Appl Bot Genet Plant Breed (Leningrad) 27 (4):1–133 (Russian with English Summary)

Rossberg M, Theres K, Acarkan A, Herrero R, Schmitt T, Schumacher K, Schmitz G, Scmidt R (2001) Comparative sequence analysis reveals extensive microcolinearlty in the *Lateral Suppressor* regions of the tomato, Arabidopsis, and Capsella genomes. Plant Cell 13:979–988

Rybka K, Miyamoto M, Ando I, Saito A, Kawasaki S (1997) High resolution mapping of the indica-derived rice blast resistance genes II. *Pi-ta2* and *Pi-ta* and a consideration of their origin. Mol Plant-Micr Interact 10:517–524

Saito A, Yano M, Kishimoto N, Nakagahra M, Yoshimura A, Saito K, Kuhara S, Ukai Y, Kawase M, Nagamine T, Yoshimura S, Ideta O, Ohsaw R, Hayano Y, Iwata N, Sugiura M (1991) Linkage map of restriction-fragment-length-polymorphism loci in rice. Jpn J Breed 41:665–670

Saito K, Miura K, Hayano-Saito Y, Kato A (2003) Analysis of quantitative trait loci for cold tolerance at the booting stage of rice. Jpn Agri Res Q 37:1–5

Saji S, Umehara Y, Antonio BA, Yamane H, Tanoue H, Baba T, Aoki H, Ishige N, Wu J, Koike K, Matsumoto T, Sasaki T (2001) A physical map with yeast artificial chromosome (YAC) clones covering 63% of the 12 rice chromosomes. Genome 44:32–37

Sallaud C, Lorieux M, Roumen E, Tharreau D, Berruyer R, Svestasrani P, Garsmeur O, Ghesquiere A, Notteghem JL (2003) Identification of five new blast resistance genes in the highly blast-resistant rice variety IR64 using a QTL mapping strategy. Theor Appl Genet 106:794–803

Sallaud C, Gay C, Larmande P, Bes M, Piffanelli P, Piegu B, Droc G, Regard F, Bourgeois E, Meynard D, Perin C, Sabau X, Ghesquiere A, Glaszmann JC, Delseny M, Guiderdoni E (2004) High throuput T-DNA insertion mutagenesis in rice: a first step towards *in silico* reverse genetics.Plant J 39:450–464

Sanchez AC, Ilag LL, Yang D, Brar DS, Ausubel F, Khush GS, Yano M, Sasaki T, Li Z, Huang N (1999) Genetic and physical mapping of *xa13*, a recessive bacterial blight resistance gene in rice. Theor Appl Genet 98:1022–1028

Sanchez AC, Brar DS, Huang N, Li Z, Khush GS (2000) Sequence tagged site marker-assisted selection for three bacterial blight resistance genes in rice. Crop Sci 40:792–797

Sano Y, Katsumata M, Omura K (1986) Genetic studies of speciation in cultivated rice. Inter- and intraspecific differentiation in the waxy gene expression of rice. Euphytica 35:1–9

Sardesai N, Rajyashri KR, Behura SK, Nair S, Mohan M (2001) Genetic, physiological and molecular interactions of rice and its major dipteran pest, gall midge. Plant Cell Tiss Org Cult 64:115–131

Sasaki A, Ashikari M, Ueguchi-Tanaka M, Itoh H, Nishimura A, Dutta S, Ishiyama K, Saito T, Kobayashi M, Khush GS, Kitano H, Matsuoka M (2002a) A mutant gibberellin-synthesis gene in rice. Nature 416:701–702

Sasaki T, Matsumoto T, Yamamoto K, Sakata K, Baba T, Katayose Y, Wu J et al (2002b) The genome sequence and structure of rice chromosome 1. Nature 420:312–316

Sasaki A, Itoh H, Gomi K, Ueguchi-Tanaka M, Ishiyama K, Kobayashi M, Jeong DH, An G, Kitano H, Ashikari M, Matsuoka M (2003) Accumulation of phosphorylated repressor for gibberellin signaling in an F-box mutant. Science 299:1896–1898

Sato Y, Sentoku N, Miura Y, Hirochika H, Kitano H, Matsuoka M (1999) Loss of function mutations in the rice homeobox gene *OSH15* affect the architechture of internodes resulting in dwarf plants. EMBO J 18:992–1002

Schena M, Shalon D, Davis RW, Brown PO (1995) Quantitative monitoring of gene expression patterns with a complementary DNA microarray. Science 270:467–470

Sebastian LS, Ikeda R, Huang N, Imbe T, Coffman WR, McCouch SR (1996) Molecular mapping of resistance to rice tungro spherical virus and green leafhopper. Phytopathology 86:25–30

Selvi A, Shanmugasundaram P, Kumar SM, Raja JAJ (2002) Molecular markers for yellow stem borer *Scirpophaga incertulas* (Walker) resistance in rice. Euphytica 124:371–377

Septiningsih EM, Trijatmiko KR, Moeljopawiro S, McCouch SR (2003a) Identification of quantitative trait loci for grain quality in an advanced backcross population derived from the *Oryza sativa* variety IR64 and the wild relative *O. rufipogon*. Theor Appl Genet 107:1433–1441

Septiningsih EM, Prasetiyono J, Lubis E, Tai TH, Tjubaryat T, Moeljopawiro S, McCouch SR (2003b) Identification of quantitative trait loci for yield and yield components in an advanced backcross population derived from the *Oryza*

sativa variety IR64 and the wild relative *O. rufipogon*. Theor Appl Genet 107:1419–1432

Sharma G, Nanda RD (1980) Excavations at Mahagara 1977–78. A Neolithic settlement in Balan valley. Archeology of Vidhyas and Ganga Valley, 6. Department of Ancient History, Culture and Archeology. University of Allahabad, India

Sharma N, Ketipearachchi Y, Murata K, Torii A, Takumi S, Mori N, Nakamura C (2003) RFLP/AFLP mapping of a brown planthopper (*Nilaparvata lugens* Stal) resistance gene *Bph1* in rice. Euphytica 129:109–117

Shen LS, He P, Xu YB, Tan ZB, Lu CFX, Zhu LH (1998) Genetic molecular linkage map construction and genome analysis of rice doubled haploid population. Acta Bot Sin 40:1115–1122

Shen L, Courtois B, McNally KL, Robin S, Li Z (2001) Evaluation of near-isogenic lines of rice introgressed with QTLs for root depth through marker-aided selection. Theor Appl Genet 103:75–83

Shizuya H, Birren B, Kim U, Mancino V, Slepak T, Tachiiri Y, Simon M (1992) Cloning and stable maintenance of 300-kilobase-pair fragments of human DNA in *Escherichia coli* using an F-factor-based vector. Proc Natl Acad Sci USA 89:8794–8797

Siangliw M, Toojinda T, Tragoonrung S, Vanavichit A (2003) Jasmine rice carrying QTLch9 (*Sub*QTL) is submergence tolerant. Ann Bot 91:255–261 Spl Iss

Singh S, Sidhu JS, Huang N, Vikal Y, Li Z, Brar DS, Dhaliwal HS, Khush GS (2001) Pyramiding three bacterial blight resistance genes (*xa5, xa13* and *Xa21*) using marker-assisted selection into indica rice cultivar PR106. Theor Appl Genet 102:1011–1015

Singh RK, Sharma RK, Singh AK, Singh VP, Singh NK, Tiwari SP, Mohapatra T (2004) Suitability of mapped sequence tagged microsatellite site markers for establishing distinctness, uniformity and stability in aromatic rice. Euphytica 135:135–143

Sirithunya P, Tragoonrung S, Vanavichit A, Pa-In N, Vongsaprom C, Toojinda T (2002) Quantitative trait loci associated with leaf and neck blast resistance in recombinant inbred line population of rice (*Oryza sativa*). DNA Res 9:79–88

Solheim II G (1972) An earlier agricultural revolution. Scientific American 226 (4):34–41

Song WY, Wang GL, Chen LL, Kim HS, Pi LY, Holsten T, Gardner J, Wang B, Zhai WX, Zhu LH, Fauquet C, Ronald P (1995) A receptor kinase-like protein encoded by the rice disease resistance gene, *Xa21*. Science 270(5243):1804–1806

Song MT, Kim KM, Jong SK, Lee JH, Cho YS, Gu JH, Lee SB, Choi SH, Hwang HG (2003) Comparison of DNA-based and pedigree-based genetic similarity among Korean rice cultivars. Kor J Genet 25:223–230

Spada A, Mantegazza R, Biloni M, Caporali E, Sala F (2004) Italian rice varieties: historical data, molecular markers and pedigrees to reveal their genetic relationships. Plant Breed 123:105–111

Spielmeyer W, Ellis MH, Chandler PM (2002) Semidwarf (*sd-1*), "green revolution" rice, contains a defective gibberellin 20-oxidase gene. Proc Natl Acad Sci USA 99:9043–9048

Sripongpangkul K, Posa GBT, Senadhira DW, Brar D, Huang N, Khush GS, Li ZK (2000) Genes/QTLs affecting flood tolerance in rice. Theor Appl Genet 101:1074–1081

Staskawicz BJ, Ausubel FM, Baker BJ, Ellis JG, Jones JD (1995) Molecular genetics of plant disease resistance. Science 268:661–667

Subudhi PK, Nguyen HT (2004) Genome mapping and genomic strategies for crop improvement. In: Nguyen HT, Blum A (eds) Physiology and Biotechnology Integration for Plant Breeding. Marcel Dekker, New York, pp 403–451

Subudhi PK, Borkakati RP, Virmani SS, Huang N (1997) Molecular mapping of a thermosensitive genetic male sterility gene in rice using bulked segregant analysis. Genome 40:188–194

Subudhi PK, Nandi S, Casal C, Virmani SS, Huang N (1998) Classification of rice germplasm: III High resolution fingerprinting of cytoplasmic genetic male sterile (CMS) lines with AFLP. Theor Appl Genet 96:941–949

Sun CQ, Wang XK, Li ZC, Yoshimura A, Iwata N (2001) Comparison of the genetic diversity of common wild rice (*Oryza rufipogon* Griff.) and cultivated rice (*O. sativa* L.) using RFLP markers. Theor Appl Genet 102:157–162

Sun X, Yang Z, Wang S, Zhang Q (2003) Identification of a 47-kb DNA fragment containing *Xa4,* a locus for bacterial blight resistance in rice. Theor Appl Genet 106:683–687

Sun X, Cao Y, Yang Z, Xu C, Li X, Wang S, Zhang Q (2004) *Xa26,* a gene conferring resistance to *Xanthomonas oryzae* pv. *oryzae* in rice, encodes an LRR receptor kinase-like protein. Plant J 37(4):517–527

Tabien RE, Li Z, Paterson AH, Marchetti MA, Stansel JW, Pinson SRM (2002) Mapping QTLs for field resistance to the rice blast pathogen and evaluating their individual and combined utility in improved varieties. Theor Appl Genet 105:313–324

Takahashi Y, Shomura A, Sasaki T, Yano M (2001) *Hd6*, a rice quantitative trait locus involved in photosensitivity, encodes the α subunit of protein kinase CK2. Proc Natl Acad Sci USA 98:7922–7927

Takeuchi Y, Hayasaka H, Chiba B, Tanaka I, Shimano T, Yamagishi M, Nagano K, Sasaki T, Yano M (2001) Mapping quantitative trait loci controlling cool-temperature tolerance at booting stage in temperate japonica rice. Breed Sci 51:191–197

Takeuchi Y, Lin SY, Sasaki T, Yano M (2003) Fine linkage mapping enables dissection of closely linked quantitative trait loci for seed dormancy and heading in rice. Theor Appl Genet 107:1174–1180

Talukder ZI, Tharreau D, Price AH (2004) Quantitative trait loci analysis suggests that partial resistance to rice blast is mostly determined by race-specific interactions. New Phytol 162:197–209

Tamura K, Fukuta Y, Hirae M, Oya S, Ashikawa I, Yagi T (1999) Mapping of the *Grh1* locus for green rice leafhopper resistance in rice using RFLP markers. Breed Sci 49:11–14

Tan XL, Vanavichit A, Amornsilpa S, Trangoonrung S (1998) Genetic analysis of rice CMS-WA fertility restoration based on QTL mapping. Theor Appl Genet 97:994–999

Tan YF, Li JX, Yu SB, Xing YZ, Xu CG, Zhang Q (1999) The three important traits for cooking and eating quality of rice grains are controlled by a single locus in an elite rice hybrid, Shanyou 63. Theor Appl Genet 99:642–648

Tan YF, Xing YZ, Li JX, Yu SB, Xu CG, Zhang QF (2000) Genetic bases of appearance quality of rice grains in Shanyou 63, an elite rice hybrid. Theor Appl Genet 101:823–829

Tan YF, Sun M, Xing YZ, Hua JP, Sun XL, Zhang QF, Corke H (2001a) Mapping quantitative trait loci for milling quality, protein content and color characteristics of rice using a recombinant inbred line population derived from an elite rice hybrid. Theor Appl Genet 103:1037–1045

Tan YF, Xing YZ, Zhang QF, Sun M, Corke H (2001b) Quantitative genetic basis of gelatinization temperature of rice. Cereal Chem 78:666–674

Tan GX, Weng QM, Ren X, Huang Z, Zhu LL, He GC (2004) Two whitebacked planthopper resistance genes in rice share the same loci with those for brown planthopper resistance. Heredity 92:212–217

Tang D, Wu W, Li W, Lu H, Worland AJ (2000) Mapping of QTLs conferring resistance to bacterial leaf streak in rice. Theor Appl Genet 101:286–291

Tanksley SD (1993) Mapping polygenes. Annu Rev Genet 27:205–233

Tanksley SD, McCouch SR (1997) Seed banks and molecular maps: unlocking genetic potential from the wild. Science 277(5329):1063–1066

Tanksley SD, Young ND, Paterson AH, Bonierbale MW (1989) RFLP mapping in plant breeding: new tools for an old science. Bio/Technology 7:257–264

Tanksley SD, Ganal MW, Martin GB (1995) Chromosome landing: a paradigm for map-based gene cloning in plants with large genomes. Trends Genet 11:63–68

Tanksley SD, Grandillo S, Fulton TM, Zamir D, Eshed T, Petiard V, Lopez J, Beck-Bunn T (1996) Advanced backcross QTL analysis in a cross between an elite processing line of tomato and its wild relative *L. pimpinellifolium*. Theor Appl Genet 92:213–224

Tarchini R, Biddle P, Wineland R, Tingey S, Rafalski A (2000) The complete sequence of 340 kb of DNA around the rice *Adh1-Adh2* region reveals interrupted colinearlity with maize chromosome 4. Plant Cell 12:381–391

Teng S, Qian Q, Zeng DL, Kunihiro Y, Fujimoto K, Huang DN, Zhu LH (2004) QTL analysis of leaf photosynthetic rate and related physiological traits in rice (*Oryza sativa* L.). Euphytica 135:1–7

Terada R, Urawa H, Inagaki Y, Tsugane K, Iida S (2002) Efficient gene targeting by homologous recombination in rice. Nat Biotechnol 20:1030–1034

Thomas G, Sreejayan JL, Kuriachan P (2001) Genetic variation and population structure in *Oryza malampuzhaensis* endemic to Western Ghats, South India. J Genet 80:141–148

Thomson MJ, Tai TH, McClung AM, Lai XH, Hinga ME, Lobos KB, Xu Y, Martinez CP, McCouch SR (2003) Mapping quantitative trait loci for yield, yield components and morphological traits in an advanced backcross population between *Oryza rufipogon* and *Oryza sativa* cultivar Jefferson. Theor Appl Genet 107:479–493

Tikhonov AP, SanMiguel PJ, Nakajima Y, Gorenstein NM, Bennetzen JL, Avramova Z (1999) Colinearity and its exceptions in orthologous *adh* regions of maize and sorghum. Proc Natl Acad Sci USA 96:7409–7414

Tinker NA, Mather DE (1995) MQTL: Software for simplified composite interval mapping of QTL in multiple environment. J Agri Genom Vol 1: http://www.cabi-publishing.org/gateways/jag/papers95/paper295/indexp295.html

Toojinda T, Siangliw M, Tragoonrung S, Vanavichit A (2003) Molecular genetics of submergence tolerance in rice: QTL analysis of key traits. Ann Bot 91:243–253 Spl Iss

Tripathy JN, Zhang J, Robin S, Nguyen TT, Nguyen HT (2000) QTLs for cell-membrane stability mapped in rice (*Oryza sativa* L.) under drought stress. Theor Appl Genet 100:1197–1202

Tyagi S, Kramer FR (1996) Molecular beacons: probes that fluoresce upon hybridization. Nat Biotechnol 14:303–308

Uchimiya H, Kidon S, Shimazaki T, Aotsuka S, Takamatsu S, Nishi R, Hashimoto H, Metsubayashi Y, Kidon N, Umeda M, Kato A (1992) Random sequencing of cDNA libraries reveals a variety of expressed genes in cultured cells of rice (*Oryza sativa* L.). Plant J 2:1005–1009

Ueda T, Sato T, Numa H, Yano M (2004) Delimitation of the chromosomal region for a quantitative trait locus, qUVR-10, conferring resistance to ultraviolet-B radiation in rice (*Oryza sativa* L.). Theor Appl Genet 108:385–391

Umehara Y, Inagaki A, Tanoue H, Yasukochi Y, Nagamura Y, Saji S, Otsuki Y, Fujimura T, Kurata N, Minobe Y (1995) Construction and characterization of a rice YAC library for physical mapping. Mol Breed 1:79–89

Uozu S, Ikehashi H, Ohmido N, Ohtsubo H, Ohtsubo R, Fukui K (1997) Repetitive sequences: cause for variation in genome size and chromosome morphology on the genus *Oryza*. Plant Mol Biol 35:791–799

Utz HF, Melchinger AE (1996) PLABQTL: a program for composite interval mapping of QTLs. J Quant Trait Loci 2 (http://probe.nalusda.gov.8000/otherdocs/jqtl/jqtl1996–01/utz.html)

Venuprasad R, Shashidhar HE, Hittalmani S, Hemamalini GS (2002) Tagging quantitative trait loci associated with grain yield and root morphological traits in rice (*Oryza sativa* L.) under contrasting moisture regimes. Euphytica 128:293–300

Virk PS, FordLloyd BV, Jackson MT, Pooni HS, Clemeno TP, Newbury HJ (1996) Predicting quantitative variation

within rice germplasm using molecular markers. Heredity 76:296–304

Virmani SS (1996) Hybrid rice. Adv Agron 57:377–462

Virmani SS (1999) Exploitation of heterosis for shifting the yield frontier in rice. In: Coors JG, Pandey S (eds) Genetics and exploitation of heterosis in crops. ASA, CSSA, SSA Inc, Madison, Wisconsin, USA, pp 423–438

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucl Acids Res 23:4407–4414

Wan JL, Zhai HQ, Wan JM, Ikehashi H (2003) Detection and analysis of QTLs for ferrous iron toxicity tolerance in rice, Oryza sativa L. Euphytica 131:201–206

Wang AY, Yu WP, Juang RH, Huang JW, Sung HY, Su JC (1992) Presence of three rice sucrose synthase genes as revealed by cloning and sequencing of cDNA. Plant Mol Biol 18:1191–1194

Wang GJ, Castiglione S, Zhang J, Fu RZ, Ma JS, Li WB, Sun YR, Sala F (1994a) Hybrid rice (Oryza sativa L)-identification and parentage determination by RAPD fingerprinting. Plant Cell Rep 14:112–115

Wang GL, Mackill DJ, Bonman JM, McCouch SR, Champoux MC, Nelson RJ (1994b) RFLP mapping of genes conferring complete and partial resistance to blast in a durably resistant rice cultivar. Genetics 136:1421–1434

Wang B, Xu WW, Wang JZ, Wu W, Zheng HG, Yang ZY, Ray JD, Nguyen HT (1995) Tagging and mapping the thermosensitive genic male-sterile gene in rice (Oryza-sativa L) with molecular markers. Theor Appl Genet 91:1111–1114

Wang FP, Mei MH, Xu CG, Zhang Q (1997) pms1 is not the locus relevant to fertility difference between the photoperiod sensitive male sterile rice Nongken 58S and normal rice Nongken58. Acta Bot Sin 39:922–925

Wang J, Liu KD, Xu CG, Li XH, Zhang Q (1998) The high level of wide-compatibility of variety 'Dular' has a complex genetic basis. Theor Appl Genet 97:407–412

Wang DL, Li Z, Paterson AH, Zhu J (1999a) Maping QTLs with epistatic effects and QTL × environment interactions by mixed linear model approach. Theor Appl Genet 99:1255–1264

Wang ZX, Yano M, Yamanouchi U, Iwamoto M, Monna L, Hayasaka H, Katayose Y, Sasaki T (1999b) The Pib gene for rice blast resistance belongs to the nucleotide binding and leucine-rich repeat class of plant disease resistance genes. Plant J 19:55–64

Wang W, Zhai W, Luo M, Jiang G, Chen X, Li X, Wing RA, Zhu L (2001) Chromosome landing at the bacterial blight resistance gene Xa4 locus using a deep coverage rice BAC library. Mol Genet Genom 265:118–125

Wang Z, Taramino G, Yang D, Liu G, Tingey SV, Miao GH, Wang GL (2001) Rice ESTs with disease-resistance gene- or defense-response gene-like sequences mapped to regions containing major resistance genes or QTLs. Mol Genet Genom 265:302–310

Wang YX, Wu P, Wu YR, Yan XL (2002) Molecular marker analysis of manganese toxicity tolerance in rice under greenhouse conditions. Plant and Soil 238:227–233

Wang CT, Tan MP, Xu X, Wen GS, Zhang DP, Lin XH (2003a) Localizing the bacterial blight resistance gene, Xa22(t), to a 100-kilobase bacterial artificial chromosome. Phytopathology 93:1258–1262

Wang YG, Xing QH, Deng QY, Liang FS, Yuan LP, Weng ML, Wang B (2003b) Fine mapping of the rice thermo-sensitive genic male-sterile gene tms5. Theor Appl Genet 107:917–921

Wang CM, Yasui H, Yoshimura A, Zhai HQ, Wan JM (2004) Inheritance and QTL mapping of antibiosis to green leafhopper in rice. Crop Sci 44:389–393

Wasaki J, Yonetani R, Kuroda S, Shinano T, Yazaki J, Fujii F, Shimbo K, Yamamoto K, Sakata K, Sasaki T, Kishimoto N, Kikuchi S, Yamagishi M, Osaki M (2003) Transcriptomic analysis of metabolic changes by phosphorus stress in rice plant roots. Plant Cell Environ 26:1515–1523

Whyte RO (1972) The Gramineae: Wild and cultivated plants of monsoonal and equatorial Asia, I. Southeast Asia. Asian Perspect 15:127–151

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey S (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl Acids Res 18:6531–6535

Wissuwa M, Yano M, Ae N (1998) Mapping of QTLs for phosphorous-deficiency tolerance in rice (Oryza sativa L.). Theor Appl Genet 97:777–783

Wissuwa M, Wegner J, Ae N, Yano M (2002) Substitution mapping of Pup1: a major QTL increasing phosphorus uptake of rice from a phosphorus-deficient soil. Theor Appl Genet 105:890–897

Wu P, Zhang G, Huang N (1996) Identification of QTLs controlling quantitative characters in rice using RFLP markers. Euphytica 89:349–354

Wu P, Luo A, Zhu J, Yang J, Huang N, Senadhira D (1997) Molecular markers linked to genes underlying seedling tolerance for ferrous iron toxicity. Plant and Soil 196:317–320

Wu P, Hu B, Liao CY, Zhu JM, Wu YR, Senadhira D, Paterson AH (1998a) Characterization of tissue tolerance to iron by molecular markers in different lines of rice. Plant and Soil 203:217–226

Wu P, Ni JJ, Luo AC (1998b) QTLs underlying rice tolerance to low-potassium stress in rice seedlings. Crop Sci 38:1458–1462

Wu P, Liao CY, Hu B, Yi KK, Jin WZ, Ni JJ, He C (2000) QTLs and epistasis for aluminum tolerance in rice (Oryza sativa L.) at different seedling stages. Theor Appl Genet 100:1295–1303

Wu J, Maehara T, Shimokawa T, Yamamoto S, Harada C, Takazaki Y, Ono N, Mukai Y, Koike K, Yazaki J, Fujii F, Shomura A Ando T, Kono I, Waki K, Yamamoto K, Yano M, Matsumoto T, Sasaki T (2002) A comprehensive rice transcript map containing 6591 expressed sequence tag sites. Plant Cell 14:525–535

Wu JL, Sinha PK, Variar M, Zheng KL, Leach JE, Courtois B, Leung H (2004) Association between molecular markers and blast resistance in an advanced backcross population of rice. Theor Appl Genet 108:1024–1032

Xiao JH, Li JM, Yuan LP, Tanksley SD (1995) Dominance is the major genetic basis of heterosis in rice as revealed by QTL analysis using molecular markers. Genetics 140:745–754

Xiao J, Grandillo S, Ahn SN, McCouch SR, Tanksley SD, Li J, Yuan L (1996a) Genes from wild rice improve yield. Nature (London) 384:223–224

Xiao J, Li J, Yuan L, McCouch SR, Tanksley SD (1996b) Genetic diversity and its relationship to hybrid performance and heterosis in rice as revealed by PCR-based markers. Theor Appl Genet 92:637–643

Xiao J, Li J, Yuan L, Tanksley SD (1996c) Identification of QTLs affecting traits of agronomic importance in a recombinant inbred population derived from a subspecific rice cross. Theor Appl Genet 92:230–244

Xiao JH, Li JM, Grandillo S, Ahn SN, Yuan LP, Tanksley SD, McCouch SR (1998) Identification of trait-improving quantitative trait loci alleles from a wild rice relative, *Oryza rufipogon*. Genetics 150:899–909

Xing YZ, Tan YF, Hua JP, Sun XL, Xu CG, Zhang Q (2002) Characterization of the main effects, epistatic effects and their environmental interactions of QTLs on the genetic basis of yield traits in rice. Theor Appl Genet 105:248–257

Xiong L, Liu KD, Dai XK, Wang SW, Xu CG, Zhang DP, Maroof MAS, Sasaki T, Zhang Q (1997) A high density RFLP map based on the F$_2$ population of a cross between *Oryza sativa* and *O. rufipogon* using Cornell and RGP markers. Rice Genet Newslett 14: 110–116

Xiong LX, Liu KD, Dai XK, Xu CG, Zhang QF (1999) Identification of genetic factors controlling domestication-related traits of rice using an F-2 population of a cross between *Oryza sativa* and *O. rufipogon*. Theor Appl Genet 98:243–251

Xu KN, Mackill DJ (1996) A major locus for submergence tolerance mapped on rice chromosome 9. Mol Breed 2:219–224

Xu J, Constantino SV, Magpantay G, Bennett J, Sarkarang S, Huang N (1998) Classification of rice germplasm. II. Discrimination of indica from japonica via analysis of amplicon length polymorphisms. Plant Cell Rep 17:640–645

Xu K, Xu X, Ronald PC, Mackill DJ (2000) A high resolution linkage map of the vicinity of the rice submergence tolerance locus *Sub1*. Mol Gen Genet 263:681–689

Xu W, Virmani SS, Hernandez JE, Sebastian LS, Redoña ED, Li Z (2002a) Genetic diversity in the parental lines and heterosis of the tropical rice hybrids. Euphytica 127:139–148

Xu XF, Mei HW, Luo LJ, Cheng XN, Li ZK (2002b) RFLP-facilitated investigation of the quantitative resistance of rice to brown planthopper (*Nilaparvata lugens*). Theor Appl Genet 104:248–253

Xu KN, Deb R, Mackill DJ (2004) A Microsatellite marker and a codominant PCR-based marker for marker-assisted selection of submergence tolerance in rice. Crop Sci 44:248–253

Yadav R, Courtois B, Huang N, McLaren G (1997) Mapping genes controlling root morphology and root distribution in a doubled haploid population of rice. Theor Appl Genet 94:619–632

Yamagishi M, Takeuchi Y, Kono I, Yano M (2002) QTL analysis for panicle characteristics in temperate japonica rice. Euphytica 128:219–224

Yamaguchi Y, Ikeda R, Hirasawa H, Minami M, Ujihara A (1997) Linkage analysis of thermosensitive genic male sterility gene, *tms-2* in rice (*Oryza sativa* L.). Breed Sci 47:371–373

Yamamoto K, Sasaki T (1997) Large-scale EST sequencing. Plant Mol Biol 35:135–144

Yamamoto T, Kuboki Y, Lin SY, Sasaki T, Yano M (1998) Fine mapping of quantitative trait loci *Hd-1, Hd-2* and *Hd-3*, controlling heading date of rice, as single Mendelian factors. Theor Appl Genet 97:37–44

Yamamoto T, Lin HX, Sasaki T, Yano M (2000) Identification of heading date quantitative trait locus *Hd6* and characterization of its epistatic interactions with *Hd2* in rice using advanced backcross progeny. Genetics 154:885–891

Yamanouchi U, Yano M, Lin H, Ashikari M, Yamada K (2002) A rice spotted leaf gene, *Spl7*, encodes a heat stress transcription factor protein. Proc Natl Acad Sci USA 99:7530–7535

Yamasaki M, Tsunematsu H, Yoshimura A, Iwata N, Yasui H (1999) Quantitative trait locus mapping of ovicidal response in rice (*Oryza sativa* L.) against whitebacked planthopper (*Sogatella furcifera* Horvath). Crop Sci 39:1178–1183

Yamasaki M, Yoshimura A, Yasui H (2000) Mapping of quantitative trait loci of ovicidal response to brown planthopper (*Nilaparvata lugens* Stal) in rice (*Oryza sativa* L.). Breed Sci 50:291–296

Yamazaki M, Tsugawa H, Miyao A, Yano M, Wu J, Yamamoto S, Matsumoto T, Sasaki T, Hirochika H (2001) The rice retrotransposon *Tos17* prefers low-copy number sequences as integration targets. Mol Genet Genom 265:336–344

Yan JQ, Zhu J, He CX, Benmoussa M, Wu P (1998) Molecular dissection of developmental behavior of plant height in rice (*Oryza sativa* L.). Genetics 150:1257–1265

Yan JQ, Zhu J, He CX, Benmoussa M, Wu P (1999) Molecular marker-assisted dissection of genotype × environment interaction for plant type traits in rice (*Oryza sativa* L.). Crop Sci 39:538–544

Yan L, Loukoianov A, Tranquilli G, Helguera M, Fahima T, Dubcovsky J (2003) Positional cloning of the wheat vernalization gene *VRN1*. Proc Natl Acad Sci USA 100:6263–6268

Yanagihara S, McCouch SR, Ishikawa K, Ogi Y, Maruyama K, Ikehashi H (1995) Molecular analysis of the inheritance of the *s-5* locus, conferring wide compatibility in indica-japonica hybrids of rice (*Oryza sativa* L). Theor Appl Genet 90:182–188

Yang DC, Magpantay GB, Mendoza M, Huang N, Brar DS (1997) Construction of a contig for a fertility restorer gene *Rf2*, in

rice using BAC library and its sequence with *Rf2* gene of maize. Rice Genet Newslett 14:116–117

Yang D, Sanchez A, Khush GS, Zhu Y, Huang N (1998) Construction of a BAC contig containing the *xa5* locus in rice. Theor Appl Genet 97:1120–1124

Yang Z, Sun X, Wang S, Zhang Q (2003) Genetic and physical mapping of a new gene for bacterial blight resistance in rice. Theor Appl Genet 106:1467–1472

Yang GX, Jan A, Shen SH, Yazaki J, Ishikawa M, Shimatani Z, Kishimoto N, Kikuchi S, Matsumoto H, Komatsu S (2004) Microarray analysis of brassinosteroids- and gibberellin-regulated gene expression in rice seedlings. Mol Genet Genom 271:468–478

Yano M, Simosaka E, Saito A, Nakagarha M (1991) Linkage analysis on gene for scent in indica rice variety, Surajmukhi, using a restriction fragment length polymorphism markers. Jpn J Breed 41 (Suppl 1) (in Japanese):338–339

Yano M, Sasaki T (1997) Genetic and molecular dissection of quantitative traits in rice. Plant Mol Biol 35:145–153

Yano M, Harushima Y, Nagamura Y, Kurata N, Minobe Y, Sasaki T (1997) Identification of quantitative trait loci controlling heading date in rice using a high-density linkage map. Theor Appl Genet 95:1025–1032

Yano M, Katayose Y, Ashikari M, Yamanouchi U, Monna L, Fuse T, Baba T, Yamamoto K, Umehara Y, Nagamura Y, Sasaki T (2000) *Hd1*, a major photoperiod sensitivity quantitative trait locus in rice, is closely related to the Arabidopsis flowering time gene *CONSTANS*. Plant Cell 12:2473–2483

Yano M, Kojima S, Takahashi Y, Lin H, Sasaki T (2001) Genetic control of flowering time in rice, a short-day plant. Plant Physiol 127:1425–1429

Yao FY, Xu CG, Yu SB, Li JX, Gao YJ, Li XH, Zhang QF (1997) Mapping and genetic analysis of two fertility restorer loci in the wild-abortive cytoplasmic male sterility system of rice (*Oryza sativa* L.). Euphytica 98:183–187

Yashitola J, Thirumurugan T, Sundaram RM, Naseerullah MK, Ramesha MS, Sarma NP, Sonti RV (2002) Assessment of purity of rice hybrids using microsatellite and STS markers. Crop Sci 42:1369–1373

Yasui H, Yoshimura A (1999) QTL mapping of antibiosis to green leafhopper, *Nephotettix virescens* Distant and green rice leafhopper, *Nephotettix cincticeps* Uhler in rice, *Oryza sativa* L. Rice Genet Newslett 16:96–97

Yazaki J, Kishimoto N, Nagata Y, Ishikawa M, Fujii F, Hashimoto A, Shimbo K, Shimatani Z, Kojma K, Suzuki K, Yamamoto M, Honda S, Endo A, Yoshida Y, Sato Y, Takeuchi K, Toyoshima K, Miyamoto C, Wu JZ, Sasaki T, Sakata K, Yamamoto K, Iba K, Oda T, Otomo Y, Murakami K, Matsubara K, Kawai J, Carninci P, Hayashizaki Y, Kikuchi S (2003) Genomics approach to abscisic acid- and gibberellin-responsive genes in rice. DNA Res 10:249–261

Yazaki J, Shimatani, Z, Hashimoto A, Nagata Y, Fujii F, Kojima K, Suzuki K, Taya T, Tonouchi M, Nelson C, Nakagawa A, Otomo Y, Murakami K, Matsubara K, Kawai J, Carninci P, Hayashizaki Y, Kikuchi S (2004) Transcriptional profiling

of genes responsive to abscisic acid and gibberellin in rice: phenotyping and comparative analysis between rice and Arabidopsis. Physiol Genom 17:87–100

Ye X, Al Babili S, Kloti A, Zhang A, Lucca P, Beyer P, Potrykus I (2000) Engineering the provitamin A (Betacareotene biosynthetic pathway) into carotenoid free rice endosperm. Science 287:303–305

Yoshida S, Ikegami M, Kuze J, Sawada K, Hashimoto Z, Ishii T, Nakamura C, Kamijima O (2002) QTL analysis for plant and grain characters of sake-brewing rice using a doubled haploid population. Breed Sci 52:309–317

Yoshimura S, Yoshimura A, Nelson RJ, Mew TW, Iwata N (1995) Tagging *xa-1*, the bacterial-blight resistance gene in rice, by using RAPD markers. Breed Sci 45:81–85

Yoshimura S, Umehara Y, Kurata N, Nagamura Y, Sasaki T, Minobe Y, Iwata N (1996) Identification of a YAC clone carrying the *Xa-1* allele, a bacterial blight resistance gene in rice. Theor Appl Genet 93:117–122

Yoshimura S, Yamanouchi U, Katayose Y, Toki S, Wang ZX, Kono I, Kurata N, Yano M, Iwata N, Sasaki T (1998) Expression of *Xa1*, a bacterial blight-resistance gene in rice, is induced by bacterial inoculation. Proc Natl Acad Sci USA 95:1663–1668

Yu ZH, Mackill DJ, Bonman JM, Tanksley SD (1991) Tagging genes for blast resistance in rice via linkage to RFLP markers. Theor Appl Genet 81:471–476

Yu ZH, Mackill DJ, Bonman JM, McCouch SR, Guiderdoni E, Notteghem JL, Tanksley SD (1996) Molecular mapping of genes for resistance to rice blast (*Pyricularia grisea* Sacc). Theor Appl Genet 93:859–863

Yu SB, Li JX, Tan YF, Gao YJ, Li XH, Zhang QF, Maroof MAS (1997) Importance of epistasis as the genetic basis of heterosis in an elite rice hybrid. Proc Natl Acad Sci USA 94:9226–9231

Yu J, Hu S, Wang J, Wong GKS, Li S, Liu B et al (2002a) A draft sequence of the rice genome (*Oryza sativa* L.ssp. indica). Science 296:79–92

Yu SB, Li JX, Xu CG, Tan YF, Li XH, Zhang QF (2002b) Identification of quantitative trait loci and epistatic interactions for plant height and heading date in rice. Theor Appl Genet 104:619–625

Yu SB, Xu WJ, Vijayakumar CHM, Ali J, Fu BY, Xu JL, Jiang YZ, Marghirang R, Domingo J, Aquino C, Virmani SS, Li ZK (2003) Molecular diversity and multilocus organization of the parental lines used in the International Rice Molecular Breeding Program. Theor Appl Genet 108:131–140

Yuan LP, Yang ZY, Yang JB (1994) Hybrid rice in China. In: Virmani SS (ed) Hybrid Rice Technology: New Developments and Future Prospects. IRRI, Manila, Philippines, pp 143–147

Zambryski P, Holsters M, Kruger K, Depicker A, Schell J, Van-Montagu M, Goodman HM (1980) Tumor DNA structure in plant cells transformed by *A. tumefaciens*. Science 209:1385–1391

Zenbayashi K, Ashizawa T, Tani T, Koizumi S (2002) Mapping of the QTL (quantitative trait locus) conferring partial re-

sistance to leaf blast in rice cultivar Chubu 32. Theor Appl Genet 104:547–552

Zeng LR, Qu S, Bordeos A, Yang C, Baraoidan M, Yan H, Xie Q, Nahm BH, Leung H, Wang GL (2004) Spotted leaf 11, a negative regulator of plant cell death and defense, encodes a U-box/armadillo repeat protein endowed with E3 ubiquitin ligase activity. Plant Cell 16:2795–2808

Zhang Q, Yu S (2000) Molecular marker-based gene tagging and its impact on rice improvement. In: Rice Breeding and Genetics: Research Priorities and Challenges. Nanda JS (ed) Science Publishers, Inc, Enfield, NH, USA, pp 241–270

Zhang QF, Shen BZ, Dai XK, Mei MH, Maroof MAS, LI ZB (1994a) Using bulked extremes and recessive class to map genes for photoperiod-sensitive genic male-sterility in rice. Proc Natl Acad Sci USA 91:8675–8679

Zhang QF, Gao YJ, Yang SH, Ragab RA, Maroof MAS, Li ZB (1994b) A diallel analysis of heterosis in elite hybrid rice-based on RFLPs and microsatellites. Theor Appl Genet 89:185–192

Zhang GY, Guo Y, Chen SL, Chen SY (1995a) RFLP tagging of a salt tolerance gene in rice. Plant Sci 110:227–234

Zhang QF, Gao YJ, Maroof MAS, Yang SH, Li JX (1995b) Molecular divergence and hybrid performance in rice. Mol Breed 1:133–142

Zhang G, Angeles ER, Abenes MLP, Khush GS, Huang N (1996a) RAPD and RFLP mapping of the bacterial blight resistance gene xa-13 in rice. Theor Appl Genet 93:65–70

Zhang Q, Zhou ZQ, Yang GP, Xu CG, Liu KD, Saghai Maroof MAS (1996b) Molecular marker heterozygosity and hybrid performance in indica and japonica rice. Theor Appl Genet 93:1218–1224

Zhang G, Bharaj TS, Lu Y, Virmani SS, Huang N (1997) Mapping of the Rf-3 nuclear fertility-restoring gene for WA cytoplasmic male sterility in rice using RAPD and RFLP markers. Theor Appl Genet 94:27–33

Zhang J, Zheng HG, Aarti A, Pantuwan G, Nguyen TT, Tripathy JN, Sarial AK, Robin S, Babu RC, Nguyen BD, Sarkarung S, Blum A, Nguyen HT (2001) Locating genomic regions associated with components of drought resistance in rice: comparative mapping within and across species. Theor Appl Genet 103:19–29

Zhao MF, Li XH, Yang JB, Xu CG, Hu RY, Liu DJ, Zhang Q (1999) Relationship between molecular marker heterozygosity and hybrid performance in intra- and inter-subspecific crosses of rice. Plant Breed 118:139–144

Zheng K, Huang N, Bennett J, Khush GS (1995) PCR-based marker-assisted selection in rice breeding. IRRI Discussion Paper Series No. 12. IRRI, Manila, Philippines

Zheng K, Zhuang J, Lu J, Qian H, Lin HX (1996) Identification of DNA markers tightly linked to blast resistance genes in rice. In: Rice Genetics III. Proc 3rd Intl Rice Genet Symp, 16–20 Oct 1995, IRRI, Manila, Philippines, pp 565–569

Zheng HG, Babu RC, Pathan MS, Ali L, Huang N, Courtois B, Nguyen HT (2000) Quantitative trait loci for root-penetration ability and root thickness in rice: Comparison of genetic backgrounds. Genome 43:53–61

Zheng BS, Yang L, Zhang WP, Mao CZ, Wu YR, Yi KK, Liu FY, Wu P (2003) Mapping QTLs and candidate genes for rice root traits under different water supply conditions and comparative analysis across three populations. Theor Appl Genet 107:1505–1515

Zhou Z, Gustafson JP (1995) Genetic-variation detected by DNA fingerprinting with a rice minisatellite probe in Oryza sativa L. Theor Appl Genet 91:481–488

Zhou H, Xie ZW, Ge S (2003a) Microsatellite analysis of genetic diversity and population genetic structure of a wild rice (Oryza rufipogon Griff.) in China. Theor Appl Genet 107:332–339

Zhou PH, Tan YF, He YQ, Xu CG, Zhang Q (2003b) Simultaneous improvement for four quality traits of Zhenshan 97, an elite parent of hybrid rice, by molecular marker-assisted selection. Theor Appl Genet 106:326–331

Zhou JH, Wang JL, Xu JC, Lei CL, Ling ZZ (2004) Identification and mapping of a blast resistance gene Pi-g(t) in the cultivar Guanchangzhan. Plant Pathol 53:191–196

Zhu LH, Chen Y, Xu YB, Xu JC, Cai HM, Ling ZZ (1993) Construction of a molecular map of rice and gene mapping using a doubled haploid population of a cross between indica and japonica varieties. Rice Genet Newslett 10:132–134

Zhu L, Lu C, Li P, Shen Li, Xu Y, He P, Chen Y (1996) Using doubled haploid populations of rice for quantitative trait locus mapping. In: Rice Genetics III. Proc 3rd Intl Rice Genet Symp, 16–20 Oct 1995, IRRI, Manila, Philippines, pp 631–635

Zhu J, Gale MD, Quarrie S, Jackson MT, Bryan GJ (1998) AFLP markers for the study of rice biodiversity. Theor Appl Genet 96:602–611

Zhu T, Budworth P, Chen WQ, Provart N, Chang HS, Guimil S, Su WP, Estes B, Zou GZ, Wang, X (2003) Transcriptional control of nutrient partitioning during rice grain filling. Plant Biotechnol J 1:59–70

Zhuang JY, Lin HX, Lu J, Qian HR, Hittalmani S, Huang N, Zheng KL (1997) Analysis of QTL × Environment interaction for yield components and plant height in rice. Theor Appl Genet 95:799–808

Zhuang JY, Ma WB, Wu JL, Chai RY, Lu J, Fan YY, Jin MZ, Leung H, Zheng KL (2002) Mapping of leaf and neck blast resistance genes with resistance gene analog, RAPD and RFLP in rice. Euphytica 128:363–370

Zou JH, Pan XB, Chen ZX, Xu JY, Lu JF, Zhai WX, Zhu LH (2000) Mapping quantitative trait loci controlling sheath blight resistance in two rice cultivars (Oryza sativa L.). Theor Appl Genet 101:569–573

2 Wheat

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2.1
Introduction

The wheats (*Triticum* spp.) belong to the Poaceae, the largest family within the monocotyledonous plants. Bread wheat (*Triticum aestivum* L. em. Thell) is one of the most important cereal grain crops of the world and is cultivated over a wide range of climatic conditions. Global production of bread wheat in 2003 was 557 Mt, with an average yield of 2.68 t/ha (http://apps.fao.org/). The world's major bread wheat-producing areas are in northern China, northern India, northern USA and adjoining areas in Canada, northern and central Europe, western Russia, southern Australia, southern Latin America and South Africa. Worldwide, wheat provides nearly 55% of the carbohydrate and 20% of the food calories consumed globally (Breiman and Graur 1995).

Wheat is one of the most extensively studied crop species, particularly in the area of cytogenetics. An extensive catalogue of genetic and cytogenetic stocks was developed in the years following the groundbreaking isolation of aneuploid lines by Sears (1954). This work led to the concept of chromosome engineering, which takes advantage of the effect of the *Ph* genes. These genes restrict pairing and recombination to homologous chromosomes (Riley and Chapman 1958). Wheat provides a model system for the study of polyploid cytogenetics because of the ease of chromosome manipulation. The pioneering cytogenetic work by Kihara, Sakamura, Sax, Sears, Riley and others (Riley and Chapman 1958; Riley 1965) showed that the species of the genus *Triticum* form a polyploid series, with a basic number of $x = 7$. Thus there are the diploid ($2n = 2x = 14$), tetraploid ($2n = 4x = 28$) and hexaploid ($2n = 6x = 42$) species. Most modern cultivated wheat varieties are hexaploid (*T. aestivum*), described as 'common' or 'bread' wheat and valued for bread making. Bread wheat is a segmental allopolyploid containing the three distinct but genetically related (homoeologous) genomes A, B and D. It also has a very large genome (1.8×10^{10} bp), making an average wheat chromosome about 25-fold larger in terms of DNA content than the average rice chromosome (Moore et al. 1995b). Thus three wheat chromosomes carry the same DNA content of the entire haploid maize genome, and half of an average wheat chromosome is equivalent to the haploid rice genome (Gill and Gill 1994). The large genome size of bread wheat is due to extensive regions of retrotransposon-type elements such that over 80% of the genome consists of repetitive DNA sequence (Schulman et al. 2004). In contrast to the suitability of bread wheat for cytogenetic studies, the application of molecular techniques has been slow (Lagudah et al. 2001; Langridge et al. 2001). Many molecular markers are unable to detect an adequate and useful polymorphism for the construction of molecular maps, and consequently applications of marker-assisted selection (MAS) applications have been limited. However, despite these problems, some success has been achieved in recent years, and molecular genetic as well as physical maps have become available for the chromosomes of all homoeologous groups (Gupta et al. 1999; Varshney et al. 2004a). Molecular markers are increasingly being used to tag genes or QTLs (quantitative trait loci) of agronomic importance, offering the possibility of their use in marker-assisted selection (MAS) for wheat breeding (Gupta et al. 1999; Jahoor et al. 2004). In addition to their use in MAS, molecular markers have begun to be used to isolate genes via map-based cloning (Stein and Graner 2004). Some molecular markers detect homoeoloci; that is, the same sequence is present

on all three members of a homoeologous group. Such homoeoloci have helped in the construction of comparative maps in different cereals, and these sometimes demonstrate the presence of major translocations thought to have occurred during speciation. In this article, we review recent progress related to the generation of genetic and physical maps in wheat and their applications for a variety of purposes including gene tagging for MAS, map-based cloning, diversity studies and comparative mapping in cereals. The impact of functional genomics and other recent approaches such as association mapping and genetical genomics on wheat breeding in the near future is also discussed.

2.2
Molecular Markers – Types and Availability

Recent advances in molecular techniques have led to the development of assays based on variation in DNA sequence, broadly referred to as DNA (or molecular) markers (Langridge and Chalmers 2004). DNA markers provide good resolution because, unlike most non-DNA-based markers (morphological, biochemical or physiological), they are (1) unlimited in number, (2) independent of environment, developmental stage and complex genetic interactions, (3) frequently free of dominant and recessive effects and (4) easy to score, analyse and interpret. The DNA markers that have been used for the construction of molecular maps are broadly classified into three groups: the first-generation markers, RFLPs (**r**estriction **f**ragment **l**ength **p**olymorphisms) and RAPDs (**r**andomly **a**mplified **p**olymorphic **D**NAs); the second-generation markers, SSRs (**s**imple **s**equence **r**epeats or microsatellites) and AFLPs (**a**mplified **f**ragment **l**ength **p**olymorphisms); and the third-generation markers, SNPs (**s**ingle **n**ucleotide **p**olymorphisms) and InDels (**in**sertion-**del**etions) (for details see Gupta et al. 2002b; Varshney et al. 2004a; Mohler and Schwarz 2004). In addition, an array of marker types have been developed amongst which are STSs (**s**equence **t**agged **s**ites), SCARs (**s**equence **c**haracterized **a**mplified **r**egions), ISSRs (**i**nter **s**imple **s**equence **r**epeats), and SAMPL (**s**elective **a**mplification of **m**icrosatellite **p**olymorphic **l**oci). More recently, EST (**e**xpressed **s**equence **t**ag)-based markers (EST-SSRs and EST-SNPs) have been developed in wheat

(Varshney et al. 2004a). Retrotransposon sequences (which are present in high-copy numbers), both alone or in combination with microsatellites or AFLPs, have been exploited to generate IRAPs (**i**nter**r**etrotransposon **a**mplified **p**olymorphisms), REMAPs (**r**etrotransposon-**m**icrosatellite **a**mplified **p**olymorphisms) and SSAPs (**s**equence-**s**pecific **a**mplified **p**olymorphisms) (Schulman et al. 2004). Each marker system has particular advantages and disadvantages (Gupta et al. 2002b) and user choice is best based on objective, convenience and cost. All these marker types, except the SNPs, have been incorporated into current molecular maps, and efforts are currently under way to construct SNP maps of wheat (Varshney et al. 2004a).

The accepted nomenclature for DNA marker loci and alleles in wheat and related species is published every 4 years in the Proceedings of the International Wheat Genetics Symposium (for the most recent edition see Proc of the 10th Int Wheat Genet Symp, Paestum, Italy, 2003), and an annual supplement is published in the Annual Wheat Newsletter (http://wheat.pw.usda.gov/ggpages/awn/). The catalogue lists all *Triticum* genes, RFLPs, SSRs, STSs, AFLPs, etc. that have been localized to a chromosome or chromosome arm, all known alleles of *Triticum* genes and prototype strains for each allele, the chromosomal locations of genetic markers, the linkage position of mapped genes, literature citations and other relevant information.

2.3
Construction of Molecular Maps

Early genetic maps were based entirely on morphological and biochemical markers. However, these maps had poor resolution, as marker number was limited and allelic variants were frequently restricted to exotic germplasm, precluding their usefulness in breeding programmes. Molecular markers detect both sequence (for example SNPs, resulting in RFLPs, RAPDs, AFLPs, etc.) and length polymorphisms (polymorphisms due to length variation of a sequence, as in SSRs and sometimes also in RFLPs). These loci usually segregate in a Mendelian manner, so that the conventional basis of linkage and recombination can be used for constructing these maps. A major advantage of molecular mapping is the possibility of analysing a large number of markers in a single mapping population. Therefore, DNA-based markers have been used

for the construction of maps with a high marker density in almost all major crops including cereals (Varshney et al. 2004a). These maps have found application for gene tagging, QTL identification, and for the characterization of germplasm collections (Gupta et al. 1999; Langridge and Chalmers 2004). The aneuploid and deletion stocks in the type variety Chinese Spring have allowed the alignment of physical and genetic maps, and this has provided an insight into the physical and genetic organization of the wheat genome.

2.3.1
Genetic Maps

RFLPs were developed for mapping in the human genome (Botstein et al. 1980). Subsequently, they were adapted for use in mapping plant genomes (Bernatzky and Tanksley 1986; Weber and Helentjaris 1989) including bread wheat (Chao et al. 1989; Liu and Tsunewaki 1991). Disappointingly, RFLPs have only been able to detect a low level of polymorphism in wheat. This has been attributed variously to its polyploid nature, its high proportion of repetitive DNA, its large genome size and its recent origin (ca. 10,000 years ago). Thus in an effort to maximize the diversity between the parents of mapping populations, a standard hexaploid variety was crossed with a synthesized hexaploid (a chromosome-doubled hybrid of the wide cross tetraploid *T. turgidum* × diploid *Aegilops tauschii*) to produce a reference mapping population known as the ITMI (International Triticea Mapping Initiative) population (Langridge et al. 2001). Alternatively, the three constituent genomes have been analysed at the diploid level. This involves generation of populations from specimen diploids *Ae. tauschii* (D genome) (Boyko et al. 1999, 2002) and *T. monococcum* (A genome) (Dubcovsky et al. 1996). Mapping populations have included F_2 populations, F_3 families, bulked F_4 families and recombinant inbred line (RIL) populations, and, in some cases, doubled haploids (DHs) and recombinant substitution lines (RSLs). RSLs, DHs and RILs have the particular advantage of being immortal, while F_2 populations, F_3 families and bulked F_4 families are easier to produce.

Using various mapping populations, a number of RFLP-based maps have been constructed both for individual chromosomes and for the entire wheat genome (Table 1). RFLP genotyping is time consuming and labour intensive and is therefore unsuitable for the rapid evaluation of large segregating populations typically encountered in commercial breeding programmes (Gale et al. 1995). The first replacement PCR-based technology was RAPDs, and these have been used for mapping many species including *Arabidopsis* (Reiter et al. 1992), barley (Giese et al. 1994) and rye (Masojć et al. 2001). In wheat, RAPDs have been of limited use, partly because of the low level of polymorphism that they uncover, but also because of poor reproducibility. Critically, RAPD alleles are usually dominant, and therefore a heterozygous genotype cannot be distinguished from one of the related homozygotes. A more profound disadvantage of the system is that a given pair of similarly sized RAPD products amplified from two genotypes may not represent homologous sequences (Devos and Gale 1992). As with RAPDs, AFLPs are commonly dominant markers. However, AFLP is a superior platform, due both to its greater robustness, and to its delivery of a far higher multiplex ratio (the number of distinct loci analysed per primer pair and per gel lane) (Ma and Lapitan 1998). AFLP has found its greatest application in fingerprinting studies (see later), but also to some extent in mapping. A number of genetic maps have incorporated AFLP loci, but usually associated with an RFLP and/or SSR backbone (Table 1). More recently, microsatellites (SSRs) have become the favoured markers. Their advantages include multiallelism, codominant inheritance, relative abundance and extensive genome coverage (Gupta and Varshney 2000). Microsatellite markers for wheat have been generated from a number of sources, including the John Innes Centre (JIC), Norwich, UK (Stephenson et al. 1998), IPK, Gatersleben, Germany (Röder et al. 1998b), the Wheat Microsatellite Consortium (WMC; Varshney et al. 2000a; Gupta et al. 2002a), Beltsville Agricultural Research Centre (BARC; Song et al. 2002a,b) and the Genoplante/INRA Wheat SSR Club (http://wheat.pw.usda.gov/ggpages/SSRclub/; Guyomarc'h et al. 2002; Nicot et al. 2004). To date the densest microsatellite-based map of wheat contains 1,238 loci covering 2,569 cM with an average interval distance of 2.2 cM (Somers et al. 2004). In addition, wheat ESTs have also been exploited to generate the microsatellite (EST-SSR) markers in wheat (see later). A detailed account on development and application of microsatellite markers in wheat is available in a recent review by Röder et al. (2004).

Emphasis in marker research is now beginning to shift to the development of SNP markers, which are biallelic and are extremely abundant. SNPs have the potential to deliver very high throughput and

Table 1. A list of some important genetic maps constructed in wheats[a]

Map type	Population used for mapping	Number of loci mapped	Genetic map length (cM)	Reference
RFLP maps				
Wheat (Group 1)	ITMI RILs (W7984 × Opata85)	98	146 to 344	Van Deynze et al. (1995a)
Wheat (Group 2)	F2/F3s (Chinese Spring × Synthetic Timgalen)	114	–	Devos et al. (1993b)
Wheat (Group 2)	ITMI RILs (W7984 × Opata85)	173	$\sim$ 600	Nelson et al. (1995b)
Wheat (Group 3)	F2/F3s (Chinese Spring × Synthetic Timgalen)	$\sim$ 60	–	Devos et al. (1992) Devos and Gale (1993)
Wheat (Group 3)	ITMI RILs (W7984 × Opata85)	160	$\sim$ 660	Nelson et al. (1995c)
Wheat (Group 4)	ITMI RILs (W7984 × Opata85)	98	–	Nelson et al. (1995a)
Wheat (Group 5)	F2/F3s (Chinese Spring × Synthetic Timgalen)	$\sim$ 50	–	Xie et al. (1993)
Wheat (Group 5)	ITMI RILs (W7984 × Opata85)	118	–	Nelson et al. (1995a)
Wheat (Group 6)	ITMI RILs (W7984 × Opata85)	154	516	Marino et al. (1996)
Wheat (Group 6)	F2/F3s (Chinese Spring × Synthetic)	62	317	Jia et al. (1996)
Wheat (Group 7)	ITMI RILs (W7984 × Opata85)	109	–	Nelson et al. (1995a)
Wheat	F2s (*T. aestivum* var. Chinese Spring × *T. spelta* var. Duha)	197	–	Liu and Tsunewaki (1991)
Wheat	DHs (Chinese Spring × Courtot)	264	1,772	Cadalen et al. (1997)
Wheat	RILs (*T. aestivum* cv. Chinese Spring × *T. spelta* var. *duhamelianum* K19-1)	320	3,451	Sasakuma and Shindo (2003)
Wheat-durum	RILs (*T. durum* var. Messapia × *T. turgidium* var. MG4343)	245	–	Blanco et al. (1998)
Wheat-diploid	F2s (*T.monococcum* KT3-5 × *T. boeoticum* KT1-1)	115	1,250	Sasakuma and Shindo (2003)
SSR maps				
Wheat	ITMI RILs (W7984 × Opata85)	279	–	Roder et al. (1998b)
Wheat	F2s (Chinese Spring × Synthetic)	53		Stephenson et al. (1998)
Wheat	ITMI RILs (W7984 × Opata85)	65		Pestsova et al. (2000)
Wheat	DHs	172	–	Harker et al. (2001)
Wheat	ITMI RILs (W7984 × Opata85)	65	–	Gupta et al. (2002a)
Wheat	4 mapping populations (W7984 × Opata85, Courtot × Chinese Spring, Eureka × Renan, Arche × Recital)	533	–	Gandon et al. (2002)
Wheat	RIL (Courtot × Chinese Spring)	84	–	Guyomarc'h et al. (2002)
Wheat	ITMI RILs (W7984 × Opata85)	168	–	Song et al. (2002a,b)
Wheat	F2:3s (ND3338 x F390)	247	3,067	Liu et al. (2003)
Wheat	3 DHs (RL4452 × AC Domain, Wuhan × Maringa, Superb × BW278) and ITMI RILs (W7984 × Opata85)	1,235	2,569	Somers et al. (2004)
Wheat	ITMI RILs (W7984 × Opata85)	825	–	Nicot et al. (2003a)
Wheat	ITMI RILs (W7984 × Opata85)	61 (eSSRs)	–	Nicot et al. (2003b)
Wheat	ITMI RILs (W7984 × Opata85)	126 (eSSRs)	–	Nicot et al. (2004)
Wheat	ITMI RILs (W7984 × Opata85)	101 (eSSRs)	–	Gao et al. (2004)
Wheat	ITMI RILs (W7984 × Opata85)	149 (eSSRs)	–	Yu et al. (2004b)
Wheat	ITMI RILs (W7984 × Opata85)	876 (eSSRs)	–	Peng et al. (2004a)
Wheat	ITMI RILs (W7984 × Opata85)	638	–	Röder et al. (2004b)
Wheat-durum	RILs (*T. durum* var. Messapia × *T. turgidium* var. MG4343)	79	–	Korzun et al. (1999)
Wheat-durum	RILs (*T. turgidum* subsp. *Durum*)	112	–	Jurman et al. (2003)

Table 1. (continued)

Map type	Population used for mapping	Number of loci mapped	Genetic map length (cM)	Reference
AFLP maps				
Wheat	DHs (Garnet × Saunders)	426	–	Penner et al. (1998)
Wheat	ITMI RILs (W7984 × Opata85)	140	–	Hazen et al. (2002)
Composite maps				
Aegilops tauschii	F2s [*Ae. tauschii* var *meyeri* (TA1691) × *Ae. tauschii* var *typical* (TA1704)]	732	–	Boyko et al. 2002
Wheat-einkorn	F2s (*T. monococcum* × *T. boeoticum* ssp. *boeoticum*)	81 (RFLPs, RAPDs, ISSRs)	–	Kojima et al. (1998)
Wheat-einkorn	F2s/ F3s (*T. monococcum* ssp. *monococcum* DV92 × *T. monococcum* ssp. Aegilopoides C3116)	335 (mainly RFLPs)	714	Dubcovsky et al. (1996)
Wheat-durum	RILs [*T. durum* (Messapia) × *T. turgidium* (MG4343)]	88 (AFLPs, RFLPs)	2,063 (total)	Lotti et al. (2000)
Wheat-durum	F2s (*T. dicoccoides* acc. Hermon H52 × *T. durum* cultivar Langdon (Ldn)	545 (AFLPs, RAPDs, SSRs)	3,169–3,180	Peng et al. (2000b)
Wheat-durum	RILs (Jennah Khetifa × Cham1)	306 (RFLPs, SSRs, AFLPs)	3,598	Nachit et al. (2001)
Wheat-durum	RILs (Omrabi5 × *T. dioccoides* 600545 × Ombrabi 5)	279 (RFLP, SSR, SSP)	2,289	Elouafi and Nachit (2004)
Wheat-emmer	RILs	549 (SSRs, AFLPs, RAPDs)		Nevo (2001)
Wheat	DHs (Schomburgk × Yarralinka)	147 (RFLPs, SSRs, AFLPs)	–	Parker et al. (1998)
Wheat	RILs (*T. aestivum* L. var. Forno × *T. spelta* L. var. Oberkulmer)	230 (RFLPs, SSRs)	2,469	Messmer et al. (1999)
Wheat	DHs (Cranbook × Halbred, CD87 × Katepwa, Sunco × Tasman)	355 to 902 (RFLPs, SSRs, AFLPs)	–	Chalmers et al. (2001)
Wheat	DHs (Courtot × Chinese Spring)	380 (RFLP, SSRs, AFLPs)	2,900	Sourdille et al. (2000b)
Wheat	DHs (Courtot × Chinese Spring)	659 (RFLP, SSRs, AFLPs)	3,685	Sourdille et al. (2003)
Wheat	F5s (Arina × Forno)	396 (RFLPs, SSRs)	3,086	Paillard et al. (2003)
Wheat	DHs (Beaver x Soissons)	241 (AFLPs, SSRs)	2,290	Verma et al. (2004)

[a]Details and updated version of these maps are available at GrainGenes (http://wheat.pw.usda.gov/GG2/maps.shtml)

automation. In the human genome, 1.8 million SNPs have been documented (http://snp.cshl.org/). In an international consortium, an attempt has been made to mine for SNPs from the massive amounts of wheat EST sequence available on public databases (http://wheat.pw.usda.gov/ITMI/2002/WheatSNP.html). Using this approach, Somers et al. (2003b) estimated SNP frequency as 1 every 540 bp, and efforts are under way to develop SNP markers in wheat (Mochida et al. 2003; Ogihara 2003).

Integrated (or 'composite') maps including more than one type of molecular marker (particularly RFLPs, SSRs and AFLPs) have also been prepared (Table 1). These maps typically have higher resolution than those based on a single marker type because they exploit a larger number of loci.

Comparisons between specific chromosomal regions across related species usually show that locus order (but not map distance) is highly conserved. Consequently, the construction of 'consensus maps' has become possible, where common markers are used as anchors and the position of other loci mapping in interstitial positions is extrapolated (for example, in barley, see Varshney et al. 2004b). In this way, 4,000 loci from 16 independent maps have been integrated into a single map (Appels 2003). This consensus map has been aligned with physical maps (see later) and has recently been put forward as the backbone for a long-range wheat genomic sequencing proposal. More rigorous consensus maps that use the linkage data from multiple populations can also be constructed using computer packages such as Join-Map (Stam and Van Ooijen 1995), but this method has not yet been used to develop a consensus map of wheat.

2.3.2
Transcript Genetic Maps or Functional Maps

A large amount of EST data has been generated in wheat, and 587,088 sequences are currently available in the public domain (http://www.ncbi.nlm.nih.gov/dbEST/dbEST_summary.html; 12 November 2004). From these, 44,630 TCs (tentative consensi) and 79,008 EST singletons have been identified (Sect. 2.5.1). The integration of these loci into genetic maps would generate a 'transcript map'/'gene map' or 'functional map' (Schuler et al. 1996). To achieve this, each EST has to be converted into an effective marker assay.

This could be in the form of RFLP, STS, CAPS (cleaved amplified polymorphic sequences), SSR or SNP. For instance, a given EST could be amplified from genomic DNA and the PCR product obtained used as an RFLP probe in a Southern hybridization (Smilde et al. 2001); or it could be tested directly for length or sequence polymorphism between the parents of a mapping population (Gilpin et al. 1997). Sequence variation between homologous PCR products can be detected directly by sequencing, indirectly by digestion with restriction enzymes (CAPS), or by heteroduplex analysis. Many ESTs contain microsatellites, which can be targeted by conventional SSR technology (Kantety et al. 2002; Varshney et al. 2002, 2004c, 2005a). Software search programmes have been developed to identify such situations, for example MISA (Thiel et al. 2003; available at http://pgrc.ipk-gatersleben.de/misa). The frequency of SSRs in wheat ESTs has been variously reported to be as high as 1 in 1.33 kb (Morgante et al. 2002) to as low as 1 in 17.42 kb (Gao et al. 2003). Discrepancies in the estimates of frequency and distribution of SSRs across different studies are probably an artefact of varying identification criteria and data quantity (Varshney et al. 2005a). Some ESTs via SSR assay (EST-SSRs) have been placed in genetic maps (Gao et al. 2004; Nicot et al. 2004; Peng et al. 2004a; Yu et al. 2004b), but they have not been integrated, to any great extent, in wheat in the way that has been done in rice (Harushima et al. 1998) and maize (Davis et al. 1999). An important feature of EST-SSR markers is their applicability across species (Holton et al. 2002; Gupta et al. 2003; Yu et al. 2004a; Varshney et al. 2005b), which makes them valuable for comparative mapping.

2.3.3
Physical Maps

Physical maps are based on the actual separation between markers, in terms of base pairs (or linear length, measured cytologically on metaphase mitotic chromosomes). This is in contrast with genetic distances, which are based on recombintaional frequencies. At the chromosome level, a physical map can be generated by hybridizing a labelled DNA *in situ* to a cytological preparation. Sites of hybridization can then be directly visualized microscopically (Schwarzacher 2003; Jiang and Gill 1994). A comparison has been made between physical and genetic distances between adjacent markers in hexaploid wheat using in situ

hybridization (ISH) with 21 RFLP probes from linkage groups 5 and 6 (Zhang et al. 2000). Although the linear order and linkage relationships between DNA probes on these physical maps were generally conserved, a significant difference between the genetic and the physical distances was observed. However, this technique is laborious and not practicable on a genome-wide scale (Varshney et al. 2004a). An alternative strategy to physically mapping single and low-copy sequences is to generate and characterize chromosomal deletion stocks (Endo and Gill 1996). Chromosomal segments defined by these deletions have been labeled 'bins', and a large number of molecular markers including functional markers have been assigned to these bins (Table 2). In the USA a National Science Foundation-funded consortium has assigned 16,099 EST loci to 159 bins (http://wheat.pw.usda.gov/NSF/progress_mapping.html, Qi et al. 2003, 2004). This 'transcriptome map' has an average of 766 loci per chromosome and an expected average of 95 loci per chromosome bin or 1 EST locus per 1 Mb of wheat DNA (Gill et al. 2003; Qi et al. 2004).

Comparing across wheat homoeologues, synteny appeared to decrease with the distance of a chromosome region from the centromere and with an increase in recombination rates along the average chromosome arm (Akhunov et al. 2003a). Furthermore, 31 paralogous sets of loci were observed with perturbed synteny. In a separate study, the physical mapping data were also used to assess organizational and evolutionary aspects of the wheat genome. It was found that recombination has played a central role in the evolution of wheat genome structure. The gradients of recombination rates along chromosome arms promoted more rapid rates of genome evolution in distal, high-recombination regions (hot spots of recombination) than in the low recombination proximal regions (Akhunov et al. 2003b; Dvořák et al. 2003).

In another project in France, a total of 725 microsatellite loci were assigned to 94 breakpoints in a homozygous (88 terminal deletions, 6 interstitial) and 5 in a heterozygous state representing 159 deletion bins with an average of 4.97 SSR/bin (Sourdille et al. 2004). Assignment of ESTs and genetically mapped SSRs to deletion bins in the above studies will be useful not only for verification of deletion stocks but also for allocating associated QTLs to deletion bins.

Physical mapping of wheat genomes using deletion lines suggests a non-random distribution of cDNA markers and ESTs (Gill et al. 1996a,b; Faris et al. 2000; Qi et al. 2003, 2004). The lower number or complete absence of cDNA markers in the centromeric region parallels the absence of recombination in these regions and suggests the presence of 85% of wheat genes in less than 10% of the genome. The small gene-rich regions are thought to be interspersed by large blocks of repetitive DNA (Gill et al. 1996a,b; Sandhu and Gill 2002a; Sandhu et al. 2003; Sidhu et al. 2003). It is believed that about three to four major and four to five minor gene-rich regions are present in each wheat chromosome (Sandhu and Gill 2002b). The gene-poor regions, in contrast, mainly contain retrotransposon-like repetitive sequences (Feuillet and Keller 1999; Schulman et al. 2004). Interestingly, physical location, structural organization and gene densities of the gene-rich regions are similar across the three genomes of hexaploid wheat (Gill et al. 1996a; for a review see Gill 2004). The resolution of this physical localization was, however, low due to a limited number of deletion lines and should improve in future with the availability of more deletion lines.

The availability of genome-wide BAC-contigs has been a prerequisite for sequencing the model genomes of *Arabidopsis* and rice (TAGI 2000, Sasaki and Burr 2000). Similar efforts are currently under way to prepare contig maps of the genomes of sorghum (Klein et al. 2000) and maize (Gardiner et al. 2004; http://www.maizemap.org/iMapDB/iMap.html). As a resource for contig construction, several large insert DNA libraries have been constructed for wheat (Stein and Graner 2004). However, the large size of the wheat genome presents serious problems for the development of a full genome contig map. Nevertheless, efforts are under way to prepare a contig map of the D genome of wheat to produce a detailed picture of gene distribution in the wheat D genome and enhance our understanding of the evolution of large genomes (http://wheat.pw.usda.gov/PhysicalMapping/). A total of 215,645 genomic fragments, cloned in BAC and BiBAC vectors, of an *Ae. tauschii* line (the D-genome progenitor of wheat) have been fingerprinted (Luo et al. 2003). As a result, 10,035 contigs were obtained at a Sulston score of 1×10^{-30} and a tolerance of 0.4 bp, corresponding to about 3,200 Mb (http://wheatdb.ucdavis.edu:8080/wheatdb/). Recent developments on construction of chromosome specific BAC library would facilitate preparation of individual physical maps of wheat in the near future (Safar et al. 2004).

As an alternative to the resource-intense development of contig maps, subgenomic physical maps of wheat can also be developed using radiation hybrid

Table 2. Some physical maps of wheat prepared after using the deletion lines

Genome	Marker loci mapped	Cytogenetic stocks used	Reference
Wheat (homoeologous group 1)	19 RFLP	18 DLs[a]	Kota et al. (1993)
Wheat (homoeologous group 1)	50 RFLPs	56 DLs	Gill et al. (1996a)
Wheat (homoeologous group 1)	2,212 loci (944 ESTs)	101 DLs	Peng et al. (2003, 2004a)
Wheat (homoeologous group 2)	30 RFLPs	21 DLs	Delaney et al. (1995a)
Wheat (homoeologous group 2)	43 SSRs	25 DLs	Röder et al. (1998a)
Wheat (homoeologous group 2)	2,600 loci (1,110 ESTs)	101 DLs	Conley et al. (2004)
Wheat (homoeologous group 3)	29 RFLPs	25 DLs	Delaney et al. (1995b)
Wheat (homoeologous group 3)	2,266 loci (996 ESTs)	101 DLs	Munkvold et al. (2004)
Wheat (homoeologous group 4)	40 RFLPs	39 DLs	Mickelson-Young et al. (1995)
Wheat (homoeologous group 4)	1,918 loci (938 ESTs)	101 DLs	Miftahudin et al. (2004)
Wheat (homoeologous group 5)	155 RFLPs	65 DLs	Gill et al. (1996b)
Wheat (homoeologous group 5)	245 RFLPs, 3 SSRs	36 DLs	Faris et al. (2000)
Wheat (homoeologous group 5)	2,338 loci (1,052 ESTs)	102 DLs	Linkiewicz et al. (2003, 2004)
Wheat (homoeologous group 5S)	100 RFLPs	17 DLs	Qi and Gill (2001)
Wheat (chromosome 5A)	22 RFLPs	19 DLs	Ogihara et al. (1994)
Wheat (homoeologous group 6)	24 RFLPs	26 DLs	Gill et al. (1993)
Wheat (homoeologous group 6)	210 RFLPs	45 DLs	Weng et al. (2000)
Wheat (homoeologous group 6)	5,154 loci (7,965 ESTs)	101 DLs	Randhawa et al. (2004)
Wheat (homoeologous group 6S)	82 RFLPs	14 DLs	Weng and Lazar (2002a)
Wheat (homoeologous group 7)	16 RFLPs	41 DLs	Werner et al. (1992)
Wheat (homoeologous group 7)	91 RFLPs, 6 RAPDs	54 DLs	Hohmann et al. (1995)
Wheat (homoeologous group 7)	2,148 loci (919 ESTs)	101 DLs	Hossain et al. (2004a)
Wheat (chromosomes 6B, 2D and 7D)	16 SSRs	13 DLs	Varshney et al. (2001)
Wheat (chromosome 1D)	32 SSRs	11 DLs	Huang and Röder (2003)
Wheat (chromosome arm 1BS)	24 AFLPs	8 DLs	Zhang et al. (2000)
Wheat (chromosome arm 4DL)	61 AFLPs, 2 SSRs, 2 RFLPs	8 DLs	Milla and Gustafson (2001)
Wheat (chromosome arm 1BS)	22 expressed sequences	DLs	Sandhu et al. (2002)
Wheat (chromosome arm 6BL)	32 AFLPs	–	Dieguez et al. (2003)
Wheat (whole genome)	121 expressed candidate resistance genes	339 DLs	Dilbirligi and Gill (2003)
Wheat (whole genome)	94 loci for genes involved in N-uptake, bread making quality or disease resistance	97 DLs	Benard et al. (2003)
Wheat (whole genome)	59 loci for 14 candidate ESTs for FHB	91 DLs	Han et al. (2003)
Wheat (whole genome)	16,099 loci (7,104 ESTs)	101 DLs	Gill et al. (2003) Qi et al. (2003, 2004)
Wheat (whole genome)	725 SSRs	159 DLs	Sourdille et al. (2004)

[a] DLs = deletion lines

(RH) populations (Cox et al. 1990) or by the so-called HAPPY (**hap**loid genome; **p**olymerase chain reaction) mapping procedure (Dear and Cook 1989). Neither method relies on the availability of BAC-contigs or cloned DNA fragments and may be suitable for the high-throughput mapping of PCR-based markers independent of the presence of polymorphism (Waugh et al. 2002; Thangavelu et al. 2003; Wardrop et al. 2002). RH mapping of one *scs*[ae] (species cytoplasm specific) gene in durum wheat is already in progress (http://cropandsoil.oregonstate.edu/cgb/projects.html). RH mapping permitted the

localization of the *scs^ae^* gene on the long arm of chromosome 1D along with eight linked markers (Kianian et al. 2003; Hossain et al. 2004b).

2.4
Application of Molecular Markers in Wheat Genetics and Breeding

In the last decade the generation of molecular markers and their mapping has offered new opportunities for plant breeding and has become a key component of what is now popularly termed *molecular breeding*. These resources allow the tracking of specific loci and alleles through the identification of markers linked to major genes, analysis of quantitative trait loci (QTLs), positional cloning of genes and characterization of genetic variation in germplasm. In addition, mapped markers can often be used in related species to analyse syntenic relationships.

2.4.1
Gene Tagging and QTL Analysis for MAS

The potential value of genetic markers, linkage groups and their association with agronomic traits has been known for more than 80 years. The usefulness of marker-assisted selection (MAS) was recognized as early as 1923 when Sax demonstrated in beans an association between seed size and seed coat pigmentation. The first molecular-marker based (RFLP) map in plants was made in tomato and consisted of 57 loci (Bernatzky and Tanksley 1986). Since then, maps have been constructed for nearly all crop plants (summarized by Philips and Vasil 2001), allowing, in principle, the application of MAS in plant breeding, as originally proposed by Sax (1923) and Thoday (1961). The concept of selection based on genotype rather than phenotype created strong interest among plant breeders (Tanksley et al. 1989; Paterson et al. 1994). The rationale relies on the discovery of phenotype/genotype associations between genome regions (as assayed by molecular markers) and traits in segregating populations (such as F_2s, RILs, DHs, etc.). These are derived by analysis of segregation of simply inherited traits and by QTL analysis for complex traits (Lee 1995). The identification of markers sufficiently tightly linked to target genes/QTLs and their conversion, if necessary, to a PCR platform has made MAS feasible in some

plant breeding programmes (Langridge and Chalmers 2004). MAS can increase the efficiency and accuracy of selection, especially for traits that are difficult to phenotype or are recessive. The time-lag between the advent of DNA-marker technologies and their practical application for MAS has been, and remains, attributable to the high unit cost in the context of a relatively low value end product (Koebner et al. 2001).

In wheat, a significant number of major genes and QTLs for different traits have been tagged. Markers for more than 36 traits were already developed by 1999 (Gupta et al. 1999). Recent progress and significant achievements in the area of mapping disease resistance genes and the identification of QTLs and major genes for some agronomically important traits are summarized in Tables 3 and 4, respectively. A variety of molecular markers (RFLP, RAPD, AFLP, SSR) have been used for gene tagging and QTL analysis, but the consensus is that SSRs are best suited for this purpose (Gupta et al. 2002b). RFLP is not readily adapted to high sample throughput and RAPD assays are not sufficiently reproducible or transferable between laboratories. While both SSRs and AFLPs are efficient in identifying polymorphisms, SSRs are more readily automated (Shariflou et al. 2003). While RFLPs and AFLPs can in principle be converted into a simple PCR assay (STS), AFLP conversion is complicated by the observation that in large genome templates, individual bands are generally composed of multiple fragments (Shan et al. 1999; Carter et al. 2003). The inclusion of many microsatellite markers on genetic maps (Röder et al. 1998b; Gandon et al. 2002; Somers et al. 2004; Peng et al. 2004a) will ease their use for tagging for marker-assisted wheat breeding.

Status of MAS in Wheat Breeding

Prior to their use in plant breeding, the markers need to be validated, a process where functionality is tested in a range of genetic backgrounds (Langridge and Chalmers 1998; Gupta et al. 1999). For instance, marker validation studies were conduced for QTL for grain protein content by using NILs (Singh et al. 2001), for *Lr10* by using 16 wheat cultivars (Blazkova et al. 2002), for QTL for Fusarium head blight (FHB) resistance by using the progeny of crosses between the FHB-resistant spring wheat line and five European wheat varieties (Angerer et al. 2003; Liu and Anderson 2003a) or NILs from existing breeding populations (Pumphrey and Anderson 2003) and in germplam

Table 3. Some examples of gene tagging or QTL identification for resisiatnce to important diseases of wheat

Disease	Gene/QTLs	Chromosome	Marker type	Reference
I. Fungal resistances				
Black (stem) rust/	*Stb1*	5BL	AFLP, RAPD	Adhikari et al. (2004b)
Septoria trici bloch (STB)	*Stb2*	3BS	SSR	Adhikari et al. (2004c)
	Stb3	6DS	SSR	Adhikari et al. (2004c)
	Stb4	7DS	AFLP, SSR	Adhikari et al. (2004a)
	Stb5	7DS	SSR	Arraiano et al. (2001)
	Stb6	3AS	SSR	Brading et al. (2002)
	Stb7	4AL	SSR	McCartney et al. (2003)
	Stb8	7BL	SSR	Adhikari et al. (2003)
	QStb.risø–2B	2BL	SSR	Eriksen et al. (2003a)
	QStb.risø–3A.1,	3AS	SSR	Eriksen et al. (2003a)
	QStb.risø–3A.2			
	QStb.risø–3B	3BL	AFLP	Eriksen et al. (2003a)
	QStb.risø–6B.1,	6B	AFLP	Eriksen et al. (2003a)
	QStb.risø–6B.2			
	QStb.risø–7B	7B	AFLP	Eriksen et al. (2003a)
	QStb	1DS	RFLP/SSR	Börner et al. (2003)
	QStb	6BS	RFLP/SSR	Börner et al. (2003)
	QStb	7BL	RFLP/SSR	Börner et al. (2003)
Powdery mildew	*Pm1*	7AL	RFLP	Ma et al. (1994)
				Hartl et al. (1995)
		7AL	STS	Hu et al. (1997)
	Pm1c	7AL	AFLP	Hartl et al. (1999)
	Pm1e	7AL	SSR	Singrün et al. (2003)
	(formerly *Pm22*)			
	Pm2	5DS	RFLP	Ma et al. (1994),
				Hartl et al. (1995)
	Pm3a, b, c	1AS	RFLP	Hartl et al. (1993)
	Pm3b	1AS	RFLP	Ma et al. (1994)
	Pm3g (Mlar)	1AS	Gliadin	Sourdille et al. (1999)
	Pm3	1AS	SSR	Bougot et al. (2002)
	Pm4a	2AL	RFLP	Ma et al. (1994)
		2AL	AFLP	Hartl et al. (1999)
		2AL	STS	Ma et al. (2003)
	Pm5e	7BL	SSR	Huang et al. (2003c)
	Pm6	2BL	RFLP	Tao et al. (2000)
	Pm8/Pm17	1BL/1RS;	STS	Mohler et al. (2001)
	(allelic)	1AL/1RS		
	Pm13	3DS	STS	Cenci et al. (1999)
	Pm18	7A	RFLP	Hartl et al. (1995)
	Pm21	6AL/6VS	RAPD	Qi et al. (1996)
		6AL/6VS	SCAR	Liu et al. (1999a)
	Pm24	1DS	SSR, AFLP	Huang et al. (2000)
	Pm25	1A	RAPD	Shi et al. (1998)
	Pm26	2BS	RFLP	Rong et al. (2000)
	Pm27	6B-6G	SSR	Järve et al. (2000)
	Pm29	7DL	RFLP	Zeller et al. (2002)
	Pm30	5BS	SSR	Liu et al. (2002b)
	Qpm.vt–1B	1B	SSR, RFLP	Liu et al. (2001a)
	Qpm.vt–2A	2A	SSR	Liu et al. (2001a)

Table 3. (continued)

Disease	Gene/QTLs	Chromosome	Marker type	Reference
	Qpm.vt–2B	2B	RFLP, SSR	Liu et al. (2001a)
	QTL	5A	RFLP	Keller et al. (1999b)
	QTL	7B	RFLP	Keller et al. (1999b)
Yellow (stripe) rust	*Yr5*	2BL	RGAP/CAPS	Yan et al. (2003a), Chen et al. (2003)
	Yr7	2BL	AFLP	Bariana et al. (2001)
	Yr9	1BL/1RS	RGAP	Shi et al. (2001)
		1BL/1RS	SCAR	Mago et al. (2002)
	Yr10/ Yr10vav	1BS	SSR	Wang et al. (2002), Bariana et al. (2002)
	Yr10	1BS	SCAR	Shao et al. (2001)
	Yr15	1BS	RFLP	Sun et al. (1997, 2002)
		1BS	SSR	Chagué et al. (1999), Peng et al. (2000a)
	Yr17	2AS	SCAR	Robert et al. (1999)
		2AS	STS	Seah et al. (2001)
		2AS	CAPS	Helguera et al. (2003)
	Yr18	7DS	RFLP	Singh et al. (2000)
		7DS	SSR, AFLP	Bariana et al. (2001)
		7DS	SSR	Suenaga et al. (2003)
	Yr26	1BS	SSR	Ma et al. (2001)
	Yr28	4DS	RFLP	Singh et al. (2000)
	Yr29	1BL	RFLP, AFLP	Bariana et al. (2001)
		1BL	AFLP	William et al. (2003c)
	Yr30	3BS	SSR	Suenaga et al. (2003)
	Yr32	2AL	AFLP, SSR	Eriksen et al. (2003b)
	YrKat	2DS	SSR	Bariana et al. (2001)
	Yrns–B1	3BS	SSR	Börner et al. (2000)
	YrH52	1BS	SSR	Peng et al. (2000a)
	YrMoro	Group 1	STS	Smith et al. (2002)
	YrQz	2B	AFLP, SSR	Deng et al. (2004)
	QTL	3BS	RFLP	Singh et al. (2000)
	QTL	3DS	RFLP	Singh et al. (2000)
	QTL	5DS	RFLP	Singh et al. (2000)
	QYR1	2BL	SSR	Boukhatem et al. (2002)
	QYR2	2AL	SSR	Boukhatem et al. (2002)
	QYR3	2BS	RFLP	Boukhatem et al. (2002)
	QYR4	7DS	RFLP	Boukhatem et al. (2002)
Brown (leaf) rust	*Lr1*	5DL	RFLP, SSR	Ling et al. (2003)
	Lr3	6BL	AFLP	Dieguez et al. (2003)
	Lr9	6B	RFLP	Autrique et al. (1995)
	Lr10	1AS	RFLP	Nelson et al. (1997)
		1AS	STS	Schachermayr et al. (1997)
	Lr19	7DL	RFLP	Autrique et al. (1995)
		7DL	STS	Prins et al. (2001)
		7DL	SCAR	Cherukuri et al. (2003)
	Lr21/Lr40	1DS	STS	Huang and Gill (2001)
	Lr23	2BS	RFLP	Nelson et al. (1997)
	Lr24	3DL	RFLP	Autrique et al. (1995)
	Lr25	4A/2R	SCAR	Procunier et al. (1995)

Table 3. (continued)

Disease	Gene/QTLs	Chromosome	Marker type	Reference
	Lr26	1BL/1RS	SCAR	Mago et al. (2002)
	Lr27	3BS	RFLP	Nelson et al. (1997)
	Lr28	4AL	STS	Naik et al. (1998)
		4AL	SSR	Vikal et al. (2004)
	Lr29	7DS	SCAR	Procunier et al. (1995)
	Lr31	4BL	RFLP	Nelson et al. (1997)
	Lr32	3DS	RFLP	Autrique et al. (1995)
	Lr34	7DS	RFLP	Nelson et al. (1997)
		7DS	SSR	Suenaga et al. (2003)
		7DS	SSR	Schnurbusch et al. (2003b)
	Lr35	2B	STS	Seyfarth et al. (1999)
		2B	SCAR	Gold et al. (1999)
	Lr37	2AS	SCAR	Robert et al. (1999)
		2AS	STS	Seah et al. (2001)
		2AS	CAPS	Helguera et al. (2003)
	Lr39	2DS	SSR	Raupp et al. (2001)
	Lr41	2D	SSR	Singh et al. (2004b)
	Lr46	1BL	SSR	Suenaga et al. (2003)
		1BL	AFLP	William et al. (2003c)
	Lr47	7AS	STS, CAPS	Helguera et al. (2000)
	Lr50	2BL	SSR	Brown–Guedira et al. (2003)
	Lr-undesignated	BSA[a]	AFLP	Craven et al. (2003)
	QTLs	7BL	RAPD	Nelson et al. (1997)
Durable broad spectrum stem rust	*Sr2*	3BS	SSR	Spielmeyer et al. (2003)
	Sr2	3BS	ESTs	Spielmeyer and Lagudah (2003)
Fusarium head blight/ Scab	QTL	1B	Glutenin	Buerstmayr et al. (2002)
	QTL	1B	SSR	Shen et al. (2003a)
	QFhs.ndsu–2A	2AL	RFLP	Waldron et al. (1999)
	QFhs.inra–2A	2A	SSR	Gervais et al. (2003)
	QTL	2BL	SSR	Zhou et al. (2002)
	QFhs.inra–2B	2B	SSR	Gervais et al. (2003)
	QTL	2DS	SSR	Shen et al. (2003b)
	QTL	2DL	SSR	Somers et al. (2003a)
	QTL	3AL	RFLP	Anderson et al. (2001)
	QTL	3AS	SSR	Bourdoncle and Ohm (2003), Shen et al. (2003a)
	QFhs.ndsu–3AS	3AS	SSR	Otto et al. (2002)
	QFhs.inra–3A	3A	RFLP	Gervais et al. (2003)
	QTL	3A	SSR	Steiner et al. (2003)
	QFhs.ndsu–3B	3BS	RFLP	Waldron et al. (1999), Liu and Anderson (2003b)
	QTLs	3BS	SSR	Anderson et al. (2001), Liu and Anderson (2003b), Buerstmayr et al. (2002, 2003), Zhou et al. (2002), Bourdoncle and Ohm (2003), Shen et al. (2003b), Somers et al. (2003a)
	QTL	3BS	STS	Guo et al. (2003)
	QTL	3BL	SSR	Bourdoncle and Ohm (2003)

Table 3. (continued)

Disease	Gene/QTLs	Chromosome	Marker type	Reference
	QFhs.inra–3B	3B	SSR	Gervais et al. (2003)
	QTL	4BS	RFLP	Anderson et al. (2001)
	QTL	4BS	SSR	Somers et al. (2003a)
	QFhs.ifa–5A	5A	SSR	Buerstmayr et al. (2002, 2003)
	QFhs.inra–5A.1, *QFhs.inra–5A.2*	5A	SSR	Gervais et al. (2003)
	QFhs.inra–5A.3	5A	Awns	Gervais et al. (2003)
	QTL	5A	SSR	Ma et al. (2003)
	QTL	5AS	SSR	Somers et al. (2003a)
	QTL	5BL	SSR	Bourdoncle and Ohm (2003)
	QFhs.inra–5D	5D		Gervais et al. (2003)
	QTL	6AS	RFLP	Anderson et al. (2001)
	QTL	6BS	RFLP	Waldron et al. (1999), Anderson et al. (2001)
	QTL	6BS	SSR	Anderson et al. (2001), Shen et al. (2003b)
	QFhs.inra–6D	6D		Gervais et al. (2003)
	QTLs (2)	3B	SSR	del Blanco et al. (2003)
	QTLS	11	AFLP	Bai et al. (1999)
	QTLs(3)	BSA	RAPD	Sun et al. (2003)
	QTLs(3)	3	AFLP	Schmolke et al. (2003)
Eyespot	*Pch1*	7D	SSR	Groenewald et al. (2003)
	Pch2	7A	RFLP	de la Pena et al. (1996, 1997)
Karnal bunt	Unspecified	4B	SSR, AFLP	Singh et al. (1999, 2003)
Loose smut	Major gene		STS from AFLP	Knox et al. (2002)
Bunt	*Bt10*	BSA	SCAR (RAPD)	Laroche et al. (2000)
Septoria nodorum	*snbTM*	BSA	SCAR (RAPD)	Cao et al. (2001)
Leaf or glume blotch	*QSng.sfr-3BS*	3B	SSR	Schnurbusch et al. (2003a)
(Stagonospora nodorum)	*QSng.sfr-34BL*	4B	SSR	Schnurbusch et al. (2003a)
	QTLs (2)	5A	SSR	Toubia-Rahme et al. (2003)
	QTLs (1)	3B	SSR	Toubia-Rahme et al. (2003)
Pyrenophora tritici	*Pti2*	1A/4A	RFLP	Faris et al. (1997)
repentis	*Pti2*	1AS	RFLP	Effertz et al. (2002)
II. Viral resistances				
Barley yellow dwarf virus	*Bdv2*		STS (RAPD)	Stoutjesdijk et al. (2001)
	BYDV	7DL	SSR	Ayala et al. (2001)
Wheat streak mosaic *virus*	*Wsm1*	Group 4	STS (RAPD)	Talbert et al. (1996)
Wheat spindle streak *mosaic virus*	*WSSMV*	2D	RFLP	Khanet al. (2000a)
		2DL	SSR	Wang et al. (2003)
III. Nematode resistances				
Cereal cyst nematode	*Cre1*	–	STS	Ogbonnaya et al. (2001)
	Cre3	–	STS	Ogbonnaya et al. (2001)
	Cre6	–	STS	Ogbonnaya et al. (2001)
Root lesion nematode	*Rlnn1*	7A	RFLP	Williams et al. (2002)
Root knot nematode	*Rkn-mn1*	TLs[b]	RAPD	Barloy et al. (2000)
		TLs	SCAR (RAPD)	Yu et al. (2003)

Table 3. (continued)

Disease	Gene/QTLs	Chromosome	Marker type	Reference
IV. Insect resistances				
Russian wheat aphid	*Dn1*	7D	SSR	Liu et al. (2001b)
		7DS	RGA	Swanepoel et al. (2003)
	Dn2	NILs	SCAR (RAPD)	Myburg et al. (1998)
		7D	STS (RFLP)	Ma et al. (1998)
		7D	SSR	Liu et al. (2001b),
				Miller et al. (2001)
	Dn4	1D	RFLP	Ma et al. (1998)
		1D	SSR,	Liu et al. (2002a),
				Arzani et al. (2003)
	Dn5	7D	SSR	Liu et al. (2001b)
	Dn6	7D	SSR	Liu et al. (2002a)
	Dn8	7D	SSR	Liu et al. (2001b)
	Dn9	7D	SSR	Liu et al. (2001b)
	Dnx	7D	SSR	Liu et al. (2001b)
	Unspecified	NILs	SCAR (RAPD)	Venter and Botha (2000)
Hessian fly	11 loci	1A, 5A	RAPD	Dweikat et al. (1997)
	H31	5BS	AFLP/STS	Williams et al. (2003)
Wheat curl mite	*Cmc3*	T1AL.1RS	SSR, RFLP	Malik et al. (2003)
	Cmc4	6D	SSR, RFLP	Malik et al. (2003)
Greenbug	*Gb3*	7DL	SSR, AFLP	Weng and Lazar (2002a)
Sawfly cutting	*Sc*	3B	SSR	Houshmand et al. (2003)

[a] BSA = bulked segregant analysis
[b] TLs = translocation lines

collections (Zhou et al. 2003). Similarly, markers associated with preharvest sprouting (Kato et al. 2001; Mares and Mrva 2001), plant height (Ellis et al. 2002), and barley yellow dwarf virus (Ayala et al. 2001) were validated and used for enriching favourable allele frequency in early generation segregating populations and tracking donor parent alleles during backcrossing (Cakir et al. 2003). Microsatellite markers were linked to two major QTLs for FHB and were subsequently used in a marker-assisted backcross scheme to transfer these QTLs from bread wheat to durum wheat (Gladysz et al. 2003). Similarly, STS markers were used in the marker-assisted introgression of *Pm13* into 18 bread wheat cultivars, where BC_5 lines had already been developed (Reffo et al. 2003). Two effective leaf rust resistance genes *Lr29+ Lr24* were also successfully transferred into registered wheat cultivars with the assistance of molecular markers (Kraic et al. 2003). Molecular markers have also facilitated the pyramiding of multiple disease resistance genes in wheat as demonstrated by Liu et al. (2000), who inte-

grated three powdery mildew resistance gene combinations (*Pm2+Pm4a*, *Pm2+Pm21*, *Pm4a+Pm21*) into an elite wheat cultivar 'Yang158'.

The use of MAS in wheat has a history of about 20 years and also involves the exploitation of non-DNA-based assays. For example, the correlation between bread-making quality and allelic status at the *Glu-1* (endosperm storage protein subunit glutenin) loci (Payne et al. 1983, 1987; Rogers et al. 1989) has been widely used in breeding programs. Some more recent examples of the utilization of MAS for glutenin alleles include Ahmad (2000), de Bustos et al. (2001), Radovanovic and Cloutier (2003), among others. More recently, a particular effort to use MAS in wheat breeding has been initiated in Australia. Over 1,000 marker assays covering five loci were performed at the University of Adelaide in the fiscal year 1999–2000 (Eagles et al. 2001), rising to >6,000 assays for 10 loci in 2002 and to ∼20,000 assays in 2003 (Kuchel et al. 2003) and around 50,000 assays in 2004 (SP Jefferies, Australia, pers. commun.). Loci

Table 4. A list of some grain quality traits of wheat for which genes or QTLs have been identified with molecular markers

Trait	Chromo-some	Molecular marker	Number of QTLs/gene identified	Per cent phenotypic variation explained	Reference
Awn length	4A	SSR	*Hd*	8.5	Sourdille et al. (2002)
	6B	SSR	*B2*	45.9	Sourdille et al. (2002)
Coleoptile length	4B	RFLP		27–45	Rebetzke et al. (2001)
Culm thickness	2A	RFLP	1		Keller et al. (1999a)
	2B	RFLP	1	13.2	Keller et al. (1999a)
	3A	RFLP	1	21	Keller et al. (1999a)
	3B	RFLP	1	11.3	Keller et al. (1999a)
	4A	RFLP	1	16	Keller et al. (1999a)
	4B	RFLP	1	12.9	Keller et al. (1999a)
	5A	RFLP	1	37.6	Keller et al. (1999a)
	5B	RFLP	1	11.1	Keller et al. (1999a)
Dormancy	2AL	RFLP	1	–	Mares et al. (2002)
	2DL	RFLP	1	–	Mares et al. (2002)
	4AL	RFLP	1	–	Mares et al. (2002)
Grain length	3B	RFLP	1	21.9	Campbell et al. (1999)
Ear compactness	2B	RFLP	*Ppd2* region	9–22	Sourdille et al. (2000a)
Floral fertility	1B	SSR	1	10	Rousset et al. (2003)
Flour colour	3A	RFLP	1	13	Parker et al. (1998)
	7A	RFLP/AFLP	1	60	Parker et al. (1998)
	7A	STS/AFLP	1	60	Parker and Langridge (2000)
Flowering time	1Am	RFLP	*Eps-Am1*	47	Bullrich et al. (2002)
	2A	SSR	1	11.5	Huang XQ et al. (2003a)
	2A	RFLP	1	14.1–16.6	Ahmed et al. (2000)
	2B	RFLP	*Esp-2BS*	13.5–13.7	Ahmed et al. (2000)
	2D	SSR	1	15	Huang XQ et al. (2003a)
	2D	RFLP	*Ppd-D1*	29–31	Li et al. (2002a)
	6A	SSR	2	13.7–16.9	Huang et al. (2003b)
	7A	RFLP	*Esp-7A*	14.5–20.9	Ahmed et al. (2000)
	2A	SSR	*Ppd-A1*	10–11	Li et al. (2002a)
Grain protein content	2A	SSR	1	20.8	Prasad et al. (2003)
	2A	SSR	1	13.4–19.6	Prasad et al. (2003)
	2D	SSR	1	18.7	Prasad et al. (1999, 2003)
	3D	SSR	1	13.9–16.2	Prasad et al. (2003)
	4A	SSR	1	8.2–13.6	Prasad et al. (2003)
	6B	RFLP	1	72	Mesfin et al. (1999), Chee et al. (2001), Distelfeld et al. (2004)
	6B	STS/SSRs	–	up to 16.4	Khan et al. (2000b), Prasad et al. (2003)
	BSA	ISSR, RAPD	9	13.4–13.5	Dholakia et al. (2001)
	5A	SSR	1	6.2	Singh et al. (2001)
	7A	SSR	1	32.4	Prasad et al. (2003)
	7D	SSR	1	15.9	Prasad et al. (2003)
Grains/spike	3A	RFLP	2	12.3–18.3	Shah et al. (1999)
	4A	RFLP	1	12–27	Araki et al. (1999)
	5A	RFLP	3	10–42	Kato et al. (2000)

Table 4. (continued)

Trait	Chromo-some	Molecular marker	Number of QTLs/gene identified	Per cent phenotypic variation explained	Reference
Grain weight	1A	RFLP	1	11.8	Campbell et al. (1999)
	1A	SSR	1	15.1	Varshney et al. (2000b)
	1B	RFLP	1	11.1	Campbell et al. (1999)
	2A	SSR	1	17.2	Huang et al. (2003b)
	2D	SSR	1	15.4	Huang et al. (2003b)
	3A	RFLP	1	12.2	Shah et al. (1999)
	3A/3B	RFLP	1	10.9	Campbell et al. (1999)
	3B	RFLP	1	12.2	Campbell et al. (1999)
	3B	AFLP	2	6	Elouafi and Nachit (2004)
	4B	AFLP	1	3	Elouafi and Nachit (2004)
	4D	SSR	1	14.3	Huang et al. (2003b)
	4D	SSR	1	6.8–13.1	Liu et al. (2003)
	5A	RFLP	1	11.0–19.0	Kato et al. (2000)
	5B	SSR	1	16	Huang et al. (2003b)
	6B	SSR	2	28	Elouafi and Nachit (2004)
	7A	SSR	1	14.5	Huang et al. (2003b)
	7B	SSR	2	20.6–25.9	Huang et al. (2003b)
	7D	SSR	1	17.3	Huang et al. (2003b)
Heading time	2BS	RFLP	Ppd-B1	23.4–44.4	Sourdille et al. (2000a)
	7BS	RFLP	*earliness per se*	7.3–15.3	Sourdille et al. (2000a)
Leaf angle	1A	RFLP	1	12.1	Keller et al. (1999a)
	3B	RFLP	1	11.1	Keller et al. (1999a)
	4A	RFLP	1	16.4	Keller et al. (1999a)
	5A	RFLP	1	11.2	Keller et al. (1999a)
	7D	RFLP	1	16.4	Keller et al. (1999a)
Leaf width	1B	RFLP	1	14	Keller et al. (1999a)
	3B	RFLP	1	19.7	Keller et al. (1999a)
	5A	RFLP	1	14.9	Keller et al. (1999a)
	5B	RFLP	1	11.2	Keller et al. (1999a)
Milling yield	3A, 7D	AFLP	2	19–22	Parker et al. (1999)
Number of spikeltes	2D	SSR	1	–	Rousset et al. (2003)
	5B	SSR	1	–	Rousset et al. (2003)
Pre-harvest sprouting tolerance (PHST)	2B	RFLP/SSR	2	4–16.2	Kulwal et al. (2004)
	2D	RFLP/SSR	1	14.9	Kulwal et al. (2004)
	3A	RFLP	1	5.6	Groos et al. (2002)
	3B	RFLP/SSR	2	24.9	Groos et al. (2002)
	3B	RFLP/SSR	5	3–20	Kulwal et al. (2004)
	3D	SSR	1	11.6	Groos et al. (2002)
	3D	RFLP	3	3.2–17.4	Kulwal et al. (2004)
	4A	rice sequence (*in GA20-oxidase-silico* analysis)			Li et al. (2004a)
	5A	RFLP	1	10.7	Groos et al. (2002)
	5B	SSR	1	–	Kulwal et al. (2004)
	5D	RFLP	1	–	Kulwal et al. (2004)
	6A	RFLP	1	–	Kulwal et al. (2004)
	6B	SSR	1	–	Roy et al. (1999)

Table 4. (continued)

Trait	Chromo-some	Molecular marker	Number of QTLs/gene identified	Per cent phenotypic variation explained	Reference
	7A	RFLP	1	5.6	Groos et al. (2002)
	7B	RFLP/SSR	1	–	Kulwal et al. (2004)
	7D	STS	1	–	Roy et al. (1999)
Plant height	1B	RFLP	1	15–30	Cadalen et al. (1998)
	1B	SSR	1	13.3	Keller et al. (1999a)
	2A	PCR	1	29.3	Keller et al. (1999a)
	2B	SSR	1	17.4	Huang et al. (2003b)
	2D	SSR	*Rht8*	∼ 100	Korzun et al. (1998)
	3A	Gene	*Eps*	42.4	Shah et al. (1999)
	3A	RFLP	1	10.4	Shah et al. (1999)
	4A	RFLP	2	20–29	Araki et al. (1999)
	4A	SSR	1	23	Keller et al. (1999a)
	4B	RFLP	2 (*Rht-B1*)	10–20	Cadalen et al. (1998)
	4B	SSR	*Rht-B1*	11.8	Huang et al. (2003b)
	4D	RFLP	*Rht-D1*	9–15	Cadalen et al. (1998)
	4D	SSR	*Rht-D1*	29.5	Huang et al. (2003b)
	5A	RFLP/SSR	*Rht-12*	–	Korzun et al. (1997b)
	5A	PCR	1	31	Keller et al. (1999a)
	5B	PCR	1	20	Keller et al. (1999a)
	6A	SSR	1	16.5	Huang et al. (2003b)
	6B	PCR	1	7	Keller et al. (1999a)
	7A	RFLP	1	10.3–11.7	Cadalen et al. (1998)
	7B	RFLP	1	7.7–16.5	Cadalen et al. (1998)
	7B	PCR	1	7	Keller et al. (1999a)
Spike length	1AL	RFLP	–	12	Sourdille et al. (2000a)
Spikes/plant	2D	*Gene-Ppd-D1*	1	16–22	Li et al. (2002a)
	4A	RFLP	1	46–52	Araki et al. (1999)
	5A	RFLP	1	26–39.1	Kato et al. (2000)
	7A	RFLP	1	16–22	Li W et al. (2002)
Test weight	6B	SSR	1	9	Elouafi and Nachit (2004)
	7A	SSR	1	17	Elouafi and Nachit (2004)
Tiller angle	2A	RFLP	1	12–14	Li et al. (2002a)
	3A	RFLP	1	14–19	Li et al. (2002a)
Tiller number	1D	RFLP	1	14–15	Li et al. (2002a)
	2D	RFLP	1	11–15	Li et al. (2002a)
	5A	RFLP	*Vrn1*	7–37	Kato et al. (2000)
	5A	RFLP	1	10–19	Kato et al. (2000)
	6A	RFLP	1	12–31	Li et al. (2002a)
Vernalization sensitivity	5B	RFLP	*Vrn1/Fr1*	–	Galiba et al. (1995)
	5B	SSR	*Vrn-B1*	–	Salina et al. (2003)
	5B	SSR/AFLP	*Vrn-B1*	–	Barrett et al. (2002)
	SB	dCAPs	*Vrn2 (=Vrn-B1)*	–	Iwaki et al. (2002)
	5B	SSR	*Vrn2 (=Vrn-B1)*	–	Iwaki et al. (2002)
	5B	AFLP	*Ppd-B1*	–	William et al. (2003b)

Table 4. (continued)

Trait	Chromo-some	Molecular marker	Number of QTLs/gene identified	Per cent phenotypic variation explained	Reference
	5D	SSR	*Vrn4* (=*Vrn-D1*)	–	Kato et al. (2003)
	7A	AFLP	*VrnA-2*	–	William et al. (2003b)
Yield	2D	SSR	1	11.5	Huang et al. (2003b)
	3B	SSR	2	9.6–21.6	Huang et al. (2003b)
	4A	RFLP	1	17–27	Araki et al. (1999)
	4D	SSR	2	10.1–12.3	Huang et al. (2003b)
	5A	Gene-*q*	1	23–27	Kato et al. (2000)
Others					
Alpha-amylase	1B	SSR	1	7.9–14.7	Zanetti et al. (2000)
	3B	RFLP	1	7–15.5	Zanetti et al. (2000)
	5A	RFLP	2	13.0–38.5	Zanetti et al. (2000)
	6A	RFLP	1	13.5–17.7	Zanetti et al. (2000)
	7B	RFLP	1	7.7–25.0	Zanetti et al. (2000)
Starch quality	4A	AS-PCR[a]	*Wx-B1*	–	McLauchlan et al. (2001)
	7A	AS-PCR	*Wx-A1*	–	McLauchlan et al. (2001)
	7D	AS-PCR	*Wx-D1*	–	McLauchlan et al. (2001)
	4A	GS-PCR[b]	GBSS[c]	–	Briney et al. (1998)
Polyphenol oxidase	2D	RFLP	1	23	Demeke et al. (2001)
	2A	RFLP	1	12–16	Demeke et al. (2001)
	3B	RFLP	1	11–14	Demeke et al. (2001)
	6B	RFLP	1	12–14	Demeke et al. (2001)
Anther culturability	5B	SSR	2	76.7	Zhang et al. (2003a)
Crossability (wheat-rye)	5B	RFLP	*Kr1*	65	Tixier et al. (1998)
Flag leaf senescence	2B	AFLP/SSR		10.2–11.4	Verma et al. (2004)
	2D	AFLP/SSR	1	21.7–32.9	Verma et al. (2004)
Glume colour	1D	SSR	*Rg2*	–	Arzani et al. (2003)
Species cytoplasm	1A	RFLP	*scs^{ti}*	–	Simons et al. (2003)
specific (scs)	1A	RH mapping	*scs^{ae}*	–	Kianian et al. (2003), Hossain et al. (2004)
Thermosensitive genic male sterlity (TGMS)	2B	AFLP/SSR	*wtms1*	–	Xing et al. (2003)
Photoperiod	–	ISSR	*ptms1*	–	Cao et al. (2003)
temperature sensitive genic malesterlity (PTSGMS)	3A	ISSR	*ptms2*	–	Cao et al. (2003)

[a] AS-PCR = allele-specific PCR

  GS-PCR = gene-specific PCR

  GBSS = granule bound starch synthetase

for which markers have been successfully tested within experimental populations in Australia include tolerance to high soil boron (*Bo1*), tolerance to late-maturity α-amylase (LMA) (7BL), barley yellow dwarf virus resistance (*Bdv2*) (7DL), cereal cyst nematode resistance *Cre1* (2BL), *Cre8* (6BL), waxy or granule-bound starch synthase (*Wx-B1*) (4A), high-molecular-weight glutenin subunits (*GluD1*)

(1DL), leaf rust resistances (*Lr46)* (1BL), (*Lr34)* (7DS), height or dwarfing genes (*Rht1)* (4BS), (*Rht2)* (4DS), (*Rht8)* (2DS), root lesion nematode resistance (*Rlnn1)* and yellow flour colour (7AL), stem rust resistances (*Sr2)* (3BS), (*Sr36)* (2B) and VPM (Ventricosa x Persicum x Marne), a source for eyespot resistance gene *Pch1*, obtained by introgrossion) segment (2AS). Additional loci for which markers are under investigation include aluminium toxicity tolerance (4B), *Glu-A3* (1AS), *Glu-B3* (1BS), *Lr1* (5DL), *Lr13* (2B), *Lr19* (7DL) and polyphenol oxidase activity (2D) (Pallotta et al. 2003). At CIMMYT (Mexico), marker implementation in wheat breeding involves the routine deployment of markers for the four genes *Cre1*, *Cre3*, BYDV resistance, *ph1b* mutant, and for the *Ae. ventricosa* segment carrying *Yr17*, *Lr37* and *Sr38* translocated 2AS. Approximately 7,000 marker assays are performed annually (William et al. 2003a).

With the availability of many more markers than in earlier years, the potential for uptake is now much greater than in the past. Reflecting this, a consortium of 12 wheat-breeding and research programmes across the US named 'MASwheat' (http://maswheat.ucdavis.edu/index.htm) has recently been launched, aiming to 'transfer new developments in wheat genomics and biotechnology to wheat production'. However, with a unit assay cost in the range of US$1–2 (Dreher et al. 2003; Koebner and Summers 2003), the widespread application of MAS must compete with alternative assay methods for the scarce funds available to most breeding programs. Although it was recently suggested that the bulk of MAS uptake remains restricted to low volume applications, such as genotype construction by backcrossing, and to the development of niche genotypes such as waxy wheats (Koebner 2004), this is clearly not the case for some breeding programmes such as the Australian programme described above. As the unit assay costs fall with the development of automated platforms and high-throughput marker systems, one can anticipate that MAS assays will become increasingly feasible for commercial wheat breeding.

2.4.2
Map-Based Cloning (MBC) of Genes in Wheat

In addition to their use for indirect selection of genes or QTLs of agronomic importance (including resistance to diseases), molecular markers offer the pos-

sibility of isolating genes of interest by positional cloning with an ultimate objective of producing transgenic plants for crop improvement. There are three major requirements for positional gene isolation: (i) a high-resolution, high-density genetic map spanning the gene or region of interest; (ii) availability of a large insert genomic YAC, BAC or PAC library for preparation of a physical map to isolate the candidate gene; and (iii) multiple independent mutant stocks, an efficient transformation system for use in functional complementation or an alternative technique for functional analysis of candidate genes. All these resources have become available in wheat (Lagudah et al. 2001; Stein and Graner 2004).

However, long-distance chromosome walking is not efficient in wheat because of the large amount of repetitive DNA and the physical size of the genome. To overcome this problem, several strategies have been developed for isolating genes from wheat.

Genome Collinearity
The gene order appears to be well conserved among various species of grass. This is referred to as synteny. Since the rice genome has been sequenced, it can be used as an intergenomic vehicle in cereals including wheat (Moore et al. 1995a; Keller and Feuillet 2000). This approach was used for the isolation of the vernalization response gene *Vrn1* from *Triticum monococcum* (Yan et al. 2003b). Complete marker/gene collinearity was observed for the putative orthologous regions on *T. monococcum* chromosome 5A^m and rice chromosome 3, and a BAC contig of the target region was constructed from a *T. monococcum* BAC library. It was collinear to two BACs representing the orthologous locus in rice. However, both physical maps showed a gap between the same two collinear genes. Interestingly, screening of a sorghum BAC library revealed a collinear BAC that bridged the gap in the other two species leading to a consensus physical map across three cereal species. The most promising candidate gene for *Vrn1* proved to be an orthologue in all three species. Similarly, using the genome collinearity approach, Sutton et al. (2003) have identified candidate meiotic genes at the *Ph2* locus of wheat. They identified the rice genomic region syntenous to the region deleted in wheat chromosome pairing mutant *ph2a*. With the help of markers known to reside within the region deleted in *ph2a* and data from wheat, barley and rice genetic maps, markers

delimiting the region deleted on wheat chromosome 3DS in the *ph2a* mutant were used to locate the syntenous region on rice chromosome 1S. A 6.58-Mb rice contig generated from 60 overlapping rice PAC clones spanning the syntenous rice region has enabled identification of 218 wheat ESTs putatively located in the region deleted in *ph2a*. The candidate gene approach may sometimes also fail, as suggested by the reports of variation in the content and order of orthologous genomic sequences from several cereal species (for a review see Bennetzen and Ramakrishna 2002; Feuillet and Keller 2002). In particular, the identification of candidate genes for race-specific disease resistance loci, which are less conserved between species and prone to genomic rearrangements (Leister et al. 1998), has proved problematic, and the earlier optimism regarding the use of the model genome strategy has diminished recently (Brueggeman et al. 2002; Bennetzen and Ma 2003).

Subgenome Chromosome Walking

In addition to the high proportion of repetitive DNA in wheat, polyploidy poses another level of complexity to positional cloning. As mentioned earlier, the three homoeologous subgenomes A, B and D are highly collinear and most of the functional loci occur as triplicate genes. Therefore, screening of a large insert library will yield two thirds of clones, which are not related to a target locus in a specific subgenome. In order to tackle this problem, large insert libraries were constructed from diploid and tetraploid wheat species (Stein and Graner 2004). Therefore an approach called 'subgenome chromosome walking', employing these libraries, has been used to isolate disease resistance genes. The first successful example of this approach involved map-based cloning of the *Lr10* leaf rust resistance locus (located on chromosome 1AS) of bread wheat. A three-step chromosome walk in a *T. monococcum* BAC library initiated from a closely linked RFLP marker allowed a BAC contig to be established, which contained the flanking markers and two candidate resistance genes (Stein et al. 2000; Wicker et al. 2001). Markers cosegregating with the gene were derived from the initial contig, and additional markers were developed from low-copy sequences obtained after low-pass shotgun sequencing of neighbouring BAC clones. All markers derived from the *T. monococcum* contig mapped to collinear segments of the *T. aestivum* genetic map. The *T. aestivum* orthologues of the two candidate genes were subsequently isolated.

One of the candidate genes, *Rga1*, proved to be *Lr10* as confirmed after sequence analysis of mutant alleles and complementation *via* transformation into a susceptible genotype (Feuillet et al. 2003).

Similarly, the powdery mildew resistance gene *Pm3b* was isolated from *T. aestivum* using a subgenomic BAC library. Since chromosome walking in *T. monococcum* was not successful due to a gap in the BAC library, a BAC library of the tetraploid relative *T. turgidum* ssp. *durum* (Cenci et al. 2003) was used, allowing construction of a contig covering *Pm3b*. Resistance conferred by transient expression was monitored in the epidermis of detached wheat leaves of a susceptible *T. aestivum* cultivar after biolistic bombardment with the homoeologue of the identified candidate gene and subsequent powdery mildew infection (Yahiaoui et al. 2003). The *Q* locus of *T. aestivum*, conferring free-threshing and square-headed spikes, was physically delimited by the same strategy (Faris et al. 2003) and should lead to the isolation of the *Q* locus.

Another gene conferring resistance to wheat leaf rust was isolated through the use of a *Aegilops tauschii* (D genome) subgenomic cosmid library (Huang et al. 2003a). *Lr21* was previously introgressed into *T. aestivum* via synthetic wheat derived from a cross between *T. turgidum* and the resistant *Ae. tauschii* accession TA1649. A closely linked RFLP probe was used to screen the cosmid library. A single cosmid clone harbouring the closely linked RFLP fragment could be isolated. The *Lr21* gene spans 4,318 bp and encodes a 1,080-amino-acid protein containing a conserved nucleotide-binding site (NBS) domain, 13 imperfect leucine-rich repeats (LRRs), and a unique 151-amino-acid sequence missing from known NBS-LRR proteins at the N terminus. The whole cosmid was used for complementation via stable transformation, and resistance was achieved.

However, unlike disease resistance, many agronomically important traits are controlled by QTLs (Table 3). In recent years, significant progress has been made in the isolation of QTLs such as those controlling fruit weight (*fw*2.2) in tomato (Alpert and Tanksley 1996) and photoperiod sensitivity (*Hd1*, *Hd3a*, *Hd6*) in rice (Yano et al. 2000; Takahashi et al. 2001; Kojima et al. 2002). Due to systematic development of resources in wheat, it is now becoming possible to clone QTLs for some important traits in wheat for crop-improvement programs.

2.4.3
Allelic Diversity

An understanding of germplasm diversity and genetic relationships among breeding materials is an invaluable aid for crop-improvement strategies. Conventional analyses of genetic diversity in germplasm accessions, breeding lines and populations have relied on pedigree information and morphological and agronomic performance data. The advent of biochemical and particularly DNA marker technology has improved the accuracy and number of lines that can be assessed in germplasm collections (Tanksley and McCouch 1997; Mohammadi and Prasanna 2003).

For practical reasons many of the early attempts to study diversity employed RAPDs (Vierling and Nguyen 1992; Joshi and Nguyen 1993). However, it soon became clear that the greater reproducibility of RFLPs was advantageous (for example, Siedler et al. 1994; Autrique et al. 1996). Now, SSRs and AFLPs have largely replaced these for genetic diversity studies (Table 4). In particular, some SSR loci can show such high levels of variability that even closely related genotypes can be distinguished from one another (Plaschke et al. 1995; Prasad et al. 2000; Stachel et al. 2000). On the other hand, AFLPs have the advantage of delivering a much higher multiplex ratio and are particularly useful for fingerprinting and the assessment of genetic diversity (Law et al. 1998; Bohn et al. 1999; Schwarz et al. 2000). As some differences in AFLP pattern have been found to be specific for particular plant organs (Donini et al. 1997), it is important to extract template DNA from physiologically uniform tissues. Recently developed genic microsatellites (or EST-SSRs) have been found to be superior to genomic SSRs due to improved quality of banding pattern (Eujayl et al. 2001; Leigh et al. 2003). Although the informativeness of genic SSRs is generally lower than for genomic SSRs, their origin from the conserved proportion of a genome have made them more suitable as a tool to assess genetic diversity across species (Gupta et al. 2003; Bandopadhyay et al. 2004; for a review see Varshney et al. 2005a). Recently, retrotransposon-based molecular markers have also been used for diversity studies in wheat by using the S-SAP (**s**equence-**s**pecific **a**mplification **p**olymorphism) assay (Queen et al. 2004). A summary of some genetic diversity studies involving with different marker systems in *Triticum* species is given in Table 5.

Molecular-marker evaluations have indicated that genetic diversity among varieties or inbred lines is higher than expected, although it is lower than that among landraces (Chen et al. 1994; Autrique et al. 1996; Zhang et al. 2002; Röder et al. 2003). For example, Röder et al. (2003) found 198 alleles across 19 SSR loci in 502 European varieties, 280 alleles in 450 European landraces and 323 alleles in 544 non-European landraces. Of the 339 alleles found in 994 landraces, 147 are present only in landraces but not in varieties, suggesting a genetic similarity of 57%.

Evidence of temporal flux in genetic diversity has been observed in wheat varieties released at different times within a country or region. Genetic diversity appears to be decreasing in Iranian wheats (Sayed-Tabatabaei and Shahnejat-Bushehri 2003), increasing in Italian durum wheats (Maccaferri et al. 2003), but has remained constant in the wheat varieties of Argentina (Manifseto et al. 2001), UK (Donini et al. 2000; Koebner et al. 2003) and the Yaqui Valley of Mexico (Souza et al. 1994). Interestingly, genetic diversity in Nordic spring wheat was enhanced by plant breeding in the first quarter of the 20th century and, following a decrease during the second quarter, increased again by plant breeding (Christiansen et al. 2002).

The use of molecular markers has also shown that diversity within a genome is largely shaped by recombination and selection and is not homogenous. In *Aegilops*, the polymorphism level of a locus has been correlated with recombination rate along the centromere to telomere axis (Dvorák et al. 1998b). Intraspecific nuclear genome variation appears lower in einkorn wheats and higher in *Ae. speltoides*, while this pattern is reversed for chloroplast DNA (Mizumoto et al. 2002). Comparison of landraces and improved varieties of Chinese wheat revealed a significant difference in the level of diversity within the D genome (Zhang et al. 2003b), indicating that high selection pressure has been applied to the D genome during the breeding process.

Genetic diversity studies involving germplasm from different countries or regions often allow separation of accessions into distinct groupings (Stachel et al. 2000; Bai et al. 2003; Pester et al. 2003). Among wild emmer wheats from Israel and Turkey, DNA polymorphisms have been associated with microclimatic stress (Fahima et al. 1999, 2002; Li et al. 1999, 2002). Associations between allelic constitutions at marker loci with agronomically important traits have been proposed in some diversity studies (Kobiljski et al. 2002; Roy et al. 2002; Bai et al. 2003). However, although suitable genotypes for hybridization were identified in this way (Roy et al. 2004), the overall ge-

Table 5. Details on some important allelic diversity studies using molecular markers in wheat

Markers	Material	Outcome	Reference
RFLP-based diversity			
58 probes	52 winter wheat, 9 spring wheat and 20 spelt (*T. spelta*) lines representing part of European breeding germplasm	A clear separation of wheat and spelt germplasm was possible. Novel spelt lines with various proportions of wheat germplasm were positioned between wheat and traditional spelt lines. The spring wheat lines formed a distinct group	Siedler et al. (1994)
39 probes	113 improved cultivars and landraces of diverse ecogeographical origin	Lower genetic distances were observed for the improved cultivars and some landraces from Morocco and Jordan while genetic distances were larger for the remaining landraces. Narrower genetic diversity in breeding lines suggested the need for the use of other sources of variation	Autrique et al. (1996)
48 probes	11 red and 11 white wheat lines from eastern USA soft wheat germplasm pool	Actual genetic similarity among unrelated lines in eastern USA soft wheat genepool appeared to be higher than that observed for unrelated landraces from southwest Asia. It also suggested that the ancestral landrace parents of this gene pool were themselves drawn from a base population where inbreeding was greater	Kim and Ward (1997)
20 probes	22 accessions representing 11 species of cultivated emmer and timopheevi wheat, 16 accessions of wild emmer wheat, 14 accessions of wild Timopheevi wheat and 1 accession of common wheat	Large genetic diversity in *T. Dicoccum*, the non-free threshing species, supported the archeological evidence that *T. dicoccum* was the earliest domesticated tetraploid wheat	Mori et al. (1997)
98 probes	124 accessions of all major Australian wheat varieties and lines	RFLP analysis can be used for the characterization and grouping of elite breeding material of wheat. Associations were derived for a range of stem rust, leaf rust and yellow rust resistance genes	Paull et al. (1998)
75 probes	Chinese accessions of *T. tauschii*, *T. aestivum* from Sichuan white (SW), Yunnan hulled (YH), Tibetan weedrace (TW) and Xinjiang rice (XR) wheat groups	Chinese landraces had a higher degree of genetic relatedness to the southwest Asian *T. tauschii*, particularly to accessions from Iran rather than to the Chinese *T. tauschii*. Chinese Spring was most related to Chendu-guang-tou, a cultivar from the southwest wheat group	Ward et al. (1998)
9 probes	202 wild wheat relative (*Ae. geniculata* Roth) genotypes belonging to 151 populations originating from different ecogeographical regions	Efficiency of RFLP markers in building core collection of *Ae. genicula* was demonstrated	Zaharieva et al. (2001)
11 probes	17 populations of wild emmer wheat sampled from southeastern Turkey	Narrow genetic variability was recorded among 17 populations studied	Tanyolac et al. (2003)

Table 5. (continued)

RAPD-based diversity

Markers	Material	Outcome	Reference
40 primer pairs	20 accessions of wild tetraploid durum wheat and 10 genotypes of cultivated tetraploid durum wheats selected from geographically diverse locations	A higher level of polymorphism among different accessions of wild emmer wheat from Israel, Turkey and Jordan than the group of cultivated American, Turkish and Syrian durum wheats	Joshi and Nguyen (1993)
26 primer pairs (182 loci)	7 accessions of Tibetan wheat, 22 cultivars of common wheat and 17 lines of spelt wheat	European spelt wheat and the Tibetan wheat showed much higher genetic diversity than Chinese common wheat which could be used to diversify the genetic basis for common wheat breeding	Sun et al. (1998)
31 primer pairs (136 loci)	29 accessions of two *Ae. tauschii* species	Divergence between the two subspecies of *Ae. tauschii* was greater than than within one subspecies from different geographical regions	Kong et al. (1998)
87 primer pairs (304 loci)		Information regarding the genetic diversity of the parental lines was not helpful for predicting F1 performance	Perenzin et al. (1998)
6 primer pairs (54 loci)	20 wheat lines	It was possible to differentiate wheat lines with different performances and the classification of parents from these markers is of predictive value for developing superior hybrids	Liu et al. (1999b)
20 primer pairs (97 loci)	118 registered individuals of wild emmer wheat	DNA polymorphisms appeared to be associated with microclimatic stress. Microclimatic selection appears to play an important role in DNA differentitation	Li et al. (1999)
10 primer pairs (59 loci)	110 genotypes of wild emmer wheat from 11 populations sampled in Israel and Turkey	Natural selection causes adaptive RAPD ecogeographical differentiation. RAPD markers are useful for estimation of genetic diversity in wild wheats and the identification of suitable parents for the development of mapping populations for the tagging of agronomically important tarits derived from wild wheat	Fahima et al. (1999)
10 primer pairs (48 loci)	15 accessions of 5 groups of hexaploid wheat: common, spelta, macha, vavilovii, and semi-wild wheat (SWW)	Common wheat is most closley related to SWW followed by spelta, vavilovii, and macha	Cao et al. (2000)
4 primer pairs (17 loci)	11 Italian local varieties of emmer wheat	High variability was found within landrace populations underlying the values of landraces as an irreplaceable bank of genetically diversified and highly co-adapted genotypes	Barcaccia et al. (2002)
17 primer pairs	35 spring wheat cultivars and lines with different levels of Fusarium resistance	A collection of unrelated genotypes can be used to identify markers linked to an agronomically important trait as three RAPD markers, significantly associated with FHB, were identified in the study	Sun et al. (2003)

Table 5. (continued)

Markers	Material	Outcome	Reference
SSR-based diversity			
23 primer pairs	40 wheat cultivars and lines comprising European elite material	Relatively small number of microsatellites can be used for estimation of genetic diversity and cultivar identification in elite material of bread wheat	Plaschke et al. (1995)
14 loci	65 wheat varieties chosen to represent the bulk of area sown in UK over past 70 years	Potential of microsatellites for high-throughput genetic diversity assesment was demonstrated	Donini et al. (1998)
23 primer pairs	21 accessions of *T. dicoccoides* (19 resistant and 2 susceptible to yellow rust) originating from centre of origin and diversity in Upper Galilee and Hermon Mountain in Israel	All the wild emmer wheat could be distinguished. Genetic diversity of wild emmer wheat is correlated with geographical distribution	Fahima et al. (1998)
20 primer pairs	55 elite exotic wheat genotypes originating in 29 countries representing 6 continents	A set of 12 primer pairs could distinguish 48 genotypes. One genotype from Portugal was found unique and diverse as it was a single member of a subcluster	Prasad et al. (2000)
42 primer pairs	60 wheat cultivars originating from three agroecological areas: Germany, Austria and Hungary	Excellent resolving power of microsatellites was demonstrated for varietal identification, which arises through breeding under specific environmental conditions and for different end use	Stachel et al. (2000)
24 primer pairs (26 loci)	15 Libyan wheat genotypes	Relatively small number of primer pairs can be used to distinguish all genotypes used	Ben Amer et al. (2001)
24 chloroplast SSR loci	43 accessions from *Triticum* and *Aegilops* species involved in wheat polyploid evolution	Results suggested that the two types of chloroplast genomes of common wheat might have independently originated from the corresponding types of wild and cultivated emmer wheat species	Ishii et al. (2001)
19 loci	124 wheat cultivars and lines	Level of genetic diversity in Australian wheat cultivars has increased over time and the introduction of semi-dwarf germplasm resulted in an increase in the overall diversity	Parker et al. (2002)
43 loci	13 wheat genotypes of diverse origin	A wide range of genomic diversity was observed among all the genotypes, providing them to be the prime candidates for selective breeding for specific traits and broadening the genetic base	Ahmad (2002)
47 primer pairs	75 Nordic spring wheat cultivars bred during 20th century	Genetic diversity in Nordic spring wheat was enhanced by plant breeding in the first quarter of the 20th century and following a decrease during the second quarter was increased again by plant breeding	Christiansen et al. (2002)

Table 5. (continued)

Markers	Material	Outcome	Reference
20 primer pairs	135 wild emmeer wheat genotypes representing 15 populations from a wide range of ecological conditions of soil, temperature and water availability in Israel and Turkey	Microsatellite analysis was found to be highly effective in distinguishing genotypes of wild emmer wheat of natural populations and for the tagging of agronomically important traits derived from wild emmer wheat	Fahima et al. (2002)
19 loci	502 recent European wheat variries, mainly of winter type	A database of 502 wheat varieties was prepared. Approximately 25% of the varieties showed some heterogeneities, with the highest level of heterogeneity in southeastern European material. Furthermore, the highest genetic diversity and the highest number of rare alleles were observed in southern European varieties	Röder et al. (2003)
24 loci	998 accessions of common wheat originating from 68 countries of 5 continents	Accessions from the Near East and the Middle East exhibited more genetic diversity than those from any other region. Greater diversity was found in south-east Europe than in northern and south-west Europe	Huang et al. (2002)
46 loci	710 wheat genotypes from Novi Sad Core Collection originating from 38 countries	Some microsatellites were found associated with 6 important traits, i.e. stem height, earliness, resistance to leaf rust and powdery mildew, sedimentation value and protein content, for wheat breeding	Kobiljski et al. (2002)
28 loci	105 individual plants of wild emmer wheat from a microsite, Yehudiyya, northeast of the Sea of Galilee, Israel	Niche-specific and niche-unique alleles and linakge disequilibria were found in the two subpopulations. Effects of ecological stresses and natural selection on SSR diversity resulted presumably in adaptive structures	Li et al. (2002b)
70 loci	58 accessions covering a wide spectrum of genetic diversity of durum wheat gene pool	Large portion of the molecular variation detected within the group of 45 modern cultivars was accounted for by SSR alleles tracking back to 10 foundation genotypes. Level of genetic diversity present in modern durum wheat germplasm was found increased over time	Maccaferri et al. (2003)
33 primer pairs	13 genotypes including 7 new lines and their parents	8 markers in combination differentiated the seven new wheat lines from each other as well as from their parents. Graphic presentation of the genetic constitution of the new plant type lines was developed which can be used as bar-coded molecular tags for identification of the respective seed samples	Mohapatra et al. (2003)
20 primer pairs	100 bread wheat varieties developed in breeding centres of Ukraine during 1912–2002	Changes were noticed in allele distribution in microsatellite loci over time. A high level of intravarietal heterogeneity was also recorded	Cheobtar et al. (2003)

Table 5. (continued)

Markers	Material	Outcome	Reference
19 loci	502 recent European wheat varities, mainly of winter type	A database of 502 wheat varieties was prepared. Approximately 25% of the varieties showed some heterogeneities, with the highest level of heterogeneity in south-eastern European material. Furthermore, the highest genetic diversity and the highest number of rare alleles were observed in southern European varieties	Röder et al. (2003)
20 primer pairs	96 random accessions of common wheat from the ten wheat regions in China including 33 modern varieties and 63 landraces	Modern varieties and landraces were grouped in two different clusters. Data suggested that one locus with good polymorphism should be detected for each 47.35 cM on average to reflect genetic relationships among varieties with more than 90% certainty	Zhang et al. (2003b)
70 SSR loci	134 durum wheat accessions comprising modern varieties and a number of founders	Genetic diversity of the exmained accessions was highly structured in a number of groups or subgroups	Maccaferri et al. (2003)
20 genomic SSRs, 22 EST-SSRs	64 durum lines, landraces and varieties	EST-SSRs produced high-quality markers but were less polymorphic than genomic SSRs. Data provided a platform to develop a genotypic database for durum wheat that will facilitate the exploitation of its genetic resources	Eujayl et al. (2001)
20 EST-SSRs	52 elite exotic wheat genotypes	EST-SSRs proved superior to genomic SSRs for diversity estimation	Gupta et al. (2003)
12 genomic SSRs, 20 EST-SSRs	56 old and new varieties of bread wheat on the UK recommended list	EST-SSRs delivered fingerprints of superior quality, amplifying clear products with few stutter bands	Leigh et al. (2003)
47 genomic SSRs, 52 EST-SSRs	68 advanced CIMMYT wheat lines targeted to different megaenvironments (MEs)	A higher number of alleles were detected for genomic SSRs than EST-SSRs, but gene diversity between MEs was similar for both type of markers. High levels of genetic diversity were found within the germplasm targeted to each ME; however, genotypes could not be separated according to their targeted MEs	Dreisigacker et al. (2003)
64 EST-SSRs	18 *Triticeae* species belonging to *Triticum-Aegilops* complex	EST-SSRs were recommended in studies on DNA polymorphism, genetic diversity, gene mapping and synteny conservation across different species of Triticeae	Bandopadhyay et al. (2004)
AFLP-based diversity			
16 primer combinations (229 loci)	54 adapted, elite wheat cultivars and 2 diploid relatives	Genetic diversity among cultivars was hierarchically arranged as cultivars nested within market class and market class nested within growth habitat	Barrett and Kidwell (1998)
16 primer combinations (229 loci)	43 spring and winter wheat lines from Pacific Northwest	Pedigree and AFLP-based genetic diversity estimates (GDEs) detected a similar hierarchical pattern of genetic diversity in 43 cultivars	Barrett et al. (1998)

Table 5. (continued)

Markers	Material	Outcome	Reference
6 primer combinations (90 loci)	55 wheat varieties commonly grown in UK over past 60 years	Results were analysed to assess the potential of AFLP for DNA profiling and plant variety registration. Higher levels of discrimination were achieved by the inclusion of greater numbers of bands in analysis	Law et al. (1998)
18 primer combinations (189 loci)	9 winter and 6 spring Turkish durum wheat cultivars	Relationships among winter and spring type durum cultivars was in accordance with the known pedigree information. The most distant and closest cultivars were selected	Incirli and Akkaya (2001)
60 primer combinations (6778 loci)	15 soft red winter wheat (SRWW) genotypes	Differences in genetic similarity were found for assessing the genetic diversity and plant variety protection use	Grunberg et al. (2001)
10 primer combinations (89 loci)	13 modern Canadian durum wheat	Level of genetic variation within the most developed cultivar is fairly substantial despite rigorous selection pressure aimed at cultivar purity	Soleimani et al. (2002a)
4 primer combinations (105 loci)	54 synthetic hexaploid wheats and their parents *T. dicoccum; Ae. tauschii*	Snythetic hexaploids had a considerably higher level of AFLP diversity than normally obersved in cultivated hexaploid wheat, suggesting their use in introducing new genetic diversity into the bread wheat gene pool	Lage et al. (2003)
6 primer combinations	87 biotypes representing 54 Strampelli varieties	Variability found by means of molecular analysis appeared not only due to the incomplete homogeneity and stability of Strampelli's material but also to the use of heterogenous local populations as parents	Boggini et al. (2003)
8 primer combinations (146 loci)	10 Italian populations of 'farro' (*T. dicoccum*) wheat	A good grouping of genotypes in each single population was possible. AFLP analysis was found suitable for an effective chracterization of *T. dicoccum* populations	Talame et al. (2003)
8 primer combinations (615 loci)	55 elite exotic wheat genotypes	A pair of genotypes was recommended for hybridization to develop superior cultivars	Roy et al. (2004)

Miscelleneous

Markers	Material	Outcome	Reference
STS-38 markers	10 elite hard red spring wheat cultivars (Montana and North Dakota), 15 hard red spring wheat cultivars and lines from North American Great Plains, 20 accessions representing a wide range of collection and morphological types	Breeding pool for hexaploid hard red spring wheat was found narrow relative to levels of diversity among and within classes in hexaploid wheat	Chen et al. (1994)
STS-12 markers	13 modern Canadian durum wheat	DNA-based markers can be used as an efficient alternative to morphological traits for cultivar identification and fingerprinting at any stage of plant development	Soleimani et al. (2002b)

Table 5. (continued)

Markers	Material	Outcome	Reference
RFLP-117 probes, AFLP-16 primer combinations, SSR-21 primer pairs	Two sets of 5 and 6 winter wheat cultivars and lines after mating the above genotypes as per factorial design	Average PIC for polymorphic bands was not significantly different between the three marker systems, whereas the marker index was low for RFLPs and SSRs but high for AFLPs and therefore AFLP was recommended for fingerprinting wheat cultivars. No common pattern between the four dendrograms by using coencestry, RFLP, SSR and AFLP data	Bohn et al. (1999)
AFLP-6 primer combinations (84 loci), SSR-14 loci, SSP-9 loci	Dominant UK winter wheat varieties from 1934 to 1994	Diversity in the time periods overlapped and the most modern group of varieties encompassed the majority of diversity found in earlier decades. Plant breeding has resulted, over time, in a qualitative, rather than quantitative, shift in the diversity of winter wheat grown in the UK	Donini et al. (2000)
SSR-10 primer pairs, AFLP-4 primer combinations (71 loci)	105 Argentine bread wheat cultivars released between 1932 and 1995	Significant differences were observed for both SSR and AFLP only between breeding programmes with large differences in number of released cultivars. The Argentine wheat germplasm has maintained a relatively constant level of genetic diversity during the last half century	Manifesto et al. (2001)
SSR-25 primer pairs, RAPD-31 primer pairs, AFLP-6 primer combination	14 varieties of wheat, 1 of durum and 1 of triticale released for general cultivation since 1920 in India	Most of the cultivars could be uniquely identified with SSR and RAPD markers but not with AFLPs	Garg et al. (2001)
RFLP-338 loci, AFLP-200 loci	40 bread wheat cultivars (central and southern Europe)	Correlations with general and specific combining ability effects for studied traits (grain yield, quality attributes) were statistically significant but too low to be predictive in practical breeding	Corbellini et al. (2002)
AFLP-8 primer combinations (633 loci), SSR-24 cp SSR loci	55 accessions of wild einkorn wheat	Intraspecific nuclear genome variation was lower in einkorn wheats and higher in Ae spletoides. In contrast, the chloroplast DNA variation was larger in einkorn wheat and the least in Ae. speltoides	Mizumoto et al. (2002)
SAMPL-2 primer combinations (87 loci)	55 elite exotic wheat genotypes	54 genotypes could be distinguished using the SAMPL banding pattern of both primers. An association of six bands with grain protein content, of seven bands with preharvest sprouting tolerance and four bands with 1,000-grain weight was observed using BSA	Roy et al. (2002)

Table 5. (continued)

Markers	Material	Outcome	Reference
AFLP-8 primer combinations, SSR-37 primer pairs	70 spring wheat accessions (32 from CIMMYT and 38 from other breeding programmes worldwide)	AFLP and SSR markers were generally in agreement with estimates of diversity measured using co-efficiency of parentage. CIMMYT accessions were found different from the worldwide group of accessions	Almanza-Pinzon et al. (2003)
AFLP-322 loci, SSR-19 loci	65 wheat cultivars from eight countries varying in head blight resistance levels	US cultivars were found more closley related to cultivars from Europe and Argentina than cultivars from Asia. Integrating FHB resistance QTLs from Asian sources into US wheat, therefore, may increase the genetic diversity in US wheat	Bai et al. (2003)
RAPD-30 primer pairs; AFLP-10 primer combinations (560 loci)	58 accessions of jointed goatgrass (*Ae. cylindrica*) and 6 accessions of the related wild species barb goatgrass	AFLP produced more scorable bands than did RAPD, but both methods revealed limited genetic diversity in jointed goatgrass. AFLPs distinguished among all but 2 of the 16 accessions surveyed	Pester et al. (2003)
SSR-21 loci, AFLP-15 primer combinations (254 loci)	140 wheat landraces, obsolete cultivars and modern cultivars of Czech Republic	A significant drift of genetic basis of modern cultivars in comparison with landraces and obsolete cultivars was recorded. Possibility of reducing number of gene bank accessions to maintain maximum number of alleles by using molecular markers was also demonstrated	Ovesna et al. (2003)
ESTs-6 loci, SSR-6 loci, ISSR-6 loci	20 accessions of Italian emmer wheat (*T. dicoccum* Schübler) populations	Study provided the correct identification of the analysis material to support its registrtaion as varieties	Pagnotta et al. (2003)
SSAP-4 primer pairs	26 *Aegilops* and 9 *Triticum* accessions	SSAP-based diversity tree for *Aegilops* spp. agreed with current classifications; however, the Triticum tree showed several significant differences which may be associcated with polyploidy in this genus	Queen et al. (2004)

netic diversity of the parental lines was inadequate for predicting either progeny variance or F_1 performance (Perenzin et al. 1998; Bohn et al. 1999; Dreisigacker et al. 2003).

Marker analysis of common wheats with presumptive wild ancestors has provided insights into the crop's domestication and guided strategies for collecting, evaluating and utilizing germplasm. AFLP fingerprinting of einkorn and emmer wheats and barley, along with their wild progenitors, indicated that both einkorn (Heun et al. 1997) and emmer wheats (Özkan et al. 2002) were domesticated in a very small area of southeastern Turkey near the Tigris and Euphrates rivers more than 10,000 years ago. More recently, microsatellite sequences have been used to generate molecular clock estimates of the dates of wheat domestication. These ranged from 9,000 to 19,000 years for the transition from *T. dicoccoides* and *T. aestivum* and 8,705 to 18,414 years between *T. dicoccoides* and *T. durum* (Fahima et al. 2003). Sequence variation at a number of D genome STS loci has suggested that multiple D genome diploid parents were involved in the origin of common wheat (Talbert et al. 1998) and that all wheats share a single D-genome gene pool, which is the *strangulata* form of *Ae. tauschii* (Dvorák et al. 1998a). The *strangulata* gene pool is larger than expected because of gene flow from the *tauschii* form of *Ae. tauschii* (Lubbers et al. 1991; Dvorák et al. 1998a).

Fig. 1. Comparative location of genes determining dwarfness (GA insensitive) on chromosomes 4B and 4D of wheat and 4H of barley using the following basic maps: (*1, 2*) Börner et al. (1997), (3) Ivandic et al. (1998). Mapped loci are marked with a *point*. The *connecting lines* between chromosomes indicate common loci which are *underlined*. Genetic distances (roughly estimated) are given in centimorgans (cM). The gene loci are *boxed*. *c* = estimated centromere position, *S* = short arm, *L* = long arm

2.4.4
Comparative Mapping and Synteny

Molecular mapping of wheat and other grass species suggested that despite more than 60 million years of evolution within the subfamily of the *Poaceae*, the individual grass genomes are characterized by large segments of conserved linkage blocks that display collinear marker orders between different species. Similar to a LEGO-model, grass genomes are considered to be made up of conserved segments (Moore 1995). This model was extended by Gale and Devos (1998), and it was shown that the grass genomes can be displayed in concentric circles in which orthologous genes, which are derived from a common ancestor locus, are located on a radial line. Some reports on comparative mapping dealing with wheat and other cereal species are listed in Table 6. These studies provide important clues about the structural organization of the cereal genomes. For instance, the com-

parative mapping of GA-insensitive dwarfing genes suggested that the dominant *Rht* genes of wheat and the codominat *Dwf2* gene of barley are members of a homoeoallelic series existing in the triticeae species (Fig. 1). In a similar way, Fig. 2 shows that the *wa1* locus (determining the waxless plant character) of rye is homoeoallelic to the glaucousness (waxiness) loci *w1* or *w2*[1] of wheat and genes/alleles for glossy sheat/spike (*gs1, gs6, gs8*) of barley. Furthermore, the alignment of the *gl2* (responsible for altering cuticle wax) gene region of maize with the *w2*[1] of wheat suggests the conservation of genes responsible for similar traits acrosss different cereal genomes. In addition to revealing evolutionary patterns within the *Poaceae* subfamily; comparative mapping provides access to the model genome of rice. An obvious strategy emerging from the concept of syntenous relationships is the transfer of the vast amount of genomic information

Table 6. A list of some important comparative mapping and genomics studies revealing the syntenic relationship of wheat with other cereal species

Species	References
Wheat, barley	Namuth et al. (1994), Hohmann et al. (1995), Dubcovsky et al. (1996), Hernandez et al. (2001), Salvo-Garrido et al. (2001), Weng and Lazar (2002b), Varshney et al. (2005b)
Wheat, maize	Devos et al. (1994)
Wheat, rice	Kurata et al. (1994), Kato et al. (1999), Sarma et al. (1998, 2000), Lamoureaux et al. (2002), Liu and Anderson (2003b), Laubin et al. (2003), Sorrells et al. (2003), Francki et al. (2003), La Rota and Sorrells (2004), Singh et al. (2004a), Li et al. (2004b), Yu et al. (2004b)
Wheat, rye	Devos et al. (1992, 1993a), Khlestkina et al. (2004)
Wheat, barley, rye	Devos et al. (1993b), Devos and Gale (1993), Börner et al. (1998), Gudu et al. (2002)
Wheat, barley, rice	Dunford et al. (1995), Gallego et al. (1998), Kato et al. (2001)
Wheat, maize, rice	Ahn et al. (1993), Moore et al. (1995b)
Wheat, maize, oat, rice	Van Deynze et al. (1995a,b)
Wheat, foxtail-millet, maize, rice	Moore et al. (1995a)

and resources available in rice genome to the wheat genome (see paragraph above, Genome Collinearity).

Our present knowledge of synteny is mainly based on comparative mapping of cross-hybridizing RFLP markers. Comparisons of genetic linkage maps are severely limited in their resolution by the number of orthologous loci detected and by population sizes. Early comparative maps (e.g., Hulbert et al. 1990; Ahn and Tanksley 1993; Ahn et al. 1993; Kurata et al. 1994; Moore et al. 1995a,b; Devos and Gale 1997; Gale and Devos 1998) greatly underestimated the complexity of genome relationships. Those low-resolution comparative maps are biased by the use of single-copy probes that do not sample multicopy regions, simplifying assumptions about collinearity and placing excessive emphasis of gene-rich regions (Bennetzen 2000; Gaut 2001, 2002). *In silico* comparison of DNA sequences among different cereals makes it possible to transfer the sequence information between species to greatly enhance the resolution of comparative maps. For instance, *in silico* comparison of 974 genetically mapped barley ESTs with 524,720 wheat ESTs provided a potential set of 934 (95.4% of the loci tested) EST-derived markers to wheat genetic maps (Varshney et al. 2004c). However, large-scale comparative DNA sequence analysis of physically mapped wheat ESTs with the rice genome suggested that there has been an abundance of rearrangements, insertions, deletions and duplications eroding the wheat-rice genome relationship that may complicate the use of rice as a model for cross-species transfer of information in non-conserved regions (Sorrells et al. 2003; La Rota and Sorrells 2004; see Sect. 2.5.2 below).

2.5
Impact of Genomics Research on Wheat Genetics and Breeding

The publication of the complete genome sequence for *Arabidopsis* (TAGI 2000) and drafts of rice genome (Goff et al. 2002; Yu et al. 2002) provides the basis for elucidating the gene and protein networks that control biological processes. These model systems provide the basis for determining the genes and the respective proteins that control key components of complex traits in crop plants like wheat (Appels et al. 2003; Gupta and Varshney 2004). A large amount of EST data has been generated for wheat, which is being used to study and analyse the transcriptome of wheat (Powell and Langridge 2004). In addition to these advances in wheat genomics, novel approaches such as linkage disequilibrium (LD) analysis and association
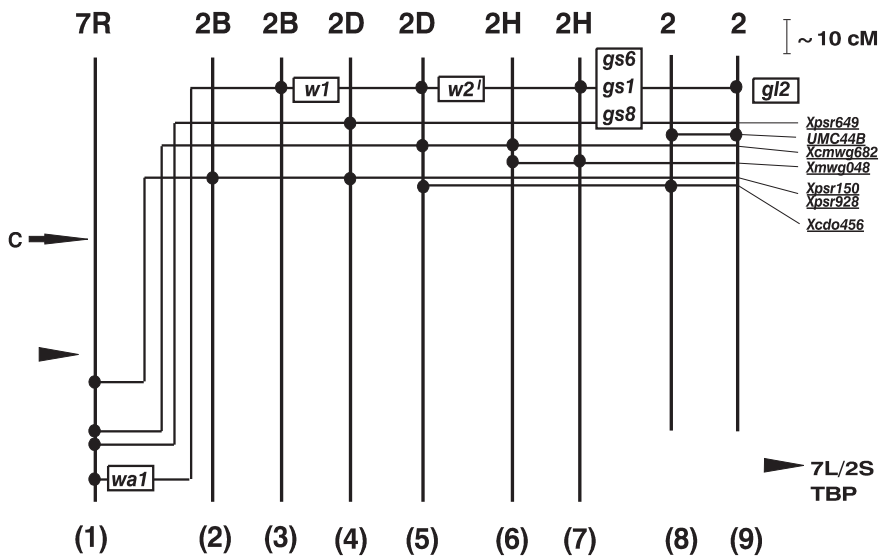
Fig. 2. Comparative location of genes determining waxless plant on chromosomes 7R of rye, 2B and 2D of wheat, 2H of barley and 2 of maize using the following basic maps: (*1*) Korzun et al. (1997a), (*2, 4*) Devos et al. (1993b), (*3*) Driscoll (1966), (*5*) Nelson et al. (1995a), (*6*) Graner et al. (1991), (*7*) Franckowiak (unpublished, cf. Börner 1999), (*8*) Ahn and Tanksley (1993), (*9*) Coe and Neuffer (1993). Mapped loci are marked with a *point*. The *connecting lines* between chromosomes indicate common loci which are underlined. Genetic distances (roughly estimated) are given in centimorgans (cM). The gene loci are *boxed*. *c* = estimated centromere position, *S* = short arm, *L* = long arm, *TPB* = translocation break point

mapping and genetical genomics would have a major impact on wheat genetics and breeding in the near future with the ultimate objective of crop improvement.

2.5.1
Transcriptomics and Functional Genomics

In order to establish an inventory of expressed genes in wheat, an international consortium (International Triticeae EST Cooperative) was established to launch the development of a wheat and barley EST database. This effort provided the first serious collection of ESTs and helped lead to other initiatives. In particular a project entitled 'The Structure and Function of Expressed Portion of Wheat Genome' involving 13 laboratories was established in 1999 and funded by the National Science Foundation (NSF), USA (http://wheat.pw.usda.gov/NSF/). The project had as its objective to decipher the chromosomal location and biological function of a large set of wheat genes, to enhance our understanding of the biology of the wheat plant and to create a new paradigm for the improvement of this important crop. To this end, a total of 117,510 ESTs (101,912 are 5′ ESTs and 15,605 are 3′ ESTs, as of July 2003) from 20 cDNA libraries

were generated (Zhang et al. 2004). Computational analysis of this dataset yielded 18,876 contigs and 23,034 singletons (http://wheat.pw.usda.gov/ NSF/curator/assembly.html; Lazo et al. 2004). In addition to these ESTs, generated in NSF-sponsored projects, other public laboratories and private organizations such as the DuPont Corporation also generated wheat ESTs and submitted them to public databases. As a result, 587,650 wheat ESTs are available in the public domain as of dbEST release 012805 (http://www.ncbi.nlm.nih.gov/dbEST/ dbEST_summary.html). A computational analysis of 554,379 wheat ESTs suggested the presence of 44,513 TCs (tentative consensi) and 83,420 singleton ESTs, as per TIGR Wheat Gene Index Release 9.0, 20 September 2004 (http://www.tigr.org).

The extensive EST databases prepared from many different tissues can be used to estimate gene expression levels by measuring the frequency of the appearance of specific sequences, employing computational tools such as Digital Differential Display (http:// www.ncbi.nlm.nih.gov/UniGene/info_ddd.shtml) or HarvEST (http://harvest.ucr.edu/). An example of the use of wheat ESTs from multiple cDNA libraries to study developmental processes was shown by Ogihara et al. (2003). After the analysis of 116,232 ESTs, generated from ten wheat tissues, the re-

searchers identified correlated expression patterns of genes across the tissues. Furthermore, relationships of gene expression profiles among the ten wheat tissues were inferred from global gene expression patterns. However, the use of EST databases to study expression profiles is limited by the availability of cDNA libraries used to develop ESTs and by the depth of EST sequencing. There are also problems in tracking genes that may be represented by several partial EST sequences.

Newer techniques allow the estimation of mRNA abundance for large numbers of genes simultaneously. The methods include serial analysis of gene expression (SAGE), microarrays, macroarrays and massively parallel signature sequencing (MPSS). These methods have not been extensively applied in wheat, although nearly all have been applied to some aspects in other cereals such as rice and maize (Milligan et al. 2004).

SAGE (serial analysis of gene expression), a logical extension of EST sequencing, can be used to study expression patterns (Velculescu et al. 1995). Unfortunately, SAGE does suffer from several problems. In particular, SAGE experiments require large amounts of RNA and can be very expensive if many samples are to be analysed, for example from a developmental series. As with MPSS (Brenner et al. 2000; http://www.lynxgen.com/), the signatures generated can be difficult to assign to particular genes when the technique is applied to wheat, where a full genome sequence is not available.

Microarrays and macroarrays offer a technique for screening the expression profile of very large numbers of genes simultaneously (Sreenivasulu et al. 2002). Both types of arrays have been used to study grain development in cereals. Macroarrays have the advantage of ease of manufacture and low cost relative to microarrays, but macroarrays do not provide the same level of gene or probe density for screening. Although macro-/microarrays have been used extensively in some cereals such as maize, rice and barley, use of these technologies in wheat has been limited. Recently cDNA microarrays containing approximately 9,000 wheat cDNAs were used to monitor gene expression during the first 28 d of grain development following anthesis (Leader et al. 2003). This study revealed 66 differentially regulated genes, which showed a sequence similar to transcription factors. Identified genes can be used for gene-specific marker development and synteny with rice to determine if any of the genes map within regions corresponding to QTL for grain yield or quality traits. Similarly, exploita-

tion of cDNA microarrays is under way to identify the genes for endosperm development (Shinbata et al. 2003), for studying the Russian wheat aphid (RWA) defense response mechanisms (Botha et al. 2003) and assessment after fungicide application (Pasquer et al. 2003).

Real-time PCR (RT-PCR) has also been used to study drought stress tolerance (Rampino et al. 2003). Such approaches hold great potential for identifying the genes corresponding to QTLs for use in breeding as recently demonstrated in barley (Potokina et al. 2004). In addition, proteomic approaches have been recently used to assess the relationship between the wheat grain transcriptome and proteome (Branlard et al. 2003). It is hoped that in the near future, the above technologies will be put to extensive use in wheat.

2.5.2
Comparative Genomics and Bioinformatics

The availability of a large number of ESTs of wheat and other cereals and the complete genome sequence of rice has allowed sequence comparisons between wheat and other cereal genomes and opened a new area of comparative genomics. Over the last decade, developments in the field of bioinformatics responded to the needs of wheat (or Triticeae) genomics researchers (Matthews et al. 2004). Several databases and Web sites including GrainGenes (http://wheat.pw.usda.gov/) for Triticeae, GRAMENE (http://www.gramene.org/) for comparative mapping in cereals, and TIGR (http://www.tigr.org/) for genome analysis have been developed. For instance, the GrainGenes database at present contains over 70 map sets and linkage data for *T. aestivum*, *T. turgidum* and diploid species (Carollo et al. 2003). The 'Genomics' page on the GrainGenes website, http://wheat.pw.usda.gov/ggpages/genomics provides resources for wheat genomics researchers such as assemblies of the ESTs, alignment of wheat ESTs to the rice genome sequence, co-operative international projects to develop wheat SNPs and SSRs, an assembly of large DNA clones (BACs) into the physical map of the D genome, and a database of repeat sequences from the Triticeae (TREP) (Matthews et al. 2003).

The use of DNA-sequence-based comparative genomics for evolutionary studies and for transferring information from model species to related large-genome species has revolutionized molecular genetics

and breeding strategies for improving these crops (Paterson 2004). Comparative sequence analysis methods provide cross-referencing of genes between species maps, enhance the resolution of comparative maps, study patterns of gene evolution, identify conserved regions of the genomes and facilitate interspecies gene cloning.

A comparison (BLASTN analysis) of 5,780 ESTs that were physically mapped in wheat chromosome bins to 3,280 ordered BAC/PAC clones of rice revealed numerous chromosomal rearrangements that will significantly complicate the use of rice as a model for cross-species transfer of information in non-conserved regions (La Rota and Sorrells 2004). In addition, the physical locations of non-conserved regions were not consistent across rice chromosomes. Some wheat ESTs with multiple wheat genome locations were found associated with the non-conserved regions. An average of 35% of the putative single-copy genes that were mapped to the most conserved bins matched rice chromosomes other than the one that was most similar.

As noted above, interruption of microcollinearity was observed in other studies when extensive comparisons were made across smaller regions between collinear chromosomes (arms) of wheat and rice. For instance, a gene-by-gene BLASTN search of 2,932 genes from rice chromosome 11 (57.3 cM to 116.2 cM) to wheat ESTs and physically mapped wheat ESTs revealed that about one-third of the genes (homologous rice genes) were mapped to the homoeologous group 4 chromosome of wheat, suggesting a common evolutionary origin (Singh et al. 2004a). Location of bin-mapped wheat contigs to chromosomes of all seven homoeologous groups was attributed to the movement of genes (transpositions) or chromosome segments (translocations) within the rice or the hexaploid wheat genomes. In another study on the investigation of microcollinearity between the rice genome and a total of 1,500 kb from physical BAC contigs on wheat chromosome 1AS, a total of 27 conserved orthologous sequences between wheat chromosome 1AS and a region of 1,210 kb located on rice chromosome 5S were identified. However, microcollinearity was found to be frequently disrupted by rearrangements (Guyot et al. 2004). Similarly, microcollinearity was disrupted between a 2.6 cM region (encompassing the grain protein content locus *Gpc-6B1*) on wheat chromosome 6B and a 350 kb region on rice chromosome 2 (Distelfeld et al. 2004). Nevertheless, the region encompassing the *Gpc-6B1* locus showed excellent conservation between the two genomes, which facilitated the saturation of the target region of the wheat genetic map with molecular markers, and the *Gpc-6B1* locus was delimited to 0.3 cM containing five candidate genes in the collinear 64-kb region in rice. Comparative genomics also facilitated the identification of genes controlling seed dormancy and preharvest sprouting in wheat, barley and rice (Li et al. 2004a).

2.5.3
Novel Approaches

AB-QTL Analysis

For the long-term sustainability of wheat production, introduction of alien or exotic genes from wild species is imperative. In the past, many useful genes were transferred from wild relatives into wheat, most of which were single genes or gene clusters conferring resistance to various diseases. Fifty-seven genes for resistance to diseases and pests were introduced into wheat from other genera of the Triticeae family via alien translocations. In many cases, the size of the alien fragments and the translocation breakpoints were precisely determined by genomic in situ hybridization (for review see Friebe et al. 1996). For transferring the QTLs of agronomically important traits from a wild species to a crop variety, an approach named 'Advanced backcross QTL analysis (ABQA)' was proposed by Tanksley and Nelson (1996). In this approach, a wild species is backcrossed to a superior cultivar, and during backcrossing cycles the transfer of a desirable gene/QTL is monitored with molecular markers. The segregating BC_2F_2 or BC_2F_3 population is then used not only for recording data on the trait of interest but also for genotyping with polymorphic molecular markers. These data are then used for QTL analysis, leading to the simultaneous discovery of QTLs, while transferring these QTLs by conventional backcrossing. This approach has been used in wheat recently (Huang et al. 2003b, 2004). After genotyping 72 preselected BC_2F_2 plants derived from a cross between a German variety and synthetic wheat, Hunag et al. (2003b) have identified 40 putative QTLs, including 11 for yield, 16 for yield components, 8 for ear emergence and 8 for plant height. Thus this approach has the potential for direct use in wheat improvement.

Association and Linkage Disequilibrium Analysis

Conventional techniques of molecular mapping require a mapping population based on the products of one (doubled haploids) or two (F_2s) cycles of recombinations, limiting the resolution of genetic maps. In addition, such populations are often not representative of the germplasm that is being actively used in breeding programmes. In contrast, association mapping, based on linkage disequilibrium (LD), does not require a conventional segregating population and may in some cases be more powerful than conventional analysis for identifying the genes responsible for the variation in a quantitative trait (Buckler and Thornsberry 2002; for review see Flint-Garcia et al. 2003). Combined with a consideration of population structure (Pritchard et al. 2000), this association mapping allows for large-scale assessment of allele/trait relationships. A high degree of LD facilitates association analysis of markers linked to a QTL but reduces the resolution of the analysis (for review see Flint-Garcia et al. 2003). For instance, in maize the rapid decay of LD provides a means of mapping candidate genes with high precision and at the same time allows one to associate alleles with phenotypic values (Thornsberry et al. 2001). For those species with high LD, comparative mapping and transcript profiling are necessary for narrowing the list of candidate genes. Various kinds of populations can be designed with the appropriate resolution. For example, segmental introgression lines would have high LD while long-term breeding populations that have been intermated for many generations would have low LD (Sorrells 2004).

In wheat, some studies on association analysis have already been conducted and provided markers linked with some traits (Paull et al. 1994, 1998). Some efforts have been expended to study the association between growth habit and haplotype using a set of 80 hexaploid cultivars and assaying a total of seven SNPs located within a 3-kb region of molecular marker PSR6001, a candidate marker for vernalization responsive gene *Vrn-A1* (Devos and Beales 2003). However, large-scale studies on the estimation of LD in the wheat genome are currently under way in several laboratories. Such high-resolution mapping of traits/QTLs to the level of individual genes will provide a new possibility for studying the molecular and biochemical basis of variation in quantitative traits and will help to identify specific targets for crop improvement in wheat. Though LD-based approaches hold great promise for accelerating fine mapping, conventional linkage mapping will continue to be useful particularly when trying to 'mendelize' QTLs and assessing the effect of QTL in isolation (Rafalski and Morgante 2004).

Genetical Genomics

Recently, a new approach, called 'genetical genomics', has also been proposed, where QTL mapping is combined with expression profiling of individual genes in a segregating (mapping) population (Jansen and Nap 2001). In this approach, total mRNA or cDNA of the organ/tissue from each individual of a mapping population is hybridized onto a microarray carrying a high number of cDNA fragments representing the species/tissue of interest and quantitative data are recorded reflecting the level of expression of each gene on the filter. Under the presumption that every gene showing transcriptional regulation is mapped within the genome of the species of interest, the expression data can be subjected to QTL analysis, thus making it possible to identify the so-called 'ExpressQTLs' (eQTLs). The recently developed software tool Expressionview for combined visualization of gene expression data and QTL mapping (Fischer et al. 2003) will be very useful in this connection. Based on segregating populations, eQTL analysis identifies gene products influencing the quantitative trait (level of mRNA expression) in *cis* (mapping of the regulated gene within the QTL) or *trans* (the gene is located outside the QTL). The latter gene product (second-order effect) is of specific interest because more than one QTL can be connected to such a *trans*-acting factor (genes acting on the transcription of other genes) (Schadt et al. 2003). The mapping of eQTLs allows multifactorial dissection of the expression profile of a given mRNA/cDNA, protein or metabolite into its underlying genetic components and also makes it possible to locate these components on the genetic map (Jansen and Nap 2001; Jansen 2003). Eventually, for each gene or gene product analysed in the segregating population (by using expression profiling methodology), eQTL analysis will underline the regions of the genome influencing its expression. This approach has been used in maize (Schadt et al. 2003) and is being investigated by several groups for wheat.

2.6
Concluding Remarks

The development of genomics and genetics resources in wheat has lagged behind that of many other plant species. This has been largely related to concerns about the large size and the polyploid nature of the wheat genome. Therefore, despite its importance as a food crop and the extensive genetic and cytogentic resources that were available for wheat, genomics programmes were slow to develop. However, over the past few years this situation has changed dramatically. Firstly, several programmes worked together to build a resource base that now allows most genomics approaches to be applied to wheat. Secondly, it has become clear that the behaviour of the wheat genome is different from that of many other species. This means that genomics-based improvement of wheat will be dependent on studies on wheat itself and also that the study of the wheat genome offers some exciting scientific challenges.

A large number of molecular markers have been generated and mapped to produce dense genetic physical maps. Based on the available marker resources, a number of agronomically important genes and an even larger number of quantitative trait loci have been tagged with molecular markers. Further progress in trait mapping will critically depend on the availability of appropriate plant material. The generation and phenotypic analysis of experimental populations (F_2, DH, RIL, etc.) is time consuming and the development of novel approaches of association genetics based on the exploitation of linkage disequilibrium (LD) may lead to the verification of candidate genes in natural populations or collections of various genotypes (Rafalski 2002).

While the isolation of a given gene is usually a prerequisite to understanding its cellular function, the identification and subsequent introgression of superior alleles will be of seminal importance to breed-improved cultivars. The launch of several new initiatives to analyse the wheat genome structure will facilitate the systematic development of wheat genetic and genomic resources.

References

Adhikari TB, Anderson JM, Goodwin SB (2003) Identification and molecular mapping of a gene in wheat conferring resistance to *Mycosphaerella graminicola.* Phytopathology 93:1158–1164

Adhikari TB, Cavaletto JR, Dubcovsky J, Gieco J, Schlatter AR, Goodwin SB (2004a) Molecular mapping of the *Stb4* gene for resistance to septoria tritici blotch in wheat. Phytopathology 94:1198–1206

Adhikari T, Yang, X, Cavaletto JR, Hu X, Buechley G, Ohm HW, Shaner G, Goodwin SB (2004b) Molecular mapping of *Stb1*, a potentially durable gene for resistance to septoria tritici blotch in wheat. Theor Appl Genet 109:944–953

Adhikari TB, Wallwork H, Goodwin SB (2004c) Microsatellite markers linked to the *Stb2* and *Stb3* genes for resistance to Septoria tritici blotch in wheat. Crop Sci 44:1403–1411

Ahmad M (2000) Molecular marker-assisted selection of HMW glutenin alleles related to wheat bread quality by PCR-generated DNA markers. Theor Appl Genet 101:892–896

Ahmad M (2002) Assessment of genomic diversity among wheat genotypes as determined by simple sequence repeats. Genome 45:646–651

Ahmed TA, Tsujimoto H, Sasakuma T (2000) Identification of RFLP markers linked with heading date and its heterosis in hexaploid wheat. Euphytica 116:111–119

Ahn SN, Tanksley SD (1993) Comparative linkage maps of the rice and maize genomes. Proc Natl Acad Sci USA 90:7980–7984

Ahn S, Anderson JA, Sorrells ME, Tanksley SD (1993) Homeologous relationships of rice, wheat and maize chromosomes. Mol Gen Genet 241:483–490

Akhunov ED, David L, Chao S, Lazo G, Anderson OD, Qi L-L, Echalier B, Gill BS, Linkiewicz AM, Dubcovsky J et al (2003a) GC composition and codon usage in genes of inbreeding and outcrossing Triticeae species. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 203–206

Akhunov ED, Goodyear AW, Geng S, Qi L-L, Echalier B, Gill BS, Miftahudin Gustafson JP, Lazo G, Chao SM et al (2003b) The organization and rate of evolution of wheat genomes are correlated with recombination rates along chromosome arms. Genome Res 13:753–763

Almanza-Pinzon MI, Khairallah M, Fox PN, Warburton ML (2003) Comparison of molecular markers and coefficients of parentage for the analysis of genetic diversity among spring bread wheat accessions. Euphytica 130:77–86

Alpert KB, Tanksley SD (1996) High-resolution mapping and isolation of a yeast artificial chromosome contig containing

fw22: a major fruit weight quantitative trait locus in tomato. Proc Natl Acad Sci USA 93:15503–15507

Anderson JA, Stack RW, Liu S, Waldron BL, Fjeld AD, Coyne C, Moreno–Sevilla B, Fetch JM, Song Q J, Cregan PB et al (2001) DNA markers for fusarium head blight resistance QTLs in two wheat populations. Theor Appl Genet 102:1164–1168

Angerer N, Lengauer D, Steiner B, Lafferty J, Loeschenberger F, Buerstmayr H (2003) Validation of molecular markers linked to two Fusarium head blight resistance QTLs in wheat. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 1096–1098

Appels R (2003) A consensus molecular genetic map for wheat- a cooperative international effort. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 211–214

Appels R, Francki M, Chibbar R (2003) Advances in cereal functional genomics. Funct Integr Genom 3:1–24

Araki E, Miura H, Sawada S (1999) Identification of genetic loci affecting amylose content and agronomic traits on chromosome 4A of wheat. Theor Appl Genet 98:977–984

Arraiano LS, Worland AJ, Ellerbrook C, Brown JKM (2001) Chromosomal location of a gene for resistance to septoria tritici blotch (*Mycosphaerella graminicola*) in the hexaploid wheat 'Synthetic 6x'. Theor Appl Genet 103:758–764

Arzani A, Peng JH, Lapitan NLV (2003) Genetic mapping of genes coding for Russian wheat aphid resistance (*Dn4*) and glume colour (*Rg2*) using microsatellite markers. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 1099–1101

Autrique E, Singh RP, Tanksley SD, Sorrells ME (1995) Molecular markers for four leaf rust resistance genes introgressed into wheat from wild relatives. Genome 38:75–83

Autrique E, Nachit MM, Monneveux P, Tanksley SD, Sorrells ME (1996) Genetic diversity in durum wheat based on RFLPs, morphophysiological traits, and coefficient of parentage. Crop Sci 36:735–742

Ayala L, Henry M, Gonzalez-de-Leon D, van Ginkel M, Mujeeb-Kazi A, Keller B, Khairallah M (2001) A diagnostic molecular marker allowing the study of *Th intermedium*-derived resistance to BYDV in bread wheat segregating populations. Theor Appl Genet 102:942–949

Bai GH, Kolb FL, Shaner G, Domier LL (1999) Amplified fragment length polymorphism markers linked to a major quantitative trait locus controlling scab resistance in wheat. Phytopathology 89:343–348

Bai GH, Guo PG, Kolb FL (2003) Genetic relationships among head blight resistant cultivars of wheat assessed on the basis of molecular markers. Crop Sci 43:498–507

Bandopadhyay R, Sharma S, Rustgi S, Singh R, Kumar A, Balyan HS, Gupta PK (2004) DNA polymorphism among 18 species of *Triticum- Aegilops* complex using wheat EST-SSRs. Plant Sci 166:349–356

Barcaccia G, Molinari L, Porfiri O, Veronesi F (2002) Molecular characterization of emmer (*Triticum dicoccom* Schrank) Italian landraces. Genet Resource Crop Evol 49:415–426

Bariana HS, Hayden MJ, Ahmed NU, Bell JA, Sharp PJ, McIntosh RA (2001) Mapping of durable adult plant and seedling resistances to stripe rust and stem rust diseases in wheat. Aust J Agric Res 52:1247–1255

Bariana HS, Brown GN, Ahmed NU, Khatkar S, Conner RL, Wellings CR, Haley S, Sharp PJ, Laroche A (2002) Characterisation of *Triticum vaviloviii*derived stripe rust resistance using genetic, cytogenetic and molecular analyses and its markerassisted selection. Theor Appl Genet 104:315–320

Barloy D, Lemoine J, Dredryver F, Jahier J (2000) Molecular markers linked to the *Aegilops variabilis*derived rootknot nematode resistance gene *Rknmn1* in wheat. Plant Breed 119:169–172

Barrett BA, Kidwell KK (1998) AFLP-based genetic diversity assessment among wheat cultivars from the Pacific Northwest. Crop Sci 38:1261–1271

Barrett BA, Kidwell KK, Fox PN (1998) Comparison of AFLP and pedigree-based genetic diversity assessment methods using wheat cultivars from the Pacific Northwest. Crop Sci 38:1271–1278

Barrett B, Bayram M, Kidwell K (2002) Identifying AFLP and microsatellite markers for vernalization response gene *Vrn-B1* in hexaploid wheat using reciprocal mapping populations. Plant Breed 121:400–406

Ben Amer IM, Borner A, Röder MS (2001) Detection of genetic diversity in Libyan wheat genotypes using wheat microsatellite markers. Genet Resource Crop Evol 48:579–585

Benard V, Boyer D, Bastide C, Rouviere C, Duranton N, Praud S, Dufour P, Murigneux A, Sourdille P, Bernard M (2003) Chromosomal location of wheat cDNA clones derived from expressed sequence tags (ESTs). In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 925–927

Bennetzen JL (2000) Comparative sequence analysis of plant nuclear genomes: Microcolinearity and its many exceptions. Plant Cell 12:1021–1029

Bennetzen JL, Ma J (2003) The genetic colinearity of rice and other cereals on the basis of genomic sequence analysis. Curr Opin Plant Biol 6:128–133

Bennetzen JL, Ramakrishna W (2002) Numerous small rearrangements of gene content, order and orientation differentiate grass genomes. Plant Mol Biol 48:821–827

Bernatzky R, Tanksley SD (1986) Toward a saturated linkage map in tomato based on isozyme and random cDNA sequences. Genetics 112:887–898

Blanco A, Bellomo MP, Cenci A, Degiovanni C, Dovidio R, Iacono E, Laddomada, B, Pagnotta MA, Porceddu E, Sciancalepore A et al (1998) A genetic linkage map of durum wheat. Theor Appl Genet 97:721–728

Blazkova V, Bartos P, Park RF, Goyeau H (2002) Verifying the presence of leaf rust resistance gene *Lr10* in sixteen wheat

cultivars by use of a PCR-based STS marker. Cereal Res Commun 30:9–16

Boggini G, Vaccino P, Brandolini A, Cattaneo M (2003) Genetic variability of Strampelli bread wheat realisations detected by storage protein composition and by AFLP. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 101–104

Bohn M, Utz HF, Melchinger AE (1999) Genetic similarities among winter wheat cultivars determined on the basis of RFLPs, AFLPs, and SSRs and their use for predicting progeny variance. Crop Sci 39:228–237

Börner A (1999) Comparative genetic mapping in triticeae. In: van Raamsdonk LWD, den Nijs JCM (eds) Plant evolution in man-made habitats, Proc VIIth Symp IOPB, Amsterdam, pp 197–210

Börner A, Röder M, Korzun V (1997) Comparative molecular mapping of GA insensititive *Rht* loci on chromosomes 4B and 4D of common wheat (*Triticum aestivum* L.). Theor Appl Genet 95:1133–1137

Börner A, Korzun V, Worland AJ (1998) Comparative genetic mapping of loci affecting plant height and development in cereals. Euphytica 100:245–248

Börner A, Röder MS, Unger O, Meinel A (2000) The detection and molecular mapping of a major gene for non-specific adult-plant disease resistance against stripe rust (*Puccinia striiformis*) in wheat. Theor Appl Genet 100:1095–1099

Börner A, Simon MR, Röder MS, Ayala FM, Cordo CA (2003) Molecular mapping of QTLs determining resistance/tolerance to biotic and abiotic stress in hexaploid wheat. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 331–333

Botha A-M, Lacock L, van Niekerk C, Matsioloko MT, du Preez FB, Myburg AA, Kunert K, Cullis CA (2003) Gene expression profiling during *Diuraphis noxia* infestation of *Triticum aestivum* cv 'Tugela DN' using microarrays. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 334–338

Botstein D, White RL, Skolnick M, Davis RW (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet 32:314–331

Bougot Y, Lemoine J, Pavoine MT, Barloy D, Doussinault G (2002) Identification of a microsatellite marker associated with *Pm3* resistance alleles to powdery mildew in wheat. Plant Breed 121:325–329

Boukhatem N, Baret PV, Mingeot D, Jacquemin JM (2002) Quantitative trait loci for resistance against yellow rust in two wheat–derived recombinant inbred populations. Theor Appl Genet 104:111–118

Bourdoncle W, Ohm HW (2003) Quantitative trait loci for resistance to Fusarium head blight in recombinant inbred wheat lines from the cross Huapei 57-2/Patterson. Euphytica 131:131–136

Boyko EV, Gill KS, Mickelson-Young L, Nasuda S, Raupp WJ, Yiegle JN, Singh S, Hassawi DS, Frity AK, Namuth D et al (1999) A high-density genetic linkage map of *Aegilops tauschii*, the D-genome progenitor of bread wheat. Theor Appl Genet 99:16–26

Boyko E, Kalendar R, Korzun V, Fellers J, Korol A, Schulman AH, Gill BS (2002) A high-density cytogenetic map of the *Aegilops tauschii* genome incorporating retrotransposons and defense-related genes: insights into cereal chromosome structure and function. Plant Mol Biol 48:767–790

Brading PA, Verstappen ECP, Kema GHJ, Brown JKM (2002) A gene–for–gene relationship between wheat and *Mycosphaerella graminicola,* the septoria tritici blotch pathogen. Phytopathology 92:439–445

Branlard G, Bancel E, Merlino M, Hamon I, Amiour N (2003) Proteome analysis of the soluble proteins of wheat kernels in ITMI progeny In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 221–224

Breiman A, Graur D (1995) Wheat evolution. Israel J Plant Sci 43:85–98

Brenner S, Johnson M, Bridgham J, Golda G, Lloyd DH, Johnson D, Luo S, McCurdy S, Foy M, Ewan M et al (2000) Gene expression analysis by massively parallel signature sequencing (MPSS) on microbead arrays. Nat Biotechnol 18:630–634

Briney A, Wilson R, Potter RH, Barclay I, Crosbie G, Appels R, Jones MGK (1998) A PCR-based marker for selection of starch and potential noodle quality in wheat. Mol Breed 1998 4:427–433

Brown-Guedira GL, Singh S, Fritz AK (2003) Performance and mapping of leaf rust resistance transferred to wheat from *Triticum timopheevii* subsp armeniacum. Phytopathology 93:784–789

Brueggeman R, Rostoks N, Kudrna D, Kilian A, Han F, Chen J, Druka A, Steffenson, B, Kleinhofs A (2002) The barley stem rust-resistance gene *Rpg1* is a novel disease- resistance gene with homology to receptor kinases. Proc Natl Acad Sci USA 99:9328–9333

Buckler ES, Thornsberry J (2002) Plant moleculer diversity and applications to genomics. Curr Opin Plant Biol 5:107–111

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

Buerstmayr H, Steiner B, Hartl L, Griesser M, Angerer N, Lengauer D, Miedaner T, Schneider B, Lemmens M (2003) Molecular mapping of QTLs for Fusarium head blight resistance in spring wheat. II. Resistance to fungal penetration and spread. Theor Appl Genet 107:503–508

Bullrich L, Appendino ML, Tranquilli G, Lewis S, Dubcovsky J (2002) Mapping of a thero-sensitive *earliness per se* gene on *Triticum monococcum* chromosome 1A^m. Theor Appl Genet 105:585–593

Cadalen T, Boeuf C, Bernard S, Bernard M (1997) An intervarietal molecular marker map in *Triticum aestivum* L em.

Thell and comparison with a map from wide cross. Theor Appl Genet 94:367–377

Cadalen T, Sourdille P, Charmet G, Tixier MH, Gay G, Boeuf C, Bernard S, Leroy P, Bernard M (1998) Molecular markers linked to genes affecting plant height in wheat using a doubled-haploid population. Theor Appl Genet 96:933–940

Cakir M, Appels R, Carter M, Loughman R, Francki M, Li C, Johnson J, Bhave M, Wilson R, McLean R et al (2003) Accelerated wheat breeding using molecular marker. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 117–120

Campbell KG, Bergman CJ, Gualberto DG, Anderson JA, Girox MJ, Hareland G, Fulcher RG, Sorrells ME Finney PL (1999) Quantitative trait loci associated with kernel traits in a soft × hard wheat cross. Crop Sci 39:1184–1195

Cao WG, Scoles G, Hucl P, Chibbar RN (2000) Phylogenetic relationships of five morphological groups of hexaploid wheat (*Triticum aestivum* L em Thell) based on RAPD analysis. Genome 43:724–727

Cao W, Hughes GR, Ma H, Dong Z (2001) Identification of molecular markers for resistance to *Septoria nodorum* blotch in durum wheat. Theor Appl Genet 102:551–554

Cao S, Guo X, Liu D, Zhang X, Zhang A (2003) Preliminary gene-mapping of photoperiod-temperature sensitive genic male sterility in wheat (*Triticum aestivum* L). In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 928–930

Carollo V, Matthews DE, Lazo GR, Anderson OD (2003) Wheat maps on GrainGenes: Past, present and coming attractions. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 225–228

Carter M, Drake-Brockman F, Cakir M, Jones M, Appels R (2003) Conversion of RFLP markers into PCR based markers in wheat. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 681–683

Cenci A, D'Ovidio R, Tanzarella OA, Ceoloni C, Porceddu E (1999) Identification of molecular markers linked to PM13, an Aegilops longissima gene conferring resistance to powdery mildew in wheat. Theor Appl Genet 98:448–454

Cenci A, Chantret N, Kong X, Gu Y, Anderson OD, Fahima T, Distelfeld A, Dubcovsky J (2003) Construction and characterization of a half million clone BAC library of durum wheat (*Triticum turgidum* ssp *durum*). Theor Appl Genet 107:931–939

Chagué V, Fahima T, Dahan A, Sun GL, Korol AB, Ronin YI, Grama A, Röder MS, Nevo E (1999) Isolation of microsatellite and RAPD markers flanking the *Yr15* gene of wheat using NILs and bulked segregant analysis. Genome 42:1050–1056

Chalmers KJ, Campbell AW, Kretschmer J, Karakousis A, Henschke PH, Pierens S, Harker N, Pallota M, Cornish GB, Shariflou MR et al (2001) Construction of three linkage maps in bread wheat, *Triticum aestivum*. Aust J Agric Res 52:1089–1119

Chao SP, Sharp PJ, Worland AJ, Warham EJ, Koebner RMD, Gale MD (1989) RFLP-based genetic maps of wheat homoeologous group 7 chromosomes. Theor Appl Genet 78:493–504

Chee PW, Elias EM, Anderson JA, Kianian SF (2001) Evaluation of a high grain protein QTL from *Triticum turgidum* L var *dicoccoides* in an adapted durum wheat background. Crop Sci 41:295–301

Chen HB, Martin JM, Lavin M, Talbert LE (1994) Genetic diversity in hard red spring wheat-based on sequence-tagged-site PCR markers. Crop Sci 34:1628–1632

Chen XM, Soria MA, Yan GP, Sun J, Dubcovsky J (2003) Development of sequence tagged site and cleaved amplified polymorphic sequence markers for wheat stripe rust resistance gene *Yr5*. Crop Sci 43:2058–2064

Cheobtar SV, Röder MS, Börner A, Sivolap YM (2003) Microsatellite analysis of Ukrainian wheat varieties cultivated in 1912–2002. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 57–60

Cherukuri DP, Gupta SK, Charpe A, Koul S, Prabhu KV, Singh RB, Haq QMR, Chauhan SVS (2003) Identification of a molecular marker linked to an *Agropyron elongatum*–derived gene *Lr19* for leaf rust resistance in wheat. Plant Breed 122:204–208

Christiansen MJ, Andersen SB, Ortiz R (2002) Diversity changes in an intensively bred wheat germplasm during the 20(th) century. Mol Breed 9:1–11

Coe EH, Neuffer MG (1993) Gene loci and linkage map of corn (maize) (*Zea mays* L.) (2n=20). In: O'Brian SJ (ed) Genetic maps, locus maps of complex genomes, 6th edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp 6.157–6.189

Conley EJ, Nduati V, Gonzalez-Hernandez JL, Mesfin A, Trudeau-Spanjers M, Chao S, Lazo GR, Hummel DD, Anderson OD, Qi LL et al (2004) A 2600-locus chromosome bin map of wheat homoeologous group 2 reveals interstitial gene-rich islands and colinearity with rice. Genetics 168:625–637

Corbellini M, Perenzin M, Accerbi M, Vaccino P, Borghi B (2002) Genetic diversity in bread wheat, as revealed by coefficient of parentage and molecular markers, and its relationship to hybrid performance. Euphytica 123:273–285

Cox DR, Burmeister M, Price ER, Kim S, Mayers RM (1990) Radiation hybrid mapping-a somatic-cell genetic method for constructing high-resolution maps of mammalian chromosomes. Science 250:245–250

Craven M, Prins R, Pretorius ZA (2003) Developement of AFLP markers for a wheat leaf rust resistance gene transferred from *Triticum monococcum*. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 1124–1126

Davis GL, McMullen MD, Baysdorfer C, Musket T, Grant D, Staebell M, Xu G, Polacco M, Koster L, Melia-Hancock S

et al (1999) A maize map standard with sequenced core markers, grass genome reference points and 932 expressed sequence tagged sites (ESTs) in a 1736-locus map. Genetics 152:1137–1172

Dear PH, Cook RR (1989) HAPPY mapping-a proposal for linkage mapping the human genome. Nucleic Acids Res 17:6795–6807

de Bustos A, Rubio P, Soler C, Garcia P, Jouve N (2001) Marker assisted selection to improve HMW-glutenins in wheat. Euphytica 119:69–73

Delaney DE, Nasuda S, Endo TR, Gill BS, Hulbert SH (1995a) Cytologically based physical maps of the group-2 chromosomes of wheat. Theor Appl Genet 91:568–573

Delaney DE, Nasuda S, Endo TR, Gill BS, Hulbert SH (1995b) Cytologically based physical maps of the group-3 chromosomes of wheat. Theor Appl Genet 91:780–782

de la Pena RC, Murray TD, Jones SS (1996) Linkage relations among eyespot resistance gene *Pch2*, endopeptidase *Ep-A1b*, and RFLP marker *Xpsr121* on chromosome 7A of wheat. Plant Breed 115:273–275

de la Pena RC, Murray TD, Jones SS (1997) Identification of an RFLP interval containing *Pch2* on chromosome 7AL in wheat. Genome 40:249–252

del Blanco IA, Frohberg RC, Stack RW, Berzonsky WA, Kianian SF (2003) Detection of QTL linked to Fusarium head blight resistance in Sumai 3-derived North Dakota bread wheat lines. Theor Appl Genet 106:1027–1031

Demeke T, Morris CF, Campbell KG, King GE, Anderson JA, Chang H-G (2001) Wheat polyphenol oxidase: distribution and genetic mapping in three inbred line populations. Crop Sci 41:1750–1757

Deng ZY, Zhang XQ, Wang XP, Jing JK, Wang DW (2004) Identification and molecular mapping of a stripe rust resistance gene from a common wheat line Qz180. Acta Bot Sin 46:236–241

Devos KM, Gale MD (1992) The use of random amplified polymorphic DNA markers in wheat. Theor Appl Genet 84:567–572

Devos KM, Gale MD (1993) Extended genetic maps of the homoeologous group-3 chromosomes of wheat, rye and barley. Theor Appl Genet 85:649–652

Devos KM, Gale MD (1997) Comparative genetics in the grasses. Plant Mol Biol 35:3–15

Devos KM, Atkinson MD, Chinoy CN, Liu CJ, Gale MD (1992) RFLP-based genetic map of the homoeologous group 3 chromosomes of wheat and rye. Theor Appl Genet 83:931–939

Devos KM, Atkinson MD, Chinoy CN, Francis HA, Harcourt RL, Koebner RMD, Liu CJ, Masojć P, Xie DX, Gale MD (1993a) Chromosomal rearrangement in the rye genome relative to that of wheat. Theor Appl Genet 85:673–680

Devos KM, Millan T, Gale MD (1993b) Comparative RFLP maps of the homoeologous group-2 chromosomes of wheat, rye and barley. Theor Appl Genet 85:784–792

Devos KM, Chao S, Li Y, Simonetti MC, Gale MD (1994) Relationship between chromosome 9 of maize and wheat homoeologous group 7 chromosomes. Genetics 138:1287–1292

Devos KM, Beales J (2003) Single nucleotide polymorphisms (SNPs) associated with the vernalization response in wheat In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 937–940

Dholakia BB, Ammiraju JSS, Santra DK, Singh H, Katti MV, Lagu MD, Tamhankar SA, Rao VS, Gupta VS, Dhaliwal HS, Ranjekar PK (2001) Molecular marker analysis of protein content using PCR-based markers in wheat. Biochem Genet 39:325–338

Dieguez MJ, Ingala L, Perera E, Sacco F, Naranjo T (2003) Physical mapping of AFLPs on chromosome 6BL of wheat, which includes the *Lr3* gene for leaf rust resistance. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 937–940

Dilbirligi M, Gill KS (2003) Identification and characterization of candidate expressed genes of wheat. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 940–942

Distelfeld A, Uauy C, Olmos S, Schlatter AR, Dubcovsky J, Fahima T (2004) Microcolinearity between a 2–cM region encompassing the grain protein content locus *Gpc-6B1* on wheat chromosome 6B and a 350 kb region on rice chromosome 2. Funct Integr Genom 4:59–66

Donini P, Elias ML, Bougourd SM, Koebner RMD (1997) AFLP fingerprinting reveals pattern differences between template DNA extracted from different plant organs. Genome 40:521–526

Donini P, Stephenson P, Bryan GJ, Koebner RMD (1998) The potential of microsatellites for high throughput genetic diversity assessment in wheat and barley. Genet Resource Crop Evol 45:415–421

Donini P, Law JR, Koebner RMD, Reeves JC, Cooke RJ (2000) Temporal trends in the diversity of UK wheat. Theor Appl Genet 100:912–917

Dreher K, Khairallah M, Ribaut JM, Morris M (2003) Money matters (I): costs of field and laboratory procedures associated with conventional and marker-assisted maize breeding at CIMMYT. Mol Breed 11:221–234

Dreisigacker S, Zhang P, Warburton ML, Van Ginkel M, Hoisington D, Bohn M, Melchinger AE (2003) SSR and pedigree analyses of genetic diversity among CIMMYT wheat lines targeted to different megaenvironments. Crop Sci 44:381–388

Driscoll CJ (1966) Gene-centromere distances in wheat by aneuploid F2 observations. Genetics 54:131–135

Dubcovsky J, Luo MC, Zhong GY, Bransteitter R, Desai A, Kilian A, Kleinhofs A, Dvorak J (1996) Genetic map of diploid wheat, *Triticum monococcum* L., and its comparison with maps of *Hordeum vulgare* L. Genetics 143:983–999

Dunford RP, Kurata N, Laurie DA, Money TA, Minobe Y, Moore G (1995) Conservation of fine-scale DNA marker order in

the genomes of rice and the Triticeae. Nucleic Acids Res 23:2724–2728

Dvorák J, Luo M-C, Yang Z-L, Zhang H-B (1998a) The structure of the *Aegilops tauschii* genepool and the evolution of hexaploid wheat. Theor Appl Genet 97:657–670

Dvorák J, Luo MC, Yang ZL (1998b) Restriction fragment length polymorphism and divergence in the genomic regions of high and low recombination in self-fertilizing and cross-fertilizing Aegilops species. Genetics 148:423–434

Dvorák J, Akhunov ED, Akhunov AR, Luo M-C, Linkiewicz AM, Dubcovsky J, Hummel D, Lazo G, Chao S, Anderson OD et al (2003) New insights into the organization and evolution of wheat genomes. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 247–253

Dweikat I, Ohm H, Patterson F, Cambron S (1997) Identification of RAPD markers for 11 Hessian fly resistance genes in wheat. Theor Appl Genet 94:419–423

Eagles HA, Bariana HS, Ogbonnaya FC, Rebetzke GJ, Hollamby GJ, Henry RJ, Henschke PH, Carter M (2001) Implementation of markers in Australian wheat breeding. Aust J Agric Res 52:1349–1356

Effertz RJ, Meinhardt SW, Anderson JA, Jordahl JG, Francl LJ (2002) Identification of a chlorosis-inducing toxin from *Pyrenophora tritici-repentis* and the chromosomal location of an insensitivity locus in wheat. Phytopathology 92:527–533

Ellis MH, Spielmeyer W, Gale KR, Rebetzke GJ, Richards RA (2002) "Perfect" markers for the *RhtB1b* and *RhtD1b* dwarfing genes in wheat. Theor Appl Genet 105:1038–1042

Elouafi I, Nachit MM (2004) A genetic linkage map of the Durum x *Triticum dicoccoides* backcross population based on SSRs and AFLP markers, and QTL analysis for milling traits. Theor Appl Genet 108:401–413

Endo TR, Gill BS (1996) The deletion stocks of common wheat. J Hered 87:295–307

Eriksen L, Afshari F, Christiansen MJ, McIntosh RA, Jahoor A, Wellings CR (2003a) *Yr32* for resistance to stripe (yellow) rust present in the wheat cultivar Carstens V. Theor Appl Genet 108:567–575

Eriksen L, Borum F, Jahoor A (2003b) Inheritance and localisation of resistance to *Mycosphaerella graminicola* causing septoria tritici blotch and plant height in the wheat (*Triticum aestivum* L) genome with DNA markers. Theor Appl Genet 107:415–527

Eujayl I, Sorrells M, Baum M, Wolters P, Powell W (2001) Assessment of genotypic variation among cultivated durum wheat based on EST-SSRS and genomic SSRs. Euphytica 119:39–43

Fahima T, Röder MS, Grama A, Nevo E (1998) Microsatellite DNA polymorphism divergence in *Triticum dicoccoides* accessions highly resistant to yellow rust. Theor Appl Genet 96:187–195

Fahima T, Sun GL, Beharav A, Krugman T, Beiles A, Nevo E (1999) RAPD polymorphism of wild emmer wheat populations, *Triticum dicoccoides*, in Israel. Theor Appl Genet 98:434–447

Fahima T, Röder MS, Wendehake K, Kirzhner VM, Nevo E (2002) Microsatellite polymorphism in natural populations of wild emmer wheat, *Triticum dicoccoides*, in Israel. Theor Appl Genet 104:17–29

Fahima T, Ramachandran S, Krugman T, Röder MS, Nevo E, Feldman MW (2003) Estimation of domestication times of wheat and barley based on microsatellite polymorphism. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 481–483

Faris JD, Anderson JA, Francl LJ, Jordahl JG (1997) RFLP mapping of resistance to chlorosis induction by Pyrenophora tritici-repentis in wheat. Theor Appl Genet 94:98–103

Faris JD, Haen KM, Gill BS (2000) Saturation mapping of a gene-rich recombination hot spot region in wheat. Genetics154:823–835

Faris JD, Fellers JP, Brooks SA, Gill BS (2003) A bacterial artificial chromosome contig spanning the major domestication locus *Q* in wheat and identification of a candidate gene. Genetics 164:311–321

Feuillet C, Keller B (1999) High gene density is conserved at syntenic loci of small and large grass genomes. Proc Natl Acad Sci USA 96:8265–8270

Feuillet C, Keller B (2002) Comparative genomics in the grass family: molecular characterization of grass genome structure and evolution. Ann Bot 89:3–10

Feuillet, C, Travella S, Stein N, Albar L, Nublat A, Keller B (2003) Map-based isolation of the leaf rust disease resistance gene *Lr10* from the hexaploid wheat (*Triticum aestivum* L) genome. Proc Nat Acad Sci USA 100:15253–15258

Fischer G, Ibrahim SM, Brockmann GA, Pahnke J, Bartocci E, Thiesen H-J, Serrano-Fernandez P, Möller S (2003) Expressionview: visualization of quantitative trait loci and gene-expression data in Ensembl. Genome Biol 4:R477

Flint-Garcia SA, Thornsberry JM, Buckler IV ES (2003) Structure of linkage disequilbrium in plants. Annu Rev Plant Biol 54:357–374

Francki MG, Appels R, Hunter A, Bellgard M (2003) Comparative organization of 3BS and 7AL using wheat-rice synteny. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 254–257

Friebe B, Jiang J, Raupp J, McIntosh RA, Gill BS (1996) Characterization of wheat-alien translocations conferring resistance to disease and pests: current status. Euphytica 91:59–87

Gale MD, Atkinson MD, Chinoy CN, Harcourt R, Jia J, Li QY, Devos KM (1995) Genetic maps of hexaploid wheat. In: Chen S (ed) Proc 8th Int Wheat Genet Symp. China Agricultural Scientech Press, Beijing, pp 29–40

Gale MD, Devos KM (1998) Comparative genetics in the grasses. Proc Natl Acad Sci USA 95:1971–1974

Galiba G, Quarrie SA, Sutka J, Morgunov A, Snape JW (1995) RFLP mapping of the vernalisation (*Vrn1*) and frost resis-

tance (*Fr1*) genes on chromosome 5A of wheat. Theor Appl Genet 90:1174–1179

Gallego F, Feuillet C, Messmer M, Penger A, Graner A, Yano M, Sasaki T, Keller B (1998) Comparative mapping of the two wheat leaf rust resistance loci *Lr1* and *Lr10* in rice and barley. Genome 41:328–336

Gandon B, Chiquet V, Guyomarc'h H, Baron C, Sourdille P, Specel S, Foisset N, Murigneux A, Dufour P, Bernard M (2002) Development of microsatellite markers for wheat genetic mapping improvement. In: Plant, Animal & Microbe Genomes X Conf, San Diego, CA. http://www.intl-pag.org/pag/10/abstracts/PAGX_P187.html

Gao LF, Tang JF, Li HW, Jia JZ (2003) Analysis of microsatellites in major crops assessed by computational and experimental approaches. Mol Breed 12:245–261

Gao LF, Jing RL, Huo NX, Li Y, Li XP, Zhou RH, Chang XP, Tang JF, Ma ZW, Jia JZ (2004) One hundred and one new microsatellite loci derived from ESTs (EST-SSRs) in bread wheat. Theor Appl Genet 108:1392–1400

Gardiner J, Schroeder S, Polacco ML, Sanchez-Villeda H, Fang ZW, Morgante M, Landewe T, Fengler K, Useche F, Hanafey M, Tingey S, Chou H, Wing R, Soderlund C, Coe EH (2004) Anchoring 9,371 maize expressed sequence tagged unigenes to the bacterial artificial chromosome contig map by two-dimensional overgo hybridization. Plant Physiol 134:1317–1326

Garg M, Singh S, Singh B, Singh K, Dhaliwal HS (2001) Estimates of genetic similarities and DNA fingerprinting of wheats (*Tritium* species) and triticale cultivars using molecular markers. Indian J Agril Sci 71:438–443

Gaut BS (2001) Patterns of chromosomal duplication in maize and their implications for comparative maps of the grasses. Genome Res 11:55–66

Gaut BS (2002) Evolutionary dynamics of grass genomes. New Phytologist 154:15–28

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

Giese H, Holm-Jensen AG, Mathiassen H, Kjær B, Rasmussen SK, Bay H, Jensen J (1994) Distribution of RAPD markers on a linkage map of barley. Hereditas 120:267–273

Gill KS (2004) Gene distribution in cereal genomes In: Gupta PK, Varshney RK (eds) Cereal Genomics. Kluwer, Dordrecht, pp 361–38

Gill KS, Gill BS (1994) Mapping in the realm of polyploidy: the wheat model. BioEssays 16:841–846

Gill KS, Gill BS, Endo TR (1993) A chromosome region-specific mapping strategy reveals gene-rich telomeric ends in wheat. Chromosoma 102:374–381

Gill KS, Gill BS, Endo TR, Taylor T (1996a) Identification and high-density mapping of gene-rich regions in chromosome group 1 of wheat. Genetics 144:1883–1891

Gill KS, Gill BS, Boyko EV (1996b) Identification and high density mapping of gene-rich regions in chromosome group 5 of wheat. Genetics 143:1001–1012

Gill BS, Qi L, Echalier B, Chao S, Lazo G, Anderson OD, Akhunov ED, Dvorak J, Linkiewicz AM, Dubcovsky J et al (2003) A transcriptome map of wheat. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 261–264

Gilpin BJ, McCallum JA, Frew GM, Timmerman-Vaughan GM (1997) A linkage map of the pea (*Pisum sativum* L) genome containing cloned sequences of known functions and expressed sequence tags (ESTs). Theor Appl Genet 95:1289–1299

Gladysz A, Steiner B, Castro M, Burestmayr H (2003) Transfer of QTLs for resistance to Fusarium head blight from bread wheat into durum wheat by marker-assisted breeding. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 715–717

Goff SA, Ricke D, Lan TH, Presting G, Wang RL, Dunn M, Glazebrook J, Sessions A, Oeller P, Varma H et al (2002) A draft sequence of the rice genome (*Oryza sativa* L ssp *japonica*). Science 296:92–100

Gold J, Harder D, Townley–Smith F, Aung T, Procunier J (1999) Development of a molecular marker for rust resistance genes *Sr39* and *Lr35* in wheat breeding lines. Electr J Biotech 2:35–40

Graner A, Jahoor A, Schondelmaier J, Siedler H, Pillen K, Fischbeck G, Wenzel G, Herrmann RG (1991) Construction of an RFLP map of barley. Theor Appl Genet 83:250–256

Groenewald JZ, Marais AS, Marais GF (2003) Amplified fragment length polymorphism-derived microsatellite sequence linked to the *Pch1* and *Ep-D1* loci in common wheat. Plant Breed 122:83–85

Groos C, Gay G, Perretant MR, Gervais L, Bernard M, Dedryver F, Charmet D (2002) Study of the relationship between pre-harvest sprouting and grain color by quantitative trait loci analysis in a whitexred grain bread-wheat cross. Theor Appl Genet 104:39–47

Grunberg AM, Costa JM, Kratochvil RJ (2001) Amplified fragment length polymorphism in a selected sample of soft red winter wheat. Cereal Res Commun 29:251–258

Gudu S, Laurie DA, Kasha KJ, Xia JJ, Snape JW (2002) RFLP mapping of a Hordeum bulbosum gene highly expressed in pistils and its relationship to homoeologous loci in other Gramineae species. Theor Appl Genet 105:271–276

Guo PG, Bai GH, Shaner GE (2003) AFLP and STS tagging of a major QTL for Fusarium head blight resistance in wheat. Theor Appl Genet 106:1011–1017

Gupta PK, Varshney RK (2000) The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. Euphytica 113:163–185

Gupta PK, Varshney RK (2004) Cereal genomics: An overview. In: Gupta PK, Varshney RK (eds) Cereal Genomics. Kluwer, Dordrecht, pp 1–18

Gupta PK, Varshney RK, Sharma PC, Ramesh B (1999) Molecular markers and their applications in wheat breeding. Plant Breed 118:369–390

Gupta PK, Balyan HS, Edwards KJ, Isaac P, Korzun V, Röder M, Gautier MF, Joudrier P, Schlatter AR, Dubcovsky J et al (2002a) Genetic mapping of 66 new microsatellite (SSR) loci in bread wheat. Theor Appl Genet 105:413–422

Gupta PK, Varshney RK, Prasad M (2002b) Molecular markers: principles and methodology. In: Jain SM, Brar DS, Ahloowalia BS (eds) Molecular Techniques in Crop Improvement. Kluwer, Dordrecht, pp 9–54

Gupta PK, Rustgi S, Sharma S, Singh R, Kumar N, Balyan HS (2003) Transferable EST-SSR markers for the study of polymorphism and genetic diversity in bread wheat. Mol Gen Genom 270:315–323

Guyomarc'h H, Sourdille P, Edwards KJ, Bernard M (2002) Studies of the transferability of microsatellites derived from Triticum tauschii to hexaploid wheat and to diploid related species using amplification, hybridization and sequence comparisons. Theor Appl Genet 105:736–744

Guyot R, Yahiaoui N, Feuillet C, Keller B (2004) In silico comparative analysis reveals a mosaic conservation of genes within a novel colinear region in wheat chromosome 1AS and rice chromosome 5S. Funct Integr Genom 4:47–58

Han F, Fedak G, Ouellet T, Somers D (2003) Isolation, characterization and physical mapping of differential clones from SSH library for Fusarium head blight (FHB) resistance In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 952–954

Harker N, Rampling LR, Shariflou MR, Hayden MJ, Holton TA, Morell MK, Sharp PJ, Henry RJ, Edwards KJ (2001) Microsatellites as markers for Australian wheat improvement. Aust J Agric Res 52:1121–1130

Hartl L, Weiss H, Zeller FJ, Jahoor A (1993) Use of RFLP markers for the identification of alleles of the Pm3 locus conferring powdery mildew resistance in wheat (Triticum aestivum L). Theor Appl Genet 86:959–963

Hartl L, Weiss H, Stephan U, Zeller FJ, Jahoor A (1995) Molecular identification of powdery mildew resistance genes in common wheat (Triticum aestivum L). Theor Appl Genet 90:601–606

Hartl L, Mohler V, Zeller FJ, Hsam SLK, Schweizer G (1999) Identification of AFLP markers closely linked to the powdery mildew resistance genes Pm1c and Pm4a in common wheat (Triticum aestivum L). Genome 42:322–329

Harushima Y, Yano M, Shomura A, Sato M, Shimono T, Kuboki Y, Yamamoto T, Lin SY, Antonio BA, Parco A et al (1998) A high density rice genetic linkage map with 2275 markers using a single F_2 population. Genetics 148:479–494

Hazen SP, Leroy P, Ward RW (2002) AFLP in Triticum aestivum L: patterns of genetic diversity and genome distribution. Euphytica 125:89–102

Helguera M, Khan IA, Dubcovsky J (2000) Development of PCR markers for wheat leaf rust resistance gene Lr47. Theor Appl Genet 101:625–631

Helguera M, Khan IA, Kolmer J, Lijavetzky D, Zhong-qi L, Dubcovsky J (2003) PCR assays for the Lr37-Yr17-Sr38 cluster of rust resistance genes and their use to develop isogenic hard red spring wheat lines. Crop Sci 43:1839–1847

Hernandez P, Dorado G, Prieto P, Gimenez MJ, Ramirez MC, Laurie DA, Snape JW, Martin A (2001) A core genetic map of Hordeum chilense and comparisons with maps of barley (Hordeum vulgare) and wheat (Triticum aestivum). Theor Appl Genet 102:1259–1264

Heun M, SchaferPregl R, Klawan D, Castagna R, Accerbi M, Borghi B, Salamini F (1997) Site of einkorn wheat domestication identified by DNA fingerprinting. Science 278:1312–1314

Hohmann U, Graner A, Endo TR, Gill BS, Herrmann RG (1995) Comparison of wheat physical maps with barley linkage maps for group 7 chromosomes. Theor Appl Genet 91:618–626

Holton TA, Christopher JT, McClure L, Harker N, Henry RJ (2002) Identification and mapping of polymorphic SSR markers from expressed gene sequences of barley and wheat. Mol Breed 9:63–71

Hossain KG, Kalavacharla V, Lazo GR, Hegstad J, Wentz MJ, Kianian PMA, Simons K, Gehlhar S, Rust JL, Syamala RR et al (2004a) A chromosome bin map of 2,148 expressed sequence tag loci of wheat homoeologous group 7. Genetics 168:687–699

Hossain KG, Riera-Lizarazu O, Kalavacharla V, Vales MI, Rust JL, Maan SS, Kianian SF (2004b) Molecular cytogenetic characterization of an alloplasmic durum wheat line with a portion of chromosome 1D of Triticum aestivum carrying the scs^{ae} gene. Genome 47:206–214

Houshmand S, Knox RE, Clarke FR, Clarke JM (2003) Microsatellite markers associated with sawfly cutting in durum wheat. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 1151–1153

Hu XY, Ohm HW, Dweikat I (1997) Identification of RAPD markers linked to the gene Pm1 for resistance to powdery mildew in wheat. Theor Appl Genet 94:832–840

Huang L, Gill BS (2001) An RGA like marker detects all known Lr21 leaf rust resistance gene family members in Aegilops tauschii and wheat. Theor Appl Genet 103:1007–1013

Huang XQ, Röder MS (2003) High-denisty genetic and physical mapping of the powdery mildew resistance gene Pm24 on chromosome 1D of wheat. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 961–964

Huang XQ, Hsam SLK, Zeller FJ, Wenzel G, Mohler V (2000) Molecular mapping of the wheat powdery mildew resistance gene Pm24 and marker validation for molecular breeding. Theor Appl Genet 101:407–414

Huang XQ, Börner A, Röder MS, Ganal MW (2002) Assessing genetic diversity of wheat (Triticum aestivum L) germplasm using microsatellite markers. Theor Appl Genet 105:699–707

Huang L, Brooks SA, Li W, Fellers JP, Trick HN, Gill BS (2003a) Map-based cloning of leaf rust resistance gene *Lr21* from the large and polyploid genome of bread wheat. Genetics 164:655–664

Huang XQ, Cöster H, Ganal MW, Röder MS (2003b) Advanced backcross QTL analysis for the identification of quantitative trait loci alleles from wild relatives of wheat (*Triticum aestivum* L). Theor Appl Genet 106:1379–1389

Huang XQ, Wang LX, Xu MX, Röder MS (2003c) Microsatellite mapping of the powdery mildew resistance gene *Pm5e* in common wheat (*Triticum aestivum* L). Theor Appl Genet 106:858–865

Huang XQ, Kempf H, Ganal MW, Röder MS (2004) Advanced backcross QTL analysis in progenies derived from a cross between a German elite winter wheat variety and a synthetic wheat (*Triticum aestivum* L.). Theor Appl Genet 109:933–941

Hulbert SH, Richter TE, Axtell JD, Bennetzen JL (1990) Genetic mapping and characterization of sorghum and related crops by means of maize DNA probes. Proc Natl Acad Sci USA 87:4251–4255

Incirli A, Akkaya MS (2001) Assessment of genetic relationships in durum wheat cultivars using AFLP markers. Genet Resource Crop Evol 48:233–238

Ishii T, Mori N, Ogihara Y (2001) Evaluation of allelic diversity at chloroplast microsatellite loci among common wheat and its ancestral species. Theor Appl Genet 103:896–904

Ivandic V, Malyshev V, Korzun V, Graner A, Börner A (1998) Comparative mapping of a gibberelic acid insensitive dwarfing gene (*Dwf2*) on chromosome 4HS of barley. Theor Appl Genet 98 :728–731

Iwaki K, Nishida J, Yanagisawa T, Yoshida H, Kato K (2002) Genetic analysis of *Vrn-B1* for vernalization requirement by using linked dCAPS markers in bread wheat (*Triticum aestivum* L). Theor Appl Genet 104:571–576

Jahoor A, Eriksen L, Backes G (2004) QTLs and genes for disease resistance in barley and wheat. In: Gupta PK, Varshney RK (eds) Cereal Genomics. Kluwer, Dordrecht, pp 199–252

Jansen RC (2003) Studying complex biological systems using multifactorial perturbation. Nature Rev Genet 4:145–151

Jansen RC, Nap J-P (2001) Genetical genomics: the added value from segregation Trends Genet 17:388–391

Järve K, Peusha HO, Tsymbalova J, Tamm S, Devos KM, Enno TM (2000) Chromosomal location of a *Triticum timopheevii*-derived powdery mildew resistance gene transferred to common wheat. Genome 43:377–381

Jia J, Devos KM, Chao S, Miller TE, Reader SM, Gale MD (1996) RFLP-based maps of the homoeologous group-6 chromosomes of wheat and their application in the tagging of *Pm12*, a powdery mildew resistance gene transferred from *Aegilops speltoides* to wheat. Theor Appl Genet 92:559–565

Jiang JM, Gill BS (1994) Nonisotopic in-situ hybridization and plant genome mapping – the first 10 years. Genome 37:717–725

Joshi CP, Nguyen HT (1993) Application of the random amplified polymorphic DNA technique for the detection of polymorphism among wild and cultivated tetraploid wheats. Genome 36:602–609

Jurman I, Castelluccio MD, Wolf M, Olivieri A, DeAmbrogio E, Morgante M (2003) Construction of an SSR-based linkage map of durum wheat. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 968–970

Kantety RV, Rota ML, Matthews DE, Sorrells ME (2002) Data mining for simple sequence repeats in expressed sequence tags from barley, maize, rice, sorghum and wheat. Plant Mol Biol 48:501–510

Kato K, Miura H, Sawada S (1999) Comparative mapping of the wheat *Vrn-A1* region with the rice *Hd-6* region. Genome 42:204–209

Kato K, Miura H, Sawada S (2000) Mapping QTLs controlling grain yield and its components on chromosome 5A of wheat. Theor Appl Genet 101:1114–1121

Kato K, Nakamura W, Tabiki T, Miura H, Sawada S (2001) Detection of loci controlling seed dormancy on group 4 chromosomes of wheat and comparative mapping with rice and barley genomes. Theor Appl Genet 102:980–985

Kato K, Yamashita M, Ishimoto K, Yoshino H, Fujita M (2003) Genetic analysis of two genes for vernalization response, the former *Vrn2* and *Vrn4*, by using PCR-based molecular markers. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 971–973

Keller B, Feuillet C (2000) Colinearity and gene density in grass genomes. Trends Plant Sci 5:246–251

Keller M, Karutz C, Schmid JE, Stamp P, Winzeler M, Keller B, Messmer MM (1999a) Quantitative trait loci for lodging resistance in a segregating wheat × spelt population. Theor Appl Genet 98:1171–1182

Keller M, Keller B, Schachermayr G, Winzeler M, Schmid JE, Stamp P, Messmer MM (1999b) Quantitative trait loci for resistance against powdery mildew in a segregating wheat × spelt population. Theor Appl Genet 98:903–912

Khan AA, Bergstrom GC, Nelson JC, Sorrells ME (2000a) Identification of RFLP markers for resistance to wheat spindle streak mosaic bymovirus (WSSMV) disease. Genome 43:477–482

Khan IA, Procunier JD, Humphreys DG, Tranquilli G, Schlatter AR, Marcucci-Poltri S, Frohberg R, Dubcovsky J (2000b) Development of PCR-based markers for a high grain protein content gene from *Triticum turgidum* ssp. *dicoccoides* transferred to bread wheat. Crop Sci 40:518–524

Khlestkina EK, Than MHM, Pestsova EG, Röder MS, Malyshev SV, Korzun V, Börner A (2004) Mapping of 99 microsatellite loci in rye (*Secale cereale* L) including 39 expressed sequence tags. Theor Appl Genet 109:725–732

Kianian SF, Hossain KG, Riera-Lizarazu O, Kalavacharla V, Vales MI, Maan SS (2003) Radiation hybrid mapping of a species cytoplasm specific (*scs^ae*)gene in wheat. In: Pogna NE, Ro-

mano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 595–597

Kim HS, Ward RW (1997) Genetic diversity in Eastern US soft winter wheat (*Triticum aestivum* L em Thell) based on RFLPs and coefficients of parentage. Theor Appl Genet 94:472–479

Klein PE, Klein RR, Cartinhour SW, Ulanch PE, Dong J, Obert JA, Morishge DT, Schlueter SD, Childs KL, Ale M et al (2000) A high throughput AFLP based method for constructing integrated genetic and physical maps: progress toward a sorghum genome map. Genome Res 10:789–807

Knox RE, Menzies JG, Howes NK, Clarke JM, Aung T, Penner GA (2002) Genetic analysis of resistance to loose smut and an associated DNA marker in durum wheat doubled haploids. Can J Plant Pathol 24:316–322

Kobiljski B, Quarrie S, Dencic S, Kirby J, Iveges M (2002) Genetic diversity of the Novi Sad Wheat Core Collection revealed by microsatellites. Cell Mol Biol Lett 7:685–694

Koebner RMD (2004) Marker-assisted selection in the cereals: The dream and the reality. In: Gupta PK, Varshney RK (eds) Cereal Genomics. Kluwer, Dordrecht, pp 317–330

Koebner R, Summers R (2003) 21st century wheat breeding: selection in plots or detection in plates? Trends Biotech 21:59–63

Koebner RMD, Powell W, Donini P (2001) The contribution of current and forthcoming DNA molecular marker technologies to wheat and barley genetics and breeding In: Janick J (ed) Plant Breed Rev 21, pp 181–220

Koebner RMD, Donini P, Reeves JC, Cooke RJ, Law JR (2003) Temporal flux in the morphological and molecular diversity of UK barley. Theor Appl Genet 106:550–558

Kojima T, Nagaoka T, Noda K, Ogihara Y (1998) Genetic linkage map of ISSR and RAPD markers in einkorn wheat in relation to that of RFLP markers. Theor Appl Genet 96:37–45

Kojima S, Takahashi Y, Kobayashi Y, Monna L, Sasaki T, Araki T, Yano M (2002) Hd3a, a rice ortholog of the Arabidopsis FT gene, promotes transition to flowering downstream of *Hd1* under short-day conditions. Plant Cell Physiol 43:1096–1105

Kong LR, Dong YC, Jia JZ (1998) Random amplified polymorphism of DNA analysis in *Aegilops tauschii*. Acta Bot Sin 40:223–227

Korzun V, Malyshev S, Voylokov A, Börner A (1997a) RFLP based mapping of three mutant loci in rye (*Secale cereale* L.) and their relation to homoeologous loci within the *Gramineae*. Theor Appl Genet 95:468–473

Korzun V, Röder M, Worland AJ, Börner A (1997b) Mapping of the dwarfing (*Rht12*) and vernalisation response (*Vrn1*) genes in wheat by using RFLP and microsatellite markers. Plant Breed 116:227–232

Korzun V, Röder MS, Ganal MW, Worland AJ, Law CN (1998) Genetic analysis of the dwarfing gene (*Rht8*) in wheat. Part I: Molecular mapping of Rht8 on the short arm of chromosome 2D of bread wheat (*Triticum aestivum* L). Theor Appl Genet 96:1104–1109

Korzun V, Röder MS, Wendehake K, Pasqualone A, Lotti C, Ganal MW, Blanco A (1999) Integration of dinucleotide microsatellites from hexaploid bread wheat into a genetic linkage map of durum wheat. Theor Appl Genet 98:1202–1207

Kota RS, Gill KS, Gill BS, Endo TR (1993) A cytogenetically based physical map of chromosome-1B in common wheat. Genome 36:548–554

Kraic J, Silkova S, Hudcovicova, Gregova E, Bartos P (2003) Leaf rust resistant wheat lines developed by marker-assisted selection. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 742–745

Kuchel H, Wraner P, Fox RL, Chalmers K, Howes N, Langridge P, Jefferies SP (2003) Whole genome based marker assisted selection strategies in wheat breeding. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 144–147

Kulwal P, Singh R, Balyan HS, Gupta PK (2004) Genetic basis of pre-harvest sprouting tolerance using single-locus and two-locus QTL analyses in bread wheat. Funct Integr Genom 4:94–101

Kurata N, Nagamura Y, Yamamoto K, Harushima Y, Sue N, Wu J, Antonio BA, Shomura A, Shimizu T, Lin et al (1994) A 300 kolobase interval genetic map of rice including 883 expressed sequences. Nat Genet 8:365–372

Lage J, Warburton ML, Crossa J, Skovmand B, Andersen SB (2003) Assessment of genetic diversity in synthetic hexaploid wheats and their *Triticum dicoccum* and *Aegilops tauschii* parents using AFLPs and agronomic traits. Euphytica 134:305–317

Lagudah ES, Dubcovsky J, Powell W (2001) Wheat genomics. Plant Physiol Biochem 39:335–344

Lamoureux D, Boeuf C, Regad F, Garsmeur O, Charmet G, Sourdille P, Lagoda P, Bernard M (2002) Comparative mapping of the wheat 5B short chromosome arm distal region with rice, relative to a crossability locus. Theor Appl Genet 105:759–765

Langridge P, Chalmers K (1998) Techniques for marker development. In: Slinkard AE (ed) Proc 9th Int Wheat Genet Symp, Vol 1. University Extension Press, University of Saskatchewan, Saskatoon, Canada, pp 107–117

Langridge P, Chalmers K (2004) The Principle: Identification and application of molecular markers. In: Lörz H, Wenzel G (eds) Biotechnology in Agriculture and Forestry, Vol 55. Molecular markers systems. Springer, Berlin Heidelberg New York, pp 3–22

Langridge P, Lagudah ES, Holton TA, Appels R, Sharp PJ, Chalmers KJ (2001) Trends in genetic and genome analyses in wheat: a review. Aust J Agric Res 52:1043–1077

Laroche A, Demeke T, Gaudet DA, Puchalski B, Frick M, McKenzie R (2000) Development of a PCR marker for rapid identification of the *Bt-10* gene for common bunt resistance in wheat. Genome 43:217–223

La Rota CM, Sorrells ME (2004) Comparative DNA sequence analysis of mapped wheat ESTs reveals complexity of genome relationships between rice and wheat. Funct Integr Genom 4:34–46

Laubin B, Nicot N, Amiour N, Sourdille P, Branlard G, Leroy P (2003) *In silico* mapping and colinearity between the homoeologous group 5 of wheat and the rice genome. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 280–283

Law JR, Donini P, Koebner RMD, James CR, Cooke RJ (1998) DNA profiling and plant variety registration. III: The statistical assessment of distinctness in wheat using amplified fragment length polymorphisms. Euphytica 102:335–342

Lazo GR, Chao S, Hummel D, Edwards H, Crosman CC, Lui N, Matthews DE, Carollo VL, Hane DL, You FM et al (2004) Development of an expressed sequence tag (EST) resource for wheat (*Triticum aestivum*): EST generation, unigene analysis, probe selection and bioinformatics for a 16,000 locus bin-delineated map. Genetics 168:585–593

Leader DJ, Cullup T, Ridley P, van Dodeweerd A-M (2003) Microarray analysis of wheat grain development: applications to trait charcterization in the field. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 287–292

Lee M (1995) DNA markers and plant breeding programs. Adv Agron 55:265–344

Leigh F, Lea V, Law J, Wolters P, Powell W, Donini P (2003) Assessment of EST- and genomic microsatellite markers for variety discrimination and genetic diversity studies in wheat. Euphytica 133:359–366

Leister D, Kurth J, Laurie DA, Yano M, Sasaki T, Devos K, Graner A, Schulze-Lefert P (1998) Rapid reorganization of resistance gene homologues in cereal genomes. Proc Natl Acad Sci USA 95:370–375

Li YC, Fahima T, Beiles A, Korol AB, Nevo E (1999) Microclimatic stress and adaptive DNA differentiation in wild emmer wheat, *Triticum dicoccoides*. Theor Appl Genet 98:873–883

Li W, Nelson JC, Chu CY, Shi LH, Huang SH, Liu DJ (2002a) Chromosomal locations and genetic relationships of tiller and spike characters in wheat. Euphytica 125:357–366

Li YC, Röder MS, Fahima T, Kirzhner VM, Beiles A, Korol AB, Nevo E (2002b) Climatic effects on microsatellite diversity in wild emmer wheat (*Triticum dicoccoides*) at the Yehudiyya microsite, Israel. Heredity 89:127–132

Li C, Ni P, Francki M, Hunter M, Zhang Y, Schibeci D, Li H, Tarr A, Wang J, Cakir M et al. (2004a) Genes controlling seed dormancy and pre-harvest sprouting in a rice-wheat-barley comparison. Funct Integr Genom 4:84–93

Li Z, Huang N, Rampling L, Wang J, Yu J, Morell M, Rahman S (2004b) Detailed comparison between the wheat chromosome group 7 short arms and the rice chromosome arms 6S and 8L with special reference to genes involved in starch biosynthesis. Funct Integr Genom 4:231–240

Ling H-Q, Zhu Y, Keller B (2003) High–resolution mapping of the leaf rust disease resistance gene *Lr1* in wheat and characterisation of BAC clones from the *Lr1* locus. Theor Appl Genet 3:875–882

Linkiewicz AM, Qi L, Echalier B, Gill BS, Chao S, Lazo G, Anderson OD, Akhunov ED, Dvorak J, Miftahudin et al. (2003) A two thousand loci physical map of wheat homoeologous group 5. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 986–988

Linkiewicz AM, Qi LL, Gill BS, Ratnasiri A, Echalier B, Chao S, Lazo G, Hummel DD, Anderson OD, Akhunov ED et al (2004) A 2,500-locus bin map of wheat homoeologous group 5 provides insights on gene distribution and colinearity with rice. Genetics 168:665–676

Liu YG, Tsunewaki K (1991) Restriction fragment length polymorphism (RFLP) analysis in wheat II Linkage maps of the RFLP sites in common wheat. Jpn J Genet 66:617–633

Liu SX, Anderson JA (2003a) Marker assisted evaluation of Fusarium head blight resistant wheat germplasm. Crop Sci 43:760–766

Liu SX, Anderson JA (2003b) Targeted molecular mapping of a major wheat QTL for Fusarium head blight resistance using wheat ESTs and synteny with rice. Genome 46:817–823

Liu Z, Sun Q, Ni Z, Yang T (1999a) Development of SCAR markers linked to the *Pm21* gene conferring resistance to powdery mildew in common wheat. Plant Breed 118:215–219

Liu ZQ, Pei Y, Pu ZJ (1999b) Relationship between hybrid performance and genetic diversity based on RAPD markers in wheat, *Triticum aestivum* L. Plant Breed 118:119–123

Liu J, Liu D, Tao W, Li W, Wang S, Chen P, Cheng S, Gao D (2000) Molecular marker-facilitated pyramiding of different genes for powdery mildew resistance in wheat. Plant Breed 119:21–24

Liu SX, Griffey CA, Maroof MAS (2001a) Identification of molecular markers associated with adult plant resistance to powdery mildew in common wheat cultivar Massey. Crop Sci 41:1268–1275

Liu XM, Smith CM, Gill BS, Tolmay V (2001b) Microsatellite markers linked to six Russian wheat aphid resistance genes in wheat. Theor Appl Genet 102:504–510

Liu XM, Smith CM, Gill BS (2002a) Identification of microsatellite markers linked to Russian, wheat aphid resistance genes *Dn4* and *Dn6*. Theor Appl Genet 104:1042–1048

Liu Z, Sun Q, Ni Z, Nevo E, Yang T (2002b) Molecular characterization of a novel powdery mildew resistance gene *Pm30* in wheat originating from wild emmer. Euphytica 123:21–29

Liu D, Gao M, Guo X, Zhang A (2003) QTL mapping for kernel weight in multiple environments. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 989–993

Lotti C, Salvi S, Pasqualone A, Tuberosa R, Blanco A (2000) Integration of AFLP markers into an RFLP-based map of durum wheat. Plant Breed 119:393–401

Lubbers EL, Gill KS, Cox TS, Gill BS (1991) Variation of molecular markers among geographically diverse accessions of *Triticum tauschii*. Genome 34:354–361

Luo M-C, Thomas CS, Deal KR, You FM, Anderson OD, Gu Y-Q, Li W, Kuraparthy V, Gill BS, McGuire PE, Dvorak J (2003) Construction of contigs of *Aegilops tauschii* genomic DNA fragments cloned in BAC and BiBAC vectors. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 293–296

Ma ZQ, Lapitan NLV (1998) A comparison of amplified and restriction fragment length polymorphism in wheat. Cereal Res Commun 26:7–13

Ma ZQ, Sorrells ME, Tanksley SD (1994) RFLP markers linked to powdery mildew resistance genes *Pm1*, *Pm2*, *Pm3* and *Pm4* in wheat. Genome 37:871–875

Ma ZQ, Saidi A, Quick JS, Lapitan NLV (1998) Genetic mapping of Russian wheat aphid resistance genes *Dn2* and *Dn4* in wheat. Genome 41:303–306

Ma JX, Zhou RH, Dong YS, Wang LF, Wang XM, Jia JZ (2001) Molecular mapping and detection of the yellow rust resistance gene *Yr26* in wheat transferred from *Triticum turgidum* L using microsatellite markers. Euphytica 120:219–226

Ma ZQ, Lin F, Kong X, Wu JZ, Zhu HL, Xie SL, Wei JB, Liu DJ (2003) Mapping QTLs associated with FHAB resistance in a Wangshuibai x Nanda2419 population. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 372–375

Maccaferri M, Sanguineti MC, Donini P, Tuberosa R (2003) Microsatellite analysis reveals a progressive widening of the genetic basis in the elite durum wheat germplasm. Theor Appl Genet 107:783–797

Mago R, Spielmeyer W, Lawrence GJ, Lagudah ES, Ellis JG, Pryor A (2002) Identification and mapping of molecular markers linked to rust resistance genes located on chromosome 1RS of rye using wheat rye translocation lines. Theor Appl Genet 104:1317–1324

Malik R, Brown-Guedira GL, Smith CM, Harvey TL, Gill BS (2003) Genetic mapping of wheat curl mite resistance genes *Cmc3* and *Cmc4* in common wheat. Crop Sci 43:644–650

Manifesto MM, Schlatter AR, Hopp HE, Suarez EY, Dubcovsky J (2001) Quantitative evaluation of genetic diversity in wheat germplasm using molecular markers. Crop Sci 41:682–690

Mares DJ, Mrva K (2001) Mapping quantitative trait loci associated with variation in grain dormancy in Australian wheat. Aust J Agric Res 52:1257–1265

Mares D, Mrva K, Tan MK, Sharp P (2002) Dormancy in white-grained wheat: Progress towards identification of genes and molecular markers. Euphytica 126:47–53

Marino CL, Nelson JC, Lu YH, Sorrells ME, Leroy P, Tuleen NA, Lopes CR, Hart GE (1996) Molecular genetic maps of the group 6 chromosomes of hexaploid wheat *Triticum aestivum* L em Thell). Genome 39:359–366

Masojć P, Myśków B, Milczarski P (2001) Extending a RFLP-based genetic map of rye using random amplified poly-morphic DNA (RAPD) and isozyme markers. Theor Appl Genet 102:1273–1279

Matthews DE, Lazo GR, Carollo V, Anderson OD (2003) Information resources for the wheat genomics In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 297–300

Matthews DE, Carollo V, Lazo G, Anderson OD (2004) Bioinformatics and Triticeae genomics: resources and future developments. In: Gupta PK, Varshney RK (eds) Cereal Genomics. Kluwer, Dordrecht, pp 425–446

McCartney CA, Brûle–Babel AL, Lamari L, Somers DJ (2003) Chromosomal location of a race–specific gene to *Mycosphaerella graminicola* in the spring wheat ST6. Theor Appl Genet 107:1181–1186

McLauchlan A, Ogbonnaya FC, Hollingsworth B, Carter M, Gale KR, Henry RJ, Holton TA, Morell MK, Rampling LR, Sharp PJ et al (2001) Development of robust PCRbased DNA markers for each homoeoallele of granulebound starch synthase and their application, in wheat breeding programs. Aust J Agric Res 52:1409–1416

Mesfin A, Frohberg RC, Anderson JA (1999) RFLP markers associated with high grain protein from *Triticum turgidum* L var *dicoccoides* introgressed into hard red spring wheat. Crop Sci 39:508–513

Messmer MM, Keller M, Zanetti S, Keller B (1999) Genetic linkage map of wheat × spelt cross. Theor Appl Genet 98:1163–1170

Mickelson-Young L, Endo TR, Gill BS (1995) A cytogenetic ladder-map of the wheat homoeologous group-4 chromosomes. Theor Appl Genet 90:1007–1011

Miftahudin, Ross K, Ma X-F, Mahmoud AA, Layton J, Rodriguez Milla MA, Chikmawati T, Ramalingam J, Feril O, Pathan MS et al (2004) Analysis of expressed sequence tag loci on wheat chromosome group 4. Genetics 168:651–663

Milla MAR, Gustafson JP (2001) Genetic and physical characterization of chromosome 4DL in wheat. Genome 44:883–892

Miller CA, Altinkut A, Lapitan NLV (2001) A microsatellite marker for tagging *Dn2*, a wheat gene conferring resistance to the Russian wheat aphid. Crop Sci 41:1584–1589

Milligan AS, Lopato S, Langridge P (2004) Functional genomics studies of seed development in cereals. In: Gupta PK, Varshney RK (eds) Cereal Genomics. Kluwer, Dordrecht, pp 447–482

Mizumoto K, Hirosawa S, Nakamura C, Takumi S (2002) Nuclear and chloroplast genome genetic diversity in the wild einkorn wheat, *Triticum urartu*, revealed by AFLP and SSLP analyses. Hereditas 137:208–214

Mochida K, Kawaura K, Ogihara Y (2003) SNPs genotyping of hexaploid wheat by the 'Allele-Specific Pyrosequencing'. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 1003–1005

Mohammadi SA, Prasanna BM (2003) Analysis of genetic diversity in crop plants – salient statistical tools and considerations. Crop Sci 43:1235–1248

Mohapatra T, Krishanpal, Singh SS, Swain SC, Sharma RK, Singh NK (2003) STMS-based DNA fingerprints of the new plant type wheat lines. Curr Sci 84:1125–1129

Mohler V, Schwarz G (2004) Genotyping tools in plant breeding: From restriction fragmnet length polymorphisms to single nucleotided polymorphisms. In: Lörz H, Wenzel G (eds) Biotechnology in Agriculture and Forestry 55: Molecular Markers Systems in Plant Breeding and Crop Improvement. Springer, Berlin Heidelberg New York, pp 23–38

Mohler V, Hsam SLK, Zeller FJ, Wenzel G (2001) An STS marker distinguishing the rye-derived powdery mildew resistance alleles at the *Pm8/Pm17* locus of common wheat. Plant Breed 120:448–450

Moore G (1995) Cereal Genome evolution-pastoral pursuits with lego genomes. Curr Opin Genet Dev 5:717–724

Moore G, Devos KM, Wang Z, Gale MD (1995a) Cereal genome evolution: grasses, line up and form a circle. Curr Biol 5:737–739

Moore G, Foote T, Helentjaris T, Devos KM, Kurata N, Gale MD (1995b) Was there a single ancestral cereal chromosome? Trends Genet 11:81–82

Mori N, Moriguchi T, Nakamura C (1997) RFLP analysis of nuclear DNA for study of phylogeny and domestication of tetraploid wheat. Genes Genet Syst 72:153–161

Morgante M, Hanafey M, Powell W (2002) Microsatellites are preferentially associated with nonrepetitive DNA in plant genomes. Nat Genet 30:194–200

Munkvold JD, Greene RA, Bermudez-Kandianis CE, La Rota CM, Edwards H, Sorrells SF, Dake T, Benscher D, Kantety R, Linkiewicz AM et al (2004) Group 3 chromosome bin maps of wheat and their relationship to rice chromosome 1. Genetics 168 :639–650

Myburg AA, Cawood M, Wingfield BD, Botha AM (1998) Development of RAPD and SCAR markers linked to the Russian wheat aphid resistance gene *Dn2* in wheat Theor Appl Genet 96:1162–1169

Nachit M, Elouafi I, Pagnotta MA, El Saleh A, Iacono E, Labhili M, Asbati A, Azrak M, Hazzam H, Benscher D et al (2001) Molecular linkage map for an intraspecific recombinant inbred population of durum wheat (*Triticum turgidum* L var *durum*). Theor Appl Genet 102:177–186

Naik S, Gill VS, Rao VSP, Gupta VS, Tamhankar SA, Pujar S, Gill BS, Ranjekar PK (1998) Identification of a STS marker linked to the *Aegilops speltoides*-derived leaf rust resistance gene *Lr28* in wheat. Theor Appl Genet 97:535–540

Namuth DM, Lapitan NLV, Gill KS, Gill BS (1994) Comparative RFLP mapping of *Hordeum vulgare* and *Triticum tauschii*. Theor Appl Genet 89:865–872

Nelson JC, Singh RP, Autrique JE, Sorrells ME (1997) Mapping genes conferring and suppressing leaf rust resistance in wheat. Crop Sci 37:1928–1935

Nelson JC, Sorrells ME, Van Deynze AE, Lu YH, Atkinson M, Bernard M, Leroy P, Faris JD, Anderson JA (1995a) Molecular mapping of wheat: major genes and rearrangements in homoeologous groups 4, 5, and 7. Genetics 141:721–731

Nelson JC, Van Deynze AE, Autrique E, Sorrells ME, Lu Y H, Merlino M, Atinkson M, Leroy P (1995b) Molecular mapping of wheat homoeologous group 2. Genome 38:516–524

Nelson JC, Van Deynze AE, Autrique E, Sorrells ME, Lu YH, Negre M, Atinkson M, Leroy P (1995c) Molecular mapping of wheat homoeologous group 3. Genome 38:525–533

Nevo E (2001) Genetic resources of wild emmer, *Triticum dicoccoides*, for wheat improvement in the third millennium. Israel J Plant Sci 49:S77–S91

Nicot N, Chiquet V, Gandon B, Specel S, Amilhat L, Leroy P, Burr B, Blewitt M, Murigneux A, Chalhoub B et al (2003a) Genetic mapping of SSR sequences isolated from wheat genomic DNA libraries. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 1014–1016

Nicot N, Chiquet V, Gandon B, Specel S, Amilhat L, Leroy P, Legeai F, Foisset N, Dufour P, Bernard M et al (2003b) SSR marker development from low copy wheat sequences. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 1017–1019

Nicot N, Chiquet V, Gandon B, Amilhat L, Legeai F, Leroy P, Bernard M, Sourdille P (2004) Study of simple sequence repeat (SSR) markers from wheat expressed sequence tags (ESTs). Theor Appl Genet 109:800–805

Ogbonnaya FC, Subrahmanyam NC, Moullet O, de Majnik J, Eagles HA, Brown JS, Eastwood RF, Kollmorgen J, Appels R, Lagudah ES (2001) Diagnostic DNA markers for cereal cyst nematode resistance in bread wheat. Aust J Agric Res 52:1367–1374

Ogihara Y (2003) SNPs analysis of homoeologous genes by computing a large scale of expressed sequence tags in the hexaploid wheat. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 301–306

Ogihara Y, Hasegawa K, Tsujimoto H (1994) High-resolution cytological mapping of the long arm of chromosome 5A in common wheat using a series of deletion lines induced by gametocidal (gc) genes of *Aegilops* speltoides. Mol Gen Genet 244:253–259

Ogihara Y, Mochida K, Nemoto Y, Murai K, Yamazaki Y, Shin-I T, Kohara Y (2003) Correlated clustering and virtual display of gene expression patterns in the wheat life cycle by large-scale statistical analyses of expressed sequence tags. Plant J 33:1001–1011

Otto CD, Kianian SF, Elias EM, Stack RW, Joppa LR (2002) Genetic dissection of a major fusarium head blight QTL in tetraploid wheat. Plant Mol Biol 48:625–632

Ovesna J, Leisova L, Kucera (2003) Evaluation of Czech wheats by DNA markers: possible applet for genetic resources preservation. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 503–505

Özkan H, Brandolini A, Schäfer-Pregl R, Salamini F (2002) AFLP analysis of a collection of tetraploid wheats indicates the origin of emmer and hard wheat domestication in south-

east Turkey. Mol Biol Evol 19:1797–1801

Pagnotta MA, Laghetti G, Mondini L, Codianni P, Volpe N, Riefolo C, Savo Sordaro ML, Perrino P, Fares C (2003) Assessment of genetic diversity and characterization of Italian emmer wheat (*Triticum dicoccum* Schübler) populations. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 506–508

Paillard S, Schnurbusch T, Winzeler M, Messmer M, Sourdille P, Abderhalden O, Keller B, Schachermayr G (2003) An integrative genetic linkage map of winter wheat (*Triticum aestivum* L). Theor Appl Genet 107:1235–1242

Pallottta MA, Warner P, Fox RL, Kuchel H, Jefferies SJ, Langridge P (2003) Marker-assisted wheat breeding in southern region of Australia. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 789–791

Parker GD, Langridge P (2000) Development of a STS marker linked to a major locus controlling flour colour in wheat (*Triticum aestivum* L). Mol Breed 6:169–174

Parker GD, Chalmers KJ, Rathjen AJ, Langridge P (1998) Mapping loci associated with flour colour in wheat (*Triticum aestivum* L). Theor Appl Genet 97:238–245

Parker GD, Chalmers KJ, Rathjen AJ, Langridge P (1999) Mapping loci associated with milling yield in wheat (*Triticum aestivum* L). Mol Breed 5:561–568

Parker GD, Fox PN, Langridge P, Chalmers K, Whan B, Ganter PF (2002) Genetic diversity within Australian wheat breeding programs based on molecular and pedigree data. Euphytica 124 (3):293–306

Pasquer F, Stein N, Isidore E, Keller B (2003) Microarray analysis of gene expression in wheat (*Triticum aestivum*) after fungicide application. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 1029–1031

Paterson AH (2004) Comparative genomics in cereals. In: Gupta PK, Varshney RK (eds) Cereal Genomics. Kluwer, Dordrecht, pp 119–134

Paterson AH, Tanksley SD, Sorrells ME (1994) DNA markers in plant breeding. Adv Agron 46:39–90

Paull JG, Pallotta MA, Langridge P (1994) The TT RFLP markers associated with *Sr22* and recombination between chromosome 7A of bread wheat and the diploid species *Triticum boeoticum*. Theor Appl Genet 89:1039–1045

Paull JG, Chalmers KJ, Karakousis A, Kretschmer JM, Manning S, Langridge P (1998) Genetic diversity in Australian wheat varieties and breeding material based on RFLP data. Theor Appl Genet 96:435–446

Payne PI, Holt LM, Thompson RD, Bartels D, Harberd NP, Harris PA, Law CN (1983) The high molecular weight subunits of glutenin: classical genetics, molecular genetics and the relationship of bread making quality. In: Sakamoto S (ed) Proc 6th Int Wheat Genet Symp, Kyoto, Japan, pp 827–834

Payne PI, Nightingale MA, Krattiger AF, Holt LM (1987) The relationship between HMW glutenin subunit composition and the breadmaking quality of British-grown wheat varieties. J Sci Food Agric 40:51–65

Peng JH, Fahima T, Röder MS, Huang QY, Dahan A, Li YC, Grama A, Nevo E (2000a) Highdensity molecular map of chromosome region harboring striperust resistance genes *YrH52* and *Yr15* derived from wild emmer wheat, *Triticum dicoccoides*. Genetica 109:199–210

Peng J, Korol AB, Fahima T, Röder MS, Ronin YI, Li YC, Nevo E (2000b) Molecular genetic maps in wild emmer wheat, Triticum dicoccoides: Genome-wide coverage, massive negative interference, and putative quasi-linkage. Genome Res 10:1509–1531

Peng JH, Zadeh H, Lazo GR, Qi LL, Echalier B, Gill BS, Chao S, Anderson OD, Sandhu D, Gill KS et al (2003) A physical map of expressed sequence tags and functional genomics in the group 1 chromosomes of wheat (*Triticum aestivum*). In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 1035–1037

Peng J, Tahir M, Wang H, Lapitan NLV (2004a) Frequency and genomic distribution of functional microsatellites in wheat, *Triticum aestivum* L. In: Plant Animal Genome XII, Jan 10–14, 2004, San Diego, P424

http://www.intl-pag.org/12/abstracts/P5c_PAG12_424.html

Peng JH, Zadeh H, Lazo GR, Gustafson JP, Chao S, Anderson OD, Qi LL, Echalier B, Gill BS, Dilbirgi M et al (2004b) Chromosome bin map of expressed sequence tags in honoeologous group 1 of hexaploid wheat and homoloeolgy with rice and *Arabidopsis*. Genetics 168:609–623

Penner GA, Zirino M, Kruger S, Townley-Smith F (1998) Accelerated recurrent parent selection in wheat with microsatellite markers. In: Slinkard AE (ed) Proc 9th Int Wheat Genet Symp Vol 1, University of Saskatchewan, Saskatoon, Canada, pp 131–134

Perenzin M, Corbellini M, Accerbi M, Vaccino P, Borghi B (1998) Bread wheat: F-1 hybrid performance and parental diversity estimates using molecular markers. Euphytica 100:273–279

Pester TA, Ward SM, Fenwick AL, Westra P, Nissen SJ (2003) Genetic diversity of jointed goatgrass (*Aegilops cylindrica*) determined with RAPD and AFLP markers. Weed Sci 51:287–293

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

Phillips RL, Vasil IK (eds) (2001) DNA-Based Markers in Plants, 2nd edn. Kluwer, Dordrecht

Plaschke J, Ganal MW, Röder MS (1995) Detection of genetic diversity in closely-related bread wheat using microsatellite markers. Theor Appl Genet 91:1001–1007

Potokina E, Caspers M, Prasad M, Kota R, Zhang H, Sreenivasulu N, Wang M, Graner A (2004) Functional association between malting quality trait components and cDNA array based expression patterns in barley (*Hordeum vulgare* L.). Mol Breed 14:153–170

Powell W, Langridge P (2004) Unfashionable crop species flourish in the 21st century. Genome Biol 5: Art. 233

Prasad M, Varshney RK, Kumar A, Balyan HS, Sharma PC, Edwards KJ, Singh H, Dhaliwal HS, Roy JK, Gupta PK (1999) A microsatellite marker associated with a QTL for grain protein content on chromosome arm 2DL of bread wheat. Theor Appl Genet 99:341–345

Prasad M, Varshney RK, Roy JK, Balyan HS, Gupta PK (2000) The use of microsatellites for detecting DNA polymorphism, genotype identification and genetic diversity in wheat. Theor Appl Genet 100:584–592

Prasad M, Kumar N, Kulwal PL, Röder MS, Balyan HS, Dhaliwal HS, Gupta PK (2003) QTL analysis for grain protein content using SSR markers and validation studies using NILs in bread wheat. Theor Appl Genet 106:659–667

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

Pritchard JK, Stephens M, Rosenberg NA, Donnelly P (2000) Association mapping in structured populations. Am J Hum Genet 67:170–181

Procunier JD, Townley–Smith TF, Fox S, Prashar S, Gray M, Kim WK, Czarnecki E, Dyck PL (1995) PCR–based RAPD/DGGE markers linked to leaf rust resistance genes *Lr29* and *Lr25* in wheat (*Triticum aestivum* L). J Genet Breed 49:87–92

Pumphrey MO, Anderson JA (2003) QTL validation via systematic development of near-isogenic wheat lines from existing breeding populations. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 1227–1229

Qi L-L, Gill BS (2001) High-density physical maps reveal that the dominant male-sterile gene *Ms3* is located in a genomic region of low recombination in wheat and is not amenable to map-based cloning. Theor Appl Genet 103:998–1006

Qi L, Cao M, Chen P, Li W, Liu D (1996) Identification, mapping, and application of polymorphic DNA associated with resistance gene *Pm21* of wheat. Genome 39:191–197

Qi L-L, Echalier B, Friebe B, Gill BS (2003) Molecular characterization of a set of wheat deletion stocks for use in chromosome bin mapping of ESTs. Funct Integr Genom 3:39–55

Qi L-L, Echalier B, Chao S, Lazo GR, Butler GE, Anderson OD, Akhunov ED, Dvorak J, Linkiewicz AM, Ratnasiri et al (2004) A chromosome bin map of 16,000 expressed sequence tag loci and distribution of genes among the three genomes of polyploid wheat. Genetics 168:701–712

Queen RA, Gribbon BM, James C, Jack P, Flavell AJ (2004) Retrotransposon-based molecular markers for linkage and genetic diversity analysis in wheat. Mol Genet Genom 271:91–97

Radovanovic N, Cloutier S (2003) Gene-assisted selection for high molecular weight glutenin subunits in wheat doubled haploid breeding program. Mol Breed 12:51–59

Rafalski A (2002) Applications of single nucleotide polymorphisms in crop genetics. Curr Opin Plant Biol 5:94–100

Rafalski A, Morgante M (2004) Corn and humans: recombination and linkage disequilibrium in two genomes of similar size. Trends Genet 20:103–111

Rampino P, Malatrasi M, Gulli M, Marmiroli N, Perrotta C (2003) Drought stress related sequences in durum wheat. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 1233–1235

Randhawa HS, Dilbriligi M, Sidgu D, Erayman M, Sandhu D, Bondareva S, Chao S, Lazo GR, Anderson OD, Miftahudin, Gustafson JP et al (2004) Deletion mapping of homoeologous group 6–specific wheat expressed sequence tags. Genetics 168:677–686

Raupp WJ, Sukhwinder-Singh, Brown-Guedira GL, Gill BS (2001) Cytogenetic and molecular mapping of the leaf rust resistance gene *Lr39* in wheat. Theor Appl Genet 102:347–352

Rebetzke GJ, Appels R, Morrison AD, Richards RA, McDonald G, Ellis MH, Spielmeyer W, Bonnett DG (2001) Quantitative trait loci on chromosome 4B for coleoptile length and early vigour in wheat (*Triticum aestivum* L.). Aust J Agric Res 52:1221–1234

Reffo G, Corbellini M, Bruschi G, Brandolini A (2003) Marker-assisted introgression of the *Pm13* powdery mildew resistance gene in Italian bread wheat cultivars. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 801–803

Reiter RS, Williams JKG, Feldmann KA, Rafalski JA, Tingey SV, Scolnik PA (1992) Global and local genome mapping in *Arabidopsis thaliana* by using recombinant inbred lines and random amplified polymorphic DNAs. Proc Natl Acad Sci USA 89:1477–1481

Riley R (1965) Cytogenetics and the evolution of wheat In: Hutchinson JB (ed) Essays on Crop Plant Evolution. Cambridge University Press, Cambridge, pp 103–118

Riley R, Chapman V (1958) Genetic control of the cytologically diploid behaviour of hexaploid wheat. Nature 182:713–715

Robert O, Abelard C, Dedryver F (1999) Identification of molecular markers for the detection of the yellow rust resistance gene *Yr17* in wheat. Mol Breed 5:167–175

Rong JK, Millet E, Manisterski J, Feldman M (2000) A new powdery mildew resistance gene: Introgression from wild emmer into common wheat and RFLP–based mapping. Euphytica 115:121–126

Röder MS, Korzun V, Gill BS, Ganal MW (1998a) The physical mapping of microsatellite markers in wheat. Genome 41:278–283

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

Röder MS, Huang X-Q, Börner A, Ganal MW (2003) Wheat microsatellite diversity of a genebank collection in comparison to registered varities. In: Pogna NE, Romano M,

Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 625–627

Röder MS, Huang X-Q, Ganal MW (2004) Wheat microsatel-lites: Potential and implications. In: Lörz H, Wenzel G (eds) Biotechnology in agriculture and forestry, Vol 55. Molecular marker systems. Springer, Berlin Heidelberg New York, pp 255–266

Rogers WJ, Payne PI, Harinder K (1989) The HMW glutenin subunit and gliadin composition of German-grown wheat varieties and their relationship with breadmaking quality. Plant Breed 103:89–100

Rousset M, Gouis JL, Heumez E (2003) A QTL analysis for spike characteristics and fertility under field conditions in a bread wheat doubled-haploid population. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 167–170

Roy JK, Prasad M, Varshney RK, Balyan HS, Blake TK, Dhaliwal HS, Singh H, Edwards KJ, Gupta PK (1999) Identification of a microsatellite on chromosomes 6B and a STS on 7D of bread wheat showing an association with preharvest sprouting tolerance. Theor Appl Genet 99:336–340

Roy JK, Balyan HS, Prasad M, Gupta PK (2002) Use of SAMPL for a study of DNA polymorphism, genetic diversity and possible gene tagging in bread wheat. Theor Appl Genet 104:465–472

Roy JK, Lakshmikumaran MS, Balyan HS, Gupta PK (2004) AFLP-based genetic diversity and its comparison with diversity based on SSR, SAMPL, and phenotypic traits in bread wheat. Biochem Genet 42:43–59

Safar J, Bartos J, Janda J, Jaroslav J, Bellec A, Kubalakova M, Valarik M, Pateyron S, Weiserova J, Tuskova R et al (2004) Dissecting large and complex genomes: flow sorting and BAC cloning of individual chromosomes from bread wheat. Plant J 39:960–968

Salina E, Dobrovolskaya O, Efremova T, Leonova I, Röder MS (2003) Microsatellite monitoring of recombination around the Vrn-B1 locus of wheat during early backcross breeding. Plant Breed 122:116–119

Salvo-Garrido H, Laurie DA, Jaffe B, Snape JW (2001) An RFLP map of diploid *Hordeum bulbosum* L and comparison with maps of barley (*H. vulgare* L) and wheat (*Triticum aestivum* L). Theor Appl Genet 103:869–880

Sandhu D, Gill KS (2002a) Gene-containing regions of wheat and the other grass genomes. Plant Physiol 128:803–811

Sandhu D, Gill KS (2002b) Structural and functional organization of the '1S08 gene-rich region' in the Triticeae. Plant Mol Biol 48:791–804

Sandhu D, Sidhu D, Gill KS (2002) Identification of expressed sequence markers for a major gene-rich region of wheat chromosome group 1 using RNA fingerprinting-differential display. Crop Sci 42:1285–1290

Sandhu D, Erayman M, Dilbirligi M, Sidhu D, Gill KS (2003) The gene rich regions of the wheat genome. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 308–312

Sarma RN, Gill BS, Sasaki T, Galiba G, Sutka J, Laurie DA, Snape JW (1998) Comparative mapping of the wheat chromosome 5A *Vrn-A1* region with rice and its relationship to QTL for flowering time. Theor Appl Genet 97:103–109

Sarma RN, Fish L, Gill BS, Snape JW (2000) Physical characterization of the homoeologous Group 5 chromosomes of wheat in terms of rice linkage blocks, and physical mapping of some important genes. Genome 43:191–198

Sasaki T, Burr B (2000) International rice genome sequencing project: the effort to completely sequence the rice genome. Curr Opin Plant Biol 3:138–141

Sasakuma T, Shindo C (2003) QTLs of heading traits in diploid and hexaploid wheat. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 1047–1049

Sax K (1923) The association of size differences with seed-coat pattern and pigmentation in *Phaseolus vulgaris*. Genetics 8:552–560

Sayed-Tabatabaei BE, Shahnejat-Bushehri AA (2003) Assesment of genetic similarity among wheat cultivars using RAPD and AFLP techniques. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 631–633

Schadt EE, Monks SA, Drake TA, Lusis AJ, Che N, Colinayo V, Ruff TG, Milligan SB, Lamb JR, Cavet G et al (2003) Genetics of gene expression surveyed in maize, mouse and man. Nature 422:297–301

Schachermayr G, Feuillet C, Keller B (1997) Molecular markers for the detection of the wheat leaf rust resistance gene *Lr10* in diverse genetic backgrounds. Mol Breed 3:65–74

Schmolke M, Zimmermann G, Ebmeyer E, Miedaner T, Schweizer G, Hart L (2003) Molecular mapping of Fusarium head blight resistance QTLs in winter wheat population using AFLP markers. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 1245–1247

Schnurbusch Th, Paillard S, Fossati D, Mesmer M, Schachermayr G, Winzeler M, Keller B (2003a) Detection of QTLs for *Stagonospora* glume blotch resistance in Swiss winter wheat. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 1248–1250

Schnurbusch Th, Paillard S, Schori A, Mesmer M, Schachermayr G, Winzeler M, Keller B (2003b) Dissection of quantitative and durable leaf rust resistance in Swiss winter wheat reveals a major resistance QTL in the *Lr34* chromosomal region. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 1254–1256

Schuler GD, Boguski MS, Stewart EA, Stein LD, Gyapay G, Rice K, White RE, Rodriguez-Tome P, Aggarwal A, Bajorek E et al (1996) A gene map of the human genome. Science 274:540–546

Schulman AH, Gupta PK, Varshney RK (2004) Organization of microsatellites and retrotransposons in cereal genomes.

In: Gupta PK, Varshney RK (eds) Cereal Genomics. Kluwer, Dordrecht, pp 83–118

Schwarz G, Herz M, Huang XQ, Michalek W, Jahoor A, Wenzel G, Mohler V (2000) Application of fluorescence based semi-automated AFLP analysis in barley and wheat. Theor Appl Genet 100:545–551

Schwarzacher T (2003) Meiosis, recombination and chromosomes: a review of gene isolation and fluorescent in situ hybridization data in plants. J Exp Bot 54:11–23

Seah S, Bariana H, Jahier J, Sivasithamparam K, Lagudah ES (2001) The introgressed segment carrying rust resistance genes *Yr17*, *Lr37* and *Sr38* in wheat can be assayed by a cloned disease resistance genelike sequence. Theor Appl Genet 102:600–605

Sears ER (1954) The aneuploids of common wheat. Missouri Agr Expt Sta Res Bull 572:59

Seyfarth R, Feuillet C, Schachermayr G, Winzeler M, Keller B (1999) Development of a molecular marker for the adult plant leaf rust resistance gene *Lr35* in wheat. Theor Appl Genet 99:554–560

Shah MM, Gill KS, Baenziger PS, Yen Y, Kaeppler SM, Ariyarathne HM (1999) Molecular mapping of loci for agronomic traits on chromosome 3A of bread wheat. Crop Sci 39:1728–1732

Shan X, Blake TK, Talbert LE (1999) Conversion of AFLP markers to sequence-specific PCR markers in barley and wheat. Theor Appl Genet 98:1072–1078

Shao YT, Niu YC, Zhu LH, Zhai WX, Xu SC, Wu LR (2001) Identification of an AFLP marker linked to the stripe rust resistance gene *Yr10* in wheat. Chinese Sci Bull 46:1466–1469

Shariflou MR, Ghannadha MR, Sharp PJ (2003) Multiplex PCR of microsatellite markers in wheat. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 1050–1052

Shen X, Ittu M, Ohm HW (2003a) Quantitative trait loci conditioning resistance to fusarium head blight in wheat line F201R. Crop Sci 43:850–857

Shen X, Zhou M, Lu W, Ohm H (2003b) Detection of fusarium head blight resistance QTL in a wheat population using bulked segregant analysis. Theor Appl Genet 106:1041–1047

Shi AN, Leath S, Murphy JP (1998) A major gene for powdery mildew resistance transferred to common wheat from wild einkorn wheat. Phytopathology 88:144–147

Shi ZX, Chen XM, Line RF, Leung H, Wellings CR (2001) Development of resistance gene analog polymorphism markers for the *Yr9* gene resistance to wheat stripe rust. Genome 44:509–516

Shinbata T, Vrinten P, Iida J, Sato M, Yonemaru J, Saito M, Mitsuse S, Nakamura T (2003) Microarray analysis of gene expression in developing endosperm from different wheat varities. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 1053–1055

Sidhu D, Sandhu D, Gill KS (2003) Genes mapping in the functional centromere of the wheat chromosomes In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Intern Wheat Genet Symp, Paestum, Italy, pp 1056–1058

Siedler H, Messmer MM, Schachermayr GM, Winzeler H, Winzeler M, Keller B (1994) Genetic diversity in European wheat and spelt breeding material based on RFLP data. Theor Appl Genet 88:994–1003

Simons KJ, Gehlhar SB, Maan SS, Kianian SF (2003) Detailed mapping of the species cytoplasm-specific (*scs*) gene in durum wheat. Genetics 165:2129–2136

Singh S, Grewal TS, Singh H, Sodhi M, Dhaliwal HS (1999) Identification of amplified fragment length polymorphism markers associated with Karnal bunt (*Neovossia indica*) resistance in bread wheat. Indian J Agric Sci 69:497–501

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

Singh H, Prasad M, Varshney RK, Roy JK, Balyan HS, Dhaliwal HS, Gupta PK (2001) STMS markers for grain protein content and their validation using near isogenic lines in bread wheat. Plant Breed 120:273–278

Singh S, Brown-Guedira GL, Grewal TS, Dhaliwal HS, Nelson JC, Singh H, Gill BS (2003) Mapping of a resistance gene effective against Karnal bunt pathogen of wheat. Theor Appl Genet 106:287–292

Singh NK, Raghuvanshi S, Srivastava SK, Gaur A, Pal K, Dalal V, Singh A, Ghazi1 IA, Bhargav A, Yadav M et al (2004a) Sequence analysis of the long arm of rice chromosome 11 for rice–wheat synteny. Funct Integ Genom 4:102–117

Singh S, Franks CD, Huang L, Brown-Guedira GL, Marshall DS, Gill BS, Fritz A (2004b) Lr41, Lr39, and a leaf rust resistance gene from Aegilops cylindrica may be allelic and are located on wheat chromosome 2DS. Theor Appl Genet 108:586–591

Singrün Ch, Hsam SLK, Hartl L, Zeller FJ, Mohler V (2003) Powdery mildew resistance gene *Pm22* in cultivar Virest is a member of the complex *Pm1* locus in common wheat (*Triticum aestivum* L em Thell). Theor Appl Genet 106:1420–1424

Smilde DW, Haluskova J, Sasaki T, Graner A (2001) New evidence for the synteny of rice chromosome 1 and barley chromosome 3H from rice expressed sequence tags. Genome 44:361–367

Smith PH, Koebner RMD, Boyd LA (2002) The development of a STS marker linked to a yellow rust resistance derived from the wheat cultivar Moro. Theor Appl Genet 104:1278–1282

Soleimani VD, Baum BR, Johnson DA (2002a) AFLP and pedigree-based genetic diversity estimates in modern cultivars of durum wheat [*Triticum turgidum* L subsp *durum* (Desf) Husn]. Theor Appl Genet 104:350–357

Soleimani VD, Baum BR, Johnson DA (2002b) Identification of Canadian durum wheat [*Triticum turgidum* L subsp *durum* (Desf) Husn] cultivars using AFLP and their STS markers. Can J Plant Sci 82:35–41

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

Somers DJ, Kirkpatrick R, Moniwa M, Walsh A (2003b) Mining single nucleotide polymorphisms from hexaploid wheat ESTs. Genome 49:431–437

Somers D, Edwards KJ, Issac P (2004) A high density microsatellite consensus map for bread wheat (*Triticum aestivum* L). Theor Appl Genet 109:1105–1114

Song QJ, Fickus EW, Cregan PB (2002a) Characterization of trinucleotide SSR motifs in wheat. Theor Appl Genet 104:286–293

Song QJ, Shi JR, Singh S, Fickus EW, Fernalld R, Gill BS, Cregan PB, Ward R (2002b) Development and mapping of wheat microsatellite markers. In: Plant, Animal and Microbe Genomes X Conf, 12–16 Jan 2002, San Diego. http://www.intl-pag.org/pag/10/abstracts/PAGX_P371.html

Sorrells ME (2004) Cereal genomics research in post-genomic era. In: Gupta PK, Varshney RK (eds) Cereal Genomics. Kluwer, Dordrecht, pp 559–584

Sorrells ME, La Rota M, Bermudez-Kandianis CE, Greene RA, Kantety R, Munkvold JD, Miftahudin, Mahmoud A, Ma X, Gustafson PJ et al (2003) Comparative DNA sequence analysis of wheat and rice genomes. Genome Res 13:1818–1827

Sourdille P, Robe P, Tixier M–H, Doussinault G, Pavoine M–T, Bernard M (1999) Location of *Pm3g*, a powdery mildew resistance allele in wheat, by using a monosomic analysis and by identifying associated molecular markers. Euphytica 110:193–198

Sourdille P, Snape JW, Cadalen T, Charmet G, Nakata N, Bernard S, Bernard M (2000a) Detection of QTLs for heading time and photoperiod response in wheat using a doubled-haploid population. Genome 43:487–494

Sourdille P, Tixier MH, Charmet G, Gay G, Cadalen T, Bernard S, Bernard M (2000b) Location of genes involved in ear compactness in wheat (*Triticum aestivum*) by means of molecular markers. Mol Breed 6:247–255

Sourdille P, Cadalen T, Gay G, Gill B, Bernard M (2002) Molecular and physical mapping of genes affecting awning in wheat. Plant Breed 121:320–324

Sourdille P, Cadalen T, Guyomarc'h H, Snape JW, Perretant MR, Charmet G, Boeuf C, Bernard S (2003) An update of the Courtot × Chinese Spring intervarietal molecular marker linkage map for the QTL detection of agronomic traits in wheat. Theor Appl Genet 106:530–538

Sourdille P, Singh S, Cadalen T, Brown-Guedira GL, Gay G, Qi L, Gill BS, Dufour P, Murigneux A, Bernard M (2004) Microsatellite-based delition bin system for the establishment of genetic-physical map relationships in wheat (*Triticum aestivum* L). Funct Integr Genom 4:12–25

Souza E, Fox PN, Byerlee D, Skovmand B (1994) Spring wheat diversity in irrigated area of 2 developing-countries. Crop Sci 34:774–783

Spielmeyer W, Lagudah ES (2003) Rice genome sequence expedites fine mapping of durable broad spectrum stem rust resistance gene *Sr2* in wheat (*Triticum aestivum*). In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 414–416

Spielmeyer W, Sharp PJ, Lagudah ES (2003) Identification and validation of markers linked to broad spectrum stem rust resistance gene *Sr2* in wheat. Crop Sci 43:333–336

Sreenivasulu N, Kavikishor PB, Varshney RK, Altschmied L (2002) Mining functional information from cereal genomes – the utility of expressed sequence tags (ESTs). Curr Sci 83:965–973

Stachel M, Lelley T, Grausgruber H, Vollmann J (2000) Application of microsatellites in wheat (*Triticum aestivum* L) for studying genetic differentiation caused by selection for adaptation and use. Theor Appl Genet 100:242–248

Stam P, Ooijen JW (1995) JoinMap version 2.0: software for the calculation of genetic linkage maps. CPRO-DLO, Wageningen, The Netherlands

Stein N, Graner A (2004) Map-based gene isolation in cereal genomes In: Gupta PK, Varshney RK (eds) Cereal Genomics. Kluwer, Dordrecht, pp 331–360

Stein N, Feuillet C, Wicker T, Schlagenhauf E, Keller B (2000) Subgenome chromosome walking in wheat: A 450-kb physical contig in *Triticum monococcum* L spans the *Lr10* resistance locus in hexaploid wheat (*Triticum aestivum* L). Proc Natl Acad Sci USA 97:13436–13441

Steiner B, Griesser M, Lemmens M, Scholz U, Buerstmayr H (2003) Molecular mapping of resistance to Fusarium head blight in the spring wheat cultivar Frontana. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 1260–1262

Stephenson P, Bryan G, Kirby J, Collins A, Devos KM, Busso C, Gale MD (1998) Fifty new microsatellite loci for the wheat genetic map. Theor Appl Genet 97:946–949

Stoutjesdijk P, Kammholz SJ, Kleven S, Matsay S, Banks PM, Larkin PJ (2001) PCRbased molecular marker for the *Bdv2 Thinopyrum intermedium* source of barley yellow dwarf virus resistance in wheat. Aust J Agric Res 52:1383–1388

Suenaga K, Singh RP, Huerta–Espino J, William HM (2003) Microsatellite markers for genes *Lr34/Yr18* and other quantitative trait loci for leaf rust and stripe rust resistance in bread wheat. Phytopathology 93:881–890

Sun GL, Fahima T, Korol AB, Turpeinen T, Grama A, Ronin YI, Nevo E (1997) Identification of molecular markers linked to the *Yr15* stripe rust resistance gene of wheat originated in wild emmer wheat, *Triticum dicoccoides*. Theor Appl Genet 95:622–628

Sun QX, Ni ZF, Liu ZY, Gao JW, Huang TC (1998) Genetic relationships and diversity among Tibetan wheat, common wheat and European spelt wheat revealed by RAPD markers. Euphytica 99:205–211

Sun Q, Wei Y, Ni Z, Xie C, Yang T (2002) Microsatellite marker for yellow rust resistance gene *Yr5* in wheat introgressed from spelt wheat. Plant Breed 121:539–541

Sun G, Bond M, Nass H, Martin R, Dong Z (2003) RAPD polymorphisms in spring wheat cultivars and lines with different level of Fusarium resistance. Theor Appl Genet 106:1059–1067

Sutton T, Whitford R, Baumann U, Dong CM, Able JA, Langridge P (2003) The *Ph2* pairing homoeologous locus of wheat (*Triticum aestivum*): identification of candidate meiotic genes using a comparative genetics approach. Plant J 36:443–456

Swanepoel E, Lacock L, Myburg AA, Botha AM (2003) A leucine-rich homolog to *Aegilops tauschii* from bread wheat line PI 137739 obtained by subtractive suppression hybridization show linkage to Russian wheat aphid resistance gene *Dn1*. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 1263–1265

TAGI, The Arabidopsis Genome Initiative (2000) The Arabidopsis Genome Initiative Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. Nature 408:796–815

Takahashi Y, Shomura A, Sasaki T, Yano M (2001) *Hd6*, a rice quantitative trait locus involved in photoperiod sensitivity, encodes the alpha subunit of protein kinase CK2. Proc Nat Acad Sci USA 98:7922–7927

Talame V, Ballardini M, Antuono FD, Maccaferri M, Tuberosa R (2003) Evaluation of genetic diversity among Italian 'Farro' (*T dicoccum*) populations using AFLP markers. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 527–529

Talbert LE, Bruckner PL, Smith LY, Sears R, Martin TJ (1996) Development of PCR markers linked to resistance to wheat streak mosaic virus in wheat. Theor Appl Genet 93:463–467

Talbert LE, Smith LY, Blake NK (1998) More than one origin of hexaploid wheat is indicated by sequence comparison of low-copy DNA. Genome 41:402–407

Tanksley SD, McChouch SR (1997) Seed banks and molecular maps: unlocking genetic potential from the wild. Science 277:1063–1066

Tanksley SD, Young ND, Patterson AH, Bonierbale MW (1989) RFLP mapping in plant breeding – new tools for an old science. Bio/Technology 7:257–264

Tanksley SD, Nelson JC (1996) Advanced backcross QTL analysis: a method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. Theor Appl Genet 92:191–203

Tanyolac B, Linton E, Ozkan H (2003) Low genetic diversity in wild emmer (*T. turgidum* L subsp *dicoccoides* (Korn ex Asch et Graebn) Thell) from South-eastern Turkey revealed by restriction fragment length polymorphism. Genet Resource Crop Evol 50:829–833

Tao W, Liu D, Liu J, Feng Y, Chen P (2000) Genetic mapping of the powdery mildew resistance gene *Pm6* in wheat by RFLP analysis. Theor Appl Genet 100:564–568

Thangavelu M, James AB, Bankier A, Bryan GJ, Dear PH, Waugh R (2003) HAPPY mapping in plant genome: reconstruction and analysis of a high-resolution physical map of 19 Mpp region of *Arabidopsis thaliana* chromosome 4. Plant Biotechnol J 1:23–31

Thiel T, Michalek W, Varshney RK, Graner A (2003) Exploiting EST databases for the developement and characterization of gene-derived SSR-markers in barley (*Hordeum vulgare* L.). Theor Appl Genet 106:411–422

Thoday JM (1961) Location of polygenes. Nature 191:368–370

Thornsberry JM, Goodman MM, Doebley J, Kresovich S, Nielsen D, Buckler ES (2001) *Dwarf8* polymorphisms associate with variation in flowering time. Nat Genet 28:286–289

Tixier MH, Sourdille P, Charmet G, Gay G, Jaby C, Cadalen T, Bernard S, Nicolas P, Bernard M (1998) Detection of QTLs for crossability in wheat using a doubled haploid population. Theor Appl Genet 97:1076–1082

Toubia-Rahme H, Steiner B, Buerstmayr H (2003) Mapping of quantitative trait loci (QTLs) for *Stagonospora* glume blotch resistance in wheat. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 1278–1280

Van Deynze AE, Dubcovsky J, Gill KS, Nelson JC, Sorrells ME, Dvorak J, Gill BS, Lagudah ES, McCouch SR, Appels R (1995a) Molecular-genetic maps for group 1 chromosomes of Triticeae species and their relation to chromosomes in rice and oat. Genome 38:45–59

Van Deynze AE, Nelson JC, Yglesias ES, Harrington SE, Braga DP, McCouch SR, Sorrells ME (1995b) Comparative mapping in gasses – wheat relationships. Mol Gen Genet 248:744–754

Varshney RK, Kumar A, Balyan HS, Roy JK, Prasad M, Gupta PK (2000a) Characterization of microsatellites and development of chromosome specific STMS markers in bread wheat. Plant Mol Biol Rep 18:5–16

Varshney RK, Prasad M, Roy JK, Harjit-Singh NK, Dhaliwal HS, Balyan HS, Gupta PK (2000b) Identification of eight chromosomes and a microsatellite marker on 1AS associated with QTL for grain weight in bread wheat. Theor Appl Genet 100:1290–1294

Varshney RK, Prasad M, Roy JK, Röder MS, Balyan HS, Gupta PK (2001) Integrated physical maps of 2DL, 6BS and 7DL carrying loci for grain protein content and pre-harvest sprouting tolerance in bread wheat. Cereal Res Comm 29:33–40

Varshney RK, Korzun V, Börner A (2004a) Molecular maps in cereals: methodology and progress. In: Gupta PK, Varshney RK (eds) Cereal genomics. Kluwer, Dordrecht, pp 35–82

Varshney RK, Prasad M, Graner A (2004b) Molecular marker maps of barley: a resource for intra- and interspecific genomics. In: Lörz H, Wenzel G (eds) Biotechnology in agriculture and forestry, Vol 55. Molecular markers systems. Springer, Berlin Heidelberg New York, pp 229–245

Varshney RK, Prasad M, Zhang H, Kota R, Sigmund R, Scholz U, Stein N, Graner A (2004c) EST-derived markers and transcript map of barley: a resource for interspecific trans-

ferability and comparative mapping in cereals. In: Spunar J, Janikova J (eds) Proc 9th Int Barley Genet Symp, Brnno, Czech Republic, pp 332–338

Varshney RK, Graner A, Sorrells ME (2005a) Genic microsatellite markers in plants: features and applications. Trends Biotechnol 23:48–55

Varshney RK, Sigmund R, Korzun V, Boerner A, Stein N, Sorrells M, Langridge P, Graner A (2005b) Interspecific transferability and comparative mapping of barley EST-SSR markers in wheat, rye and rice. Plant Sci 168:195–202

Varshney RK, Thiel T, Stein N, Langridge P, Graner A (2002) *In silico* analysis on frequency and distribution of microsatellites in ESTs of some cereal species. Cell Mol Biol Lett 7:537–546

Velculescu Ve, Zhang L, Vogelstein B, Kinzler KW (1995) Serial analysis of gene expression. Science 270:484–487

Venter E, Botha AM (2000) Development of markers linked to *Diuraphis noxia* resistance in wheat using a novel PCRRFLP approach. Theor Appl Genet 100:965–970

Verma V, Foulkes MJ, Worland AJ, Sylvester-Bradley R, Caligari PDS, Snape JW (2004) Mapping quantitative trait loci for flag leaf senescence as a yield determinant in winter wheat under optimal and drought-stressed environments. Euphytica 135:255–263

Vierling RA, Nguyen HT (1992) Use of RAPD markers to determine the genetic diversity of diploid, wheat genotypes. Theor Appl Genet 84:835–838

Vikal Y, Chhuneja P, Singh R, Dhaliwal HS (2004) Tagging of an Aegilops speltoides derived leaf rust resistance gene Lr 28 with a microsatellite marker in wheat. J Plant Biochem Biotechnol 13:47–49

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

Wang LF, Ma JX, Zhou RH, Wang XM, Jia JZ (2002) Molecular tagging of the yellow rust resistance gene *Yr10* in common wheat, PI178383 (*Triticum aestivum* L). Euphytica 124:71–73

Wang X-E, Zhang Q-P, Wang Y-N, Chen P-D, Chu C-G, Qi Z-J, Zhuang L-F, Liu D-J (2003) Identification and genetic analysis of new germplasms with wheat spindle streak mosaic bymovius (WSMMV) resistance. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 1284–1286

Ward RW, Yang ZL, Kim HS, Yen C (1998) Comparative analyses of RFLP diversity in landraces of Triticum aestivum and collections of T-tauschii from China and southwest Asia. Theor Appl Genet 96:312–318

Wardrop J, Snape JW, Powell W, Machray GC (2002) Constructing plant radiation hybrid panels. Plant J 31:223–228

Wardrop J, Fuller J, Powell W, Machray GC (2004) Exploiting plant somatic radiation hybrids for physical mapping of expressed sequence tags. Theor Appl Genet 108:343–348

Waugh R, Dear PH, Powell W, Machray GC (2002) Physical education-new technologies for mapping plant genomes. Trends Plant Sci 7:521–523

Weber D, Helentjaris T (1989) Mapping RFLP loci in maize using B – A translocations. Genetics 121:583–590

Weng Y, Lazar MD (2002a) Amplified fragment length polymorphism and simple sequence, repeatbased molecular tagging and mapping of greenbug resistance gene *Gb3* in wheat. Plant Breed 121:218–223

Weng Y, Lazar MD (2002b) Comparison of homoeologous group-6 short arm physical maps of wheat and barley reveals a similar distribution of recombinogenic and generich regions. Theor Appl Genet 104:1078–1085

Weng Y, Tuleen NA, Hart GE (2000) Extended physhical map of the homoeologous group-6 chromosomes of wheat (*Triticum aestivum* L). Theor Appl Genet 100:519–527

Werner JE, Endo TR, Gill BS (1992) Toward a cytogenetically based physical map of the wheat genome. Proc Natl Acad Sci USA 89:11307–11311

Wicker T, Stein N, Albar L, Feuillet C, Schlagenhauf E, Keller B (2001) Analysis of a contiguous 211 kb sequence in diploid wheat (*Triticum monococcum* L) reveals multiple mechanisms of genome evolution. Plant J 26:307–316

William HM, Crosby M, Trethovan R, van Ginkel M, Mujeeb-Kazi A, Pfeiffer W, Khairallah M, Hoisington D (2003a) Molecular markers service laboratory at CIMMYT: an interface between the laboratory and the field. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 852–854

William HM, Garcia V, Ortiz-Islas S, van Beem J, Worland AJ (2003b) Progress in molecular markers characterization for phasic development genes in wheat. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 855–857

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

Williams KJ, Taylor SP, Bogacki P, Pallotta M, Bariana HS, Wallwork H (2002) Mapping of the root lesion nematode (*Pratylenchus neglectus*) resistance gene *Rlnn1* in wheat. Theor Appl Genet 104:874–879

Williams CE, Collier CC, Sardesai N, Ohm HW, Cambron SE (2003) Phenotypic assessment and mapped markers for H31, a new wheat gene conferring resistance to Hessian fly (Diptera: Cecidomyiidae). Theor Appl Genet 107:1516–1523

Xie DX, Devos KM, Moore G, Gale MD (1993) RFLP-based genetic maps of the homoeologous group 5 chromosomes of bread wheat (*Triticum aestivum* L). Theor Appl Genet 87:70–74

Xing QH, Ru ZG, Zhou CJ, Xue X, Liang CY, Yang DE, Jin DM, Wang B (2003) Genetic analysis, molecular tagging and mapping of the thermo-sensitive genic male-sterile gene (*wtms1*) in wheat. Theor Appl Genet 107:1500–1504

Yahiaoui N, Srichumpa P, Dudler R, Keller B (2003) Genome analysis at different ploidy levels allows cloning of the powdery mildew resistance gene *Pm3b* from hexaploid wheat Plant J 37:528–538

Yan GP, Chen XM, Line RF, Wellings CR (2003a) Resistance gene–analog polymorphism markers co–segregating with the *Yr5* gene for resistance to wheat stripe rust. Theor Appl Genet 106:636–643

Yan L, Loukoianov A, Tranquilli G, Helguera M, Fahima T, Dubcovsky J (2003b) Positional cloning of the wheat vernalization gene *VRN1*. Proc Natl Acad Sci USA 100:6263–6268

Yano M, Katayose Y, Ashikari M, Yamanouchi U, Monna L, Fuse T, Baba T, Yamamoto K, Umehara Y, Nagamura Y et al (2000) *Hd1*, a major photoperiod sensitivity quantitative trait locus in rice, is closely related to the *Arabidopsis* flowering time gene *CONSTANS*. Plant Cell 12:2473–2483

Yu J, Hu S, Wang J, Wang G, Li SG, Wong KSG, Liu B, Deng Y, Dai L, Zhou Y, Zhang X et al (2002) A draft sequence of the rice genome (*Oryza sativa* L ssp. *indica*). Science 296:79–92

Yu M-Q, Yan NH, Ma XR, Deng GB, Yang XJ, Chen J (2003) Development of SCAR marker for root-knot nematode resistance gene *Rkn-mn1* in wheat. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 1292–1294

Yu J-K, La Rota M, Kantety RV, Sorrells ME (2004a) EST-derived SSR markers for comparative mapping in wheat and rice. Mol Gen Genom 271:742–751

Yu J-K, Dake TM, Singh S, Benscher D, Li W, Gill BS, Sorrells ME (2004b) Development and mapping of EST-derived simple sequence repeat markers for hexaploid wheat. Genome 47:805–818

Zaharieva M, Santoni S, David J (2001) Use of RFLP markers to study genetic diversity and to build a core-collection of the wild wheat relative Ae-geniculata Roth (= Ae-ovata L). Genet Selec Evol 33:S269–S288

Zanetti S, Winzeler M, Keller M, Keller B, Messmer M (2000) Genetic analysis of pre-harvest sprouting resistance in a wheat × spelt cross. Crop Sci 40:1406–1417

Zeller FJ, Kong L, Hartl L, Mohler V, Hsam SLK (2002) Chromosomal location of genes for resistance to powdery mildew in common wheat (*Triticum aestivum* L em Thell) 7. Gene *Pm29* in line Pova. Euphytica 123:187–194

Zhang HN, Nasuda S, Endo TR (2000) Identification of AFLP markers on the satellite region of chromosome 1BS in wheat. Genome 43:729–735

Zhang XY, Li CW, Wang LF, Wang HM, You GX, Dong YS (2002) An estimation of the minimum number of SSR alleles needed to reveal genetic relationships in wheat varieties. I. Information from large-scale planted varieties and cornerstone breeding parents in Chinese wheat improvement and production. Theor Appl Genet 106:112–117

Zhang L, Sourdille P, Bernard M, Madeore A, Bernard S (2003a) QTL mapping for anther culturability in wheat using a doubled-haploid mapping population. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 1078–1080

Zhang XY, You GX, Wang LF (2003b) An estimation of the minimum number of SSR alleles needed to reveal genetic relationships in wheat varieties: information from 96 random accessions with maximized genetic diversity. In: Pogna NE, Romano M, Pogna EA, Galterio G (eds) Proc 10th Int Wheat Genet Symp, Paestum, Italy, pp 545–548

Zhang D, Choi DW, Wanamaker S, Fenton RD, Chin A, Malatrasi M, Turuspekov Y, Walia H, Akhunov ED, Kianain P et al (2004) Construction and evaluation of cDNA libraries for large-scale expressed sequence tag sequencing in wheat (*Triticum aestivum* L.). Genetics 168:595–608

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

Zhou WC, Kolb FL, Bai GH, Domier LL, Boze LK, Smith NJ (2003) Validation of a major QTL for scab resistance with SSR markers and use of marker-assisted selection in wheat. Plant Breed 122:40–46

3 Maize

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3.1
Introduction

3.1.1
Brief History of the Crop

Maize or corn (*Zea mays* L.) is the world's third leading cereal crop, after wheat and rice. It most probably originated in Central America, specifically Mexico, and spread northward to Canada and southward to Argentina. The oldest maize known, about 7,000 years old, was found by archaeologists in Teotihuacán, Mexico, but it is possible that there were other secondary centers of origin in the Americas. At the end of the 15th century, after the arrival in the Americas of Christopher Columbus, maize was introduced into Europe through Spain. It then spread through the warmer climates of the Mediterranean. Evidence now suggests that maize was secondarily introduced into northern Europe via North American trade routes. Following these introductions, maize spread rapidly throughout Europe.

All the main types of maize known today were apparently already being grown by the native populations when the Spanish arrived. All cultivated maize is classified as *Zea mays* ssp. *mays*. Evidence from botany, genetics, and cytology has pointed to a common origin for every existing type of maize. Most researchers believe that maize was developed from the annual teosinte (*Zea* spp.). Others, however, believe that maize originated as a wild maize species that is now extinct. Teosinte is native to Mexico and Guatemala and in its native habitat may be found growing wild in cultivated fields of maize. The closeness of teosinte to maize is suggested by the fact that both have ten chromosomes that are homologous or partially homologous. There is still discussion as to whether maize originated by a single domestication from the basal-branching teosinte subspecies *Z. mays* L. ssp. *parviglumis*, from the lateral branching subspecies *Z. mays* L. ssp. *mexicana*, or by a dual domestication and subsequent hybridization from the two subspecies (Galinat 1988, 1992).

Today, most of the modern cultivars of maize have been derived from materials developed in the southern United States of America, Mexico, and Central and South America.

3.1.2
Botanical Description

Botanically, maize belongs to the grass family (Poaceae) and is a tall annual plant with an extensive fibrous root system. It is a cross-pollinating species, with the female (ear) and male (tassel) flowers in separate places on the plant. The grain develops in the ears, or cobs, often one on each stalk; each ear has about 300 to 1,000 kernels, weighing between 190 and 300 g per 1,000 kernels, in a variable number of rows (12 to 16).

Mangelsdorf and Reeves (1939) have shown that maize is grown in every suitable agricultural region of the world and that a crop of maize is being harvested somewhere around the globe every month of the year. Maize grows from latitude 58° N in Canada and the former Soviet Union to latitude 40° S in the southern hemisphere. Maize crops are harvested in regions below sea level in the Caspian Plain and at altitudes of more than 4,000 m in the Peruvian Andes.

Maize is a diploid species with a chromosome number of $2n = 2x = 20$, and it has a moderate genome size of about 2,400 Mb.

3.1.3
Economic Importance

Maize is the most important cereal grain in the world after wheat and rice. The United States is the largest producer, accounting for nearly 40% of the total world production, followed by China and Brazil (Table 1).

Table 1. Top 20 in maize production (Mt) and area of harvest (ha) (average of 2000–2003 data from FAOSTAT (http://apps.fao.org/default.jsp))

Country	Production (Mt)	Country	Area Harvest (Ha)
United States of America	244,762,128.00	United States of America	28,500,357.50
China	114,026,556.25	China	23,894,511.00
Brazil	39,394,229.00	Brazil	12,157,779.25
Mexico	19,160,713.00	Mexico	7,460,658.50
Argentina	15,546,611.75	India	6,713,775.00
France	15,191,146.75	Nigeria	4,307,500.00
India	12,792,575.00	South Africa	3,434,180.00
Italy	10,123,352.75	Indonesia	3,316,855.50
Indonesia	9,897,102.25	Romania	2,911,458.00
South Africa	9,735,673.75	Argentina	2,632,341.50
Canada	8,482,250.00	Philippines	2,469,347.00
Romania	7,998,494.75	France	1,794,691.75
Egypt	6,554,190.00	Ethiopia	1,683,860.00
Hungary	5,874,244.75	Tanzania	1,623,316.75
Nigeria	4,702,750.00	Kenya	1,535,000.00
Ukraine	4,642,275.00	Malawi	1,479,982.25
Serbia and Montenegro	4,580,414.75	Congo, Democratic Republic	1,460,010.50
Philippines	4,458,387.25	Ukraine	1,385,950.00
Spain	4,434,463.25	Zimbabwe	1,336,274.00
Thailand	4,414,500.00	Canada	1,216,275.00

Maize provides nutrients for humans and animals and serves as a basic raw material for the production of starch, oil, protein, alcoholic beverages, food sweeteners, and, more recently, fuel. The green plant, made into silage, has been used with much success in the dairy and beef industries. After the grain is harvested, the dried leaves and upper part, including the flowers, are used to provide relatively good forage for ruminant animals owned by many small farmers in developing countries. The erect stalks, which in some cultivars are strong, have been used as long-lasting fences and walls. The husks are also used to make various craft items in China and elsewhere.

There are clear differences in the chemical composition of the main parts of the maize kernel. The seed coat, or pericarp, is characterized by a high crude fiber content of about 87%. On the other hand, the endosperm contains a high level of starch (87.6%) and about 8% protein. The crude fat content (ether extract) in the endosperm is relatively low. In contrast, the embryo has a high crude fat content, averaging about 33%. It also contains a relatively high level of protein (18.4%) and high levels of minerals (Watson 1987).

3.1.4
Breeding Objectives

Maize is an open-pollinated species. Hybrid cultivars are grown most often. By the 1940s hybrid corn had replaced most of the open-pollinated forms throughout the United States Corn Belt and was being introduced to other leading corn-producing areas of the world. The production of elite maize hybrid cultivars depends not only on good inbred lines but also on the ability of inbred lines to combine.

The objectives of maize breeding are as follows:

1) Grain yield
2) Adaptation: including maturity duration, response to soil fertility, cold tolerance, and resistance to heat and drought.
3) Traits suitable for mechanical harvesting: including stalk quality (lodging resistance, etc.), resistance to ear dropping, husk covering, and rapid dry-down.
4) Disease and insect resistance: there are many important corn diseases and insects, including northern corn leaf blight, southern corn leaf blight, rust, southern corn rust, and earworm.

5) Quality: including high protein content, high oil content, and high protein quality.

In the breeding of special-purpose hybrids, such as sweet corn, popcorn, waxy corn, and cob pipe corn, other objectives are important.

3.1.5
Classical Mapping Efforts

Emerson et al. (1935) presented the first comprehensive maps, linkage data, and genetic descriptions of maize. These maps included a total of 62 phenotypic variants and a few reciprocal translocations. One disease resistance gene, *rp1*, which confers resistance to *Puccinia sorghi*, had been placed on a chromosome arm by deletion analysis, but no biochemically defined loci such as isozymes or protein markers were identified.

A cytological map based on B-A translocations (Roman and Ullstrup 1951) and phenotypic markers is also available (Beckett 1991).

From the 1960s to the 1980s, many isozyme markers were identified and mapped on the maize chromosomes. Goodman and Stuber (1983) reported 37 isozyme loci on nine chromosomes.

In the classic map presented by Neuffer at al. (1997) in the book *Mutants of Maize*, a total of 674 morphological, biochemical, and cytological markers were mapped on the ten maize chromosomes.

3.1.6
Classical Breeding Achievements

After corn hybrids replaced open-pollinated cultivars, the development and use of genetically improved hybrids, combined with improved cultural practices, resulted in a 585% increase in corn yields in the United States between 1866 and 2003 (Fig. 1), especially after single-cross hybrids replaced double-cross hybrids in the 1960s.

The concept of "heterotic patterns" or "heterotic groups" was based on the results of several studies (Beal 1877; Richey 1922; reviewed in Hallauer and Miranda 1981). Heterotic patterns are of great concern to corn breeders because in most cases heterosis (hybrid vigor) is expected in hybrids between different heterotic groups. Some useful heterotic patterns are given in Table 2.

A very famous plant selection program was begun by C.G. Hopkins of the University of Illinois in 1896 to improve the concentrations of oil and protein in corn. After 90 cycles of selection, the oil concentration was increased from 4.7% to 19.3% (Dudley and Lambert 1992). The protein concentration reached 25% by cycle 76. A breeding program to increase oil content was started in the late 1940s by C.M. Woodworth and continued by R.W. Jugenheimer and D.E. Alexander; 26 high-oil inbred lines were developed (Lambert 2001).

In breeding for disease resistance, *Ht* (resistant to northern corn leaf blight), *Rpp* (resistant to southern corn rust), and other important resistance genes were found and introduced into elite inbred lines for use in maize-breeding programs.

3.1.7
Limitations of Classical Endeavors and Utility of Molecular Mapping

Although classical breeding methods are still very useful in maize breeding, most agriculturally important traits, including yield, quality, lodging resistance, and cold or drought tolerance, are quantitatively inherited and controlled by quantitative trait loci (QTL). The use of molecular markers to assist the breeding process will allow breeding goals to be reached more efficiently and may reduce the need for field assay by inoculation.

3.2
Construction of Genetic Maps

3.2.1
Brief History of Mapping Efforts

In 1983 and 1985, two US groups projected the use of restriction indexGenetic mapfragment length polymorphism (RFLP) markers, combined with isozymes and visible genes, to map quantitative traits and to contribute to plant breeding (Burr et al. 1983; Burr and Burr 1985; Helentjaris et al. 1985). Subsequently, one of the groups definitively oriented maps of molecular markers relative to the chromosome arms and to established gene maps by monosomy (Helentjaris et al. 1986c) and with B-A translocations (Weber and Helentjaris 1989).
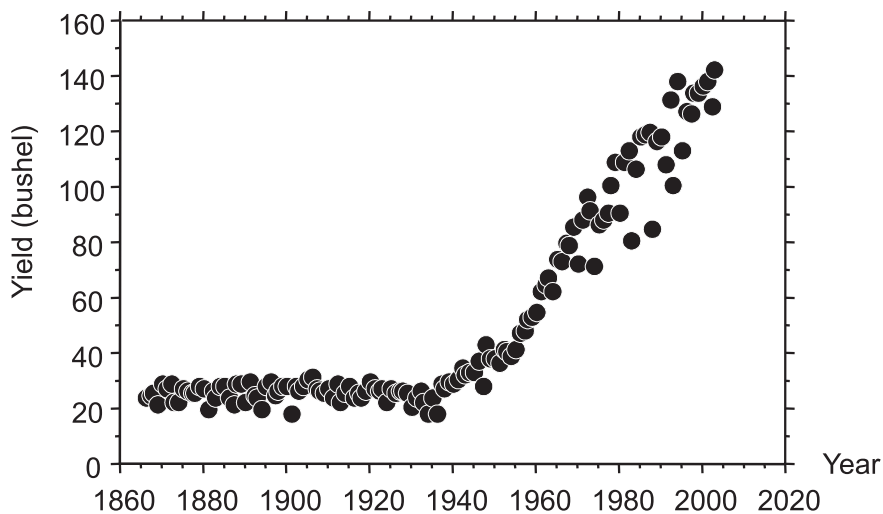
Fig. 1. Increase of average US corn yields from 1866 to 2003. Data from Agricultural Statistics Database (http://www.nass.usda.gov:8080/QuickStats/index2.jsp)

Gardiner et al. (1993) proposed designating "bins" for intervals along the molecular linkage maps to simplify defining locations of genes. A total of 90 RFLP markers distributed throughout the ten chromosomes and flanking segments (bins) of approximately 20 cM each in length were selected as "core" markers . Bins are now used routinely to define the locations of loci.

In the 1990s, simple sequence repeat (SSR) markers were developed and mapped on linkage maps. In October 2001, 1,855 SSR primer pairs (1,797 distinct loci) were developed and published in the MaizeGDB database (http://www.maizegdb.org). One third of them were derived from cDNA sequences. Because the SSR markers have an advantage over other markers such as RFLP, amplified fragment length polymorphism (AFLP), and random amplified polymorphic DNA (RAPD) markers in being easy to detect, codominant, and highly polymorphic, most recently constructed maize linkage maps are based on SSR markers.

3.2.2
First-Generation Maps

The first-generation maize linkage maps were mostly based on RFLP markers. The first molecular-marker-based maize map was published by Helentjaris et al. (1986a,b). It contained 116 loci and used cDNA and random genomic clones as probes. Maps based on

publicly available maize RFLP probes were presented by Coe et al. (1987) and Burr et al. (1988), who used random genomic clones in an F_2 population and in a set of recombinant inbred lines (RILs), respectively. Gardiner et al. (1993) published an updated version of the earlier F_2 map based on an immortalized version of the same F_2 population. It contained 214 loci and the first group of "core" markers. Beavis and Grant (1991) reported a linkage map based on information from four F_2 populations. Beavis et al. (1992) developed a mapping population based on random mating that provided improved resolution compared with F_2 or RI populations of similar size. More recently, a composite map based on four mapping populations, containing 275 loci representing both expressed sequence tagged sites (ESTs) and anonymous sequences, was published (Causse et al. 1996). A linkage map using doubled haploid lines was also constructed (Bentolila et al. 1992; Murigneux et al. 1993a,b; Dufour et al. 2001). A number of other groups have also produced RFLP maps in maize, most with the intention of mapping quantitative traits relative to the molecular markers (see details in Sect. 3.4, Analysis of QTL). Polymerase chain reaction (PCR)-based DNA markers composed of tandem-repeated, short di- or tri-nucleotide repeats known as SSR markers and AFLP markers have also been used to map genes in maize (Senior and Heun 1993; Senior et al. 1996; Taramino and Tingey 1996; Castiglioni et al. 1999). Most linkage map information is listed in the MaizeGDB public database (http://www.maizegdb.org).

Table 2. Some useful heterotic patterns in maize (from Troyer 2001)

Inbred 1	Inbred 2	RM*	Inbred 1 Family	Inbred 2 Family
A509	× Idt's early	90	NWest dent/Minn. #13	Iodent
A619	× A632	102	Lancaster/Minn. #13	Stiff stalk (B14)
A632	× B37s early	105	Stiff stalk (B14)	Stiff stalk (B37)
A632s early	× F2	85	Stiff stalk (B14)	Lacaune flint
A634	× Mo17	105	Stiff stalk (B14)	Lancaster/Krug
A634	× W153R	100	Stiff stalk (B14)	US133 (Minn. #13)
B14	× Oh43	115	Stiff stalk	Lancaster/Minn. #13
B37	× B73	112	Stiff stalk	Stiff stalk
B37	× C103	118	Stiff stalk	Lancaster
B37s early	× Idt's early	105	Stiff stalk (B37)	Iodent
B37	× Idt's late	115	Stiff stalk	Iodent
B37	× Oh43	115	Stiff stalk	Lancaster/Minn. #13
B73	× C103	117	Stiff stalk	Lancaster
B73s early	× CO255	100	Stiff stalk (B73)	Inra 258, four way
B73	× Idt	105	Stiff stalk	Iodent
B73	× Mo17	115	Stiff stalk	Lancaster/Krug
B73	× Oh07	120	Stiff stalk	Learning
B73	× Oh43	111	Stiff stalk	Lancaster/Minn. #13
C103	× Oh43	112	Lancaster	Lancaster/Minn. #13
C103	× WF9	118	Lancaster	Reid
C103s early	× WF9s early	105	Lancaster (C103)	Reid (WF9)
CM105	× F2	80	Stiff stalk (B14)	Lacaune flint
CM105	× CO255	80	Stiff stalk (B14)	Inra 258, four way
CM7	× F2	75	Ottawa flint/Mixed dent	Lacaune flint
C109	× WF9s early	90	Early Butler	Reid (WF9)
EP1, F7	× F115, W33	80	Euro. flint	I153; Golden glow, Minn. #13
F2	× Idt's early	85	Lacaune flint	Iodent
F2	× Mo17s early	90	Lacaune flint	Lancaster/Krug
F2, F7	× W401	82	Lacaune flint	Minn. #13/Golden glow
Idt's late	× WF9s late	120	Iodent	Reid (WF9)
Oh43	× W64A	110	Lancaster/Minn. #13	Reid (WF9)/Krug
W117	× W64A	95	Minn. #13	Reid (WF9)/Krug

*RM: Relative maturity

Several studies pointed out that the maize genome contains extensive chromosomal duplications (Wendel et al. 1986; Helentjaris et al. 1988; Ahn and Tanksley 1993; Gaut 2001). Comparative mapping studies have identified roughly ten duplicate (or homologous) chromosomal regions in maize, all of which share homology with a rice chromosome (Ahn and Tanksley 1993; Moore et al. 1995; Gale and Devos 1998; Wilson et al. 1999).

3.2.3
Second-Generation Maps

A high-density maize molecular linkage map was reported by Davis et al. (1999). In this map, more than 1736 loci, including sequenced core markers, grass genome reference points, and ESTs, were mapped by using an immortalized F_2 population . The total map length was 1,727.4 cM, with an average of 1.0 cM between adjacent markers. Chromosome 1 had the longest map distance of 245.2 cM, and chromosome 10 had the shortest length of 138.6 cM. The largest remaining gap (22.8 cM) in this map occurs
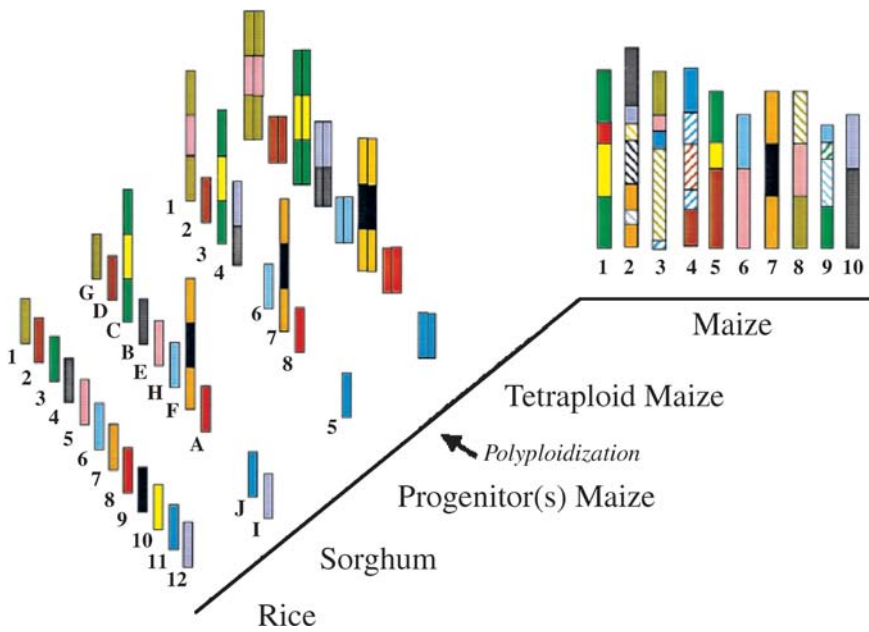
Fig. 2. Genome structures of domesticated grasses along continuum of maize evolution relative to rice. The rice genome, with 12 basic chromosome structures represented as *colored blocks*, marks the divergence of the Orzyoideae and Panicoideae subfamilies. Sorghum, progenitor maize, duplicated progenitor maize, and modern maize are drawn relative to rice chromosome structures. Chromosome inversions inferred in modern maize are indicated as *hatched shading of blocks* representing inverted linkage segments. To facilitate structural comparisons, sorghum mapping data from Pereira et al. 1994 were included and compared to rice on the basis of ISU markers (from Wilson et al. 1999, with author's permission)

in the telomere region of the short arm of chromosome 7.

The widely used public-domain maize-mapping populations were produced with minimal opportunities for recombination. The genetic resolution of the maize linkage maps constructed by using F_2 or RI populations would be improved markedly by the provision of additional opportunities for recombination before the development of the mapping progeny (Lee et al. 2002). The potential of this approach was demonstrated by Beavis et al. (1992) and Liu et al. (1996). An SSR-based high-resolution linkage map was constructed by using an intermated B73 × Mo17 (IBM) population (Sharopova et al. 2002). The newest version of this map provided in the MaizeGDB includes 2,006 markers and has a total map length of 7,308 cM, giving an average interval of 3.6 cM. The current version of the IBM linkage map is presented at http://www.maizegdb.org.

Conservation of gene/locus order across distant species is called synteny. Synteny was found not only in RFLP locus order in the maps but also at the gene level (Bennetzen and Freeling 1993; Moore et al. 1993, reviewed by Gale and Devos 1998). Synteny between maize and other grass species was reported by several authors: in sorghum and maize (Hulbert et al. 1990; Whitkus et al. 1992; Pereira et al. 1994), rice and maize (Ahn and Tanksley 1993), maize and wheat (Devos et al. 1994), rice, wheat, and maize (Ahn et al. 1993), and sugarcane, sorghum, and maize (Guimarães et al. 1997). Wilson et al. (1999) indicated a possible sequence of development of maize relative to rice (Fig. 2).

Table 3 lists some maize molecular marker linkage maps constructed from different populations and markers.

3.3
Gene Mapping

In the 1980s, RFLP markers (sometimes combined with near-isogenic lines, or NILs) were mostly used in locating single genes. The use of RFLP markers does have some disadvantages, such as a limited number of markers, the fact that analysis requires a large amount of genomic DNA, and the fact that it is time-

Table 3. Some maize molecular marker linkage maps based on different types of population and markers

Population name	Population type*	Population size	Marker type	No. of loci	Total map length (cM)	Average marker interval (cM)	Reference
H427 X 761	F_2	50	RFLP	117	703.0	6.0	Helentjaris et al. (1986a,b)
T232 X CM37	RI	46	RFLP	134	–	–	Burr et al. (1988)
CO159 X Tx303	RI	38	RFLP	136	–	–	Burr et al. (1988)
Tx303 X CO159	Immortalized F_2	56	RFLP, Isozyme	215	1,859.0	8.6	Gardiner et al. (1993)
B73 X G35	F_2	112	RFLP	106	–	–	Beavis and Grant (1991)
B73 X Mo17	F_2	112	RFLP	148	–	–	Beavis and Grant (1991)
K05 X W65	F_2	144	RFLP	78	–	–	Beavis and Grant (1991)
J40 X V94	F_2	144	RFLP	68	–	–	Beavis and Grant (1991)
Io X F2	F_2	95	RFLP, Protein	108	1,869.0	17.3	Causse et al. (1996)
Io X F2	RI	145	RFLP, Protein	142	1,843.0	13.0	Causse et al. (1996)
F252 X F2	RI	129	RFLP, Protein	139	1,604.0	11.5	Causse et al. (1996)
Io X F252	RI	152	RFLP, Protein	145	1,588.0	11.0	Causse et al. (1996)
DH5 X DH7	DH	71	RFLP	101	1,299.6	12.9	Murigneux et al. (1993b)
A188 X DH7	DH	109	RFLP	104	1,266.4	12.2	Murigneux et al. (1993b)
A188 X DH7	RI	60	RFLP	100	1,159.2	11.6	Murigneux et al. (1993b)
R6 X DH89.1	DH	72	RFLP	94	1,049.5	11.2	Bentolila et al. (1992)
R6 X DH89.1	F_2	100	RFLP	94	1,142.9	12.2	Bentolila et al. (1992)
T232 X CM37	RI	42	RFLP, SSR	220	1,499.9	6.8	Taramino and Tingey (1996)
B37 X A7	F_2	232	RFLP, AFLP	312	2,057.0	6.6	Castiglioni et al. (1999)
Tx303 X CO159	Immortalized F_2	54	RFLP, SSR	1,736	1,727.4	1.0	Davis et al. (1999)
B73 X Mo17	Intermated	302	RFLP, SSR	2,006	7,308.0	3.6	MaizeGDB (2004)

* RI: Recombinant inbred lines, DH: Doubled haploid

consuming and costly. The AFLP technique (Vos et al. 1995) provides a marker system that enables, with modest effort, the rapid evaluation of many thousands of polymorphic loci. AFLPs have been used widely in linkage map construction and marker identification. Bulked segregant analysis (BSA) was proposed by Michelmore et al. (1991) to overcome the problems associated with a lack of availability of NILs. The use of BSA in combination with the AFLP method has been proved to be a very useful and powerful technique for identifying markers tightly linked to or cosegregating with genes underlying monogenic traits in various plant species (Meksem et al. 1995; Simons et al. 1997; Yang et al. 1997; Tai et al. 1999; Xu et al. 1999; von Malek et al. 2000; Ouedraogo et al. 2001). Recently, resistance gene analog markers have also been used in targeting resistance genes (Collins et al. 1998, 1999, 2001; Rostoks et al. 2002).

Bentolila et al. (1991) identified an RFLP marker tightly linked to the *Ht1* (northern corn leaf blight) gene using four pairs of NILs and an F_2 population of the DF20 × LH146*Ht* cross. *Ht1* was located on chromosome 2 (Hooker 1963). Of 26 probes of chro-

mosome 2 tested, six, all located on the long arm, exhibited a variable pattern among the four NILs, in the F_2 segregating population. Finally, *Ht1* was found to be linked with an RFLP marker, umc105B, at a distance of <1.0 cM. The second gene for resistance to northern corn leaf blight, *Ht2*, was mapped on chromosome 8, and RFLP marker umc48 was found to be linked with *Ht2* by using 375 F_2 plants from the A619*Ht2* × W64A cross (Zaitlin et al. 1992).

Southern corn leaf blight is a serious disease widely distributed in warm-temperate and tropical corn-producing areas throughout the world. It is caused by the fungus *Bipolaris maydis* (Nisikado) Shoemaker (= *Helminthosporium maydis* Nisikado and Miyake). The O-race-specific resistance gene *rhm* was shown to be located on the short arm of chromosome 6 (Smith and Hooker 1973). By the use of isogenic line pairs and linkage analysis using an $F_{2:3}$ population, the RFLP probe umc85 and marker agrp144 were found to be closely linked to the *rhm* locus, and agrp144 was found to be tightly linked to *rhm* at 0.5 cM (Zaitlin et al. 1993). Zaitlin et al. (1993) also developed a sequence tagged site (STS) marker from

the sequence of agrP144, to distinguish *rhm* and non-*rhm* lines. However, this primer pair could not detect the difference between H95 and H95*rhm*. Cai et al. (2003) applied a combination of the AFLP technique and BSA to a large F_2 population (720 plants) to identify molecular markers linked to the *rhm*. One codominant AFLP marker, p7m36, was linked to *rhm* at 1.0 cM apart, and it was converted to an STS marker. Combining the newly found p7m36 marker and the previously identified agrp144 marker may be useful in map-based cloning of the *rhm* gene and in marker-assisted selection (MAS) for breeding with *rhm*.

Lehmensiek et al. (2001) detected three QTLs for the gray leaf spot resistance gene in maize. By using BSA and AFLP markers in 230 F_2 plants, the researchers detected 11 polymorphisms and converted them to sequence-specific PCR markers. Five of the 11 converted AFLPs were linked to three gray leaf spot resistance QTLs. Of these, a QTL on chromosome 1 with a logarithm of odds (LOD) score of 21 was localized in bin 1.05/06 and accounted for 37% of total variance. Agrama et al. (2002) identified four AFLP markers linked to genes for resistance to sorghum downy mildew in an RI population in maize. Of those, three were mapped on chromosomes 1 and 9. The other marker was associated with disease susceptibility but could not be linked to any chromosome. These four AFLP fragments were isolated, cloned, and sequenced, and sequence-characterized amplified region markers were developed.

Wise and Schnable (1994) mapped the *rf1* and *rf2* nuclear-fertility restorer loci of Texas (T) cytoplasm using RFLP and visible markers. *rf1* was mapped on chromosome 3, linked to two RFLP markers, umc97 and umc10, with a spacing of 1.1 cM, on the basis of RFLP and visible marker data from five mapping populations. *rf2* was mapped on the consensus map of maize chromosome 9, linked to an RFLP marker, umc153, with a spacing of 3.8 cM. The gene *Teosinte crossing barrier 1* (*tcb1*), governing hybridization of teosinte with maize, was fine-mapped on chromosome 6 by using SSR markers in a BC_1 (backcross) population; *tcb1* cosegregated with SSR marker MMC0471 and lay 0.5 cM from the SSR marker Bnlg490 (Evans and Kermicle 2001). Table 4 summarizes the results of gene mapping using molecular markers.

3.4
Quantitative Trait Loci (QTL) Analysis

There are many reports on QTL mapping in maize, including QTLs for yield and related traits (Edwards et al. 1987; Stuber et al. 1992; Ajmone-Marsan et al. 1994; Beavis et al. 1994; Veldboom and Lee 1994; Ajmone-Marsan et al. 1995; Ragot et al. 1995; Austin and Lee 1996b; Graham et al. 1997; Lübberstedt et al. 1997; Ribaut et al. 1997; Eta-Ndu and Openshaw 1999; Frova et al. 1999; Jiang et al. 1999; Austin et al. 2000; Ajmone-Marsan et al. 2001), for disease and insect resistance (Pe et al. 1993; Schön et al. 1993; Jung et al. 1994; Bohn et al. 1996; Byrne et al. 1996; Holland et al. 1998; Lübberstedt et al. 1998; Marçon et al. 1999; Pernet et al. 1999; Welz et al. 1999; Brown et al. 2001; Krakowsky et al. 2004), for quality traits (Kahler 1985; Sughroue and Rocheford 1994; Goldman et al. 1994; Berke and Rocheford 1995), for stress tolerance (Frova and Sari-Gorla 1994; Lebreton et al. 1995; Agrama and Moussa 1996; Sibov et al. 1999), and for other morphological and physiological traits (Beavis et al. 1991; Doebley and Stec 1991; Edwards et al. 1992; Doebley and Stec 1993; Koester et al. 1993; Damerval et al. 1994; Doebley et al. 1994; Quarrie et al. 1994; Sari-Gorla et al. 1994; Veldboom et al. 1994; Berke and Rocheford 1995; Causse et al. 1995; Austin and Lee 1996a; Veldboom and Lee 1996; Berke and Rocheford 1999; Vlădutu et al. 1999; Austin et al. 2001; Cardinal et al. 2003; Flint-Garcia et al. 2003a–c). Examples of QTL mapping in maize follow.

Stuber et al. (1992) reported the results of QTLs for heterosis and genotype-by-environment (G × E) interaction using a cross between two widely used elite maize inbred lines, B73 and Mo17. For the traits evaluated, 264 BC families (backcrossed to B73 or Mo17) and 264 F_4 families were used. Using the data of 76 isozymes and RFLP markers that represented 90 to 95% of the maize genome, they detected six QTLs explaining 60.9% of total variance for grain yield in the backcross to B73 families and eight QTLs explaining 59.1% of total variance in the backcross to Mo17 families. On the other hand, although the plants were grown and measured in six diverse environments, there was little evidence for G × E interaction for most QTLs.

Holland et al. (1998) mapped the QTLs for southern corn rust using two $F_{2:3}$ families. Using 11 markers mapped on chromosomes 3, 4, and 10, on which common rust resistance genes are located, a single locus

Table 4. Some genes targeted by molecular markers

Trait	Gene	Chromo-some	Marker type	Nearest marker	Converted to PCR marker	Reference
Resistance to northern corn leaf blight	*Ht1*	2	RFLP	1.0 cM to UMC105B	No	Bentolila et al. (1991)
Resistance to northern corn leaf blight	*Ht2*	8	RFLP	4.9 cM to UMC48a	No	Zaitlin et al. (1992)
Resistance to southern corn leaf blight	*rhm*	6	RFLP	0.5 cM to agrp144	STS marker	Zaitlin et al. (1993)
Resistance to southern corn leaf blight	*rhm*	6	AFLP	1.0 cM to p7m36	STS marker	Cai et al. (2003)
Resistance to gray leaf spot		1	AFLP	Close to us44	STS marker	Lehmensiek et al. (2001)
Resistance to gray leaf spot		5	AFLP	Close to us40	STS marker	Lehmensiek et al. (2001)
Resistance to sorghum downy mildew		1	AFLP	Close to EM227	SCAR marker	Agrama et al. (2002)
Resistance to sorghum downy mildew		9	AFLP	Close to EM159	SCAR marker	Agrama et al. (2002)
Nuclear-fertility restorer locus	*rf1*	3	RFLP	1.1 cM to UMC97	No	Wise and Schnable (1994)
Nuclear-fertility restorer locus	*rf2*	9	RFLP	3.8 cM to UMC153	No	Wise and Schnable (1994)
Teosinte crossing barrier 1	*Tcb1*	4	SSR	0.5 cM to MMC0471		Evans and Kermicle (2001)

on 10S, bnl3.04, was associated with 82 to 83% of the variation among field resistance scores of selected $F_{2:3}$ families. Two loci, on chromosomes 3 (*umc26*) and 4 (*umc31*), were significantly associated with resistance only in one family accounting for 13 to 15% of the phenotypic variation in $F_{2:3}$ field scores. A recent study by Chen et al. (2004) also found a major gene for rust resistance on chromosome 10, in agreement with the results of Holland et al. (1998).

Doebley and Stec (1993) compared the results of QTLs for morphological differences between maize and teosinte in two F_2 populations to reveal the mechanisms of maize evolution. They detected 50 significant associations (putative QTLs) between the molecular marker loci and nine key traits that distinguish maize and teosinte. The results indicated that probably a relatively small number of loci with larger effects were involved in the early evolution of the key traits that distinguish maize and teosinte.

H.W. Cai and coworkers (unpubl. obs.) mapped the QTLs for oil concentration in maize using an F_2 and two BC_1 populations. The linkage maps were constructed with Joinmap software (Stam 1993) from AFLP and SSR markers. The putative QTLs for oil concentration were detected by using the interval mapping method in MapQTL software (van Ooijen and Maliepaard 1996). A total of 13 QTLs for oil con-

centration were detected on five chromosomes and in two unknown linkage groups; three of the QTLs (qOC4-1, qOC6-2, and qOC7-1) showed a positive dominance effect, and the others showed a negative dominance effect. This suggests that heterosis for the trait of oil concentration might not be apparent in high-oil-maize breeding programs. Of the 13 QTLs, five showed mainly an additive effect, and only one (qOCun-1) showed mainly a dominance effect.

Vlădutu et al. (1999) detected two linked QTLs affecting the timing of pollen shed on chromosome 8L by using selected recombinant lines from F_3 and F_4 plants derived from crosses between E20 variants (an early maturing derivative of N28) and N28. From the phenotype, they assumed that each QTL harbors a single gene, and they named the respective genes *Vegetative to generative transition 1* (*Vgt1*, at the major QTL) and 2 (*Vgt2*, at the minor QTL). *Vgt1* affected the timing of the transition of the apical meristem, and *Vgt2* affected the global extent of internode elongation.

3.5
Marker-Assisted Breeding

There are numerous reports on the genetic diversity of maize inbred lines (Melchinger et al. 1991; Messmer et al. 1991, 1992; Livini et al. 1992; Dubreuil et al. 1996; Smith et al. 1997; Pejic et al. 1998; Benchimol et al. 2000; Lu and Bernardo 2001; Enoki et al. 2002; Liu et al. 2003) and on maize-breeding populations (Doebley et al. 1986; Dubreuil and Charcosset 1998; Rebourg et al. 2001, 2003; Gauthier et al. 2002; Reif et al. 2004) based on isozyme, RFLP, and SSR data. Most studies of the genetic diversity of maize inbred lines based on RFLP or SSR data showed that classification by molecular distance was convenient for (a) identifying heterotic groups and (b) assigning origins to unknown or broadly based inbreds. That information should prove useful for choosing (a) combinations of inbreds to be evaluated in hybrid trials and (b) parents for breeding programs.

Stuber (1994) reported success in the use of molecular markers for yield enhancement. On the basis of the results of QTLs for heterosis, they transferred the identified segments of maize inbred lines of T x 303 and Oh43 (six fragments each) to B73 and Mo17 by backcrossing. The yields of hybrids between enhanced B73 and enhanced Mo17 were higher yield than that of the normal hybrid B73 × Mo17 (Table 5). Bouchez et al. (2002) also reported the results of marker-assisted introgression of QTLs of favorable alleles between maize elite lines. By the use of marker analysis and backcrossing, they transferred the target fragments for three QTLs for earliness and grain yield to other elite lines. After the fragments were transferred, the QTL positions were generally sustained in the introgression background. The magnitude and sign of the QTL effects on earliness were in good agreement with those expected from initial RIL analysis. Conversely, important discrepancies were observed in the magnitude and sign of the QTL effects on yield observed after introgression, relative to the initial RIL analysis. The authors indicated that these discrepancies were probably due to important genotype-by-environment interactions. Flint-Garcia et al. (2003a) compared the efficiency of phenotypic selection (PS) versus MAS for rind penetrometer resistance (a parameter for stalk strength) and resistance to second-generation European corn borer. MAS for high or low penetrometer resistance was effective in the three populations studied. PS for both high and low penetrometer resistance

was more effective than MAS in two of the populations. However, in a third population, MAS for high penetrometer resistance using QTL effects from the same population was more effective than PS, and using QTL effects from a separate population was just as effective as PS. MAS for resistance and susceptibility to European corn borer using QTL effects from the same populations was effective in increasing susceptibility but not in increasing resistance. MAS using QTL effects from a separate population was effective in both directions of selection.

We have analyzed the gene frequency of *rhm* (gene for resistance to southern corn leaf blight) using STS marker p7m36 (Cai et al. 2003); three alleles of p7m36 were detected among the 15 inbred lines used. The resistance or susceptibility of public inbred lines and some private companies' inbred lines indicates that resistant inbred lines H95*rhm*, B73*Htrhm*, and H84 have allele 1, the susceptible inbred lines 957L and H95 have allele 2, and the susceptible inbred lines 914L and Mo17*Ht* have allele 3. Therefore, allele 1 seems to correspond to the resistant allele of *rhm* and alleles 2 or 3 to the susceptible alleles of *rhm*. From these results we concluded that the *rhm*-unknown inbred lines TD16 and TD40, which have allele 1, would be resistant to southern corn leaf blight; whereas the inbred lines TD34, TD35, and TD43 have allele 2, and TD25 and TD26 have allele 3, and so would be susceptible to southern corn leaf blight (Cai et al. unpubl. obs.).

3.6
Map-Based Cloning

Map-based cloning has been reported in several major plant species, such as rice (Song et al. 1995; Yoshimura et al. 1998; Ashikari et al. 1999; Yano et al. 2000), barley (Büschges et al. 1997; Kilian et al. 1997; Simons et al. 1997; Han et al. 1999; Wei et al. 1999; Brueggeman et al. 2002), wheat (Stein et al. 2000; Huang et al. 2003), tomato (Martin et al. 1993; Jones et al. 1994; Dixon et al. 1996; Salmeron et al. 1996), *Arabidopsis* (Bent et al. 1994; Mindrinos et al. 1994; Grant et al. 1995), tobacco (Whitham et al. 1994), potato (Bendahmane et al. 1999), and flax (Lawrence et al. 1995). However, unlike in wheat, which has three subgenomes, allowing subgenome mapping (Stein et al. 2000), and unlike in barley, which has high synteny with rice (Kilian et al. 1997; Brunner et al. 2003), map-based

Table 5. Grain yields of 5 high-yielding, single-cross hybrids developed by crossing "enhanced" B73 lines with "enhanced" Mo17 lines (from Stuber 1994)

Enhanced lines	Segment	Grain yield (bushels/acre)		
		1993	1994	Average
B73 (248-6)	5S, 6L (Tx303)			
Mo17 (284-7)	3S, 10S (Oh43)	178.7	170.9	174.8
B73 (257-1)	6L (Tx303)			
Mo17 (271-8)	3S, 4S, 10S (Oh43)	178.1	169.5	173.8
B73 (198-2)	1S, 5S, 6L (Tx303)			
Mo17 (41-27)	4S, 9S (Oh43)	162.8	191.2	177.0
B73 (82-06)	3S, 5S (Tx303)			
Mo17 (271-9)	4S, 10S (Oh43)	160.8	189.3	175.1
B73 (198-2)	1S, 5S, 6L (Tx303)			
Mo17 (278-8)	3S, 4S, 10S (Oh43)	173.5	185.5	179.5
Checks-B73 × M17		154.8	165.8	160.3
-P3165		156.4	169.7	163.1
S.E.D*		6.4	5.1	4.5

*Standard error of mean difference

cloning in maize is very difficult. Maize has a moderate genome size (about 2,400 Mb), and 1 cM on the genetic map corresponds to 1,500 Kb on the physical map (Civardi et al. 1994). Therefore, in order to use chromosome walking to isolate a gene, a very large mapping population (>2,000 individuals) is needed. In addition, maize has the *Ac-Ds* transposon system, *Spm*, and Robertson's mutator, *Mu*, which are usually used in gene isolation in maize (Chomet 1994; Cone 1994; Dellaporta and Moreno 1994).

However, the basic tools for map-based cloning in maize are complete. Bacterial artificial chromosome (BAC) libraries (different varieties with different vector and restriction enzyme combinations) exist (http://genome.arizona.edu/). Many RFLP and SSR markers are mapped on the chromosomes, and more than 100,000 ESTs are under development by various institutes (Coe et al. 2001; O'Sullivan et al. 2001).

Examples of map-based gene cloning in maize were reported by Pei et al. (2000) and Salvi et al. (2002). Pei et al. (2000) attempted to clone a maize T-cytoplasm restorer gene, *rf1*. Two B73 BAC libraries were screened using tightly linked RFLP markers and low-copy-number probes designed from the *rf1* candidate cDNA. Two BAC contigs were constructed. The authors tried to isolate a major QTL for flowering time in maize, *Vgt1* (vegetative to generative transition 1), by using positional cloning. An AFLP marker closely linked to *Vgt1* (ca. 0.3 cM) was found and has been used to screen new markers (Salvi et al. 2002).

3.7
Future Scope of Works

3.7.1
Maize Genome Sequencing

So far, the genomes of two plant species, *Arabidopsis* and rice, have been completely sequenced (Arabidopsis Genome Initiative 2000; Goff et al. 2002; Yu et al. 2002). Compared to *Arabidopsis* and rice, the genome size of maize is much larger (19 times that of *Arabidopsis* and 6 times that of rice). To sequence the maize genome has been estimated to require about US$52 million and 4 years (Bennetzen et al. 2001). The maize genome sequencing project sponsored by the National Science Foundation (http://www.nsf.gov/bio/pubs/awards/genome02.htm) will focus on the gene-rich, low-copy fraction of the genome, and the inbred line B73 will be the primary focus of the project.

3.7.2
Next-Generation Marker Development: SNP

The single nucleotide polymorphism (SNP) is the next-generation genetic marker. It is capable of very-high-throughput genotyping for genetic mapping. Marker-assisted breeding and plant germplasm pro-

tection are made possible by high polymorphism and easy automatic analysis. The results of an SNP project at DuPont and Pioneer showed that alignment and analysis of amplification products from 20 loci randomly distributed in the genome among 30 inbred lines revealed very frequent polymorphisms averaging one nucleotide change per 70 bp; 60% of the SNPs were transitions and 40% were transversions. One insertion or deletion was detected per 160 bp. The researchers selected eight inbred lines to catalog SNP alleles at 1,000 loci selected from ESTs and at genes of interest (Bhattramakki et al. 2000).

3.7.3
Map-Based Cloning Using Information of Sorghum and Rice Genome Sequences

Comparative genome analysis has demonstrated extensive conservation of gene content and order at the level of the overall genetic map among rice, maize, sorghum, and wheat (Gale and Devos 1998). However, as many as 15,000 local rearrangements differentiate the maize and rice genomes (Tikhonov et al. 1999; Dubcovsky et al. 2001). Therefore, rice may be too distant a model to facilitate rapid map-based cloning in maize and other important cereals such as wheat, barley, sorghum, and oats. For this reason, another well-studied crop species, sorghum, will be useful in map-based cloning studies. Sorghum has about twice the genome size of rice, and high-resolution genetic maps based on STSs, RFLPs, AFLPs, and SSRs have been constructed (Menz et al. 2002; Bowers et al. 2003). Moreover, sorghum is closely related to maize. The basic tools for map-based cloning, BAC libraries, have been constructed by several authors (Woo et al. 1994; Klein et al. 2000; Draye et al. 2001). A complete set of maize chromosome addition lines to oat has been developed (Kynast et al. 2001) and will be useful in the map-based cloning of maize genes.

References

Agrama HAS, Moussa ME (1996) Mapping QTLs in breeding for drought tolerance in maize (*Zea mays* L.). Euphytica 91:89–97

Agrama HA, Houssin SF, Tarek MA (2002) Cloning of AFLP markers linked to resistance to *Peronosclerospora sorghi* in maize. Mol Genet Genom 267:814–819

Ahn S, Tanksley SD (1993) Comparative linkage maps of the rice and maize genomes. Proc Natl Acad Sci USA 90:7980–7984

Ahn S, Anderson JA, Sorrells ME, Tanksley SD (1993) Homoeologous relationships of rice, wheat and maize chromosomes. Mol Gen Genet 241:483–490

Ajmone-Marsan P, Monfredini G, Ludwig WF, Melchinger AE, Franceschini P (1994) Identification of genomic regions affecting plant height and their relationship with grain yield in an elite maize cross. Maydica 39:133–139

Ajmone-Marsan P, Monfredini G, Ludwig WF, Melchinger AE, Franceschini P, Pagnotto G, Motto M (1995) In an elite cross of maize, a major quantitative trait locus controls one-fourth of the genetic variation for grain yield. Theor Appl Genet 90:415–424

Ajmone-Marsan P, Gorni C, Chittò A, Redaelli R, van Vijk R, Stam P, Motto M (2001) Identification of QTLs for grain yield and grain-related traits of maize (*Zea mays* L.) using an AFLP map, different testers, and cofactor analysis. Theor Appl Genet 102:230–243

Arabidopsis Genome Initiative (2000) Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. Nature 408:796–815

Ashikari M, Wu, J, Yano M, Sasaki T, Yoshimura A (1999) Rice gibberellin-insensitive dwarf mutant gene *Dwarf1* encodes the alpha-subunit of GTP-binding protein. Proc Natl Acad Sci USA 96:10284–10289

Austin DF, Lee M (1996a) Genetic resolution and verification of quantitative trait loci for flowering and plant height with recombinant inbred lines of maize. Genome 39:957–968

Austin DF, Lee M (1996b) Comparative mapping in $F_{2:3}$ and $F_{6:7}$ generations of quantitative trait loci for grain yield and yield components in maize. Theor Appl Genet 92:817–826

Austin DF, Lee M, Veldboom LR, Hallaur AR (2000) Genetic mapping in maize with hybrid progeny across testers and generations: grain yield and grain moisture. Crop Sci 40:30–39

Austin DF, Lee M, Veldboom LR (2001) Genetic mapping in maize with hybrid progeny across testers and generations: plant height and flowering. Theor Appl Genet 102:163–176

Beal WJ (1877) Report of the professor of botany and horticulture. Michigan Board of Agriculture, Lansing, MI

Beavis WD, Grant D (1991) A linkage map based on information from four F_2 populations of maize (*Zea mays* L.). Theor Appl Genet 82:636–644

Beavis WD, Grant D, Albertsen M, Fincher R (1991) Quantitative trait loci for plant height in four maize populations and

their associations with qualitative genetic loci. Theor Appl Genet 83:141–145

Beavis WD, Lee M, Grant D, Hallauer AR, Owens T (1992) The influence of random mating on recombination among RFLP loci. Maize Gene Coop Newslett 66:52–53

Beavis WD, Smith OS, Grant D, Fincher R (1994) Identification of quantitative trait loci using a small sample of topcrossed and F_4 progeny from maize. Crop Sci 34:882–896

Beckett JB (1991) Cytogenetic, genetic, and plant breeding applications of B-A translocations in maize. In: Gupta PK, Tsuchiya T (eds) Chromosome Engineering in Plants: Genetics, Breeding, and Evolution, Part A. Elsevier, Amsterdam, pp 493–529

Benchimol LL, de Souza CL Jr, Garcia AAF, Kono PMS, Mangolin CA, Barbosa AMM et al (2000) Genetic diversity in tropical maize inbred lines: heterotic group assignment and hybrid performance determined by RFLP markers. Plant Breed 119:491–496

Bendahmane A, Kanyuka K, Baulcombe DC (1999) The *Rx* gene from potato controls separate virus resistance and cell death responses. Plant Cell 11:781–792

Bennetzen JL, Freeling M (1993) Grasses as a single genetic system: genome composition, colinearity, and compatibility. Trends Genet 9:259–261

Bennetzen JL, Chandler VL, Schnable P (2001) National Science Foundation-sponsored workshop report. Maize genome sequencing project. Plant Physiol 127:1572–1578

Bent AF, Kunkel BN, Dahlbeck D, Brown KL, Schmidt RL, Giraudat J et al (1994) *RPS2* of *Arabidopsis thaliana*: a leucine-rich repeat class of plant disease resistance genes. Science 265:1856–1860

Bentolila S, Guitton C, Bouvet N, Sailland A, Nykaza S, Freyssinet G (1991) Identification of an RFLP marker tightly linked to the *Ht1* gene in maize. Theor Appl Genet 82:393–398

Bentolila S, Hardy T, Guitton C, Freyssinet G (1992) Comparative genetic analyses of F_2 plants and anther culture derived plants of maize. Genome 35:575–582

Berke TG, Rocheford TR (1995) Quantitative trait loci for flowering, plant and ear height, and kernel traits in maize. Crop Sci 35:1542–1549

Berke TG, Rocheford TR (1999) Quantitative trait loci for tassel traits in maize. Crop Sci 39:1439–1443

Bhattramakki D, Ching A, Morgante M, Dolan M, Register J, Smith H, Tingey S, Rafalski JA (2000) Conserved single nucleotide polymorphism (SNP) haplotypes in maize. In: Plant and Animal Genome VIII Conf, San Diego

Bohn M, Khairallah M, Gonzalez de Leon D, Hoisington DA, Utz H (1996) QTL mapping in tropical maize. 1. Genomic regions affecting leaf feeding resistance to sugarcane borer and other traits. Crop Sci 36:1352–1361

Bouchez A, Hospital F, Causse M, Gallais A, Charcosset A (2002) Marker-assisted introgression of favorable alleles at quantitative trait loci between maize elite lines. Genetics 162:1945–1959

Bowers JE, Abbey C, Anderson S, Chang C, Draye X, Hoppe AH et al (2003) A high-density genetic recombination map of sequence-tagged sites for *sorghum*, as a framework for comparative structural and evolutionary genomics of tropical grains and grasses. Genetics 165:367–386

Brown AHD, Allard RW (1969) Further isozyme differences among the inbred parents of a reciprocal recurrent selection population of maize. Crop Sci 9:643–644

Brown AF, Juvik JA, Pataky JK (2001) Quantitative trait loci in sweet corn associated with partial resistance to Stewart's wilt, northern corn leaf blight, and common rust. Phytopathology 91:293–300

Brueggeman R, Rostoks N, Kudrna D, Kilian A, Han F (2002) The barley stem rust-resistance gene *Rpg1* is a novel disease-resistance gene with homology to receptor kinases. Proc Natl Acad Sci USA 99:9328–9333

Brunner S, Keller B, Feuillet C (2003) A large rearrangement involving genes and low-copy DNA interrupts the micro-collinearity between rice and barley at the *Rph7* locus. Genetics 164:673–683

Burr B, Burr FA (1985) Towards a molecular characterization of multiple factor inheritance. In: Zaitlin M et al (eds) Biotechnology in Plant Science. Academic, New York, pp 277–284

Burr B, Evola SV, Burr FA, Beckman J (1983) The application of restriction fragment length polymorphism to plant breeding. In: Setlow JK, Hollaender A (eds) Genetic Engineering: Principles and Methods. Plenum, New York, pp 45–58

Burr B, Burr SA, Thompson KH, Albertsen MC, Stuber CW (1988) Gene mapping with recombinant inbreds in maize. Genetics 118:519–526

Büschges R, Hollricher K, Panstruga R, Simons G, Wolter M (1997) The barley *Mlo* gene: a novel control element of plant pathogen resistance. Cell 88:695–705

Byrne PF, McMullen MD, Snook ME, Musket TA, Theuri JM, Widstrom NW et al (1996) Quantitative trait loci and metabolic pathways: genetic control of the concentration of maysin, a corn earworm resistance factor, in maize silks. Proc Natl Acad Sci USA 93:8820–8825

Cai HW, Gao ZS, Yuyama N, Ogawa N (2003) Identification of AFLP markers closely linked to the *rhm* gene for resistance to southern corn leaf blight in maize by using bulked segregant analysis. Mol Gen Genom 269:299–303

Cardinal AJ, Lee M, Moore KJ (2003) Genetic mapping and analysis of quantitative trait loci affecting fiber and lignin content in maize. Theor Appl Genet 106:866–874

Castiglioni P, Ajmone-Marsan P, van Wijk R, Motto M (1999) AFLP markers in a molecular linkage map of maize: codominant scoring and linkage group distribution. Theor Appl Genet 99:425–431

Causse M, Rocher JP, Henry AM, Charcosset A, Prioul JL (1995) Genetic dissection of the relationship between carbon metabolism and early growth in maize, with emphasis on key enzyme loci. Mol Breed 1:259–272

Causse M, Santoni S, Damerval C, Maurice A, Charcosset A (1996) A composite map of expressed sequences in maize. Genome 39:418–432

Chen CX, Wang ZL, Yang DE, Ye CJ, Zhao YB, Jin DM et al (2004) Molecular tagging and genetic mapping of the disease resistance gene *RppQ* to southern corn rust. Theor Appl Genet 108:945–950

Chomet PS (1994) Transposon tagging with Mutator. In Freeling M, Walbot V (eds) The Maize Handbook. Springer, Berlin Heidelberg New York, pp 243–249

Civardi L, Xia Y, Edwards KJ, Schnable PS, Nikolau BJ (1994) The relationship between genetic and physical distances in the cloned *a1-sh2* interval of the *Zea mays* L. genome. Proc Natl Acad Sci USA 91:8268–8272

Coe EH, Hoisington DA, Neuffer MG (1987) Linkage map of corn (maize) (*Zea mays* L.). Maize Genet Coop Newslett 61:116–147

Coe EH, Polacco M, Davis GL, McMullen MD (2001) Maize molecular maps: markers, bins, and database. In: Phillips RL, Vasil IK (eds) DNA-Based Markers in Plants. Kluwer, Dordrecht, pp 255–284

Collins NC, Webb CA, Seah S, Ellis JG, Hulbert SH, Pryor A (1998) The isolation and mapping of disease resistance gene analogs in maize. Mol Plant-Micr Interact 11:968–978

Collins NC, Drake J, Ayliffe M, Sun Q, Ellis J, Hulbert S, Pryor T (1999) Molecular characterization of the maize Rp1-D rust resistance haplotype and its mutants. Plant Cell 11:1365–1376

Collins N, Park R, Spielmeyer W, Ellis J, Pryor AJ (2001) Resistance gene analogs in barley and their relationships to rust resistance genes. Genome 44:375–381

Cone K (1994) Transposon tagging with Spm. In: Freeling M, Walbot V (eds) The Maize Handbook. Springer, Berlin Heidelberg New York, pp 240–242

Damerval C, Maurice A, Josse JM, de Vienne D (1994) Quantitative trait loci underlying gene product variation: a novel perspective for analyzing regulation of genome expression. Genetics 137:289–301

Davis GL, McMullen MD, Baysdorfer C, Musket T, Grant D, Staebell M et al (1999) A maize map standard with sequenced core markers, grass genome reference points and 932 expressed sequence tagged sites (ESTs) in a 1736-locus map. Genetics 152:1137–1172

Dellaporta SL, Moreno MA (1994) Gene tagging with Ac/Ds elements in maize. In: Freeling M, Walbot V (eds) The Maize Handbook. Springer, Berlin Heidelberg New York, pp 219–233

Devos KM, Chao S, Li QY, Simonetti MC, Gale MD (1994) Relationship between chromosome 9 of maize and wheat homeologous group 7 chromosomes. Genetics 138:1287–1292

Dixon MS, Jones DA, Keddie JS, Thomas CM, Harrison K, Jones JDG (1996) The tomato *Cf-2* disease resistance locus comprises two functional genes encoding leucine-rich repeat proteins. Cell 84:451–459

Doebley J, Stec A (1991) Genetic analysis of the morphological differences between maize and teosinte. Genetics 129:285–295

Doebley J, Stec A (1993) Inheritance of the morphological differences between maize and teosinte: comparison of results for two F_2 populations. Genetics 134:559–570

Doebley JF, Goodman MM, Stuber CW (1986) Exceptional genetic divergence of northern flint corn. Am J Bot 73:64–69

Doebley J, Bacigalupo A, Stec A (1994) Inheritance of kernel weight in two maize-teosinte hybrid populations: implications for crop evolution. J Hered 85:191–195

Draye X, Lin YR, Qian XY, Bowers JE, Burow GB, Morrell PL et al (2001) Toward integration of comparative genetic, physical, diversity, and cytomolecular maps for grasses and grains, using the sorghum genome as a foundation. Plant Physiol 125:1325–1341

Dubcovsky J, Ramakrishna W, SanMiguel PJ, Busso CS, Yan L, Shiloff BA, Bennetzen JL (2001) Comparative sequence analysis of collinear barley and rice bacterial artificial chromosomes. Plant Physiol 125:1342–1353

Dubreuil P, Charcosset A (1998) Genetic diversity within and among maize populations: a comparison between isozyme and nuclear RFLP loci. Theor Appl Genet 96:577–587

Dubreuil P, Dufour P, Krejcj E, Causse M, de Vienne D, Gallais A, Charcosset A (1996) Organization of RFLP diversity among inbred lines of maize representing the most significant heterotic groups. Crop Sci 36:790–799

Dudley JW, Lambert RJ (1992) Ninety generations of selection for oil and protein in maize. Maydica 37:81–87

Dufour P, Johnsson C, Antoine-Michard S, Cheng R, Murigneux A, Beckert M (2001) Segregation distortion at marker loci: variation during microspore embryogenesis in maize. Theor Appl Genet 102:993–1001

Edwards MD, Stuber CW, Wendel JF (1987) Molecular-marker-facilitated investigation of quantitative-trait loci in maize. I. Numbers, genomic distribution and types of gene action. Genetics 116:113–125

Edwards MD, Helentjaris T, Wright S, Stuber CW (1992) Molecular-marker-facilitated investigations of quantitative trait loci in maize. 4. Analysis based on genome saturation with isozyme and restriction fragment length polymorphism markers. Theor Appl Genet 83:765–774

Emerson RA, Beadle GW, Fraser AC (1935) A summary of linkage studies in maize. Cornell Univ Agric Exp Stn Mem 180:1–83

Enoki H, Sato H, Koinuma K (2002) SSR analysis of genetic diversity among maize inbred lines adapted to cold regions of Japan. Theor Appl Genet 104:1270–1277

Eta-Ndu JT, Openshaw SJ (1999) Epistasis for grain yield in two F_2 populations of maize. Crop Sci 39:346–352

Evans MMS, Kermicle JL (2001) Teosinte crossing barrier 1, a locus governing hybridization of teosinte with maize. Theor Appl Genet 103:259–265

Flint-Garcia SA, Darrah LL, McMullen MD, Hibbard BE (2003a) Phenotypic versus marker-assisted selection for stalk

strength and second-generation European corn borer resistance in maize. Theor Appl Genet 107:1331–1336

Flint-Garcia SA, Jampatong C, Darrch LL, McMullen MD (2003b) Quantitative trait locus analysis of stalk strength in four maize populations. Crop Sci 43:13–22

Flint-Garcia SA, McMullen MD, Darrch LL (2003c) Genetic relationship of stalk strength and ear height in maize. Crop Sci 43:23–31

Frova C, Sari-Gorla M (1994) Quantitative trait loci (QTLs) for pollen thermotolerance detected in maize. Mol Gen Genet 245:424–430

Frova C, Krajewski P, di Fonzo N, Villa M, Sari-Gorla M (1999) Genetic analysis of drought tolerance in maize by molecular markers. I. Yield components. Theor Appl Genet 99:280–288

Gale MD, Devos KM (1998) Comparative genetics in the grasses. Proc Natl Acad Sci USA 95:1971–1974

Galinat WC (1988) The origin of corn. In: Sprague GF, Dudley JW (eds) Corn and Corn Improvement (3rd ed). ASA/CSSA/SSSA Publishers, Madison, WI, pp 1–31

Galinat WC (1992) Evolution of corn. Adv Agron 47:203–231

Gardiner J, Coe E, Melis-Hancocks S, Hoisington DA, Chao S (1993) Development of a core RFLP map in maize using an immortalized-F_2 population. Genetics 134:917–930

Gaut BS (2001) Patterns of chromosomal duplication in maize and their implications for comparative maps of the grasses. Genome Res 11:55–66

Gauthier P, Gouesnard B, Dallard J, Redaelli R, Rebourg C, Charcosset A, Boyat A (2002) RFLP diversity and relationships among traditional European maize populations. Theor Appl Genet 105:91–99

Goff SA, Ricke D, Lan TH, Presting G, Wang R, Dunn M et al (2002) A draft sequence of the rice genome (*Oryza sativa* L. ssp. *japonica*). Science 296:92–100

Goldman IL, Rocheford TR, Dudley JW (1994) Molecular markers associated with maize kernel oil concentration in an Illinois high protein × Illinois low protein cross. Crop Sci 34:908–915

Goodman MM, Stuber CW (1983) Maize. In: Tanksley SD, Orton TJ (eds) Isozymes in Plant Genetics and Breeding, Part B. Elsevier, Amsterdam, pp 1–33

Graham GI, Wolff DW, Stuber CW (1997) Characterization of a yield quantitative locus on chromosome five of maize by fine mapping. Crop Sci 37:1601–1610

Grant MR, Godiard L, Straube E, Ashfield T, Lewald J, Sattler A et al (1995) Structure of the *Arabidopsis RPM7* gene enabling dual specificity disease resistance. Science 269:843–846

Guimarães CT, Sills GR, Sobral BWS (1997) Comparative mapping of Andropogoneae: *Saccharum* L. (sugarcane) and its relation to sorghum and maize. Proc Natl Acad Sci USA 94:14261–14266

Hallauer AR, Miranda JB (1981) Quantitative genetics in maize breeding. Iowa State University Press, Ames, IA

Han F, Kilian A, Chen JP, Kudrna D, Steffenson B, Tamamoto K et al (1999) Sequence analysis of a rice BAC covering the syntenous barley *Rpg1* region. Genome 42:1071–1076

Helentjaris T, King G, Slocum M, Siedenstrang C, Wegman S (1985) Restriction fragment polymorphism as probes for plant diversity and their development as tools for applied plant breeding. Plant Mol Biol 5:109–118

Helentjaris T, Slocum M, Wright S, Schaefer A, Nienhuis J (1986a) Construction of genetic linkage maps in maize and tomato using restriction fragment length polymorphisms. Theor Appl Genet 72:761–769

Helentjaris T, Wright S, Weber D (1986b) Construction of a genetic linkage map in maize using restriction fragment length polymorphisms. Maize Genet Coop Newslett 60:118–120

Helentjaris T, Weber DF, Wright S (1986c) Use of monosomics to map cloned DNA fragment in maize. Proc Natl Acad Sci USA 83:6035–6039

Helentjaris T, Weber D, Wright S (1988) Identification of the genomic locations of duplicate nucleotide sequences in maize by analysis of restriction fragment length polymorphisms. Genetics 118:353–363

Holland JB, Uhr DV, Jeffers D, Goodman MM (1998) Inheritance of resistance to southern corn rust in tropical-by-corn-belt maize populations. Theor Appl Genet 96:232–241

Hooker AL (1963) Inheritance of chlorotic-lesion resistance to *Helminthosporium turcicum* in seedling corn. Phytopathology 53:660–662

Huang L, Brooks SA, Li W, Fellers JP, Trick HN, Gill BS (2003) Map-based cloning of leaf rust resistance gene *Lr21* from the large and polyploid genome of bread wheat. Genetics 164:655–664

Hulbert SH, Richter TE, Axtell JD, Bennetzen JL (1990) Genetic mapping and characterization of sorghum and related crops by means of maize DNA probes. Proc Natl Acad Sci USA 87:4251–4255

Jiang C, Edmeades GO, Armstead I, Lafitte HR, Hayward MD, Hoisington D (1999) Genetic analysis of adaptation differences between highland and lowland tropical maize using molecular markers. Theor Appl Genet 99:1106–1119

Jones DA, Thomas CM, Hammond-Kosack KE, Balint-Kurti PJ, Jones JDG (1994) Isolation of the tomato Cf-9 gene for resistance to *Cladosporium fulvum* by transposon tagging. Science 266:789–793

Jung M, Weldekidan T, Schaff D, Paterson A, Tingey S, Hawk J (1994) Generation-means analysis and quantitative trait locus mapping of anthracnose stalk rot genes in maize. Theor Appl Genet 89:413–418

Kahler AL (1985) Association between enzyme marker loci and agronomic traits in maize. In: Proc 40th Annu Corn and Sorghum Res Conf, Am Seed Trade Assoc, Washington, DC, pp 66–89

Kilian A, Chen J Han F, Steffenson B, Kleinhofs A (1997) Towards map-based cloning of the barley stem rust resistance

genes *Rpg1* and *Rpg4* using rice as an intergenomic cloning vehicle. Plant Mol Biol 35:187–195

Klein PE, Klein RR, Cartinhour SW, Ulanch PE, Dong JM, Obert JA et al (2000) A high-throughput AFLP-based method for constructing integrated genetic and physical maps: progress toward a sorghum genome map. Genome Res 10:789–807

Koester RP, Sisco PH, Stuber CW (1993) Identification of quantitative trait loci controlling days to flowering and plant height in two near isogenic lines of maize. Crop Sci 33:1209–1216

Krakowsky MD, Lee M, Woodman-Clikeman WL, Long MJ, Sharopova N (2004) QTL mapping of resistance to stalk tunneling by the European corn borer in RILs of maize population B73 × De811. Crop Sci 44:274–282

Kynast RG, Riera-Lizarazu O, Vales MI, Okagaki RJ, Maquieira SB, Chen G et al. (2001) A complete set of maize individual chromosome additions to the oat genome. Plant Physiol 125:1216–1227

Lambert RJ (2001) High-oil corn hybrids. In: Hallauer AR (ed) Specialty Corns, 2nd edn. CRC Press, Boca Raton, FL, pp 131–154

Lawrence GJ, Finnegan EJ, Ayliffe MA, Ellis JG (1995) The *L6* gene for flax rust resistance is related to the *Arabidopsis* bacterial resistance gene *RPS2* and the tobacco viral resistance gene *N*. Plant Cell 7:1195–1206

Lebreton C, Lazic-Jancic V, Steed A, Pekic S, Quarrie SA (1995) Identification of QTL for drought responses in maize and their use in testing causal relationships between traits. J Exp Bot 46:853–865

Lee M, Sharopova N, Beavis WD, Grant D, Katt M, Blair D, Hallauer A (2002) Expanding the genetic map of maize with the intermated B73 × Mo17 (IBM) population. Plant Mol Biol 48:453–461

Lehmensiek A, Esterhuizen AM, van Staden D, Nelson SW, Retief AE (2001) Genetic mapping of gray leaf spot (GLS) resistance genes in maize. Theor Appl Genet 103:797–803

Liu SC, Kowalsky SP, Lan TH, Feldmann KA, Paterson AH (1996) Genome-wide high-resolution mapping by recurrent intermating using *Arabidopsis thaliana* as a model. Genetics 142:247–258

Liu K, Goodman M, Muse S, Smith JS, Buckler E, Doebley J (2003) Genetic structure and diversity among maize inbred lines as inferred from DNA microsatellite. Genetics 165:2117–2128

Livini C, Ajmone-Marsan P, Nelchinger AE, Messmer MM, Motto M (1992) Genetic diversity of maize inbred lines within and among heterotic groups revealed by RFLPs. Theor Appl Genet 84:17–25

Lu H, Bernardo R (2001) Molecular marker diversity among current and historical maize inbreds. Theor Appl Genet 103:613–617

Lübberstedt T, Melchinger AE, Schon CC, Utz H, Klein D (1997) QTL mapping in testcrosses of European flint lines of maize.

1. Comparison of different testers for forage yield traits. Crop Sci 37:921–931

Lübberstedt T, Klein D, Melchinger AE (1998) Comparative QTL mapping of resistance to *Ustilago maydis* across four populations of European flint-maize. Theor Appl Genet 97:1321–1330

Mangelsdorf PC, Reeves RG (1939) The origin of Indian corn and its relatives. Bull No. 574, Texas Agri Exp Stn, College Station, TX

Marçon A, Kaeppler SM, Jensen SG, Senior L, Stuber C (1999) Loci controlling resistance to high plains virus and wheat streak mosaic virus in a B73 × Mo17 population of maize. Crop Sci 39:1171–1177

Martin GB, Brommonschenkel SH, Chunwongse J, Frary A, Ganal MW, Spivey R et al (1993) Map-based cloning of a protein kinase gene conferring disease resistance in tomato. Science 262:1432–1436

Meksem K, Leister D, Paleman J, Zabeau M, Salamini F, Gebhardt C (1995) A high-resolution map of the vicinity of the *R1* locus on chromosome V of potato based on RFLP and AFLP markers. Mol Gen Genet 249:74–81

Melchinger AE, Messmer MM, Lee M, Woodman WL, Lamkey KR (1991) Diversity and relationships among U.S. maize inbreds revealed by restriction fragment length polymorphisms. Crop Sci 31:669–678

Menz MA, Klein RR, Mullet JE, Obert JA, Unruh NC, Klein PE (2002) A high-density genetic map of *Sorghum bicolor* (L.) Moench based on 2926 AFLP, RFLP and SSR markers. Plant Mol Biol 48:483–499

Messmer MM, Melchinger AE, Lee M, Woodman WL, Lee EA, Lamkey KR (1991) Genetic diversity among progenitors and elite lines from the Iowa Stiff Stalk Synthetic (BSSS) maize population: comparison of allozyme and RFLP data. Theor Appl Genet 83:97–107

Messmer MM, Melchinger AE, Boppenmaier J, Brunklaus-Jung E, Herrmann RG (1992) Relationships among early European maize inbreds. I. Genetic diversity among flint and dent lines revealed by RFLPs. Crop Sci 32:1301–1309

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

Mindrinos M, Katagiri F, Yu G-L, Ausubel FM (1994) The *Arabidopsis thaliana* disease resistance gene *RPS2* encodes a protein containing a nucleotide-binding site and leucine-rich repeats. Cell 78:1089–1099

Moore G, Gale MD, Kurata N, Flavell RB (1993) Molecular analysis of small grain cereal genomes: current status and prospects. Bio/Technology 11:594–599

Moore G, Devos KM, Wang Z, Gale MD (1995) Cereal genome evolution – grasses, line up and form a circle. Curr Biol 5:737–739

Murigneux A, Barloy D, Leroy P, Beckert M (1993a) Molecular and morphological evaluation of doubled haploid lines in

maize. 1. Homogeneity within DH lines. Theor Appl Genet 86:837–842

Murigneux A, Baud S, Beckert M (1993b) Molecular and morphological evaluation of doubled-haploid lines in maize. 2. Comparison with single-seed-descent lines. Theor Appl Genet 87:278–287

Neuffer MG, Coe E, Wessler S (1997) Mutants of Maize. Cold Spring Harbor Laboratory, New York

O'Sullivan DM, Ripoll PJ, Rodgers M, Edwards KJ (2001) A maize bacterial artificial chromosome (BAC) library from the European flint inbred line F2. Theor Appl Genet 103:425–432

Ouedraogo JT, Maheshwari V, Berner DK, St-Pierre C-A, Belzile F, Timko MP (2001) Identification of AFLP markers linked to resistance of cowpea (*Vigna unguiculata* L.) to parasitism by *Striga gesnerioides*. Theor Appl Genet 102:1029–1036

Pe ME, Gianfranceschi L, Taramino G, Tarchini R, Angelini P (1993) Mapping quantitative trait loci (QTLs) for resistance to *Gibberella zeae* infection in maize. Mol Gen Genet 241:11–16

Pei DQ, Gobelman-Werner K, Wise R (2000) Physical characterization of the maize *rf1* genomic region. In: Plant & Animal Genome VIII Conf, San Diego

Pejic I, Ajmone-Marsan P, Morgante M, Kozumplick V, Castiglioni P, Taramino G, Motto M (1998) Comparative analysis of genetic similarity among maize inbred lines detected by RFLPs, RAPDs, SSRs, and AFLPs. Theor Appl Genet 97:1248–1255

Pereira MG, Lee M, Bramel-Cox P, Woodman W, Doebley J, Whitkus R (1994) Construction of an RFLP map in sorghum and comparative mapping in maize. Genome 37:236–243

Pernet A, Hoisington D, Franco J, Isnard M, Jewell D, Jiang C et al (1999) Genetic mapping of maize streak virus resistance from the Mascarene source. I. Resistance in line D211 and stability against different virus clones. Theor Appl Genet 99:524–539

Quarrie SA, Steed A, Lebreton C, Gulli M, Calestani C (1994) QTL analysis of ABA production in wheat and maize and associated physiological traits. Russian J Plant Physiol 41:565–571

Ragot M, Sisco PH, Hoisington DA, Stuber CW (1995) Molecular-marker-mediated characterization of favorable exotic alleles at quantitative trait loci in maize. Crop Sci 35:1306–1315

Rebourg C, Gouesnard B, Charcosset A (2001) Large scale molecular analysis of traditional European maize populations. Relationships with morphological variation. Heredity 86:574–587

Rebourg C, Chastanet M, Gouesnard B, Welcker C, Dubreuil P, Charcosset A (2003) Maize introduction into Europe: the history reviewed in the light of molecular data. Theor Appl Genet 106:895–903

Reif JC, Xia XC, Melchinger AE, Warburton ML, Hoisington DA, Beck D et al (2004) Genetic diversity determined within and among CIMMYT maize populations of tropical, subtropical, and temperate germplasm by SSR markers. Crop Sci 44:326–334

Ribaut JM, Jiang C, Gonzalez-de-Leon D, Edmeades GO, Hoisington DA (1997) Identification of quantitative trait loci under drought condition in tropical maize. 2. Yield components and marker-assisted selection strategies. Theor Appl Genet 94:887–896

Richey FD (1922) The experimental basis for the present status of corn breeding. J Am Soc Agron 14:1–17

Roman H, Ullstrup AJ (1951) The use of A-B translocations to locate genes in maize. Agron J 43:450–454

Rostoks N, Zale JM, Soule J, Brueggeman R, Druka A, Kudrna D et al (2002) A barley gene family homologous to the maize rust resistance gene *Rp1-D*. Theor Appl Genet 104:1298–1306

Salmeron JM, Oldroyd GED, Rommens CMT, Scofield SR, Kim HS, Lavelle DT et al (1996) Tomato *Prf* is a member of the leucine-rich repeat class of plant disease resistance genes and lies embedded within the *Pto* kinase gene cluster. Cell 86:123–133

Salvi S, Tuberosa R, Chiapparino E, Maccaferri M, Veillet S, van Beuningen L et al (2002) Toward positional cloning of *Vgt1*, a QTL controlling the transition from the vegetative to the reproductive phase in maize. Plant Mol Biol 48:601–613

Sari-Gorla M, Pe ME, Rossini L (1994) Detection of QTLs controlling pollen germination and growth in maize. Heredity 72:332–335

Schön CC, Lee M, Melchinger AE, Guthrie WD, Woodman WL (1993) Mapping and characterization of quantitative trait loci affecting resistance against second-generation European corn borer in maize with the aid of RFLPs. Heredity 70:648–659

Senior ML, Heun M (1993) Mapping maize microsatellites and polymerase chain reaction confirmation of the targeted repeats using a CT primer. Genome 36:884–889

Senior ML, Chin ECL, Lee M, Smith JSC, Stuber CW (1996) Simple sequence repeat markers developed from maize sequences found in the GenBank database: map construction. Crop Sci 36:1676–1683

Sharopova N, McMullen MD, Schultz L, Schroeder S, Sanchez-Villeda H, Gardiner J et al (2002) Development and mapping of SSR markers for maize. Plant Mol Biol 48:463–481

Sibov ST, Gaspar M, Silva MJ, Ottoboni LMM, Arruda P, Souza AP (1999) Two genes control aluminum tolerance in maize: genetic and molecular mapping analyses. Genome 42:475–482

Simons G, van der Lee T, Diergaarde P, van Daelen R, Groenendijk J, Frijters A et al (1997) AFLP-based fine mapping of the *Mlo* gene to a 30-kb DNA segment of the barley genome. Genomics 44:61–70

Smith DR, Hooker AL (1973) Monogenic chorotic-lesion resistance in corn to *Helminthosporium maydis*. Crop Sci 13:330–331

Smith JSC, Chin ECL, Shu H, Smith OS, Wall SJ, Senior ML et al (1997) An evaluation of utility of SSR loci as molecular markers in maize (*Zea mays* L.): comparisons with data from RFLPs and pedigree. Theor Appl Genet 95:163–173

Song W-Y, Wang G-L, Chen L-L, Kim H-S, Pi LY, Holsten T et al (1995) A receptor kinase-like protein encoded by the rice disease resistance gene *Xa21*. Science 270:1804–1806

Stam P (1993) Construction of integrated genetic linkage maps by means of a new computer package, JoinMap. Plant J 3:739–744

Stein N, Feuillet C, Wicker T, Schlagenhauf E, Keller B (2000) Subgenome chromosome walking in wheat: a 450-kb physical contig in *Triticum monococcum* L. spans the *Lr10* resistance locus in hexaploid wheat (*Triticum aestivum* L.). Proc Natl Acad Sci USA 97:13436–13441

Stuber CW (1994) Success in the use of molecular markers for yield enhancement in corn. In: Proc 49th Annu Corn and Sorghum Ind Res Conf, Am Seed Trade Assoc, 49:232–238

Stuber CW, Lincoln SE, Wolff DW, Helentjaris TH, Lander ES (1992) Identification of genetic factors contributing to heterosis in a hybrid from two elite maize inbred lines using molecular markers. Genetics 132:823–839

Sughroue JR, Rocheford TR (1994) Restriction fragment length polymorphism differences among Illinois long-term selection oil strains. Theor Appl Genet 87:916–924

Tai T, Dahlbeck D, Stall RE, Peleman J, Staskawicz BJ (1999) High-resolution genetic and physical mapping of the region containing the *Bs2* resistance gene of pepper. Theor Appl Genet 99:1201–1206

Taramino G, Tingey S (1996) Simple sequence repeats for germplasm analysis and mapping in maize. Genome 39:277–287

Tikhonov AP, SanMiguel PJ, Nakajima Y, Gorenstein NM, Bennetzen JF, Avramova Z (1999) Colinearity and its exceptions in orthologous *adh* regions of maize and sorghum. Proc Natl Acad Sci USA 96:7409–7414

Troyer F (2001) Temperate corn – background, behavior, and breeding. In: Hallauer AR (ed) Specialty Corns, 2nd edn. CRC Press, Boca Baton, FL, pp 393–466

van Ooijen JW, Maliepaard C (1996) MapQTL version 3.0: Software for the calculation of QTL positions on genetic maps. Plant Genome IV Conf, San Diego

Veldboom LR, Lee M (1994) Molecular-marker-facilitated studies of morphological traits in maize. 2. Determination of QTLs for grain yield and yield components. Theor Appl Genet 89:451–458

Veldboom LR, Lee M (1996) Genetic mapping of quantitative trait loci in maize in stress and nonstress environments. II. Plant height and flowering. Crop Sci 36:1320–1327

Veldboom LR, Lee M, Woodman WL (1994) Molecular marker-facilitated studies in an elite maize population. 1. Linkage analysis and determination of QTL for morphological traits. Theor Appl Genet 88:7–16

Vlădutu C, McLaughlin J, Philips RL (1999) Fine mapping and characterization of linked quantitative trait loci involved in the transition of the maize apical meristem from vegetative to generative structures. Genetics 153:993–1007

von Malek B, Weber WE, Debener T (2000) Identification of molecular markers linked to *Rdr1*, a gene conferring resistance to blackspot in roses. Theor Appl Genet 101:977–983

Vos P, Hogers R, Bleekers M, Reijans M, van der Lee T, Hornes M et al (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23:4407–4414

Watson SA (1987) Structure and composition. In: Watson SA, Ramstad PE (eds) Corn: Chemistry and Technology. Am Assoc Cereal Chem, St Paul, MN, pp 53–82

Weber DF, Helentjaris T (1989) Mapping RFLP loci in maize using B-A translocations. Genetics 121:583–590

Wei F, Gobelman-Werner K, Morroll SM, Kurth J, Mao L, Wing R et al (1999) The *Mla* (powdery mildew) resistance cluster is associated with three *NBS-LRR* gene families and suppressed recombination within a 240-kb DNA interval on chromosome 5S (1HS) of barley. Genetics 153:1929–1948

Welz HG, Xia XC, Bassetti P, Melchinger AE, Lünnerstedt T (1999) QTLs for resistance to *Setosphaeria turcica* in an early maturing dent × flint maize population. Theor Appl Genet 99:649–655

Wendel JF, Stuber CW, Edwards MD, Goodman MM (1986) Duplicated chromosome segments in maize (*Zea mays* L.): further evidence from hexokinase isozymes. Theor Appl Genet 72:178–185

Whitham S, Dinesh-Kumar SP, Choi D, Hehl R, Corr C, Baker B (1994) The product of the tobacco mosaic virus resistance gene *N*: similarity to *Toll* and the interleukin-1 receptor. Cell 78:1101–1115

Whitkus R, Doebley J, Lee M (1992) Comparative genome mapping of sorghum and maize. Genetics 132:1119–1130

Wilson WA, Harrington SE, Woodman WL, Lee M, Sorrells ME, McCouch SR (1999) Inferences on the genome structure of progenitor maize through comparative analysis of rice, maize and the domesticated panicoids. Genetics 153:453–473

Wise RP, Schnable PS (1994) Mapping complementary genes in maize: positioning the *rf1* and *rf2* nuclear-fertility restorer loci of Texas (T) cytoplasm relative to RFLP and visible markers. Theor Appl Genet 88:785–795

Woo SS, Jiang J, Gill BS, Paterson AH, Wing RA (1994) Construction and characterization of a bacterial artificial chromosome library of *Sorghum bicolor*. Nucleic Acids Res 22:4922–4931

Xu ML, Melchinger AE, Xia XC, Lubberstedt T (1999) High-resolution mapping of loci conferring resistance to sugarcane mosaic virus in maize using RFLP, SSR, and AFLP markers. Mol Gen Genet 261:574–581

Yang HY, Korban SS, Kruger J, Schmidt H (1997) The use of a modified bulk segregant analysis to identify a molecular

marker linked to a scab resistance gene in apple. Euphytica 94:175–182

Yano M, Katayose Y, Ashikari M, Yamanouchi U, Monna L, Fuse T et al (2000) *Hd1*, a major photoperiod sensitivity quantitative trait locus in rice, is closely related to the *Arabidopsis* flowering time gene *CONSTANS*. Plant Cell 12:2473–2484

Yoshimura S, Yamanouchi U, Katayose Y, Toki S, Wang ZX (1998) Expression of *Xa1*, a bacterial blight-resistance gene in rice, is induced by bacterial inoculation. Proc Natl Acad Sci USA 95:1663–1668

Yu J, Hu S, Wang J, Wong GKS, Li S, Liu B et al (2002) A draft sequence of the rice genome (*Oryza sativa* L. ssp. *indica*). Science 296:79–92

Zaitlin D, DeMars SJ, Gupta M (1992) Linkage of a second gene for NCLB resistance to molecular markers in maize. Maize Gene Coop Newslett 66:69–70

Zaitlin D, DeMars S, Ma Y (1993) Linkage of *rhm*, a recessive gene for resistance to southern corn leaf blight, to RFLP marker loci in maize (*Zea mays*) seedlings. Genome 36:555–564

4 Barley

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4.1
Introduction

Cultivation of barley traces back to the earliest remains of agricultural activity in the Old World about 10,000 years ago. Only a few other species, such as einkorn and emmer wheat, lentils, peas and chickpeas, bitter vetches, and flax, have been found to have accompanied barley during the transitional phase from preferential reaping of wild plants to purposely performed cultivation.

4.1.1
Genus *Hordeum*

Barley, *Hordeum vulgare* L., is placed in *Hordeum*, which is a moderately sized genus with ca. 32 species and altogether ca. 45 taxa (von Bothmer et al. 1995, for review and references). All species in *Hordeum* have a similar set of diagnostic, morphological characters, particularly with three, one-flowered spikelets at each rachis node, called a triplet. The two lateral florets are pedunculate, or sessile, and may be sterile (as in two-rowed barley) or fertile (as in six-rowed barley). The glumes are setaceous or flattened and placed on the adaxial side of (and not surrounding) the spikelet.

Despite the seemingly homogeneous structure in basic morphology and specialization, *Hordeum* shows a high degree of biological diversity. Some species are annuals often with more or less strict inbreeding, like *H. marinum* Huds., *H. murinum* L., and *H. pusillum* Nutt. Some species are perennials with a self-incompatibility system, like *H. bulbosum* L. and *H. brevisubulatum* (Trin.) Link. The majority of species are perennials with a versatile reproductive system.

Most species, like cultivated barley, are diploids ($2n = 2x = 14$), but tetraploids ($2n = 4x = 28$) and hexaploids ($2n = 6x = 42$) are also frequent. Autoploidy is found in two species, *H. bulbosum* and *H. brevisubulaturn*. The majority of polyploids are segmental alloploids.

4.1.2
Taxonomic Position of Barley

Together with wheat (*Triticum aestivum* L.), rye (*Secale cereale* L.), and several important forages, like Russian wild rye [*Psathyrostachys fragilis* (Boiss.) Nevski] and crested wheatgrass [*Agropyron cristatum* (L.) Gaertn.], barley (*Hordeum vulgare*) belongs to the tribe (tribus) *Triticeae*. This tribe represents a highly successful evolutionary branch in the grass family (*Poaceae*) and comprises a vast number of species and genera. The numerous wild species are thus potential gene sources for cereal breeding. The *Triticeae* comprises very complex modes of speciation, including polyploidy and interspecific and intergeneric hybridizations, that have resulted in a reticulate pattern of relationships. There are still major disagreements among taxonomists especially with regard to generic delimitations. No comprehensive systematic review of *Triticeae* has been presented in recent years (cf. Löve 1984).

4.1.3
Gene Pools of Barley

When applied to barley and its wild allies, the gene pool concept of Harlan and de Wet (1971) presents a very clear-cut picture (Fig. 1). In addition to elite
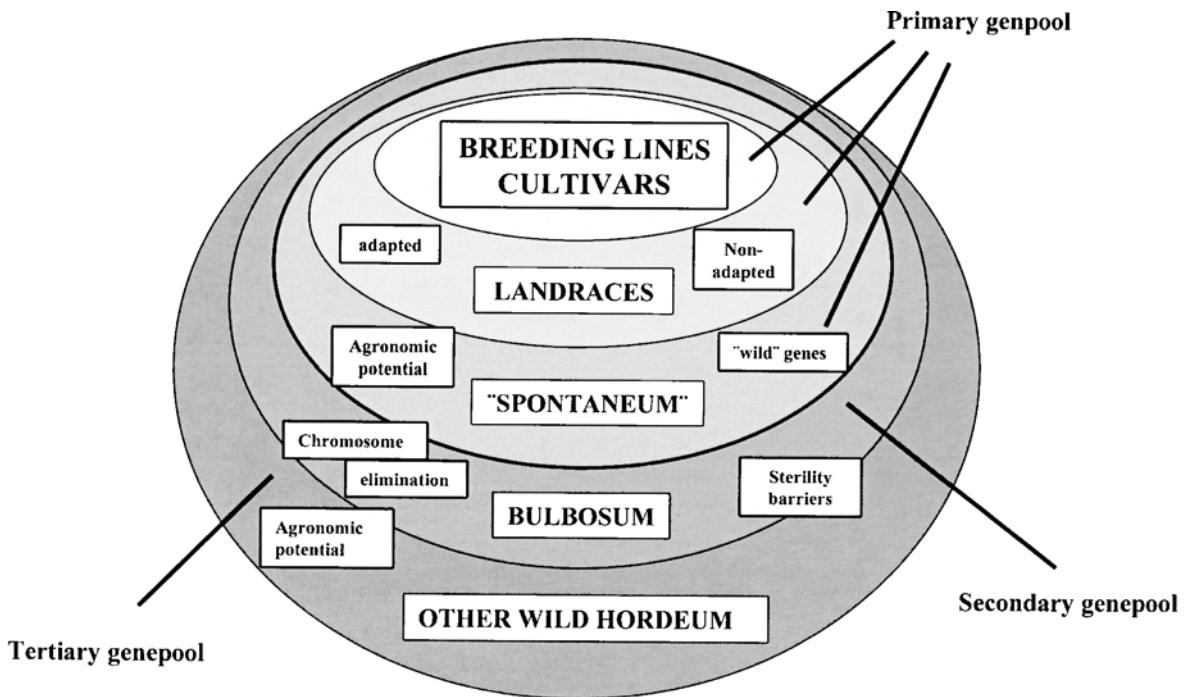
Fig. 1. Gene pools in cultivated barley (*Hordeum vulgare*)

material, varieties, and landraces, the progenitor of domesticated barley, *H. vulgare* ssp. *spontaneum*, also belongs to the primary gene pool of barley. Crossing ability of cultivated barley with this form shows no incompatibility barriers, hence there is a full capacity for gene transfer.

The secondary gene pool includes only a single species, *H. bulbosum*, sharing the basic *H* genome with barley (*H. vulgare*). However, crossing between these two species is difficult but not impossible.

However, in recent years, it has been demonstrated that genes from *H. butbosum* can be transferred to cultivated barley, thus providing a new source for breeding (Pickering 2000, for review and references). Formerly, *H. bulbosum* was used for the production of doubled haploids in barley breeding through chromosome elimination (Kasha and Kao 1970; Pickering 1984, 2000; Subrahmanyam and von Bothmer 1987).

All the remaining species of *Hordeum* are classified into the tertiary gene pool. They cross with barley only with difficulty, and backcrossing to the crop is even more difficult (von Bothmer et al. 1983; von Bothmer and Linde-Laursen 1989).

4.1.4
The Wild Progenitor of Barley

The immediate ancestor of cultivated barley is still abundant in nature. It was first discovered in Turkey by the German botanist Carl Koch and described by him as a separate species, *Hordeum spontaneum*. However, based on several criteria, the progenitor form is now regarded as a subspecies [ssp. *spontaneum* (C. Koch) Thell.] within the same major species, *H. vulgare* L., as cultivated barley (ssp. *vulgare*).

The center of distribution for ssp. *spontaneum* lies in southwest Asia, particularly in the Middle East. The natural distribution includes the Eastern Mediterranean area with eastern Greece and Turkey and the Cyrenaica area of Libya and Egypt, and the taxon extends eastwards to Afghanistan, Turkmenia, and Baluchistan in western Pakistan (Fig. 2) (Giles and von Bothmer 1985; Zohary and Hopf 1993)

Brittle rachis types also occur outside this area (Bekele 1983; Molina-Cano et al. 1987), and there are ongoing disputes whether they are true wild forms or represent weedy types from segregation products (Frost et al. 1975; Giles and Leftkovitch 1984, 1985).

Ssp. *spontaneum* has large ecological amplitude. It grows in natural habitats in arid or semiarid biotopes,

but also on segetal habitats on disturbed ground. It is an important annual component in open, herbaceous vegetation and is particularly common in the summer in the deciduous oak forests in the western part of the Middle East (Zohary and Hopf 1993). Outside this area it occurs in drier steppes and semideserts representing more weedy types (Harlan and de Wet 1971). It may also be an aggressive weed in man-made habitats, e.g., in cultivated fields, edges of fields, and roadsides. In the central parts of the distribution area ssp. *spontaneum* often occurs in very dense stands in large populations. In the more marginal parts of its native distribution area it is less abundant, scattered, and even rare.

It may sometimes be difficult to distinguish between true, wild *spontaneum* and primitive forms and landraces of cultivated barley. The two taxa (ssp. *spontaneum* and ssp. *vulgare*) are morphologically similar but can be distinguished by a combination of a number of characters. ssp. *spontaneum* is always two-rowed and often taller than ssp. *vulgare* of the same area, but lower types may occur in certain areas or habitats. The wild subspecies has a brittle rachis, but this character alone cannot identify the wild form since mutations and segregation products of crosses may also occur within ssp. *vulgare*. ssp. *spontaneum* is usually more open-flowering and hence has a higher frequency of cross-pollination than the cultivated form. Outbreeding of up to 10% has been reported (Brown et al. 1978; Nevo 1992). The dispersal is adapted to zoochory (seeds are attached to, for example, furs of animals) and these traits are still intact in ssp. *spontaneum* but modified under domestication in ssp. *vulgare*. The wild traits include long and tough bristles on rachis segments and on the rachilla as well as a tough (nonbrittle) awn. The kernels are often shrunken, not plump, as in cultivated barley. Apart from the visible, morphological traits, the wild ssp. *spontaneum* also has a number of specific adaptive traits, such as a well-developed dormancy system and high drought tolerance (Nevo 1992; van Rijn et al. 2000).

No crossing barriers have been developed between the wild and the cultivated forms. Spontaneous and artificial crosses are easily obtained (cf. Asfaw and von Bothmer 1990). However, in some populations of ssp. *spontaneum* chromosome translocations may occur, resulting in a reduced fertility in some crosses (Ahokas 1999). There has certainly been a high frequency of introgression in areas where the wild and the cultivated forms are in close contact. The wild

form is thus an excellent source of useful alleles for barley breeding, as has been demonstrated in several current national and international projects (Jahoor and Fischbeck 1987; Schönfeld et al. 1996; Lehmann et al. 1998; Backes et al. 2003).

4.1.5
Domestication of Barley

The identified area for the dawn of agriculture lies in the particular area of the Eastern Mediterranean called the Fertile Crescent, which comprises the arch from present-day central Israel, over western Jordan, Lebanon, Syria, South East Turkey, North Iraq, and to the Zagros Mountains in western Iran (Fig. 2). It constitutes mainly a mountainous or hilly area with relatively dry steppes and dry woodlands (oak forests). Early settlement remains have shown that in the Fertile Crescent humans went from being hunter-gatherers to sedentary farmers. The welfare of the early societies and the basis for our current civilization was created by the development of agriculture and the gradual domestication of a number of plant and animal species, like einkorn and emmer wheat, barley, flax, vetches, lens, and peas and goat, sheep, cattle, and pigs (cf. Zohary and Hopf 1993; Smith 1995; Lev-Yadun et al. 2000).

Barley, einkorn, and emmer wheat are probably the crops presenting the best archaeological and biological evidence for the process of domestication. Remains of prehistoric cereal cultures are often composed of barley mixtures. As a result of intensive archaeological research during the second half of the 20th century, carbonized kernel imprints in pottery and mud bricks have appeared among the remains showing the transition from the wild to a domesticated state (Zohary and Hopf 1993; Smith 1995; Ladizinsky 1999).

Gathering of wild barley seeds from nature seems to have occurred in great quantities as early as 17,000 to 19,000 years ago (Harlan 1992, 1995; Kislev et al. 1992; Zohary and Hopf 1993; Ladizinsky 1999). Gradually, barley was adapted to cultivation and there is clear evidence of early cultivation as well as signs of initial domestication dating from ca. 10,000 years ago (Zohary and Hopf 1993; Harlan 1995; Smith 1995; Ladizinsky 1999). Evident remains of nonbrittle barley in a low frequency (10 to 12%) are found in the eighth millennium BC usually in mixtures with brittle ssp. *spontaneum* types. Suitable mutations were given
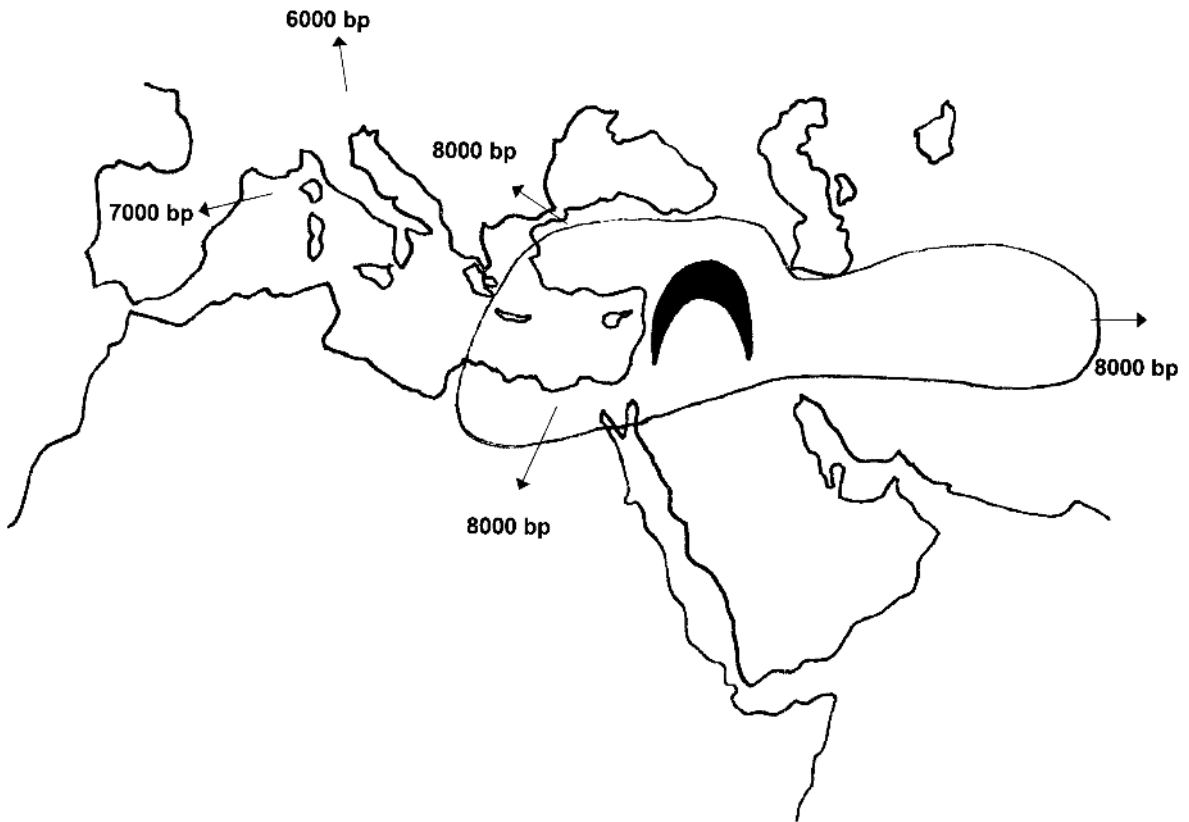
Fig. 2. Fertile Crescent, area of early domestication of cultivated barley (*Hordeum vulgare* ssp. *vulgare*) in Middle East, distribution of wild progenitor of barley (*H. vulgare* ssp. *spontaneum*) (within s*olid line*), and approximate time (year before present) for cultivated barley to reach different areas

the chance to survive in seed mixtures from nonfragile ear types soon after the beginning of barley cultivation. The earliest type to appear was two-rowed barley. Six-rowed types appeared somewhat later (ca. 9,500 years ago) and from ca. 6000 BC naked forms occurred.

There is a strong tendency of higher percentages of cultivated wheat at sites with better soil fertility even in the early stages of agricultural activities within the Fertile Crescent. Barley regained dominance at lower soil fertility, harsher climates, and shorter vegetation periods.

It has been a matter of discussion whether barley was taken into cultivation only once in the Fertile Crescent or whether it was the subject of repeated domestication in space and time in the area (cf. Harlan 1992). For the other original crops of the Fertile Crescent, a single domestication event is assumed. However, according to several authors, barley is the single species assumed to have undergone multiple domestication events, also outside the Fertile Cres-

cent. Especially the occurrence of brittle rachis types outside the core area of the Fertile Crescent has been taken as evidence of more than one region of domestication (Åberg 1938; Molina-Cano et al. 1982; Bekele 1983). More recently, some studies included RFLP markers (Molina-Cano et al. 1999) and DNA sequence data (El Rabey and Salamini 2000). So far, no comprehensive study has been made that unambiguously proves more than one domestication area outside the Fertile Crescent (Yasuda et al. 1993; Badr et al. 2000; Blattner and Badany Méndez 2001), apart from the allelic substitution originating from true *H. spontaneum* types during the migration of domesticated barley from the Near East to South Asia, which determines the Himalayas as of "domesticated barley diversification".
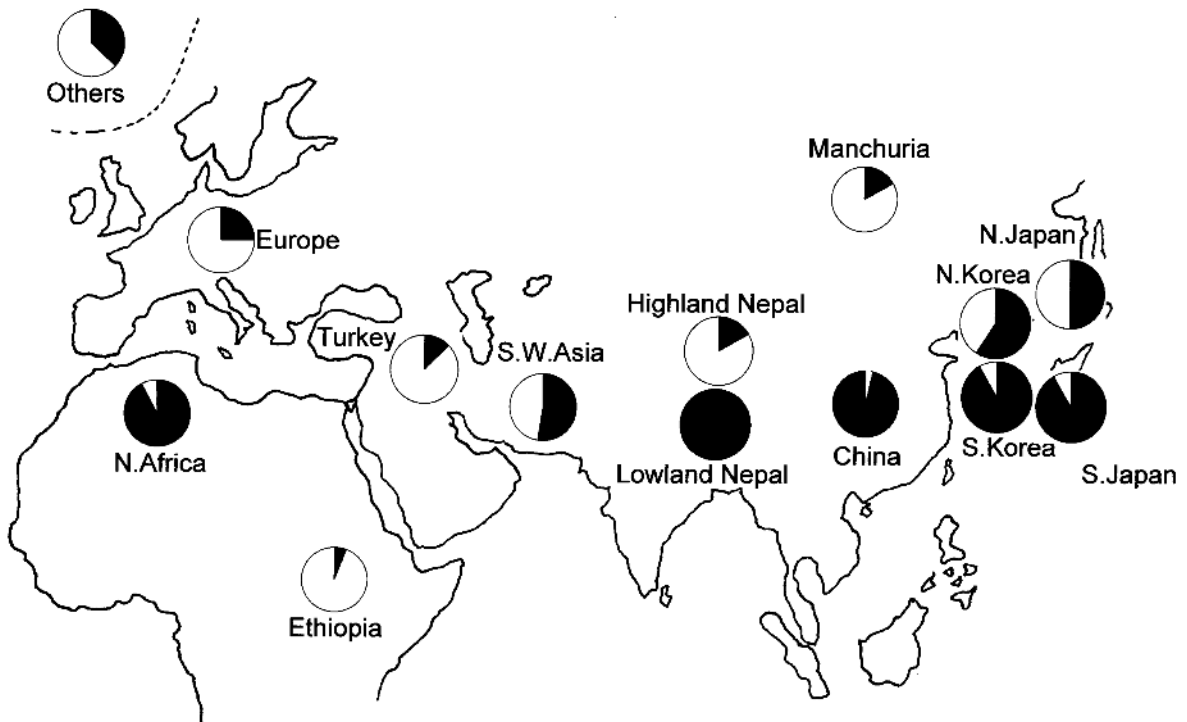
Fig. 3. Geographical difference in ratio of two nonbrittle barley genotypes *BtrlBtrl btr2btr2* (type E: *black*) and *btrlbtrl Btr2Btr2* (type W: *white*)

4.1.6
Important Traits for Domestication

The domestication of barley was a gradual process with accumulation of genes particularly suited for cultivation. The selections may have been unconscious, i.e., as a result of the action of edaphic or climatic factors, or conscious, that is, as a result of deliberate choices of desired traits by humans. Some traits were of particular importance for domestication itself or for connecting closely with early cultivation systems.

Brittleness of Rachis

Shattering is a characteristic of natural adaptation in wild plants. The brittleness of rachis in barley promotes the spreading of seeds together with the rough awn, which may easily attach to animals for effective dispersal. One of the most important traits for the domestication of barley is probably the nonbrittleness of rachis, which is of benefit for an efficient harvest without loss of grains.

Takahashi and Yamamoto (1949) clarified the genetic system of nonbrittleness of barley occurring in

ssp. *spontaneum*. When they made crosses among cultivated barleys, brittleness of rachis occurred only in crosses between East Asian and European cultivars. Two recessive genes, *btr1* and *btr2*, each responsible for nonbrittle rachis, had been independently established by natural mutations in the wild progenitor, which had a brittle rachis due to two dominant, complementary genes, *Btr1* and *Btr2* (Takahashi 1987). The double recessive genotype, *btrlbtrl btr2btr2*, is not found in any landrace material. According to Takahashi and Hayashi (1964), the two genes *Btrlbtrl* and *Btr2btr2* are pseudoallelic. The complementary action of *Btr1* and *Btr2* in the heterozygote suggested that these two were situated on different loci, but no recombinants were recovered from a cross between the two different genotypes *BtrlBtrI btr2btr2* and *btrlbtrl Btr2Btr2*.

Takahashi and his coworkers made two-way testcrosses of a world collection using two kinds of pollen donors, typical East Asian and European cultivars. The relative frequencies of type E cultivars and type W cultivars in various geographical regions is shown in Fig. 3. Two-rowed barleys are mostly type W, with some exceptions. Type W is dominant in occidental, six-rowed barleys but not as frequent

as in the two-rowed varieties. In East Asia, varieties with type W are rather frequent in the northern parts of Japan, North Korea, and Manchuria (Takahashi 1955; Takahashi et al. 1963, 1983).

Kernel Row Type

There is a wide variation in kernel row type with a detailed classification system (Lundquvist et al. 1996). The row type is basically controlled by the gene *vrs1* and six-rowed is recessive to two-rowed. Several genes showing imperfect six-rowed, such as *vrs2* or *vrs3*, have been found mainly in artificially induced mutants. Tanno et al. (1999, 2002) studied DNA sequences of some 900 bp closely linked to the *vrsl* locus, which showed that ssp. spontaneum has a larger variation than ssp. vulgare. Within ssp. vulgare the two-rowed form had a larger variation than the six-rowed form and the study showed that there are two distinct lineages of six-rowed barley (type I and II). Type I, including the majority of six- rowed barley in the world, had an identical DNA sequence with that of a strain of var. proskowetzii from Turkmenistan; type II, being less frequent and distributed in the Mediterranean region, had an identical DNA sequence as that of brittle rachis types from Morocco. The molecular study thereby showed a diphyletic origin of six-rowed barley.

Two-rowed forms analyzed included four types of DNA sequences. The major type showed only one-nucleotide difference from that of type I of six-rowed barley. The DNA sequence of the major type was identical to that of some materials of Iranian var. *spontaneum* (Sayed et al. 2000; Tanno et al. 2002), whereas the three remaining types were distributed in the Mediterranean region and Ethiopia. As the two-rowed spike is very similar in cultivated and wild forms, any introgression between the two forms can not be excluded.

Covered and Naked Kernel

Naked kernel is a single recessive character from the covered wild type. Naked barley is distributed widely in the world, but there is a higher preference for naked barleys in East Asian countries such as China, Korea, and Japan, and it is especially high in Tibet and the northern parts of Nepal, India, and Pakistan. It has, however, become clear that naked barley was grown in Anatolia (Turkey) and in northern Europe already in ancient times (Hunter 1952; Helbaek 1969).

Dormancy

Dormancy is a natural adaptation system controlling the seed germination in semi-arid areas where barley was domesticated. Takeda (1995) evaluated the dormancy of more than 4,000 cultivars and 177 wild (ssp. *spontaneum*) accessions. All wild material tested was highly dormant. Compared to other characters, which might have been important for domestication, the genetic system of dormancy seems more complicated. Takeda (1995) reported quantitative inheritance of dormancy as a result of a diallele analysis and several quatitative trait loci (QTL) from the cross of Harrington × TR306. A certain level of dormancy is also useful in cultivated material to prevent pre-harvest sprouting or unnecessary starch degradation during the storage period.

Growth Habit

One of the prerequisites for expansion of the cultivation area for barley must have been differentiation of spring habit. In high latitudes and in mountainous regions, barley is sown in spring to avoid damage by a severely cold winter. Accordingly, in these regions spring type cultivars are needed in order to grow and head normally. At low latitudes, on the other hand, air temperature is too high to induce vernalization in a winter type. Spring type cultivars prevail in these regions. In mid-latitudinal regions including North Africa, southern Europe, Nepal, China, and Japan, both spring and winter barley cultivars are generally sown in autumn.

The genes *sghl, Sgh2,* and *Sgh3* all regulate spring habit, and their allelic genes regulate winter habit. Because of the epistatic effect among these genes, only a single genotype, *Sghlsgh2sgh3*, exhibits winter habit. Linkage studies of the three spring type genes *sghl, Sgh2,* and *Sgh3* have shown that they are located on chromosomes 4H, 5H, and 1H, respectively (Takahashi and Yasuda 1956, 1958; Laurie et al. 1995). It has also been determined that different degrees of vernalization requirement are controlled by the multiple allelic genes *Sgh2I* and *Sgh2II* denoting the spring genes with high and moderate degrees of spring habit, respectively. The *Sgh2* locus has been hypothesized to be homoeologous with the *Vrn1* locus of wheat (Karsai et al. 1997).

Almost all strains of *Hordeum vulgare* ssp. *spontaneum* are of winter habit with the exception of a few strains, which are regarded as cross products with spring cultivars (Takahashi et al. 1963, 1968). Con-

sequently, the first barley types to be domesticated might have been of winter habit type, but a dominant mutation occurred first in the *sgh2* locus, resulting in a spring barley type.

Subsequent mutations at the *Sghl* and *sgh3* loci and crosses among the resulting genotypes explain the existence of five genetically different types of spring growth habit, which are differently distributed throughout across barley growing regions, as explored by Yasuda (1992) and Yasuda et al. (1993).

Based on additional mutations, spontaneous hybridization between different types of cultivated barley as well as accompanying weedy forms of *Hordeum spontaneum* probably allowed a multitude of barley genotypes to survive within mixed stands that differed in morphological as well as in physiological traits, but that also were subjected to the forces of natural selection for adaptation to the prevailing growing conditions. Such forces gained even more in importance with the expansion of agriculture into new environments beyond the Fertile Crescent. In addition, drift effects from the small population size of the seed lots that were taken along might have played a role. Further, effects of mass selection for desired genotypes might have also received preferential attention from different farming communities.

4.1.7
Migration and History of Barley Cultivation

As is documented from archaeological research, it was already during the sixth and fifth millennium BC that cultivated barley accompanied emmer and einkorn wheat, often together with weedy forms of wild barley, into the Aegean region and subsequently into all other regions of the eastern part of the Mediterranean Basin (Zohary and Hopf 1993).

Early remains of cultivated barley from Egypt date back to the fifth millennium BC and to sites in the Nile Delta (Darby et al. 1977). Later phases of ancient civilization in the Nile Valley credited barley as a gift of the goddess Isis, and germinating barley kernels symbolized the resurrection of goddess Osiris. Most probably, the mixture of barley types that characterizes the remains of cultivated barley from Egypt was carried from the Nile and ultimately may have reached the highlands of Ethiopia. Continuous cultivation practices under a wide range of ecological conditions finally developed into the secondary center of genetic diversity, which still exists within the

Ethiopian landraces of barley (Lakev et al. 1997) that impressed and puzzled N.I. Vavilov and his coworkers so much.

Expansion of agriculture in the eastern direction apparently took place at the same time, as is documented from early remains of the major crops. Not only the Caucasus and Transcaucasus regions were reached during the fifth millennium BC (Lisitsina 1984); remains of cultivated barley also indicate that the Old World type of agriculture was practiced even in the highlands of the Indian subcontinent (Costantini 1984).

Expansion into the western parts of the Mediterranean Basin is witnessed from remains that date back to the fourth millennium BC (Hopf 1991), and there is an ongoing scientific debate as to whether Morocco represents a secondary center in which barley was domesticated independently from the Fertile Crescent sources. Another pathway of expanding agriculture originating from the Aegean region turned to the north, moving upward along the riverbeds of the Danube, throughout the Balkan region, and upward along the Dniester from Ukraine into Poland. With further expansion, cultivated barley reached Central and Northern Europe during the third millennium BC (Körber-Grohne 1987).

From present-day knowledge it appears that cultivation of barley reached China only during the second half of the second millennium BC (Ho 1977). It may have been the exchange of seeds from the Old World type of agriculture with the rice-based agriculture in the Far East civilization that established it there and, further, on the Korean Peninsula and the islands of Japan.

Considering the changes in frequency of barley within the remains from ancient cereal cultures and the presence of different barley types among them (Zohary and Hopf 1993), it appears that during the earliest phases of agricultural activity barley occurred in rather large percentages, and very often as a mixture of two-row and six-row ear types as well as hulled and naked kernels. A characteristic change during the later phases and development of agriculture throughout the Old World is associated with the preference given to free-threshing types of wheat. In the beginning this was restricted to the tetraploid progenies of emmer wheat (*T. turgidum* ssp. *durum* and others) but later on included the hexaploid common wheat (*T. aestivum*) that is supposed to have originated from spontaneous hybridizations of tetraploid cultivated wheat with *T. squarrosa*, a wild wheat-grass species that occurred

most probably within the Caucasus/Transcaucausus region when the tetraploid wheats entered there (Zohary and Hopf 1993). With reference to barley, the exceptions from the general rule of increased preference for wheat deserve special interest, and it has already been observed (Hillman 1975) that barley occupied the prime position mainly at locations characterized by less favorable growing conditions.

During the fourth millennium BC, the Mesopotamian Basin was almost completely covered with agricultural activity, and it was during this period that six-row, hulled barley types outnumbered not only other types of barley but also cultivated wheats. It is assumed that tolerance against nutritional depletion of the cultivated soils and/or salinity problems was the reason for it (Zohary and Hopf 1993).

Six-row ear types of barley also played a major role during later and more far-reaching expansion of agriculture, for example, into the western part of the Mediterranean Basin. Only six-row ear types of barley reached Central and Northern Europe during the third and fourth millennia BC, and it is also documented (Körber-Grohne 1987) that barley represents only small percentages among the remains found from sites located on better soils, but its presence increases dramatically in the remains from settlements located on very light, sandy soils, which are widely distributed across the more northern lowlands of Central Europe, together with the percentages of naked barley kernels. In fact, two-row ear types of barley remained virtually unknown to cereal cultivation practices in Central and Northern Europe throughout the first millennium BC. Without well-documented proof, it is assumed that two-row ear types were introduced only with seeds brought along by crusaders who fought and lived in Near East countries during the 12th and 13th centuries.

Much more recent in time and well documented is the spread of cultivated barley into the Americas and Oceania. Settlers following the Spanish conquerors introduced barley seeds into Mesoamerica and the southern parts of the United States (Poehlman 1959). Mainly six-row, lax ear types originating from North Africa were found to grow well and gave rise to the ecotypes cultivated in this region and, later on, to cultivars still in use today. Furthermore, six-row barley types became naturalized members of cultivated crops in the Andean Highlands. Later introductions of two-row barley from Australia and Europe gained

limited importance in the southern plains regions of Uruguay and Argentina.

During the 18th and 19th centuries, immigrants from many Western, Central, and Eastern European countries who settled in New England and the Midwest brought along barley seeds from landraces that originally were grown in their European homesteads. Clear and sometimes surprising differences concerning the level of adaptation to the new environment were noted. These gave rise to the preferential distribution of regionally well-adapted types, such as Manchuria and Oderbrucker in the Midwest. Together with similar experiences with other immigrant-introduced crop plants, this eventually resulted in the U.S. Plant Introduction Service (Moseman and Smith 1981), which implemented more systematic approaches after assembling crop plants from other countries and evaluating their potential for crop production in the United States.

Introduction of barley into Oceania was closely related, and in the beginning was also restricted to its ties with the British Empire. Later on, Australian scientists (Finlay and Wilkinson 1963) used the results of comparative yield trials with a large set of barley introductions under Australian growing conditions to develop a method that can be applied to quantify differences in adaptation between the accessions tested and to evaluate their potential for crop improvement.

4.2
Construction of Genetic Maps

The following list presents genetic maps published for barley. Besides M 8, a physical map based on translocations, only linkage maps are presented. The maps M 13, M 16, M 17, M 35, M 56, and M 65 are consensus maps based on the joint mapping from two to more populations (Fig. 4), while the other maps are based on single populations. References to the mapping programs are given at the end of the list (Table 1).

M 1: *Population:* Igri × Franka, 71 DH lines
Reference: Graner et al. 1991, 1994
Marker: 92 loci (88 RFLP, 2 protein, 2 phenotypic), later (Graner et al. 1994): 273 loci
Method: MAPMAKER
Characteristics: Coverage: 870 cM (1,433 cM); mean marker distance: 5.2 cM

Table 1. Linkage mapping programs used, references, number of maps in list, and references of the first use

Program	Reference	Maps	Reference of first use
MAPMAKER/EXP	Lander et al. 1987	28	M 1 (Graner et al. 1991)
GMENDEL	Liu and Knapp 1990	12	M 5 (Hayes et al. 1993a)
JOINMAP	Stam 1993	20	M 13 (Langridge et al. 1995)
MapManager QT	Manly and Olson 1999	8	M 24 (Barr et al. 1998)
MapManager QTX	Manly et al. 2001	3	M 61 (Read et al. 2003)
MAPL	Ukai et al. 1995	2	M 50 (Hori et al. 2003)

M 2: *Population:* Vada × 1B-87, 135 F$_{2:3}$ families
Reference: Graner et al. 1991
Marker: 163 RFLP loci
Method: MAPMAKER
Characteristics: Coverage: 1,408 cM

M 3: *Population:* Proctor × Nudinka, 91 DH lines
Reference: Heun et al. 1991
Marker: 157 loci (155 RFLP, 2 phenotypic)
Method: MAPMAKER
Characteristics: Coverage: 1,096 cM

M 4: *Population:* Magnum × Goldmarker, 105 DH lines
Reference: Laurie et al. 1993
Marker: 33 markers, including morphological markers and RFLP markers, later 78 markers (Pan et al. 1994)
Method: MAPMAKER

M 5: *Population:* Dicktoo × Morex, 100 DH lines
Reference: Hayes et al. 1993a; Pan et al. 1994
Marker: 33 markers, including morphological markers and RFLP markers, later 78 markers (Pan et al. 1994)
Method: GMENDEL
Remark: Only 5H (8 markers) is presented in Hayes et al. 1993a, 2H presented in Sanguineti et al. 1994, complete map in Pan et al. 1994

M 6: *Population:* Vogelsanger Gold × Alf, 90 DH lines
Reference: Giese et al. 1994
Marker: 80 loci (28 RFLP, 23 RAPD, 29 phenotypic)
Method: Single-marker ML
Characteristics: Coverage: 680 cM

M 7: *Population:* Steptoe × Morex, 150 DH lines
Reference: Kleinhofs et al. 1993, 1994
Marker: 295 loci (166 RFLP, 5 isozyme, 3 phenotypic and 7 SAP markers)
Method: MAPMAKER, GMENDEL

Characteristics: Coverage: 1,259 cM, mean marker distance: 4.2 cM

M 8: *Population:* 676 translocation lines, and Igri × Franka (M 1; Graner et al. 1991, 1994)
Reference: Marthe and Künzel 1994; Sorokin et al. 1995; Korzun and Künzel 1996
Marker: See M1 (Graner et al. 1991, 1994)
Method: Translocation lines
Characteristics: Physical map

M 9: *Population:* Harrington × TR306, 150 DH lines
Reference: Kasha and Kleinhofs 1994
Marker: 191 loci (RFLP, RAPD, phenotypic markers)
Method: GMENDEL v.3.0

M 10: *Population:* Igri × Danilo, 249 DH lines
Reference: Backes et al. 1995, 1996
Marker: 54 RFLP loci, later 67 RFLP loci
Method: MAPMAKER

M 11: *Population:* Igri × Triumph, 94 DH lines
Reference: Laurie et al. 1995
Marker: 94 loci (92 RFLP, 2 protein)
Method: MAPMAKER

M 12: *Population:* Blenheim × E224/3, 59 DH lines
Reference: Thomas et al. 1995
Marker: 101 loci (54 RFLP, 39 RAPD, 5 Isozyme, 1 STS, 1 morphological, 1 disease resistance marker)
Method: MAPMAKER, JOINMAP
Characteristics: Coverage: 675 cM

M 13: *Population:* Joined map of the populations Igri × Franka (M 1), Proctor × Nudinka (M 3), Steptoe × Morex (M 7); additional information from Clipper × Sahara 371 (M 59), Galleon × Haruna Nijo (M 58), Chebec × Harrington (M 57), and Shannon × Proctor
Reference: Langridge et al. 1995
Marker: 587 loci
Method: JOINMAP

M 14: *Population:* Vogelsanger Gold × Tystofte Prentice, 90 DH lines
Reference: Kjær et al. 1995
Marker: 97 loci (85 RFLP, 8 protein, 4 phenotypic)
Method: MAPMAKER
Characteristics: Coverage: 1,100 cM

M 15: *Population:* Blenheim × Kym, 99 DH lines
Reference: Bezant et al. 1996
Marker: 99 loci (93 RFLP, 2 storage proteins, 4 isozymes)
Method: JOINMAP v. 1.4

M 16: *Population:* Joined map of populations Igri × Franka (M 1), Proctor × Nudinka (M 3), Steptoe × Morex (M 7), and Harrington × TR306 (M 9)
Reference: Qi et al. 1996
Marker: 898 loci
Method: JOINMAP

M 17: *Population:* Consensus map
Reference: Franckowiak 1997
Marker: 244 morphological loci
Characteristics: Coverage: 1,187 cM

M 18: *Population:* (Steptoe × Morex) × Steptoe, 210 BC_2F_1 lines, derived from a DH line from Steptoe × Morex (Kleinhofs et al. 1993)
Reference: Han et al. 1997
Marker: 32 loci on 7H centromeric region (29 RFLP, 2 protein, 1 morphological)
Method: MAPMAKER, GMENDEL
Characteristics: Coverage: 27 cM

M 19: *Population:* Harrington × Morex, 140 DH lines
Reference: Hayes et al. 1997
Marker: 296 loci (RFLP, AFLP, SSR, isozymes, phenotypic); skeleton map with 106 loci.
Method: MAPMAKER

M 20: *Population:* Tadmor × Er/Apm, 167 RILs (F_8)
Marker: 99 loci (73 RFLP, 26 RAPD), later (Teulat et al. 2001a): 118 loci (93 RFLP, 38 AFLP, 2 RAPD, 1 SSR, 1 morphological), later (Teulat et al. 2002) 170 markers (99 RFLP, 47 SSR, 15 AFLP, 6 RAPD, 2 STS, 1 phenotypic)
Reference: Teulat et al. 1998, 2001a, 2002
Method: MAPMAKER
Characteristics: Coverage: 1,056 cM (1,101 cM); mean marker distance: 14.5 cM

M 21: *Population:* L94 × Vada, 103 RILs (F_9)
Reference: Qi et al. 1998a
Marker: 556 loci (AFLP)
Method: JOINMAP v.2.0

Characteristics: Coverage: 1,026 cM; mean marker distance: 1.9 cM

M 22: *Population:* Derkado × B83-12/215, 160 DH lines
Reference: Thomas et al. 1998
Marker: 178 loci (119 AFLP, 36 SSR, 20 S-SAP, 3 phenotypic): later (Ellis et al. 2002) 241 loci (128 AFLP, 90 SSR, 20 S-SAP, 4 phenotypic)
Method: JOINMAP v.2.0
Characteristics: Coverage: 1,384 cM; mean marker distance: 10.9 cM

M 23: *Population:* Apex × Prisma, 94 RILs (F_8)
Reference: Yin et al. 1999b
Marker: 191 loci (190 AFLP, 1 phenotypic)
Method: JOINMAP v.2.0
Characteristics: Coverage: 965.3 cM

M 24: *Population:* Galleon × Haruna Nijo, 112 DH lines
Reference: Barr et al. 1998; Karakousis et al. 2003a
Marker: 435 loci (281 RFLP, 98 AFLP, 43 SSR, 13 others)
Method: MapManager QT
Characteristics: Coverage: 1360 cM

M 25: *Population:* Gobernadora × CMB643, 144 DH lines
Reference: Zhu et al. 1999
Marker: 97 RFLP loci
Method: GMENDEL v.3.0
Characteristics: Coverage: 1,306 cM; mean marker distance: 13.5 cM

M 26: *Population:* Chevron × M69, 101 $F_{4:7}$ families
Reference: de la Peña et al. 1999, revised by Canci et al. 2003
Marker: 94 RFLP loci (+ 45 SSR loci in revised version)
Method: MAPMAKER; GMENDEL for revised version.

M 27: *Population:* Lerche × BGR41936, 182 F_2 plants
Reference: Pillen et al. 2000
Marker: 51 SSR loci
Method: MAPMAKER
Characteristics: Coverage: 840 cM

M 28: *Population:* L94 × 116-5, 117 RILs (F_8)
Reference: Qi et al. 2000
Marker: 280 loci (278 AFLP, 2 morphological)
Method: JOINMAP v.2.0
Characteristics: Coverage: 857 cM; mean marker distance: 3 cM

M 29: *Population:* Krona × HOR1063, 220 DH lines
Reference: Kicherer et al. 2000

Marker: 59 loci (58 RFLP, 1 morphological)
Method: MAPMAKER

M 30: *Population:* Ko A × Mokusekko 3, 120 F_2 plants
Reference: Miyazaki et al. 2000
Marker: 222 loci (220 RFLP, 1 protein, 1 morphological)
Method: MAPMAKER
Characteristics: Coverage: 1,389 cM; mean marker distance: 6.5 cM

M 31: *Population:* Chevron × Stander, 147 DH lines
Reference: Ma et al. 2000
Marker: 211 loci (RFLP)
Method: MAPMAKER
Characteristics: Coverage: 1,026 cM

M 32: *Population:* Lina × *H. spontaneum* Canada Park
Reference: Ramsay et al. 2000
Marker: 299 SSR loci
Method: JOINMAP v.2.0
Characteristics: Coverage: 1,173 cM

M 33: *Population:* Shyri × Galena, 94 DH lines
Reference: Toojinda et al. 2000
Marker: 810 loci (562 RFLP, 155 RGAP, 51 SSR, 41 RFLP, 1 morphological)
Method: GMENDEL v.3.0
Characteristics: Coverage: 1,317 cM

M 34: *Population:* Rolfi × Botnia, 200 DH lines
Reference: Manninen 2000
Marker: 111 loci (94 RAPD, 14 RFLP, 3 SSR)
Method: JOINMAP
Characteristics: Coverage: 694 cM

M 35: Coordinator's consensus maps (BGN)
Marker: 172 loci
Reference: Jensen 2002; Franckowiak 2001; Forster 2001
Remark: Chromosomes 1H, 2H, 4H only

M 36: *Population:* Wolfe Multiple Dominant × Wolfe Multiple Recessive, 94 DH lines
Reference: Costa et al. 2001
Marker: 111 loci (RFLP, RAPD, AFLP, SSR, morphological)
Method: GMENDEL
Characteristics: Coverage: 1,387 cM; mean marker distance: 1.9 cM

M 37: *Population:* Post × Vixen, 70 DH lines
Reference: Scheurer et al. 2001
Marker: 117 loci (56 AFLP, 33 RAPD, 25 SSR, 1 morphological, 1 CAP, 1 STS)
Method: MAPMAKER

Characteristics: Coverage: 1,328 cM; mean marker distance: 12.1 cM

M 38: *Population:* Post × Nixe, 70 DH lines
Reference: Scheurer et al. 2001
Marker: 725 loci (70 AFLP, 28 RAPD, 23 SSR)
Method: MAPMAKER
Characteristics: Coverage: 959 cM; mean marker distance: 8.4 cM

M 39: *Population:* PB1 (*H. bulbosum*) × PB11 (*H. bulbosum*), 111 F_1 full-sib families
Reference: Salvo-Garrido et al. 2001
Marker: 131 RFLP loci
Method: JOINMAP v.2.0
Characteristics: Coverage: 616 cM; mean marker distance: 8.4 cM
Remark: Outcrossing species; therefore separate maps for each parental lines that were combined

M 40: *Population:* Karl × Lewis, 146 RILs (F_5)
Reference: See et al. 2002
Marker: 110 loci (86 AFLP, 12 STS, 7 SSR, 3 morphological, 2 protein)
Method: MAPMAKER
Characteristics: Coverage: 1,294 cM

M 41: *Population:* Alexis × Regatta, 110 DH lines
Reference: Jensen et al. 2002
Marker: 150 loci (62 AFLP, 40 R FLP, 22 RAPD, 19 SSR, 5 STS, 2 phenotypic)
Method: Proprietary Maximum Likelihood program

M 42: *Population:* L94 × C123, 111 RI lines (F_8)
Reference: Arru et al. 2002
Marker: 260 loci
Method: MAPMAKER
Characteristics: Coverage: 911 cM; mean marker distance: 3.5 cM

M 43: *Population:* Abyssinian × Ingrid, 50 DH lines
Reference: Grønnerød et al. 2002
Marker: 335 loci (280 AFLP, 30 SSR, 20 RFLP, 4 STS, 1 phenotypic)
Method: JOINMAP v.2.0
Characteristics: Coverage: 844.7 cM; mean marker distance: 4.3 cM
Remark: Skeletal map comprised of 195 markers

M 44: *Population:* Azumamugi × Kanto Nagate Gold, 99 RI lines (F_9)
Reference: Mano and Komatsuda 2002
Marker: 272 loci, skeletal map with 100 loci
Method: MAPMAKER
Characteristics: Mean marker distance: 6.5 cM

M 45: *Population:* Angora × W704/137, 99 DH lines
Reference: Buck-Sorlin 2002
Marker: 171 loci (150 AFLP, 15 SSR, 6 phenotypic)
Method: JOINMAP v.2.0
Characteristics: Coverage: 1,900 cM; mean marker distance: 11.2 cM

M 46: *Population:* 1B-87 × Vada, 121 RI lines
Reference: Backes et al. 2003
Marker: 221 loci (134 AFLP 58 RFLP, 16 SSR, 13 RGA)
Method: JOINMAP v.2.0, GMENDEL
Characteristics: Coverage: 1,264 cM; mean marker distance: 5.8 cM

M 47: *Population:* Frederickson × Stander, 130 $F_{4:5}$ plants
Reference: Mesfin et al. 2003
Marker: 143 loci (85 RFLP, 57 SSR, 1 phenotypic)
Method: GMENDEL
Characteristics: Coverage: 1,170 cM

M 48: *Population:* H7 × H1 (both *Hordeum chilense*), 100 F_2 plants
Reference: Hernàndez et al. 2001; Vaz Patto et al. 2003
Marker: 113 loci (79 RAPD, 15 RFLP, 10 SSR, 3 SCAR, 1 STS, 2 protein), later (Vaz Patto et al. 2003): 466 loci
Method: JOINMAP v.2.0
Characteristics: Coverage: 694 cM; mean marker distance: 5.7 cM

M 49: *Population:* MNBrite × M96, 98 $F_{4:6}$ families
Reference: Canci et al. 2003
Marker: 114 loci (87 RFLP, 27 SSR)
Method: GMENDEL
Remark: Only 6H is presented

M 50: *Population:* Russia 6 × H.E.S. 495 RI lines (F_9)
Reference: Hori et al. 2003
Marker: 1,172 loci (1,134 AFLP, 34 SSR, 3 STS, 1 morphological)
Method: MAPMAKER, MAPL98
Characteristics: Coverage: 1,596 cM; mean marker distance: 1.4 cM

M 51: *Population:* Kinuyutaka × Yoshikei 15, 150 DH lines
Reference: Kai et al. 2003
Marker: 55 loci (RFLP, RAPD, STS, SSR)
Method: MAPL98
Characteristics: Coverage: 547 cM

M 52: *Population:* Arta × H.spontaneum 41-1, 190 RI lines (F_7)
Reference: Baum et al. 2003
Marker: 189 loci (158 AFLP, 30 SSR, 1 morphological); skeletal map: 129 marker loci (106 AFLP, 22 SSR, 1 morphological)
Method: MAPMAKER, JOINMAP
Characteristics (Skeletal): Coverage: 890 cM, mean marker distance: 7.3 cM

M 53: *Population:* (ND9712 × Zhedar 2) × Foster, 75 barley lines
Reference: Dahleen et al. 2003
Marker: 214 loci (123 AFLP, 53 RFLP, 29 SSR, 7 RGA, 2 morphological)
Method: MAPMAKER
Characteristics: Coverage: 1,331 cM, mean marker distance: 6.2 cM

M 54: *Population:* WI-2875-1 × Alexis, 153 RI lines
Reference: Barr et al. 2003a
Marker: 291 loci (187 AFLP, 553 RFLP, 50 SSR, 1 morphological)
Method: MapManager QT

M 55: *Population:* Alexis × Sloop, 111 DH lines
Reference: Barr et al. 2003a
Marker: 274 loci (177 AFLP, 51 RFLP, 45 SSR, 1 morphological)
Method: MapManager QT

M 56: *Population:* Consensus: WI-2875-1 × Alexis, 153 RI lines; Alexis × Sloop, 111 DH lines
Reference: Collins et al. 2001
Marker: 167 AFLP, 68 RFLP, 46 SSR
Method: MapManager QT

M 57: *Population:* Chebec × Harrington, 120 DH lines
Reference: Barr et al. 2003b
Marker: 348 loci (258 RFLP, 47 AFLP, 41 SSR, 2 morphological)
Method: MapManager QT
Characteristics: Coverage: 1,330 cM

M 58: *Population:* Galleon × Haruna Nijo, 112 DH lines
Reference: Karakousis et al. 2003a
Marker: 435 loci (281 RFLP, 98 AFLP, 43 SSR, 13 other)
Method: MapManager QT
Characteristics: Coverage: 1,360 cM

M 59: *Population:* Clipper × Sahara 371, 150 DH lines
Reference: Karakousis et al. 2003b
Marker: 348 loci (174 RFLP, 35 SSR, 6 morphological)
Method: MapManager QT
Characteristics: Coverage: 1,330 cM

M 60: *Population:* Amagi Nijo × WI2585, 139 DH lines
Reference: Pallotta et al. 2003
Marker: 100 loci (91 RFLP, 9 SSR)
Method: MapManager QT

M 61: *Population:* Sloop × Halycon, 166 DH lines
Reference: Read et al. 2003
Marker: 257 marker (151 SFLP, 78 RFLP, 27 SSR, 1 SNP)
Method: MapManager QTX
Characteristics: Coverage: 1,280 cM, mean marker distance: 5 cM

M 62: *Population:* Tallon × Kaputar, 65 DH lines
Reference: Cakir et al. 2003
Marker: 177 loci (AFLP, SSR)
Method: MAPMAKER, MapManager QTX

M 63: *Population:* Mundah × Keel, 110 DH lines
Reference: Long et al. 2003
Marker: 54 loci (28 SSR, 14 AFLP, 12 RFLP)
Method: MapManager QTX

M 64: *Population:* VB9524 × NB11231*12, 180 DH lines
Reference: Emebiri et al. 2003
Marker: 270 loci (197 AFLP, 43 SSR, 23 RFLP, 6 STS, 1 RAPD)
Method: GMENDEL

M 65: *Population:* Joined map of the populations Galleon × Haruna Nijo (M 58), Chebec × Harrington (M 57), Clipper × Sahara 371 (M 59), Alexis × Sloop (M 55), Amagi Nijo × WI2585 (M 60)
Reference: Karakousis et al. 2003c
Marker: 773 loci (136 SSR)
Method: JOINMAP v.2.0
Characteristics: Coverage: 933 cM

M 66: *Population:* Tadmor × WI2991, 71 DH lines
Reference: Sayed et al. 2004
Marker: 182 loci (144 AFLP, 32 SSR, 5 RFLP, 1 phenotypic)
Method: JOINMAP v.2.0
Characteristics: Coverage: 521 cM, mean marker distance: 2.8 cM
Remark: Only 2H, 3H, 4H presented

M 67: *Population:* Tankard × Livet, 184 RI lines
Reference: Rajasekaran et al. 2004
Marker: 114 loci (AFLP, SSR, REMAP, IRAP, STS)
Method: JOINMAP v.2.0
Characteristics: Coverage: 1,095 cM

M 68: *Population:* Nure × Tremois, 136 DH lines
Reference: Francia et al. 2004
Marker: 128 loci (AFLP, SSR, STS, protein, morphological)
Method: MAPMAKER v.3.0
Characteristics: Coverage: 1,182 cM, mean marker distance: 10 cM

M 69: *Population:* H.v. ssp *spontaneum* Ashquelon × H.v. ssp *spontaneum* Mehola, 140 $F_{2:3}$ families
Reference: Verhoeven et al. 2004a
Marker: 90 AFLP loci

M 70: *Population:* IPZ24727 × Barke, 86 DH lines
Reference: Behn et al. 2004
Marker: 195 loci (164 AFLP, 30 SSR, 1 phenotypic)
Method: JOINMAP v.2.0
Characteristics: Coverage: 1,092 cM, mean marker distance: 2.3 cM

M 71: *Population:* Triumph × Morex, 107 DH lines
Reference: Prada et al. 2004
Marker: 147 loci (95 AFLP, 45 SSR, 2 STS, 5 morphological)
Method: MAPMAKER v.3.0
Characteristics: Coverage: 1,125 cM, mean marker distance: 7.5 cM

4.3
Gene Mapping

Qualitative traits are usually controlled by one or two (and rarely a few) genes; moreover, qualitative genes are inherited according to classical Mendelian genetics. Their allelic forms give qualitatively distinct phenotypes that clearly represent their genotypes. Mapping a gene to a certain location on the chromosome demands a linkage map of the whole genome using a segregating population that can be F_2, backcross, doubled-haploid, or recombinant inbreed lines. The phenotypes in a segregating progeny can be scored in a similar ratio as molecular markers.

Recently, different marker systems such as restriction fragment length polymorphism (RFLP), random amplified polymorphism DNAs (RAPDs), amplified fragment length polymorphism (AFLP), sequence tagged sites (STS), simple sequence repeats (SSRs or microsatellites), single nucleotide polymorphisms (SNPs), and others have been developed and used for tagging qualitative traits such as resistance genes in barley.
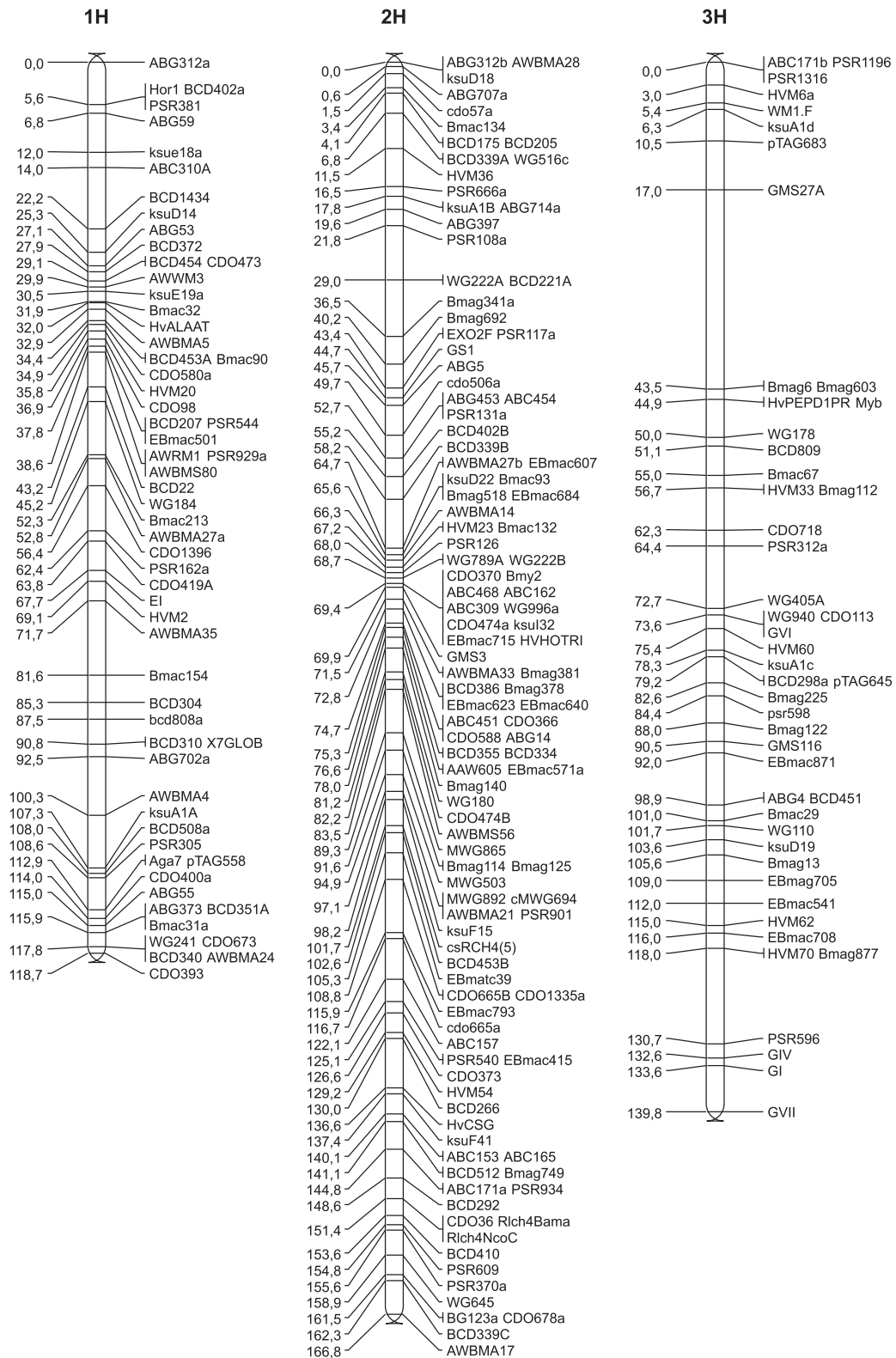
1H

Position	Marker
0,0	ABG312a
5,6	Hor1 BCD402a
	PSR381
6,8	ABG59
12,0	ksue18a
14,0	ABC310A
22,2	BCD1434
25,3	ksuD14
27,1	ABG53
27,9	BCD372
29,1	BCD454 CDO473
29,9	AWWM3
30,5	ksuE19a
31,9	Bmac32
32,0	HvALAAT
32,9	AWBMA5
34,4	BCD453A Bmac90
34,9	CDO580a
35,8	HVM20
36,9	CDO98
37,8	BCD207 PSR544
	EBmac501
	AWRM1 PSR929a
	AWBMS80
38,6	
43,2	BCD22
45,2	WG184
52,3	Bmac213
52,8	AWBMA27a
56,4	CDO1396
62,4	PSR162a
63,8	CDO419A
67,7	EI
69,1	HVM2
71,7	AWBMA35
81,6	Bmac154
85,3	BCD304
87,5	bcd808a
90,8	BCD310 X7GLOB
92,5	ABG702a
100,3	AWBMA4
107,3	ksuA1A
108,0	BCD508a
108,6	PSR305
112,9	Aga7 pTAG558
114,0	CDO400a
115,0	ABG55
115,9	ABG373 BCD351A
	Bmac31a
117,8	WG241 CDO673
	BCD340 AWBMA24
118,7	CDO393

2H

Position	Marker
0,0	ABG312b AWBMA28
	ksuD18
0,6	ABG707a
1,5	cdo57a
3,4	Bmac134
4,1	BCD175 BCD205
6,8	BCD339A WG516c
11,5	HVM36
16,5	PSR666a
17,8	ksuA1B ABG714a
19,6	ABG397
21,8	PSR108a
29,0	WG222A BCD221A
36,5	Bmag341a
40,2	Bmag692
43,4	EXO2F PSR117a
44,7	GS1
45,7	ABG5
49,7	cdo506a
52,7	ABG453 ABC454
	PSR131a
55,2	BCD402B
58,2	BCD339B
64,7	AWBMA27b EBmac607
	ksuD22 Bmac93
65,6	Bmag518 EBmac684
66,3	AWBMA14
67,2	HVM23 Bmac132
68,0	PSR126
68,7	WG789A WG222B
	CDO370 Bmy2
	ABC468 ABC162
	ABC309 WG996a
69,4	CDO474a ksuI32
	EBmac715 HVHOTRI
	GMS3
69,9	AWBMA33 Bmag381
71,5	BCD386 Bmag378
	EBmac623 EBmac640
72,8	ABC451 CDO366
	CDO588 ABG14
74,7	BCD355 BCD334
75,3	AAW605 EBmac571a
76,6	Bmag140
78,0	WG180
81,2	CDO474B
82,2	AWBMS56
83,5	MWG865
89,3	Bmag114 Bmag125
91,6	MWG503
94,9	MWG892 cMWG694
	AWBMA21 PSR901
97,1	ksuF15
98,2	csRCH4(5)
101,7	BCD453B
102,6	EBmatc39
105,3	CDO665B CDO1335a
108,8	EBmac793
115,9	cdo665a
116,7	ABC157
122,1	PSR540 EBmac415
125,1	CDO373
126,6	HVM54
129,2	BCD266
130,0	HvCSG
136,6	ksuF41
137,4	ABC153 ABC165
140,1	BCD512 Bmag749
141,1	ABC171a PSR934
144,8	BCD292
148,6	CDO36 Rlch4Bama
	Rlch4NcoC
151,4	BCD410
153,6	PSR609
154,8	PSR370a
155,6	WG645
158,9	BG123a CDO678a
161,5	BCD339C
162,3	AWBMA17
166,8	

3H

Position	Marker
0,0	ABC171b PSR1196
	PSR1316
3,0	HVM6a
5,4	WM1.F
6,3	ksuA1d
10,5	pTAG683
17,0	GMS27A
43,5	Bmag6 Bmag603
44,9	HvPEPD1PR Myb
50,0	WG178
51,1	BCD809
55,0	Bmac67
56,7	HVM33 Bmag112
62,3	CDO718
64,4	PSR312a
72,7	WG405A
73,6	WG940 CDO113
	GVI
75,4	HVM60
78,3	ksuA1c
79,2	BCD298a pTAG645
82,6	Bmag225
84,4	psr598
88,0	Bmag122
90,5	GMS116
92,0	EBmac871
98,9	ABG4 BCD451
101,0	Bmac29
101,7	WG110
103,6	ksuD19
105,6	Bmag13
109,0	EBmag705
112,0	EBmac541
115,0	HVM62
116,0	EBmac708
118,0	HVM70 Bmag877
130,7	PSR596
132,6	GIV
133,6	GI
139,8	GVII

Fig. 4. Consensus linkage map M65 (Karakousis et al. 2003c, AFLP loci not shown)

4H

Position	Marker
0,0	XGermin
3,8	BCD339d
5,9	HvOLE
6,0	HVM40
14,0	bcd808b BCD265a
17,8	BCD351B
22,9	ABG3a GMS89
26,0	Bmag375
26,9	Bmac181 EBmac906
28,7	PSR141
30,1	BCD453c WG622a
30,8	Pm AWBMS62
	CDO795 WG232
32,2	CDO650 Mne1a
	AWBMS90
35,5	ABG54
37,8	EBmac635 EBmac701
43,6	Mne1b
45,0	EBmac679
46,2	ABG472
51,5	WG719
57,5	BCD15a
58,0	CDO1406
63,0	HvBTAI3 TaCMD
64,9	WG114 EBmac788
66,3	ABG498 PSR164
68,7	ksuE2
69,5	CDO1312 HVMLOHIA
71,9	CDO63
72,5	BCD1130
73,0	CDO677
86,0	Bmag419
101,6	EXO2a
102,0	HVM67
103,3	Bmy1 HvBAMY
104,4	ABG312c

5H

Position	Marker
0,0	ksuD4
3,0	ksuA1e
3,6	ABC483
5,1	AWWM1a HVTL
11,4	ABG708
14,7	cdo665c CDO1335b
15,7	ksuH7
	PSR156 ksuD16
19,0	PSR123
20,6	CDO989
21,7	ABG705 ABC164
	CDO749 BCD21a
22,7	Bmag105
26,5	AWBMA12 AWBMS54
	BG123b AWPCB7b
27,0	pTAG354a
	ABC156A ksuA1f
27,9	PSR162b PSR360
	AWBMS53
	BCD1072 PSR1204
	BCD276 psr161
	ABG3b WG405B
28,5	BCD147a ksuA3
	PSR117b Bmac113
	Bmac286 Bmag387
29,3	BCD508B
29,6	Bmag337
	PSR128 ABG702B
30,1	AWBMS70 AWBMS75
	AWBMS88
30,4	CDO504
31,4	MWG514 HVM3
	AWBMS31 HvACLIa
	EXO1 EXO2b
33,6	Bmac96
36,0	psr326 PSR426
38,5	ABG712
39,7	bcd808c BCD265B
42,0	WG181 EBmac85
43,5	WG564
48,2	AWBMA3 X-I
49,9	GMS27B
50,9	Ebmatc3
51,8	BCD339f
56,0	BCD351C
58,4	WG364 Bmag222
59,4	Bmag223
63,3	psr131
66,8	ABG391 CDO419B
	PSR370b
	ABG496 BCD298B
68,1	PSR911
74,1	WG1026
75,5	PSR637a
	psr115 ABG707b
78,6	BCD339e
79,5	ABG390 ABC310b
83,5	GS2
	CDO400b CDO678b
84,2	PSR394
84,8	AWBMA32
88,8	HVM6b
89,5	csRCH4(1)
90,4	CDO460 ABG463
	ABG460 HvLOXC
93,1	cdo506b
	ABG57 pTAG354b
93,6	Rlch5Bamb Rlch5Ncoa
95,7	ABC254
105,7	GMS1

6H

Position	Marker
0,0	AWBMA36
3,0	ABG62
4,7	psr119a GMS6
5,8	PSR899
7,2	HVM11a BCD342
	PSR167 PSR312b
8,4	Bmac316
10,3	BCD102
12,1	NAR7a
15,3	BCD21B
17,5	TAM10 HVM14
18,8	HVM74
19,4	PSR611
22,0	ABG654
23,8	WG405C Bmag9
28,7	ksuA1g
45,0	EBmac874
56,1	EBmac806
56,7	WG996b
57,2	MWG820
58,3	ABC163
59,3	ABC175
60,2	WG286
60,9	ABC156b
67,2	CDO580B
68,1	Amy1 PSR149
71,8	Bmac251
72,9	NAR7b
	ABG458 BCD269
75,5	EBmac602
76,5	PSR666b
77,1	BCD453d
	cMWG669 BCD339g
83,0	EBmac571b HvACLIb
85,9	BCD1
90,9	PSR154 BCD221B
94,0	ABC154
109,4	PSR627
116,9	CDO836 BCD15b
118,4	MWG897 WG622b
127,4	AWBMA20
128,8	CDO347a

Fig. 4. (continued)

7H

Position	Marker
0,0	ABG312d
7,6	PSR160
8,2	ABG704
9,4	PSR637b
10,4	ABG75
11,3	psr119b
13,6	AWWM1b
14,7	ABC255
16,7	CDO1400 BCD15c
	Bmag206
17,9	ABC465 BCD129
18,7	BCD351D
19,8	ABG320
21,7	BCD339h
22,2	BCD135
24,3	HvWaxy4a HvWaxyG
26,7	ABC152
31,7	WG789B
38,5	ABG380
39,4	ABG497a
54,8	LD
64,8	Bmac167 Bmag359a
	Bmag359b EBmac827
66,7	Bmac31b
67,8	HvA22S
69,9	Bmag321
70,8	CDO676
74,0	HVCMA Bmag507
75,2	PSR929b
	ABG701 ABG11
76,4	AWBMA26 CDO665d
	PSR108b
77,0	EBmac764
77,8	Bmag217
78,6	HvSS1 Bmag183
79,6	ABC308a Bmag369
	cdo57b ABG652a
80,1	AWBMS37
80,9	ABG476
81,4	BCD263 Bmag341b
	CDO595 CDO358
82,0	ksuA1h WG789c
83,1	BCD147b
93,0	AWBMA15
94,9	AWBMA16
101,2	WG996c Bmag120
106,5	ksuE19b
112,8	AWBMS22
114,0	PSR637c
114,9	PSR117c
117,1	AWBMA11
120,6	WG686
125,9	ABC306 CDO583
	EBmac757
126,5	ABC305 psr547
	ksuF37
134,6	ABC321
135,4	ksue18b
137,3	ksuE18c
140,6	PSR129 ABG497b
145,1	EBmac755
148,4	ABG461
149,7	WG420
153,1	ABG652b ABG714b
156,1	HVM11b
159,9	AaWBl Bmac156
161,4	HVM49
164,6	pTAG732
166,7	PSR680
167,3	ABC308b M501
170,1	CDO347b Bmag135

Fig. 4. (continued)

4.3.1
Resistance Genes

Diseases caused by pathogens not only reduce the yield but also lower the quality of the produce. The most effective way to control diseases is to introduce resistant cultivars. The new and effective resistance genes have to be identified and introduced in the newly developed cultivars. Such resistance genes are frequently found either in exotic material or in the wild relatives of cultivated crop plants. Therefore, several resistance genes have been mapped for different diseases with DNA markers in barley in order to accelerate the incorporation of resistance genes from exotic material into highly adapted material.

Powdery Mildew Caused by the Fungus *Blumeria* (syn. *Erysiphe*) graminis f. sp. *hordei*

Intensive studies have been conducted concerning powdery mildew, which considered the most important disease on barley especially in temperate climatic zones. Many resistance genes for powdery mildew were found and mapped on barley chromosomes (Table 2). Five major powdery mildew resistance genes have been identified and localized on chromosome 1H: *Mlra, Mla, Mlk,* and *Mlnn* on the short arm of this chromosome and *MlGa* on the long arm. Briggs and Stanford (1938) illustrated for the first time that the cv. Algerian (CI 1179) possesses the *Mla* gene for powdery mildew resistance. The *Mla* locus contains the highest number of different alleles among all known barley powdery mildew resistance genes identified so far. Until now more than 32 alleles have been detected in this locus (Weibull et al. 2003). This locus was mapped with molecular markers by Graner et al. (1991) near the RFLP marker cMWG645 in a cross between wild barley *Hordeum vulgare* ssp. *spontaneum* and the susceptible cultivar Vada. Schüller et al. (1992) found a very close linkage (only 0.7 ± 0.7 cM) between *Mla* locus and the RFLP marker MWG036. Finally, Schwarz et al. (1999) detected a cosegregation between the RFLP marker MWG2197 and the resistance locus in a cross between near isogenic lines P01 containing *Mla1* allele and P10 with the *Mla12* allele. The *Mlra* gene originated from the landrace Ragusa b and described for the first time by Wiberg (1974) in the line Weihenstephan 41/145. The gene was localized by Doll and Jensen (1986) in the cross Vogelsanger Gold

× Alf between the hordin genes *Hor1* and *Hor2*, while Jensen (2002), in contradiction to that, placed the gene distal from *Hor2* and near the leaf rust resistance gene *Rph4* (*Pa4*). Two genes such as *Mlk* and *Mlnn* are localized on the short arm of chromosome 1H but more proximally than the *Mla*. The *Mlk* locus was described for the first time together with the *Mla locus* by Briggs and Stanford (1938) in the line Kwan (CI 1016). Giese (1981) was able to localize the *Mlk* gene on 1H. According to Jensen (2002) this gene was flanked by the two RFLP markers MWG2083 and ABA004, while the distance between this gene and *Mla6* was only 0.5 cM. The *Mlnn* powdery mildew resistance gene has been detected in the line Nigrinudum (C.I. 11549) by Hiura (1960). This gene is mapped between the RFLP markers CD099 and ABG053 (Jensen 2002). In a cross between Galleon with three susceptible cultivars (Weeah, Clipper, and Sonja), the *MlGa* was found to be linked loosely with the *Hor1* (C-hordein) locus with a recombination percentage of 35.7 ± 4.5 and very loosely linked to *Hor2* (B-hordein) with a recombination percentage of 40.9 ± 4.6. The order of location of the three loci studied was found to be *Hor-2 – Hor-1 – M1-(Ga),* with the latter toward the centromere (Hossain and Sparrow 1991a,b). Jensen (2002) placed the gene in tight linkage with the RFLP marker ABR377 with a distance of 0.03 cM.

The *MlLa* locus is the only major powdery mildew resistance gene known on chromosome 2H, with the resistance originating from *Hordeum laevigatum*. The locus was localized in a cross between Alf, which bear the resistance allele, and Vogelsanger Gold. This locus is flanked by the RFLP loci cMWG660 and MWG97 (Hilbers et al. 1992).

Two resistance genes were localized on chromosome 4H: *Mlg* and *mlo*. The *Mlg* powdery mildew resistance gene was described first in the line Weihenstephan (CP 127422) by Honecker (1931). Briggs and Stanford (1943) identified this gene in the variety Goldfoil. Fifty years later, Görg et al. (1993) found the gene to cosegregate with the RFLP marker MWG032 near the centromere. However, the more famous resistance gene on 4H is *mlo*. It gives a leaf lesion phenotype and broad-spectrum resistance. The resistance was found both in artificial mutants (Freisleben and Lein 1942) and barley landraces from Ethiopia (Negassa 1985). Hinze et al. (1991) found that the RFLP marker bAL88/2 was cosegregating with this resistance locus at a distance of 2.4 cM.

From wild barley lines (*H. vulgare* ssp. s*pontaneum*) Schönfeld et al. (1996) localized

one powdery mildew resistance gene on 5HL, *Mlj*, and two powdery mildew resistance genes on 7H, *mlt* and *Mlf*. The *Mlj* was found to be flanked by two RFLP markers, MWG592 and MWG999. On 7HL, the RFLP markers MWG035and MWG555a flanked the *mlt* locus, while *Mlf* was mapped on the short arm of chromosome 7H between the RFLP loci MWG053 and MWG539.

Leaf or Brown Rust Caused by *Puccinia hordei* Otth

The leaf rust resistance gene *Rph4* (*Pa4*) was described by Roane and Starling (1967) on chromosome 1H. McDaniel and Hathcock (1969) detected this gene in the varieties Gold and Lechtaler and localized it at a distance of 17 cM from the *Mla* locus in a cross between the resistance variety Gold and the cultivar Cebada Capa.

On chromosome 2H, two leaf rust resistance genes were localized; (i) *Rph1* (*Pa, Pa1*) was found in the varieties Oderbrucker originally from Germany, Speciale from USA, and Sudan from Sudan (Roane and Starling 1967) and was localized by Tuleen and McDaniel (1971). (ii) *Rph16* was localized from two wild barley (*H. vulgare* ssp. *spontaneum*) lines in a cross with susceptible line L94 (Ivandic et al. 1998). The gene *Rph16* was mapped on the short arm of chromosome 2H and found to be cosegregated with the RFLP markers MWG874 and MWG2133.

At least four leaf rust resistance genes were reported on chromosome 3H. Zhong et al. (2003) localized *Rph6* on the short arm of the chromosome in the cross Bolivia (resistance and carrying *Rph6* gene) × Bowman (susceptible). The gene was localized at a distance of 4.4 cM distal from RFLP marker MWG2021 and 1.2 cM proximal from RFLP marker BCD907. They found as well that the gene was allelic to the previously localized resistance gene *Rph5* (Mammadow et al. 2003) and closely linked to *Rph7*, at 1.3 cM distal to the RFLP marker MWG691 (Brunner et al. 2000; Graner et al. 2000). The gene *Rph7* was first detected in the cultivar Cebada Capa (Parlevliet 1976). Brunner et al. (2000) mapped this gene in the cross Cebada Capa × Bowman between the two AFLP markers Xabc171 and Xs1543.Another resistance gene, *Rph10*, was detected on 3H by Feuerstein et al. (1990) and mapped with the isozyme locus *Est2* in near-isogenic lines of wild barley (*H. vulgare* ssp. *spontaneum*) in a background of the variety Clipper. Later the gene was mapped to the interval between

Table 2. Qualitative gene mapped or targeted with molecular markers in barley

Gene	Trait	Material used	Chromosome	Nearest marker(s)	Marker type	Reference
Mlra	P.M.	Vogelsanger Gold × Alf	1HS	Between *Hor1* & *Hor2*	Protein	Doll and Jensen 1986
		Crosses with Wolf barley		Act8A, Gle1 Near Rph4	AFLP	Jensen 2002
Mla	P.M.	VADA × H. v. subsp. *spontaneum*	1HS	CMWG645	RFLP	Graner et al. 1991
		Pallas × Siri		MWG036	RFLP	Schüller et al. 1992
		P01 × P10		MWG2191	RFLP	Schwarz et al. 1999
Mlk	P.M.	Crosses with Wolf barley	1HS	MWG2083, ABA004	RFLP	Jensen 2002
Mlnn	P.M.	Crosses with Wolf barley	1HS	CD99 & ABG053	RFLP	Jensen 2002
MlGa	P.M.	Galleon Weeah, Clipper and Sonja	1HL	*Hor-1* & *Hor-2*	SDS-PAGE	Hossain and Sparrow 1991a,b
		Crosses with Wolf barley		ABR377	RFLP	Jensen 2002
MlLa	P.M.	Alf × Vogelsanger Gold	2H	cMWG660 & MWG97	RFLP	Hilbers et al. 1992
Mlg	P.M.	Ingrid × Pallas	4H	MWG032	RFLP	Görg et al. 1993
mlo	P.M.	H. v. subsp. *vulgare* and H. v. subsp. *spontaneum*	4H	BAL88/2	RFLP	Hinze et al. 1991
Mlj	P.M.	H. v. subsp. *spontaneum* HSY-78 × Amir	5HL	MWG592 & MWG999	RFLP	Schönfeld et al. 1996
mlt	P.M.	H. v. subsp. *spontaneum* RS42-6 × Orial	7HL	Flanked by MWG035 & MWG555a	RFLP	Schönfeld et al. 1996
Mlf	P.M.	H. v. subsp. *spontaneum* RS137-28 × Elgina	7HS	Between MWG053 & MWG539	RFLP	Schönfeld et al. 1996
Rph4 (Pa4)	L.R	Gold × Cebada Capa	1H	Morphological		McDaniel and Hathcock 1969
Rph1 (Pa, Pal)	L.R	Sudan	2H	Morphological		Tuleen and McDaniel 1971
Rph16	L.R	H. v. subsp. *spontaneum* × L94	2HS	MWG874 & MWG2133	RFLP	Ivandic et al. 1998
Rph6	L.R allelic to *Rph5*	Bolivia × Bowman	3HS	MWG2021, BCD907	RFLP	Zhong et al. 2003
Rph10	L.R	H. v. subsp. *spontaneum* × Clipper	3HL	*Est2*	Isozyme	Feuerstein et al. 1990
RphQ	L.R allelic to *Rph2*	Q21861 × SM89010	5HS	CDO749 & ITS1	RFLP	Borovkova et al. 1997
Rph2	L.R	Q21861 × SM89010	5HS	CDO749 & ITS1	RFLP	Borovkova et al. 1997
Rph9	L.R	Bowman × Hor 2596	5HL	ABC155 & ABG3	STS	Borovkova et al. 1998
Rph12	L.R	Triumph/I91-533-va	5HL	ABC155	STS	Borovkova et al. 1998

Table 2. (continued)

Gene	Trait	Material used	Chromosome	Nearest marker(s)	Marker type	Reference
Rph11	L.R	H. v. subsp. spontaneum	6H	Acp3 & Dip2	Isozyme	Feuerstein et al. 1990
Rphx	L.R	LBIran/UNA8271//Gloria/Come × Bowman	7H	ABC310a & ABC461	RFLP	Hayes et al. 1996
Rph3	L.R	Bowman	7HL	Morphological		Jin et al. 1993
Rph19	L.R	Chebec × Harrington	7H	HVM 49 & HVM 11	SSR	Park and Karakousis 2002
Rph7.g	L.R	Cebada Capa Bowman	3HS	Xabc171, Xs1543	AFLP	Brunner et al. 2000
Rpg1	S.R	Steptoe × Morex	7HS	ABG704 & ABG312	RFLP	Kleinhofs et al. 1993
rpg4	S.R	Q21861	5H	MWG740 & ABG390	RFLP	Borovkova et al. 1995
rpg4	S.R	Steptoe × Q21861	5HL	ARD009.1	RFLP	Druka et al. 2000
Rpsx	Y.R	C.I. 10587 × Galena	7HL	Ris44, ABG461	RFLP	Castro et al. 2003
Rrs14	Sc	Clipper ×H.v.subsp. spontaneum	1H	Hor1 & Hor2	RFLP	Garvin et al. 2000
Rrs1 (Rh, Rh1, Rh3, Rh4, rh16)	Sc	Igri × Triton	3HL	Co-seg. CMWG680, MWG582, ABG462, BCD263 & BCD828	RFLP	Graner and Tekauz 1996
Rrs4 (Ryh, rrsx)	Sc	Blenheim × lineSCRI	3H	Linked CDO1174	RFLP	Barua et al. 1993
	Sc	Ingrid × Nigrinudum (CI 11549)		HVM60, HVM36b	SSR	Patil et al. 2003
Rrs13	Sc	Back. Cross H. v. subsp. spontaneum	6H	Cxp3 & ABG458	RFLP	Abbott et al. 1995
Rrs2 (Rh2)	Sc	Atlas × Steffi	1H	Co-seg. CDO545	RFLP	Schweizer et al. 1995
Rdg1a	L.S.	Alf × Vogelsanger	2HL	MSU21, Xris45b	RFLP	Thomsen et al. 1997
Rdg2a	L.S.	Thibaut × Mirco	7HS	OPQ-9$_{(700)}$, MWG 2018	RFLP	Tacconi et al. 2001
Pt,,a	N.B.	Igri × Franka	3HL	BCD828, MWG2138	RFLP	Graner et al. 1996
Rpt4	N.B.	Galleon × Haruna Nijo	7H	Xpsr117 (D) Xcdo673	RFLP	Williams et al. 1999
Dhn1, 2	Dhd.	Wheat-barley addition lines	5HL	Gene-specific primers	*Dicktoo AF043087, AF043088	Choi et al. 1999
Dhn3, 4, 5, 7, 8	Dhd.	Wheat-barley addition lines	6HL	Gene-specific primers	*Dicktoo AF043089, AF043090, AF043096, AF043092, AF043093	Choi et al. 1999
Dhn6	Dhd.	Wheat-barley addition lines	4HS	Gene-specific primers	*Dicktoo AF043091	Choi et al. 1999
Dhn9	Dhd.	Wheat-barley addition lines	5H	Gene-specific primers	*Dicktoo AF043094	Choi et al. 1999
Dhn10, 11	Dhd.	Wheat-barley addition lines	3H	Gene-specific primers	*Dicktoo AF043095, AF043086	Choi et al. 1999
Dhn12	Dhd.	Wheat-barley addition lines	6HL	Gene-specific primers	*Morex 141	Choi and Close 2000
rym3	BaYMV	Ishuku Shirazu × Ko A	5HS	MWG28 and ABG705A	RFLP	Saeki et al. 1999

Table 2. (continued)

Gene	Trait	Material used	Chromosome	Nearest marker(s)	Marker type	Reference
BSMV	BSMV	Steptoe × Morex	1HS	ABC455. Marker ABG011	RFLP	Edwards and Steffenson 1996
Yd2	BYDV	Ethiopian (*H. vulgare* L.)	3HL	Xwg889 and XYlp	RFLP	Collins et al. 1996
Rym14^{Hb}	BaMMV1,-2	Borwina × *H. bulbosum*	6HS	MWG2318 and ABG466	RFLP	Ruge et al. 2003
rym3 (ym3)	BaYMV	Ishuku Shirazu × Ko A	5HS	MWG28 and ABG705A	RFLP	Saeki et al. 1999
Ha2	CCN	Chebec × Harrington Clipper × Sahara	2H	AWBMA 21 and MWG 694	RFLP	Kretschmer et al. 1997
Ha4	CCN	Haruna Nijo × Galleon	5HL	XYL	RFLP	Barr et al. 1998
Vrs1	2- /6-row ear type	Kanto Nakate Gold × Azumamugi	2HL	CMNA-38$_{700}$, OPJ-09$_{850}$ & OPP-02$_{700}$	RAPD	Komatsuda et al. 1997
Int-c	F.L.S.	Azumamugi × Kanto Nakate Gold	4HS	MWG2033	RFLP	Komatsuda and Mano 2002
btr1	Brittle rachis	Azumamugi × Kanto Nakate Gold	3HS	e14m27.4.1 & e15m19.7	AFLP	Komatsuda and Mano 2002
nud	N.C.	Kobinkatagi × Triumph	7HL	KT2 and KT4	AFLP	Kikuchi et al. 2003
denso	D.W.	Magnum × Goldmarker	3HL	Xpsb177, Xpsr170	RFLP	Laurie et al. 1993
gai	D.W.	Hv287 × Betzes	2H	Xmwg2058, Xmwg2287	RFLP	Borner et al. 1999
gal	D.W.	Hv288 × Monte Cristo	2HL	Xmwg581, Xmwg882, Xmwg2212, XksuG5	RFLP	Borner et al. 1999

P.M. = powdery mildew, L.R. = leaf rust, S.R. = stem rust, Y.R. = yellow rust, Sc. = scald, L.S. = barley leaf stripe, N.B. = net blotch, Dhn. = dehydrin, BaYMV = barley yellow mosaic virus, BSMV = barley stripe mosaic virus, BYDV = barley yellow dwarf virus, BaMMV = barley mosaic virus complex, CCN = cereal cyst nematode, F.L.S. = fertility of lateral spikelets, N.C. = naked caryopsis, D.W. = dwarfing gene

* Accession number

the RFLP loci ABG495b and MWG838 (Collins et al. 2001).

The two loci *RphQ* and *Rph2* were localized in the cross Q21861 × SM89010 on the short arm of 5H, near the centromere, and found to be either closely linked or allelic. They were localized in an interval between the RFLP marker CDO749 and ITS1 (Borovkova et al. 1997). Two more leaf rust resistance genes were localized on 5HL *Rph9* and *Rph12*. The *Rph9* was mapped in the cross Bowman × Hor 2596 and localized between two STS markers, ABC155 and ABG3 (Borovkova et al. 1998). The same group mapped *Rph12* in the cross Triumph × I91-533-va. The gene was linked to the STS marker ABC155 (Borovkova et al. 1998).

On chromosome 6H, Feuerstein et al. (1990) localized the *Rph11* leaf rust resistance gene *originating* again from wild barley *H. vulgare* ssp. *spontaneum*, and they were linked to the isozyme loci *Acp3* and *Dip2*.

On 7H, three leaf rust resistance loci, *Rph3, Rph19,* and *Rphx,* were localized. *Rph3* was mapped on the long arm of chromosome 7H. It was detected in the cultivar Estate (Roane and Starling 1967) at a distance of 9.7 cM from *Rphx* in a more distal position (Jin et al. 1993). The gene *Rph19* was described in the cultivar Reka 1 between two SSR markers, HVM49 and HVM11 (Park and Karakousis 2002). *Rphx* was mapped in the cross (LBIran/UNA8271//Gloria/Come × Bowman) and localized between the RFLP loci ABC310a and ABC461 (Hayes et al. 1996)

Stem Rust Caused by *Puccinia graminis* f. sp. *tritici*

Stem rust had been one of the most serious diseases of barley till 1942 when the resistance cultivars carrying the gene *Rpg1* were released for the first time. *Rpg1* is dominant and considered to be durable because it has remained effective for a long time (Brueggeman et al. 2002). *Rpg1* was mapped on the short arm of chromosome 7H. Kleinhofs et al. (1993) localized it in the cross Steptoe × Morex between the two RFLP markers ABG704 and ABG312. The second known resistance gene against stem rust is *rpg4*. This gene was mapped on chromosome 5H between the RFLP markers MWG740 and ABG390 (Borovkova et al. 1995). Druka et al. (2000) mapped the RFLP marker ARD009.1 about 0.6 cM proximal to *rpg4*t in the cross Steptoe × Q21861 on the long arm of chromosome 5H.

Stripe Rust Caused by *Puccinia striiformis* Westend. f. sp. *hordei* Erikss (*P. s. hordei*)

Four qualitative resistance genes, *Yr1* to *Yr4*, were described. Of these genes, only the *Yr4* locus has been mapped so far. Jensen and Jørgensen (1997) localized *Rps4* (*Yr4*) conferring resistance to stripe rust in the variety Abed Deba on chromosome 1H close to the *Mla* locus. Hayes et al. (1999) mapped an adult plant resistance gene, *Yr4*, for stripe rust on the long arm of chromosome 7H in CI10587. However, the precise nomenclature of gene symbol for this locus is missing. In an attempt to pyramid quantitatively and qualitatively inherited resistance genes against stripe rust, Castro et al. (2003) mapped another qualitative resistance gene *Rpsx* originating from the line C.I.10587 in a cross with Galena to 7HL between the two RFLP markers Ris44 and ABG461.

Scald Caused by *Rynchosporium secalis*

Several symbols were used to describe different alleles of the same locus. Recently, the nomenclature of the scald resistance genes in barley was revised by Bjørnstad et al. (2002). In this review, both old and new symbols are included.

Garvin et al. (1997) backcrossed the scald susceptible barley cultivar Clipper with different Iranian and Turkish wild barley lines and detected the scald resistance gene *Rrs14*. Later they localized the gene on chromosome 1H between two hordein loci, *Hor1* and *Hor2*. The gene was mapped ca. 1.8 cM from the *Hor2* locus, in the cross between Clipper and a third backcross (BC3) line homozygous for the *Rrs14* that originally came from *Hordeum vulgare* ssp. *spontaneum* (Garvin et al. 2000).

The complex locus *Rrs1* was localized near the centromere and *Rrs4* about 20 cM more distal on the long arm of chromosome 3H. *Rrs1* was earlier described as *Rh, Rh1, Rh3,* or *Rh4* (Dyck and Schaller 1961; Habgood and Hayes 1971) and *rh6* (Baker and Larter 1963). The gene was mapped in the cross between the susceptible cultivar Igri and Trition, which is resistant and carries *Rrs1*. The gene was found to cosegregate with the RFLP markers cMWG680, MWG582, ABG462, BCD563, and BCD828 (Graner et al. 1996). The second gene on 3H is *Rrs4,* which was described earlier as *Rhy* (Barua et al. 1993) and *rrsx* (Patil 2001). Barua et al. (1993) linked the gene to the RFPL marker CDO1174 (6.8 cM) in a cross between the scald susceptible cultivar Blenheim and scald resistance SCRI breeding line. Patil et al. (2003) mapped this gene be-

tween the two SSR loci HVM60 and HVM36b in the Ingrid population (susceptible) × Nigrinudum (C.I. 11549, resistance).

On chromosome 6H the scald resistance gene *Rrs13* was identified in a backcross line carrying resistance genes obtained from wild barley (*H. vulgare* ssp. *spontaneum*). The gene was mapped between the RFLP marker loci Cxp3 and ABG458 (Abbott et al. 1995). Dyck and Schaller (1961) described the scald resistance gene *Rrs2* (formally *Rh2*). Later the gene was localized by Schweizer et al. (1995) on chromosome 1H in the cross between the resistant cultivar Atlas (C.I. 4118) and susceptible cultivar Steffi. They found the gene to be cosegregated with the RFLP marker CDO545.

Barley (Leaf) Stripe Caused by *Pyrenophora graminea*

Two barley leaf stripe resistance genes are known. The first one is *Rdg1a*, which is also known as Vada-resistance, and it was localized between the RFLP loci MSU21 and Xris45b on chromosome 2HL using the cross Alf (*Rdg1a*) × Vogelsanger Gold (*rdg1a*) (Thomsen et al. 1997). *Rdg2a*, the second gene for resistance against the same disease, was identified in the cultivar Thibaut and localized in near-isogenic lines obtained by introgressing the cultivar Thibaut (resistance) into the genetic background of the susceptible cultivar Mirco. The gene was mapped to the telomeric region of barley chromosome 7HS and was flanked by the RFLP markers OPQ-9$_{(700)}$ and MWG 2018 at distances of 3.1 and 3.5 cM, respectively (Tacconi et al. 2001). This gene is designated as *Rdg2a*.

Net Blotch Caused by *Pyrenophora teres*

Two resistance genes acting against *Pyrenophora teres* have been localized in barley until now. The first one was preliminarily named *Pt,,a* and mapped to the long arm of the chromosome 3H close to the centromere, between the RFLP markers BCD828 and MWG2138, using the cross Igri (resistance) × Franka (susceptible) (Graner et al. 1996). The other gene *Rpt4* was originated from the cultivar Galleon and provides resistance against the spot form of net blotch (*Pyrenophora teres* f. *maculate*). This gene was localized on the long arm of 7H using a DH population derived from a cross with susceptible cultivar

Haruna Nijo and the resistant cultivar Galleon. The gene was flanked by the RFLP loci Xpsr-117 (D) and Xcdo673 at distances of 6.9 cM and 25.9 cM, respectively (Williams et al. 1999).

Resistance Genes for Viral Diseases

Several viral diseases cause serious damage on barley production all over the world. Saeki et al. (1999) localized the gene *rym3* conferring resistance to barley yellow mosaic virus (BaYMV) in a cross between Ishuku Shirazu, which carries *rym3,* and the susceptible cultivar Ko A. The gene was mapped on the short arm of 5H and flanked by two RFLP markers, MWG28 and ABG705A, at a distance of 7.2 and 11.7 cM, respectively. Resistance gene for barley stripe mosaic virus (BSMV) was localized on the 1HS in the cross Steptoe × Morex. The gene was found to be flanked by two RFLP markers ABC455 and ABG011. The marker ABG011 was located at 6.1 cM distal to the resistance locus (Edwards and Steffenson 1996). Collins et al. (1996) identified a gene in Ethiopian barley against barley yellow dwarfluteovirus (BYDV). They designated the gene as *Yd2*, which was mapped on chromosome 3H and cosegregated with the RFLP loci Xwg889 and XYlp, which were located on the long arm, 0.5 cM from the centromere.

Soil-borne barley yellow mosaic virus disease (caused by several viruses such as, barley mild mosaic virus (BaMMV), barley yellow mosaic virus (BaYMV) and (BaYMV-2)) is a serious disease of winter barley cultivars. Several genes were described and mapped against this disease. Bauer and Graner (1995) mapped the resistance gene *ym4* against BaMMV on chromosome 3H. The gene was derived from the cultivar Ragusa and was localized with RFLP markers on the long arm of chromosome 3H. The *rym3* gene conferring resistance to barley yellow mosaic virus (BaYMV) was mapped by Saeki et al. (1999) in a cross between BaYMV-resistant cultivar Ishuku Shirazu carrying *rym3* and susceptible cultivar Ko A on the short arm of chromosome 5H. Two RFLP markers MWG28 and ABG705A flanked the gene at a distances of 7.2 and 11.7 cM, respectively. The SSR markers WMS6 and HVM67 have been tested to facilitate marker-assisted selection for the resistance gene *rym9* because the gene and the markers are closely linked on chromosome 4HL (Werner et al. 2000). The gene *rym13* was derived from the cultivar Taihoku A and localized based on an AFLP marker in the telomeric

region of chromosome 4HL with the closest linkage of 5.9 cM (Werner et al. 2003). Recently, the gene *yrm5* was mapped in the cross Plaisant and Chikurin Ibaraki 1. The resistance gene was flanked by two markers near the centromeric region of chromosome 6HS, Bmag0173, at 0.6±1.2 cM, and EBmac0874, at 5.8 ± 3.4 cM (Le Gouis et al. 2004).

Cereal Cyst Nematode (CCN) Caused by *Heterodera avenae* Well

Cereal cyst nematode is an economically damaging pest of barley in many regions of the world's cereal-growing areas. *Ha2* and two other resistance genes against CCN have been mapped on the chromosome 2H. Kretschmer et al. (1997) mapped *Ha2* in two crosses and found two RFLP markers flanking this gene. The markers AWBMA21 and MWG694 mapped 4.1 and 6.1 cM, respectively, from the *Ha2* locus in the cross Chebec × Harrington and 4.0 and 9.2 cM, respectively, in the cross Clipper × Sahara cross. *Ha4* has been mapped in a cross between an Australian barley variety Galleon derived from the landrace CI3576 and Haruna Nijo. The gene was localized on the long arm of chromosome 5H and estimated to be 6.2 cM from the RFLP marker XYL (Barr et al. 1998).

4.3.2
Genes Related to Abiotic Stresses

Dehydrins are water-soluble lipid-associating proteins that are accumulated during low-temperature or water-deficit conditions and are thought to play a role in freezing- and drought-tolerance in plants (Choi and Close 2000). Many dehydrin genes have been found in barley. Choi et al. (1999) identified 11 unique *Dhn* genes in the cultivar Dicktoo; 7 out of 11 appear to be alleles of *Dhn* genes identified previously in other barley cultivars, while *Dhn9* appears to be orthologous to a *Triticum durum Dhn* gene. The genes were mapped with wheat-barley addition lines using gene-specific primers. Genetic mapping established the presence of *Dhn* genes on four different barley chromosomes (3H, 4H, 5H, 6H). Choi and Close (2000) identified an additional *Dhn* gene in the variety Morex 141. They designated this gene as *Dhn12,* which is located on chromosome 6H and has embryo-specific expression. *HVA1* is a single-copy barley gene encoding a class 3 late embryogenesis-abundant protein. This gene can be induced by either treatment of abscisic acid (ABA) or by stress conditions such as drought, cold, heat, or salinity. The gene was isolated and found to contain about 400 bp of 5′-upstream sequence, a single 109-bp intron, and the full coding sequence (Straub et al. 1994).

4.3.3
Traits Important for Domestication

A number of "key" genes were involved in the domestication of barley. Two- and six-rowed ear type is one of the most important traits in barley, which refers to the fertility of the lateral spikelets. The lateral spikelets are female-sterile in two-rowed barleys, while all spikelets are fertile in six-rowed barleys. The formation of the two- and six-rowed spike is controlled predominantly by the *V* and *v* (*vrs1*) alleles, respectively. The recessive allele is responsible for six-rowed types, while the dominant allele controls the two-rowed type. A cross between Kanto Nakate Gold (two-rowed) and Azumamugi (six-rowed) showed tight linkage between the RAPD marker CMNA-38$_{(700)}$ and *vrs1* locus with a recombination frequency of zero, while OPJ-09$_{(850)}$ and OPP-02$_{(700)}$ were linked to the *vrs1* locus at a map distance of 1.4 cM (Komatsuda et al. 1997). The brittleness of the rachis was lost during the domestication. Nonbrittle rachis is controlled by alleles at two tightly linked loci, *btr1* and *btr2*. The locus *btr1* was mapped on the short arm of chromosome 3H and found to be flanked between two AFLP loci, e14m27.4.1 and e15m19.7, with a map distances of 3.1 cM and 4.2 cM, respectively. *Vrs1* is modified by the locus *int-c*, which was mapped in the cross Azumamugi × Kanto Nakate Gold. The locus *int-c* was mapped to the end of chromosome 4HS, 8.2 cM distally from the RFLP marker MWG2033 locus (Komatsuda and Mano 2002).

The naked caryopsis character of barley (*H. vulgare* L.) is an important trait for edibility that allows one to trace its domestication process. The gene responsible for naked caryopsis is designated as *nud* and its inheritance is recessive; this gene is located on the long arm of chromosome 7H. Kikuchi et al. (2003) mapped the locus in a cross between Kobinkatagi (naked type) and Triumph. The *nud* locus cosegregated with KT3 and KT7 and was flanked by two additional AFLP markers, KT2 and KT4, at the 0.3-cM proximal and the 1.2 cM distal, respectively.

Lines with the *denso* gene showed a distinctive prostrate juvenile growth habit and tended to have later ear emergence times and lower (plant grain weights, ear grain weights, and 1,000 grain weights). The dwarfing gene *denso* was mapped in a cross between the barley varieties Magnum and Goldmarker and was located on the long arm of chromosome 3H, ca. 8 cM distal to the RFLP locus Xpsr170 (Laurie et al. 1993). Two more dwarfing genes were mapped, the *gai* gibberellic acid insensitivity (GA-ins) gene in the centromeric region and the *gal* (GA-less) on the long arm of chromosome 2H. The gene *gai*, which determines reduced plant height and gibberellic acid (GA) insensitivity, is assumed to be pleiotropic. This gene was found to cosegregate with the two RFLP markers Xmwg2058 and Xmwg2287. The GA-sensitive dwarfing gene *gal* was found to be cosegregating with three RFLP markers, Xmwg581, Xmwg882, and Xmwg2212 (Borner et al. 1999).

4.4
Analysis of Quantitative Trait Loci

The following list presents localization of quantitative trait loci (QTL) published for barley. For each localization, the title of the article is given, followed by the reference, the population used, the map according to the previous list (if available), and the mode of phenotyping, as well as a list of the detected QTLs. For the QTLs the chromosome localization is shown. If several QTLs for one trait were mapped to the same chromosome, the number of loci is indicated in parentheses after the name of the chromosome. References to the mapping programs are given at the end of the list (Table 3).

Q 1:   *Title:* Associations between 23 quantitative traits and 10 genetic markers in a barley cross
Reference: Kjær et al. 1991
Population: Mona × Tystofte Prentice, 63 DH lines
Phenotyping: 1 year × 1 location
QTLs: Grain yield: 1H, 2H; grain weight: 1H, 2H, 6H; spike number: 1H, 2H; kernel/spike: 1H, 2H; lodging: 1H, 2H, 3H; straw length: 1H, 2H, 3H; top internode length: 3H; length of internode 2 & 3: 1H, 2H, 3H; length of internode 4: 1H, 3H; length of basal internode: 1H, 2H; straw diameter: 1H, 3H, 6H; N-content

in grain: 5H, 6H; P-content in grain: 1H, 2H, 5H; Na-content in grain: 1H, 2H; K-content in grain: 1H, 2H; Ca-content in grain: 6H; Mg-content in grain: 1H; N-content in straw: 1H, 3H, 6H; P-content in straw: 1H, 2H, 3H; Na-content in straw: 1H; K-content in straw: 1H, 2H; Ca-content in straw: 1H; Mg-content in straw: 1H, 2H
Remark: Analysis based on 3 Giemsa-bands, 2 isozyme loci, and 4 phenotypic markers

Q 2:   *Title:* Statistical analysis of a linkage experiment in barley involving quantitative trait loci for height and ear-emergence time and two genetic markers on chromosome 4
Reference: Hackett et al. 1992
Population: Gerbel × Heriot, 25 F_2 plants
Phenotyping: 1 year × 1 location
Method: Maximum likelihood calculations
QTLs: Height: 4HL, Ear-emergence time: 4HL
Remark: Analysis based on 2 isozyme loci known to be on 4HL

Q 3:   *Title:* Mapping quantitative powdery mildew resistance of barley using a restriction fragment length polymorphism map
Reference: Heun 1992
Population: Proctor × Nudinka, 113 DH lines
Map: M 3 (Heun et al. 1991)
Phenotyping: Detached leaves on agar
Method: MAPMAKER/QTL
QTLs: Powdery mildew resistance: 5HS, 7HS

Q 4:   *Title:* Quantitative trait loci on barley (*Hordeum vulgare* L.) chromosome 7 associated with components of winterhardiness
Reference: Hayes et al. 1993a
Map: M 5 (Hayes et al. 1993a; Pan et al. 1994)
Population: Dicktoo × Morex, 100 DH lines
Phenotyping: 1 year × 1 to 2 locations
Method: QTL-STAT (least-squares interval mapping)
QTLs: Field survival under cold stress: 7HL; heading date: 7HL; fructan content: 7HL
Remark: Only 7H was analyzed

Q 5:   *Title:* Genetic mapping of a quantitative trait locus (QTL) that enhances the shoot differentiation rate in *Hordeum vulgare* L.
Reference: Komatsuda et al. 1993
Population: Azumamugi × Kanto Nagate Gold, 119 BC_1F_1
Phenotyping: Tissue culture (30 embryos per line)
Method: Maximum likelihood calculations

Table 3. QTL mapping programs used, references, number of maps in list, and references of first use

Program	Reference	Analyses	Reference of first use
MAPMAKER/QTL	Paterson et al. 1988	35	Q 3 (Heun 1992)
QTL-STAT	Liu and Knapp unpublished	3	Q 4 (Hayes et al. 1993a)
MQTL	Tinker and Mather 1995	16	Q 20 (Tinker et al. 1996)
MAPQTL	Van Oijen and Maliepaard 1996	15	Q 35 (Qi et al. 1998b)
QGENE	Nelson 1997	6	Q 42 (Williams et al. 1999)
RI MANAGER	Manly 1993	1	Q 42 (Williams et al. 1999)
PLABQTL	Utz and Melchinger 1996	15	Q 47 (de la Peña et al. 1999)
QTL Cartographer	Basten et al. 1997	3	Q 50 (Kandemir et al. 2000)
MultiQTL	http://esti.haifa.ac.il/~web/QTL/	1	Q 69 (Castro et al. 2002)
MapManager QTX	Manly et al. 2001	11	Q 88 (Cakir et al. 2003)
R/qtl	Broman et al. 2003	1	Q 106 (Emebiri et al. 2004)

QTLs: Shoot differentiation: 2H
Remark: Analysis based on 4 isozyme loci

Q 6: *Title:* Quantitative trait locus effects and environmental interaction in a sample of North American barley germ plasm
Reference: Hayes et al. 1993b
Population: Steptoe × Morex, 150 DH lines
Map: M 6 (Kleinhofs et al. 1993), but reduced to a 123-marker skeleton map
Phenotyping: 1 year × 5 locations
Method: QTL-STAT (least squares interval mapping)
QTLs: Yield: 2H, 3H, 6H, 7H; lodging: 2H, 3H, 4H, 6H, 7H; plant height: 1H, 2H, 3H, 4H, 5H, 6H, 7H; heading date: 2H, 3H, 4H, 6H, 7H; grain protein content: 2h, 3H, 4H, 5H; α-amylase activity: 1H, 2H, 3H, 4H, 5H, 6H, 7H; diastatic power: 1H, 2H, 4H, 5H, 6H, 7H; malt extract: 1H, 2H, 4H, 6H, 7H

Q 7: *Title:* Identification of RAPD markers linked to genetic factors controlling the milling energy requirement of barley
Reference: Chalmers et al. 1993
Population: Blenheim × E224/3, 59 DH lines
Map: M 12: Partial map of 5HS (4 RAPD markers)
Phenotyping: 3 years
Method: MAPMAKER/QTL
QTLs: Milling energy: 5HS

Q 8: *Title:* Mapping genes for resistance to barley stripe rust (*Puccinia striiformis* f. sp. *hordei*)
Reference: Chen et al. 1994
Population: Lblran/UNA8271//Gloria/Come × Bowman, 110 DH lines

Map: 16 loci, chromosomes 4H and 5H only
Phenotyping: 2 years in 1 to 2 rows
Method: QTL-STAT, MAPMAKER-QTL
QTLs: resistance against stripe rust: 4H, 5H (major)

Q 9: *Title:* Utilization of a recombinant inbred population to localize QTLs for abscisic-acid content in leaves of drought-stressed barley (*Hordeum vulgare* L.)
Reference: Sanguineti et al. 1994
Population: Dicktoo × Morex, 30 DH lines
Map: M 5 (Hayes et al. 1993a)
Phenotyping: Greenhouse experiment with 2 pots per line
Method: MAPMAKER/QTL
QTLs: ABA-content after drought stress: 2HS

Q 10: *Title:* Utilization of a recombinant inbred population to localize QTLs for abscisic-acid content in leaves of drought-stressed barley (*Hordeum vulgare* L.)
Reference: Pan et al. 1994
Population: Dicktoo × Morex, 100 DH lines
Map: M 5 (Hayes et al. 1993a) with additional markers
Phenotyping: See Hayes et al. (1993a), additionally controlled-environment experiments with different light/temperature conditions
Method: MAPMAKER/QTL
QTLs: Field survival under cold stress: 7HL; heading date short day with vernalization: 1H, 3H, 7H; heading date short day without vernalization: 1H, 3H, 5H, 7H; heading date long day with vernalization: 2H, 5H; heading date long day without vernalization: 2H, 5H;

heading date permanent light with vernalization: 2H, 5H; heading date permanent light without vernalization: 2H, 5H

Q 11: *Title:* Localization of quantitative trait loci (QTLs) for agronomic important characters by the use of a RFLP map in barley (*Hordeum vulgare* L.)
Reference: Backes et al. 1995
Population: Igri × Danilo, 249 DH lines
Map: M 10 (Backes et al. 1995)
Phenotyping: 3 years × 2 locations
Method: MAPMAKER/QTL
QTLs: Scald resistance: 2HL, 7H; powdery mildew resistance: 7H; Lodging: 4H; ear breaking: 2H, 4H, 6H; physical state before harvest: 4H; plant height: 5H; heading date: 2H, 7H; kernel weight: 4H

Q 12: *Title:* RFLP mapping of 5 major genes and 8 quantitative trait loci controlling flowering time in a winter × spring barley (*Hordeum vulgare* L.) cross
Reference: Laurie et al. 1995
Population: Igri × Triumph, 94 DH lines
Map: M 11 (Laurie et al. 1995)
Phenotyping: One field experiment with autumn and one with spring sowing; glasshouse experiment with 0, 3 and 6 weeks of vernalization and 10, 13, and 16 h of natural daylight
Method: MAPMAKER/QTL
QTLs: Vernalization response: 2HS, 3HL, 4HL, 5HL, 6HL(2), 7HS, 7HL
Remark: Significant effect of *Ppd-H2* on chromosome 1H with autumn sowing

Q 13: *Title:* Detection of quantitative trait loci for agronomic, yield, grain and disease characters in spring barley (*Hordeum vulgare* L.)
Reference: Thomas et al. 1995
Population: Blenheim × E224/3, 59 DH lines
Map: M 12 (Thomas et al. 1995)
Phenotyping: field experiments with 2 to 4 years at 2 locations
Method: Least-squares interval mapping
QTLs: Heading date: 3H, 5H, 6H; plant height: 3H, 5H, 7H; grain yield: 3H, 5H; kernel weight: 3H, 6H, 7H; specific kernel weight: 3H(2), 6H; kernel size: 1H, 2H, 3H(2), 6H; powdery mildew resistance: 1H; scald resistance: 3H(2); brown rust resistance: 5HL; yellow rust resistance: 1H, 5H, 7H

Q 14: *Title:* Mapping of *β*-glucan content and *β*-glucanase activity loci in barley grain and malt
Reference: Han et al. 1995
Population: Steptoe × Morex, 150 DH lines
Map: M 6 (Kleinhofs et al. 1993) but reduced to a 123-marker skeleton map Hayes et al. 1993b
Phenotyping: 1 year × 4 locations
Method: MAPMAKER/QTL
QTLs: *β*-Glucan content barley: 1H, 2H(2); *β*-glucan content malt: 1H(2), 3H, 4H, 7H; *β*-glucanase activity green malt: 1H, 4H, 5H; *β*-glucanase activity finished malt: 1H, 4H, 5H, 7H

Q 15: *Title:* Quantitative trait loci or heading date and straw characters in barley
Reference: Kjær et al. 1995
Population: Vogelsanger Gold × t Tystofte Prentice, 90 DH lines
Map: M 14 (Kjær et al. 1995)
Phenotyping: 2 years × 1 location
Method: MAPMAKER/QTL
QTLs: Heading date: 2H(3); straw length: 2H(2); length of top internode: 2H(2), 6H; length of basal internode: 2H(2); straw diameter: 2H(2), 5H; ear length: 2H(2)

Q 16: *Title:* Quantitative trait loci for germination and malting quality characters in a spring barley cross
Reference: Thomas et al. 1996
Population: Blenheim × E224/3, 59 DH lines
Map: M 12 (Thomas et al. 1995)
Phenotyping: 1 to 4 years × 2 locations
Method: Least-squares interval mapping
QTLs: Germinative capacity in 4 ml, 4 weeks after harvest: 6H; germinative energy in 8 ml 4 weeks after harvest: 2H(2), 3H; germinative capacity in 8 ml 4 weeks after harvest: 2H, 3H; germinative energy in 8 ml 8 weeks after harvest: 7H(2); germinative capacity in 8 ml 8 weeks after harvest: 2H, 5H, 7H

Q 17: *Title:* Genetics of seedling and adult plant resistance to net blotch (*Pyrenophora teres* f. *teres*) and spot blotch (*Cochliobolus sativus*) in barley
Reference: Steffenson et al. 1996
Population: Steptoe × Morex, 150 DH lines
Map: M 6 (Kleinhofs et al. 1993) reduced to a 123-marker skeleton map (Hayes et al. 1993b)
Phenotyping: Field experiment in 2 locations × 1 year and grow-chamber experiments

Method: MAPMAKER/QTL

QTLs: Net blot resistance seedling stage: 4H, 6H; net blot adult plants: 2H; 3H(2), 4H; 5H, 6H; spot blotch resistance seedling stage: 7H, spot blotch resistance adult plants: 1H, 7H

Q 18:   *Title:* Application of quantitative trait locus mapping to the development of winter-habit malting barley

Reference: Oziel et al. 1996

Population: Dicktoo × Morex, 100 DH lines

Map: M 6 (Pan et al. 1994)

Phenotyping: 1 field experiment with autumn and one with spring sowing

Method: MAPMAKER/QTL

QTLs: Grain protein: 1H, 4H, 5H, 6H, 7H; soluble total protein: 3H, 5H, diastatic power: 5H, 7H; α-amylase activity: 5H, 7H; malt extract: 2H, 5H; fine-coarse difference: 5H, 7H; wort β-glucan: 5H

Remark: On 5H and 7H QTL for several traits

Q 19:   *Title:* Marker regression mapping of QTL controlling flowering time and plant height in a spring barley (*Hordeum vulgare* L.) cross

Reference: Bezant et al. 1996

Population: Blenheim × Kym, 99 DH lines

Map: M 15 (Bezant et al. 1996)

Phenotyping: 1 location × 2 years

Method: Least-squares interval mapping

QTLs: Heading date: 1H, 3H, 5H, 6H, 7H(2); Plant height: 3H, 5H, 7H

Q 20:   *Title:* Regions of genome that affect agronomic performance in two-row barley

Reference: Tinker et al. 1996

Population: Harringon × TR306, 150 DH lines

Map: M 9 (Kasha and Kleinhofs 1994), reduced to a 127-marker skeleton map

Phenotyping: 17 locations × 1 to 2 years (30 environments)

Method: MQTL

QTLs: Kernel yield: 1H, 2H, 3H, 4H(2), 5H(2), 6H, 7H; heading date: 2H, 3H(2), 4H, 5H, 6H, 7H(2); maturity: 2H, 3H, 4H, 5H(2), 7H(2); plant height: 1H, 2H, 3H, 4H, 5H(2), 6H, 7H(2); lodging: 2H,3H, 4H(3), 5H(2), 6H; kernel weight: 2H(2), 3H, 4H, 5H, 6H, 7H; specific kernel weight: 3H, 4H(2), 5H(2), 6H(2), 7H

Q 21:   *Title:* Mapping genes for callus growth and shoot regeneration in barley (*Hordeum vulgare* L.)

Reference: Mano et al. 1996

Population: Steptoe × Morex, 150 DH lines

Map: M 6 (Kleinhofs et al. 1993) reduced to a 123-marker skeleton map (Hayes et al. 1993b)

Phenotyping: Tissue culture (10 to 15 embryos per line)

Method: MAPMAKER/QTL

QTLs: Callus growth: 2HL, 3HL; Shoot regeneration: 2HS, 3HS, 5HL, 6HL

Q 22:   *Title: Amy2* polymorphism as a possible marker of β-glucanase activity in barley (*Hordeum vulgare* L)

Reference: Zwickert-Menteur et al. 1996

Population: Steptoe × Morex, 150 DH lines

Map: M 6 (Kleinhofs et al. 1993) reduced to a 123-marker skeleton map (Hayes et al. 1993b)

Phenotyping: 3 locations × 1 to 2 years (4 environments)

Method: MAPMAKER/QTL

QTLs: α-Amylase activity: 2H, 4H, 5H; β-glucanase activity in green malt: 1H, 2H, 4H, 7H; β-glucanase-activity in kilned malt: 1H(2), 5H, 7H(3)

Q 23:   *Title:* Quantitative trait loci for grain yield and yield components in a cross between a six-rowed and a two-rowed barley

Reference: Kjær and Jensen 1996

Population: Vogelsanger Gold × Tystofte Prentice, 90 DH lines

Map: M 14 (Kjær et al. 1995)

Phenotyping: 1 location × 2 years

Method: MAPMAKER/QTL

QTLs: Grain yield: 2H(2); grain yield, 6-rowed: 4H; kernel weight: 2H(2), 4H; kernel weight 2-rowed: 4H; kernels/ear: 2H; ears/m²: 2H, 7H; ears/m² 2-rowed: 4H, 6H

Q 24:   *Title:* Quantitative resistance to barley leaf stripe (*Pyrenophora graminea*) is dominated by one major locus

Reference: Pecchioni et al. 1996

Population: Proctor × Nudinka, 91 DH lines

Map: M 3 (Heun et al. 1991)

Phenotyping: Greenhouse experiment with 4 × 30 plants

Method: MAPMAKER/QTL

QTLs: Resistance to barley leaf stripe: 2H, 4H, 7H (major)

Q 25:   *Title:* Marker regression mapping of QTL controlling flowering time and plant height in a spring barley (*Hordeum vulgare* L.) cross

Reference: Bezant et al. 1997b

Population: Blenheim × Kym, 99 DH lines
Map: M 15 (Bezant et al. 1996)
Phenotyping: Field experiment with 1 location × 3 years
Method: Least-squares interval mapping, MAPMAKER/QTL
QTLs: Ear grain weight: 1H, 2H(3), 3H, 4H(2), 5H, 6H(2), 7H(4); kernel weight: 2H, 3H, 4H, 5H, 6H, 7H(2); plant grain yield: 1H, 2H(2), 3H(2), 4H, 5H(2), 6H, 7H(2); plot yield: 2H(2), 3H, 4H, 5H, 6H, 7H; ear grain number: 1H, 3H, 4H, 5H, 6H

Q 26: *Title:* Comparison between QTL analysis of powdery mildew resistance in barley based on detached primary leaves and on field data
Reference: Backes et al. 1996
Population: Alexis × Regatta, 249 DH lines
Map: M 10 (Backes et al. 1996)
Phenotyping: Field experiments with 2 locations × 3 years (Q 11, Backes et al. 1995) and experiments on detached leaves
Method: MAPMAKER/QTL
QTLs: Powdery mildew resistance seedlings: 6H, 7H; powdery mildew resistance adult plants: 7H

Q 27: *Title:* Regions of genome that affect grain and malt quality in a North American two-row barley cross
Reference: Mather et al. 1997
Population: Harringon × TR306, 150 DH lines
Map: M 9 (Kasha and Kleinhofs 1994) reduced to a 127-marker skeleton map
Phenotyping: 6 locations × 1 year
Method: Simplified composite interval mapping
QTLs: Kernel plumpness: 1H, 3H, 4H(3), 5H(2), 6H, 7H; kernel weight: 1H, 2H(3), 3H 5H, 6H, 7H(4); grain protein content: 4H, 5H(3), 7H; fine-grind extract: 1H, 4H, 5H(2); fine-coarse difference: 2H, 3H, 5H, 6H; soluble protein: 1H(2), 4H,5H, 6H, 7H; β-glucan content: 1H, 3H, 5H(2), 6H; viscosity: 1H, 3H, 4H(2), 5H(2), 6H(2), 7H; diastatic power: 1H(2), 2H(2), 3H, 4H(2), 5H, 6H; α-amylase activity: 4H, 5H, 6H, 7H

Q 28: *Title:* Mapping resistance to cereal aphids in barley
Reference: Moharramipour et al. 1997
Population: Harrington × TR306, 150 DH lines
Map: M 9 (Kasha and Kleinhofs 1994) reduced to a 127-marker skeleton map

Phenotyping: Field experiment with 1 location × 2 years
Method: MQTL
QTLs: Resistance to cereal aphids: 1H, 7H

Q 29: *Title:* Mapping of QTL controlling NIR predicted hot water extract and grain nitrogen content in a spring barley cross using marker regression
Reference: Bezant et al. 1997a
Population: Blenheim × Kym, 99 DH lines
Map: M 15 (Bezant et al. 1996)
Phenotyping: Field experiment with 1 location × 1 year
Method: least squares interval mapping
QTLs: Hot-water extract: 1H(2), 2H(2), 3H, 4H, 5H, 6H; nitrogen content: 1H(2), 2H, 4H, 5H, 6H, 7H(2)

Q 30: *Title:* Mapping quantitative trait loci for salt tolerance at germination and seedling stage in barley (*Hordeum vulgare* L)
Reference: Mano and Takeda 1997
Population: Steptoe × Morex (SM), 150 DH lines; Harrington × TR306 (HT), 150 DH lines
Map: SM: M 6 (Kleinhofs et al. 1993) reduced to a 123-marker skeleton map (Hayes et al. 1993b); HT: M 9 (Kasha and Kleinhofs 1994) reduced to a 127-marker skeleton map
Phenotyping: Seedling tests on filter paper and greenhouse tests
Method: MAPMAKER/QTL
QTLs: SM: Salt tolerance: 4H, 5H, 6H; ABA response: 1H, 2H, 3H, 5H; germination speed: 2H, 3H, 5H(2); HT: salt tolerance: 1H, 5H; ABA response: 2H, 5H; germination speed: 5H, 6H, 7H

Q 31: *Title:* Molecular-marker-assisted selection for malting quality traits in barley
Reference: Han et al. 1997
Population: (Steptoe × Morex) × Steptoe, 210 BC$_2$F$_1$ lines; derived from a DH line from Steptoe × Morex (Kleinhofs et al. 1993)
Map: M 18 (Han et al. 1997)
Phenotyping: 5 locations × 1 year
Method: ANOVA of isogenic lines
QTLs: Malt extract: 7H(2); α-amylase: 7H(2); diastatic power: 7H(2-3).

Q 32: *Title:* Potential of doubled-haploid lines and localization of quantitative trait loci (QTL) for partial resistance to bacterial leaf streak (*Xanthomonas campestris* pv. *hordei*) in barley
Reference: El Attari et al. 1998

Population: Steptoe × Morex, 119 DH lines
Map: M 6 (Kleinhofs et al. 1993)
Phenotyping: Growth chamber experiments (20 × 3 seedlings)
Method: MQTL
QTLs: Resistance against bacterial leaf streak: 3H(2)

Q 33: *Title:* Genetic analysis of components of winterhardiness in barley (*Hordeum vulgare* L.)
Reference: Karsai et al. 1997
Population: Dicktoo × Morex, 100 DH lines
Map: M 5 (Hayes et al. 1993a; Pan et al. 1994)

Phenotyping: Field experiments (Hayes et al. 1993a) and growth chamber experiments (2 repeats)
Method: MAPMAKER/QTL
QTLs: Winter survival field: 5H; heading date with vernalization and 8h light: 1H(2), 3H, 7H; heading date without vernalization and 8 h light: 1H(2), 3H, 7H; heading date with vernalization and 24h light: 2H, 5H; heading date without vernalization and 24h light: 2H, 5H

Q 34: *Title:* Several QTLs involved in osmotic adjustment trait variation in barley (*Hordeum vulgare* L.)
Reference: Teulat et al. 1998
Population: Tadmor × Er/Apm, 167 RIL (F$_8$)
Map: M 20 (Teulat et al. 1998)
Phenotyping: Growth chamber experiments with 5 replicates
Method: MAPMAKER/QTL
QTLs: Relative water content under water-stress: 6H, 7H(2); leaf osmotic potential under water stress: 2H(2), 6H, 7H; leaf osmotic potential at full turgor under water stress: 1H, 6H; Relative water content with irrigation: 7H; leaf osmotic potential at full turgor with irrigation: 6H; osmotic adjustment: 6H

Q 35: *Title:* Identification of QTLs for partial resistance to leaf rust (*Puccinia hordei*) in barley
Reference: Qi et al. 1998b
Population: L94 × 'Vada', 103 RILs (F$_9$)
Map: M 21 (Qi et al. 1998a) reduced to a skeletal map
Phenotyping: Field experiment (1 location × 1 year) and growth chamber experiments with seedlings as well as adult plants
Method: MAPQTL v.3.0

QTLs: Leaf rust resistance field: 2H, 4H, 5H, 6H; leaf rust resistance seedlings: 2H, 6H, 7H; leaf rust resistance adult plants: 2H, 4H, 5H, 6H; heading date: 2H(2), 7H; plant height: 2H, 3H, 7H

Q 36: *Title:* Genetic variation in barley of crossability with wheat and its quantitative trait loci analysis
Reference: Taketa et al. 1998
Population: Steptoe × Morex, 119 DH lines
Map: M 6 (Kleinhofs et al. 1993) reduced to a 222-marker skeleton map
Phenotyping: 6 to 9 spikes per line crossed with wheat
Method: MAPMAKER/QTL
QTLs: Crossability with wheat: 2H, 3H, 5H, 7H

Q 37: *Title:* Identification of a QTL decreasing yield in barley linked to *Mlo* powdery mildew resistance
Reference: Thomas et al. 1998
Population: Derkado × B83-12/215; 160 DH lines
Map: M 22 (Thomas et al. 1998)
Phenotyping: Field experiments (1 to 2 locations × 3 years, 4 environments)
Method: MQTL
QTLs: Kernel yield: 2H, H, 4, 5H, 7H; fertile stems: 4H; grain number on main stem: 4H(2); grain weight; 4H; main stem yield: 4H; single plant yield: 4H; heading date: 4H
Remark: Beside for grain yield, only 4H was analyzed to explore the effect of the *mlo*-locus on yield

Q 38: *Title:* Mapping quantitative trait loci for starch granule traits in barley
Reference: Borém et al. 1999
Population: Steptoe × Morex, 150 DH lines
Map: M 6 (Kleinhofs et al. 1993) reduced to a 223-marker skeleton map
Phenotyping: Field experiment (1 location × 1 year)
Method: MQTL
QTLs: Overall mean granule volume: 2H; proportion of A granules: 2H; mean volume of A granules: 2H(2); mean maximum diameter of A granules: 2H, 5H; mean F-shape of B granules: 2H, 4H; heading date: 2H; plant height: 2H.
Remark: For heading date and plant height, only 2H was analyzed

Q 39:   *Title:* Barley-*Pyrenophora graminea* interaction: QTL analysis and gene mapping
Reference: Pecchioni et al. 1999
Population: Proctor × Nudinka, 91 DH lines
Map: M 3 (Heun et al. 1991) with additional 6 PR genes
Phenotyping: Greenhouse experiments (4 replications)
Method: MAPMAKER/QTL
QTLs: Resistance against barley leaf stripe: 2H, 3H, 7H(2)

Q 40:   *Title:* Inheritance and fine mapping of a major barley seed dormancy QTL
Reference: Han et al. 1999b
Population: (Steptoe × Morex) × Morex, 53 BC_2F_1 lines; and [(Steptoe × Morex) × Morex] × Morex, 11 BC_3F_1 lines (NILs); all derived from a DH line from Steptoe × Morex (Kleinhofs et al. 1993)
Map: M 6 (Kleinhofs et al. 1993)
Phenotyping: Germination chambers experiments (3 × 50 seeds)
Method: ANOVA
QTLs: Seed dormancy; 5H(3)

Q 41:   *Title:* AFLP mapping of quantitative trait loci for yield-determining physiological characters in spring barley
Reference: Yin et al. 1999b
Population: Apex × Prisma, 94 RILs (F_8)
Map: M 23 (Yin et al. 1999b)
Phenotyping: Field experiment with 2 locations × 1 year
Method: MAPQTL v.3.0
QTLs: Plant height: 1H, 3H; preflowering duration: 1H, 2H, 3H; postflowering duration: 1H, 2H, 3H; leaf nitrogen content at flowering time: 1H, 2H, 3H; specific leaf area t flowering: 2H, 3H, 4H; fraction of biomass to leaves (FBL) at developmental stage (DS) 0.27: 7H; FBL at DS 0.47: 3H; FBL at DS 0.59: 3H; fraction of biomass to ears at DS 1.15: 3H; yield: 2H, 3H, 6H

Q 42:   *Title:* Role of ecophysiological models in QTL analysis: example of specific leaf area in barley
Reference: Yin et al. 1999a
Population: Apex × Prisma, 94 RILs (F_8)
Map: M 23 (Yin et al. 1999b)
Phenotyping: Field experiment with 1 location × 1 year
Method: MAPQTL v.3.0
QTLs: Specific leaf area (SLA) 17 d after emergence (DAE): 1H, 3H, 7H; SLA 27 DAE: 3H; SLA 38 DAE: 3H; SLA at flowering: 4H; SLA 14 d after flowering: 3H

Q 43:   *Title:* Identification and mapping of a gene conferring resistance to the spot form of net blotch (*Pyrenophora teres* f *maculata*) in barley
Reference: Williams et al. 1999
Population: Galleon × Haruna Nijo, 95 DH lines
Map: M 24 (Barr et al. 1998)
Phenotyping: Growth chamber experiments with 4 × 5 plant-clumps/line
Method: QGENE, RI MANAGER
QTLs: Spot form net blotch: 7H

Q 44:   *Title:* Resistance to powdery mildew in a doubled-haploid barley population and its association with marker loci
Reference: Falak et al. 1999
Population: Harrington × TR306, 145 DH lines
Map: M 9 (Kasha and Kleinhofs 1994) reduced to a 127-marker skeleton map
Phenotyping: Field experiments (1 environment) and experiments on leaf segments
Method: MQTL
QTLs: Powdery mildew resistance: 4H(*Mlg*), 5H, 6H

Q 45:   *Title:* Isolate-specific QTLs for partial resistance to *Puccinia hordei* in barley
Reference: Qi et al. 1999
Population: L94 × Vada, 103 RILs (F_9)
Map: M 21 (Qi et al. 1998a) reduced to a skeletal map
Phenotyping: Growth chamber experiments with seedlings (4 to 5 per line) and adult plants (3 to 6 flag leaves per line)
Method: MAPQTL
QTLs: Leaf rust resistance: 2H(2); 4H(2), 5H(2), 6H, 7H(3)

Q 46:   *Title:* Does function follow form? Principal QTLs for *Fusarium* head blight (FHB) resistance are coincident with QTLs for inflorescence traits and plant height in a doubled-haploid population of barley
Reference: Zhu et al. 1999
Population: Gobernadora × CMB643, 144 DH lines
Map: M 25 (Zhu et al. 1999)
Phenotyping: Field experiments (4 locations × 2 years)

Method: MQTL

QTLs: Type-I resistance against *Fusarium*: 3H, 4H, 7H; type-II resistance and DON concentration: 2H, 3H, 4H; plant height: 4H, seeds per inflorescence: 2H, 3H,4H(2); inflorescence density: 3H(2); lateral floret size: 2H, 4H

Q 47: *Title:* Quantitative trait loci associated with resistance to *Fusarium* head blight and kernel discoloration in barley
Reference: de la Peña et al. 1999
Population: Chevron × M69, 101 $F_{4:7}$ families
Map: M 26 (de la Peña et al. 1999)
Phenotyping: Field experiments on 3 to 5 locations × 1 to 3 years (8 environments)
Method: PLABQTL
QTLs: Fusarium head blight: 1H, 2H(3), 3H, 4H, 5H, 7H(3); kernel discoloration: 2H(2), 3H(2), 4H(2), 5H, 6H(2), 7H(2); DON accumulation: 2H(2), 5H, 7H; heading date: 2H(3), 4H ,5H, 7H(2)

Q 48: *Title:* The evidence for abundance of QTLs for partial resistance to *Puccinia hordei* on the barley genome
Reference: Qi et al. 2000
Population: Lo4 × 116-5, 117 RILs (F_8)
Map: M 28 (Qi et al. 2000)
Phenotyping: Field experiments (1 location × 2 years)
Method: MAPQTL v.3.0
QTLs: Seedling resistance to leaf rust: 2H(2), 6H; field resistance to leaf rust: 4H, 6H, 7H

Q 49: *Title:* Localising QTLs for leaf rust resistance and agronomic traits in barley (*Hordeum vulgare* L.)
Reference: Kicherer et al. 2000
Population: Krona × HOR1063, 220 DH lines
Map: M 29 (Kicherer et al. 2000)
Phenotyping: Field experiments (1 location × 2 years)
Method: PLABQTL
QTLs: Leaf rust resistance: 2H(3), 4H; heading date: 2H(2); plant height: 2H, 3H; kernel weight: 2H, 5H, 6H

Q 50: *Title:* Molecular-marker-assisted genetic analysis of head shattering in six-rowed barley
Reference: Kandemir et al. 2000
Population: Steptoe × Morex, 119 DH lines
Map: M 6 (Kleinhofs et al. 1993) reduced to a 149-marker skeleton map
Phenotyping: Field experiments (1 location × 2 years)

Method: QTL Cartographer
QTLs: Head shattering: 2H, 3H; spike density: 3H; number of rachis node: 2H, 3H, 6H; peduncle curve: 2H, 5H

Q 51: *Title:* Mapping of quantitative trait loci for *Fusarium* head blight resistance in barley
Reference: Ma et al. 2000
Population: Chevron × Stander, 147 DH lines
Map: M 31 (Ma et al. 2000)
Phenotyping: Field experiments with 4 locations × 1 to 2 years (7 environments)
Method: MAPQTL
QTLs: Fusarium head blight resistance: 1H, 2H(2), 3H(3), 4H, 5H(2), 6H, 7H; DON accumulation: 1H, 2H(2), 3H(3), 5H, 6H, 7H; plant height: 2H, 3H(2), 4H(2), 5H(2), 6H, 7H(2); heading date: 2H, 3H, 5H, 7H; spike angle: 2H; kernel plumpness: 6H; nodes per cm rachis: 1H, 2H, 3H, 4H, 7H

Q 52: *Title:* Mapping quantitative and qualitative disease resistance genes in a doubled-haploid population of barley (*Hordeum vulgare*)
Reference: Toojinda et al. 2000
Population: Shyri × Galena, 94 DH lines
Map: M 33: (Toojinda et al. 2000)
Phenotyping: Field experiments with 1 to 3 years (4 environments)
Method: MQTL
QTLs: Stripe rust resistance: 1H, 2H, 3H, 6H; leaf rust resistance: 7H; barley Yellow dwarf virus tolerance: 1H, 3H, 4H, 7H

Q 53: *Title:* Associations between anther-culture response and molecular markers on chromosomes 2H, 3H, and 4H of barley (*Hordeum vulgare* L.)
Reference: Manninen 2000
Population: Rolfi × Botnia
Map: M 34 (Manninen 2000)
Phenotyping: Anther culture (500 anthers per genotype)
Method: Kruskal-Wallis, Mann-Whitney U
QTLs: Percentage of responsive anthers: 2H(2), 4H(3); plants per responsive anther: 2H(3), 3H(2); percentage of diploid green plants: 4H

Q 54: *Title:* Genetic markers associated with green and albino plant regeneration from embryogenic barley callus
Reference: Bregitzer and Campbell 2001
Population: Steptoe × Morex, 77 DH lines
Map: M 6 (Kleinhofs et al. 1993)
Phenotyping: Tissue culture (10 to 20 petri

plates)

Method: MAPMAKER/QTL

QTLs: Green plant regeneration: 2H, 3H(2), 4H, 5H(2), 6H, 7H; albino plants: 1H, 3H

Q 55: *Title:* New QTLs identified for plant water status, water-soluble carbohydrate and osmotic adjustment in a barley population grown in a growth chamber under two water regimes

Reference: Teulat et al. 2001a

Population: Tadmor × Er/Apm, 167 RILs (F$_8$)

Map: M 20 (Teulat et al. 1998, 2001a)

Phenotyping: Growth chamber experiments with 5 replicates (Q 34; Teulat et al. 1998)

Method: MAPMAKER/QTL, QGENE

QTLs: Relative water content under water stress: 6H, 7H(2); leaf osmotic potential under water stress:1H, 2H, 4H, 5H, 6H, 7H; leaf osmotic potential at full turgor under water stress: 1H, 2H, 4H, 5H(2), 6H; water-soluble carbohydrates under water stress: 2H; relative water content with irrigation: 2H, 7H; leaf osmotic potential with irrigation: 1H, 5H, 7H; leaf osmotic potential at full turgor with irrigation: 1H, 5H; osmotic adjustment: 4H, 5H, 6H; net solute accumulation contributing to OA: 2H, 4H; contribution to a change in water content to OA: 2H, 4H, 5H

Q 56: *Title:* QTL analysis of tolerance to a German strain of BYDV-PAV in barley (*Hordeum vulgare* L.)

Reference: Scheurer et al. 2001

Population: Post × Vixen (PV), 70 DH lines; Post × Nixe (PN), 70 DH lines

Map: M 37, M 38 (Scheurer et al. 2001)

Phenotyping: Field experiments (12 plants per line, 1 location × 3 years) and greenhouse experiments

Method: PLABQTL

QTLs: Relative kernel yield: PV: 2H, 3H; PN: 2H, 4H, 7H; relative ears per plant: PV: 3H, 4H, 5H, PN: 2H, 5H, 7H; relative kernel weight: PV: 2H, 3H; PN: 2H; relative kernel per ear: PV: 3H, 4H, 7H; relative plant height: PV: 3H, PN: 2H; relative heading date: PV: H, PN: 2H

Remark: The traits were calculated as infected plants relative to healthy control

Q 57: *Title:* QTL mapping for enzyme activity and thermostability of *β*-amylase in barley (*Hordeum vulgare* L.)

Reference: Kaneko et al. 2001

Population: Steptoe × Morex (SM), 150 DH

lines; Harrington × TR306 (HT), 146 DH lines

Map: M 6 (Kleinhofs et al. 1993); M 9 (Kasha and Kleinhofs 1994)

Phenotyping: Field experiments (1 year × 1 location)

Method: MAPMAKER/QTL

QTLs: *β*-amylase activity: SM: 1H, 2H; HT: 5H; *β*-amylase activity: SM: 2H, 4H

Q 58: *Title:* QTL analysis of malting quality in barley based on the doubled-haploid progeny of two elite North American varieties representing different germplasm groups

Reference: Marquez-Cedillo et al. 2000

Population: Harrington × Morex, 140 DH lines

Map: M 19 (Hayes et al. 1997)

Phenotyping: Field experiments in 3 to 5 locations × 2 years (8 environments)

Method: MQTL

QTLs: Kernel plumpness: 2H; specific kernel weight: 2H, 4H, 5H; grain protein content: 2H, 4H, 5H; S/T protein ratio: 2H, 4H, 5H; *α*-amylase activity: 4H, 5H; diastatic power: 2H, 5H, 7H; malt extract: 1H(2)

Q 59: *Title:* QTLs for agronomic traits from a Mediterranean barley progeny grown in several environments

Reference: Teulat et al. 2001b

Population: Tadmor × Er/Apm, 167 RILs (F$_8$)

Map: Teulat et al. 1998, Teulat et al. 2001a

Phenotyping: 2 locations × 1 to 3 years × 1 to 2 treatments (6 environments)

Method: MAPMAKER/QTL

QTLs: Plant height: 2H, 3H(2), 4H, 5H, 6H(2), 7H; number of grains per ear: 3H, 4H; number of fertile tillers: 3H, 4H, 6H; grain weight: 1H, 2H, 3H, 4H, 5H(3), 6H(2); dry aerian biomass: 4H, 6H, plant grain yield:4H,5H, 7H; harvest index: 3H, 4H, 5H, 7H; heading date: 1H, 2H, 3H(3), 5H, 6H, 7H

Q 60: *Title:* Mapping genes for deep-seeding tolerance in barley

Reference: Takahashi et al. 2001

Population: Steptoe × Morex (SM), 146H lines; Harrington × TR306 (HT), 146 DH lines

Map: M 6 (Kleinhofs et al. 1993); M 9 (Kasha and Kleinhoffs 1994)

Phenotyping: Growth chamber experiments (2 × 50 kernels)

Method: MAPMAKER/QTL

QTLs: Deep-seedling tolerance: SM: 7H; HT:

1H, 5H; first internode length: SM: 7H; HT: 5H, 7H; coleoptile length: HT: 5H; kernel weight: SM. 7H; HT: 5H, 7H(2)

Q 61: *Title:* Mapping genes controlling variation in barley grain protein concentration
Reference: See et al. 2002
Population: Karl × Lewis, 146 RILs (F$_5$)
Map: M 40 (See et al. 2002)
Phenotyping: Field experiments with 1 location × 4 years
Method: MAPMAKER/QTL
QTLs: Grain protein concentration: 2H, 6H(2)

Q 62: *Title:* Quantitative trait loci for scald resistance in barley localized by a noninterval mapping procedure
Reference: Jensen et al. 2002
Population: Alexis × Regatta, 110 DH lines
Map: M 41 (Jensen et al. 2002)
Phenotyping: Field experiments with 1 location × 2 years
Method: Proprietary Maximum Likelihood program
QTLs: Scald resistance; 3H, 4H(2)

Q 63: *Title:* Hordoindolines are associated with a major endosperm-texture QTL in Barley (*Hordeum vulgare*)
Reference: Beecher et al. 2002
Population: Steptoe × Morex, 150 DH lines
Map: M 6: (Kleinhofs et al. 1993)
Phenotyping: Field experiment with 1 location × 2 years
Method: MAPMAKER/QTL
QTLs: Grain hardness: 1H, 4H, 5H, 7H

Q 64: *Title:* Genomic regions determining resistance to leaf stripe (*Pyrenophora graminea*) in barley
Reference: Arru et al. 2002
Population: L94 × Vada (LV), 103 RILs (F$_9$); L94 × C123 (LC), 111 RI lines (F$_8$)
Map: M 21 (Qi et al. 1998a); M 42 (Arru et al. 2002)
Phenotyping: Greenhouse experiment (3 × 30 pots per line)
Method: MAPMAKER/QTL
QTLs: Resistance to leaf stripe: LV: 2H, 7H; LC: 2H, 7H
Remark: The QTL on 2H was different for the two populations

Q 65: *Title:* Phenotype/genotype associations for yield and salt tolerance in a barley mapping population segregating for two dwarfing genes
Reference: Ellis et al. 2002
Population: Derkado × B83-12/215, 160 DH lines
Map: M 22 (Thomas et al. 1998; Ellis et al. 2002)
Phenotyping: Field experiments with 2 locations × 2 to 4 years (6 environments) and glasshouse experiment with salt treatments in hydroponic pots.
Method: MQTL
QTLs: Grain nitrogen concentration: 3H, 4H, 7H; grain yield: 4H, 5H, 6H; main stem leaves: 2H, 3H; tiller number: 7H; root nitrogen stable isotope ratio: 3H; root weight: 5H; shoot carbon stable isotope ratio: 3H, 5H; shoot reaction to GA: 5H; shoot weight: 4H, 5H

Q 66: *Title:* Genetic analysis of resistance to barley scald (*Rhynchosporium secalis*) in Ethiopian line Abyssinian (CI668)
Reference: Grønnerød et al. 2002
Population: Abyssinian × Ingrid, 50 DH lines
Map: M 43 (Grønnerød et al. 2002)
Phenotyping: Growth chamber experiment with infection with different isolates
Method: PLABQTL
QTLs: Resistance to scald: 2H(2), 3H(3), 6H

Q 67: *Title:* Use of component analysis in QTL mapping of complex crop traits: a case study on yield in barley
Reference: Yin et al. 2002
Population: Apex × Prisma, 94 RILs (F$_8$)
Map: M 23 (Yin et al. 1999b)
Phenotyping: Field experiments with 1 location × 2 years
Method: MAPQTL
QTLs: Spikes per area: 2H, 3H, 4H(3), 7H; kernels per spike: 1H(2), 2H(2), 3H(2), 4H(2), 5H, 7H; grain weight: 1H, 2H, 3H(3), H, 6H(2), 7H; kernel yield: 1H, 2H(2), 3H, 4H, 6H, 7H

Q 68: *Title:* QTLs affecting kernel size and shape in a two-row by six-row barley cross
Reference: Ayoub et al. 2002
Population: Harrington × Morex, 140 DH lines
Map: M 19 (Hayes et al. 1997)
Phenotyping: Field experiments in 3 to 5 locations × 2 years (8 environments, Marquez-Cedillo et al. 2000)
Method: MQTL
QTLs: Kernel weight: all lines: 2H, 5H; kernel

width: 2H, 4H; kernel length: 1H, 2H, 6H; kernel perimeter: 1H, 2H, 3H; kernel area: 4H, 2H; F-shape: 2H, 3H, 5H; F-circle: 1H, 5H, 7H; 2-row lines only; kernel width: 1H, 4H,5H, 7H; kernel length: 1H, 3H; kernel perimeter: 1H, 3H; kernel area: 5H; F-shape: 3H, 7H; F-circle: 1H, 3H, 5H, 6H, 7H

Q 69:   *Title:* Coincident QTLs that determine seedling and adult plant resistance to stripe rust in barley
Reference: Castro et al. 2002
Population: Shyri × Galena, 94 DH lines
Map: M 33 (Toojinda et al. 2000)
Phenotyping: Growth-chamber experiments
Method: MQTL, MultiQTL
QTLs: Stripe rust resistance seedlings: 1H, 6H
Remark: Results of seedlings were compared with results from field experiments (Q 52, Toojinda et al. 2000)

Q 70:   *Title:* Identification of QTLs controlling tissue-culture traits in barley (*Hordeum vulgare* L.)
Reference: Mano and Komatsuda 2002
Population: Azumamugi × Kanto Nagate Gold, 99 RI lines (F_9)
Map: M 44 (Mano and Komatsuda 2002)
Phenotyping: Tissue culture experiments in F_7 and F_{10}
Method: QTL Cartographer v.1.14
QTLs: Callus growth: 2H(2), 5H; shoot differentiation: 1:H, 2H, 3H; green shoot ratio: 7H(2)

Q 71:   *Title:* Search for QTL in barley (*Hordeum vulgare* L.) using a new mapping population
Reference: Buck-Sorlin 2002
Population: Angora × W704/137, 99 DH lines
Map: M 45 (Buck-Sorlin 2002)
Phenotyping: Field and greenhouse experiments (both 1 location × 2 years)
Method: QGENE
QTLs: Tillering: 3H(2), 6H; number of grains: 6H, 7H
Remark: The QTLs were integrated into a phenotype model to predict the traits in the respective lines

Q 72:   *Title:* QTLs for grain carbon isotope discrimination in field-grown barley
Reference: Teulat et al. 2002
Population: Tadmor × Er/Apm, 167 RILs (F_8)
Map: M 20 (Teulat et al. 1998, 2001a, 2002)
Phenotyping: 1 to 2 locations × 1 to 2 years ×

1 to 2 treatments [3 of the environments used in Q 1 (Teulat et al. 2001a)]
Method: MQTL, PLABQTL
QTLs: Carbon isotope discrimination: 1H(2), 2H(2), 4H, 5H, 6H(2), 7H(2)

Q 73:   *Title:* Localization of genes for resistance against *Blumeria graminis* f. sp. *hordei* and *Puccinia graminis* in a cross between a barley cultivar and a wild barley (*Hordeum vulgare* ssp. *spontaneum*) line
Reference: Backes et al. 2003
Population: 1B-87 × Vada, 121 RI lines
Map: M 46 (Backes et al. 2003)
Phenotyping: Field experiments with 1 year × 1 location
Method: MAPQTL (Kruskal-Wallis)
QTLs: Powdery mildew resistance: 1H, 2H, 3H, 4H, 6H, 7H; leaf rust resistance: 2H, 5H

Q 74:   *Title:* Mapping of QTL associated with nitrogen storage and remobilization in barley (*Hordeum vulgare* L.) leaves
Reference: Mickelson et al. 2003
Population: Karl × Lewis, 146 RILs (F_8, F_9)
Map: M 40 (See et al. 2002)
Phenotyping: Field experiment with 1 location × 2 years
Method: PLABQTL
QTLs: Leaf nitrogen at anthesis: 1H, 6H; leaf nitrogen at mid-grain fill: 1H, 3H(2), 5H, 6H(2); leaf nitrogen at maturity: 1H, 3H(2), 4hs, 5H(2), 6H(2); difference in leaf nitrogen content between anthesis and mid-grain fill: 5H(2), 6H; leaf NO3 at anthesis: 3H(2), 4H, 5H, 6H, 7H; leaf NO3 at mid-grain fill: 6H; leaf α-NH2 at anthesis: 2H; leaf α-NH2 at mid-grain fill: 3(4); 4H, 5H, 6H(2)

Q 75:   *Title:* Quantitative trait loci for *Fusarium* head blight resistance in barley detected in a two-row by six-row population
Reference: Mesfin et al. 2003
Population: Frederickson × Stander, 130 $F_{4:6}$ plants
Map: M 47 (Mesfin et al. 2003)
Phenotyping: Field experiments with 1 to 3 locations × 2 years (4 environments), with different infection methods and greenhouse experiments
Method: PLABQTL
QTLs: Fusarium head blight resistance: 1H, 2H(7), 3H(2), 6H(2), 7H

Q 76: *Title:* Isolate specific QTLs of resistance to leaf stripe (*Pyrenophora graminea*) in the Steptoe x Morex spring barley cross
Reference: Arru et al. 2003
Population: Steptoe × Morex, 143 DH lines
Map: M 6 (Kleinhofs et al. 1993, 1994)
Phenotyping: Greenhouse experiment with 3 × 30 plants per line (2 isolates)
Method: PLABQTL
QTLs: Leaf stripe resistance 2H(2), 3H(2), 5H

Q 77: *Title:* Comparative mapping of *β*-amylase activity QTLs among three barley crosses
Reference: Clancy et al. 2003
Population: Steptoe × Morex (S/M), 146H lines; Harrington × TR306 (H/T), 150 DH lines; Harrington × Morex (H/M), 144 DH lines
Map: M 6 (Kleinhofs et al. 1993); M 9 (Kasha and Kleinhofs 1994); M 19 (Hayes et al. 1997)
Phenotyping: Field experiments with 1 year × 1 location
Method: MAPMAKER/QTL
QTLs: *β*-amylose activity per g flour: S/M: 1H(2), 4H, 5H, 6H(2); H/T: 1H, 3H; H/M: 2H, 7H; *β*-amylose activity per g protein: S/M: 1H(2), 4H, 6H(2), 7H; H/T: 1H, 2H, 3H; H/M: 2H, 7H; diastatic power: S/M: 1H(2), 2H, 3H, 4H, 5H, 6H(2), 7H; H/T: 1H, 5H, 6H; H/M: 2H, 7H

Q 78: *Title:* QTL mapping provides evidence for lack of association of avoidance of leaf rust in *Hordeum chilense* with stomata density
Reference: Vaz Patto et al. 2003
Population: H7 × H1 (both *Hordeum chilense*), 100 F$_2$ plants
Map: M 48 (Hernàndez et al. 2001; Vaz Patto et al. 2003)
Phenotyping: Greenhouse experiments with 2 clones per line.
Method: MAPQTL
QTLs: Leaf rust avoidance: 1H, 3H; stoma density: 3H, 5, 7H

Q 79: *Title:* Advanced backcross QTL analysis in barley (*Hordeum vulgare* L.)
Reference: Pillen et al. 2003
Population: (Apex × ISR101-23) × Apex, 136 BC$_2$F$_2$ plants
Map: M 32 (Ramsay et al. 2000); M 27 (Pillen et al. 2000)
Phenotyping: Field experiments with 3 locations × 2 years (BC$_2$F$_{2:5}$, BC$_2$F$_{2:6}$)

Method: ANOVA
QTLs: Heading date: 1H(4), 2H(4), 4H(4), 5H(5), 7H(5); plant height: 1H(2), 4H(5), 5H(2), 7H(7); lodging at flowering: 5H(2); lodging at harvest: 1H(2), 4H; kernel per ear: 1H; kernel weight: 2H(3), 4H(3), 5H(4), 7H(2); kernel yield: 1H, 2H(2), 3H, 4H(2), 5H(4), 7H(3); aboveground biomass: 7H; harvest index: 4H(2), 5H, 7H(2); protein content: 4H, 5H(2); water absorption: 4H(2), 5H, 7H(3); malt tenderness: 5H, 7H

Q 80: *Title:* Genetic relationship between kernel discoloration and grain protein concentration in barley
Reference: Canci et al. 2003
Population: Chevron × M69 (C/M), 101 F$_{4:7}$ families; MNBrite × M96 (M/M), 98 F$_{4:6}$ families
Map: M 26 (de la Peña et al. 1999); M 49 (Canci et al. 2003)
Phenotyping: C/M: Field experiments on 3–5 locations × 1–3 years (8 environments, de la Peña et al. 1999); M/M: Field experiments on 2 locations × 2 years
Method: PLABQTL
QTLs: C/M: Kernel discoloration: 2H(2), 4H, 5H, 6H(3), 7H(2); grain protein concentration: 3H, 4H, 6H; M/M: 6H(5)
Remark: Reexamines the results from C/M (de la Peña et al. 1999) and tests the QTLs from 6H in M/M

Q 81: *Title:* Quantitative genetic analysis of acid detergent fibre content in barley grain
Reference: Han et al. 2003
Population: Steptoe × Morex, 150 DH lines
Map: M 6 (Kleinhofs et al. 1993)
Phenotyping: Field experiments with 3 locations × 1–2 years (4 environments)
Method: MAPMAKER/QTL
QTLs: Acid Detergent Fibre content: 1H, 2H(3), 4H

Q 82: *Title:* Efficient construction of high-density linkage map and its application to QTL analysis in barley
Reference: Hori et al. 2003
Population: Russia 6 × H.E.S. 4, 95 RI lines (F$_9$)
Map: M 50 (Hori et al. 2003)
Phenotyping: Field experiment with 1 location × 1 year
Method: MAPMAKER/QTL

1H, 2H, 5H, 6H; α-amylase activity: 2, 7H; hot-water extract: 6H; protein content: 2H, 5H

Q 89: *Title:* Mapping and QTL analysis of barley population Mundah × Keel
Reference: Long et al. 2003
Population: Mundah × Keel, 110 DH lines
Map: M 63 (Long et al. 2003)
Phenotyping: Field experiments on 1 to 5 locations × 1 to 3 years (different for specific traits)
Method: MapManager QTX
QTLs: Grain yield: 1H; 2H(2), 4H(2); kernel weight: 1H(2), 2H(3), 5H; kernel size: 1H, 2H(3), 3H; early dry matter production: 1H, 2H, 5H, 7H; early vigour: 5H; growth habit: 1H, 3H, 5H, 7H; early maturity: 1H, 3H, 7H; net blotch resistance: 2H

Q 90: *Title:* Identification of QTLs associated with variations in grain protein concentration in two-row barley
Reference: Emebiri et al. 2003
Population: VB9524 × NB11231*12, 180 DH lines
Map: M 64 (Emebiri et al. 2003)
Phenotyping: Field experiment with 1 location × 1 year × 4 treatments (with and without nitrogen fertilisation, and dryland/irrigated)
Method: QTL Cartographer 2.0
QTLs: Grain protein concentration: 2H(2), 4H, 5H(2), 7H(2); grain yield: 4H, 7H(2); heading date: 2H, 4H, 7H

Q 91: *Title:* Mapping and validation of chromosome regions associated with high malt extract in barley (*Hordeum vulgare* L.)
Reference: Collins et al. 2001
Population: Consensus WI-2875-1 × Alexis and Alexis × Sloop (A/S), 153 RI lines, 111 DH lines; Galleon × Haruna Nijo (G/H), 112 DH lines; Chebec × Harrington (C/H), 120 DH lines
Map: M 54, M 55 (Barr et al. 2003a); M 56 (Collins et al. 2001); M 58 (Karakousis et al. 2003a); M 57 (Barr et al. 2003b)
Phenotyping: Field experiments with 1 to 3 locations × 1 to 2 years (different for each population)
Method: QGENE
QTLs: Hot-water extract: A/S: 1H, 2HL, 4HL(2), 5HL; G/H: 2HS, 2HL, 5HS, 6HS, C/H: 1HL, 5HL

Q 92: *Title:* Quantitative trait loci controlling kernel discoloration in barley (*Hordeum vulgare* L.)
Reference: Li et al. 2003a
Population: WI-2875-1 × Alexis (W/A), 153 RI lines; Alexis × Sloop (A/S), 111 DH lines; Galleon × Haruna Nijo G/H), 112 DH lines; Chebec × Harrington (C/H), 120 DH lines; Sloop × Halycon (S/H), 166 DH lines; Arapiles × Franklin (A/F), 168 DH lines; VB9104 × Dash (V/D), 182 DH lines
Map: M 54, M 55 (Barr et al. 2003a), M 58 (Karakousis et al. 2003a); M 57 (Barr et al. 2003b); M 61 (Read et al. 2003); Moody et al. (unpublished)
Phenotyping: 1 to 2 locations × 1 year
Method: MapManager QTX
QTLs: Kernel brightness: 2HLa (W/A, A/S, G/H, C/H, S/H, A/F), 2HLb (V/D), 3HS (G/H, C/H, A/F), 3HL (W/A, A/S, S/H), 4HS (S/H), 5HS(V/D), 7HS (W/A, A/S, C/H, S/H)a; kernel redness: 2HL (W/A, A/S, C/H), 4HS (S/H), 5HL (W/A, A/S), 7HS (C/H); kernel yellowness: 2HLa (A/S, C/H, S/H, A/F), 2HLb (V/D, S/A), 3HL (S/H, A/F), 4H (S/H), 5HS (V/D), 5HL (G/H, A/F)

Q 93: *Title:* Conventional and molecular genetic analysis of factors contributing to variation in the timing of heading among spring barley (*Hordeum vulgare* L.) genotypes grown over a mild winter growing season
Reference: Boyd et al. 2003
Population: Steptoe × Morex (S/M), 150 DH lines; Dicktoo × Morex (D/M), 100 DH lines; Alexis × Sloop (A/S), 111 DH lines; Chebec × Harrington (C/H), 120 DH lines; Sloop × Halycon (S/H), 166 DH lines; Tallon × Kaputar (T/K), 65 DH lines; Arapiles × Franklin (A/F), 168 DH lines
Map: M 6 (Hayes et al. 1993b; Pan et al. 1994); M 5 (Kleinhofs et al. 1993, 1994); M 55 (Barr et al. 2003a); M 57 (Barr et al. 2003b); M 61 (Read et al. 2003); M 62 (Cakir et al. 2003); Moody et al. (unpublished)
Phenotyping: Field experiments with 1 year × 1 location and growth-chamber experiments
Method: MapManager QTX
QTLs: Minimum duration to heading, growth chamber: 2HC (C/H, A/S, H/S), 2HS (S/M, D/M, C/H, A/S, H/S, T/K), 3HS (T/K), 5HL (D/M, H/S), 6HL: (D/M, T/K), 7HS (C/H), minimum duration to heading, field, 18 h

photoperiod: 1HL (S/M, D/M), 2HC (C/H, A/S, H/S, A/F), 2HS (S/M, C/H, A/S, H/S, A/F), 5HL (D/M, H/S, A/F), 6HL (D/M, C/H), 7HL (S/M); duration of heading, field: 1HL (S/M, D/M,H/S), 2HC (C/H, A/S, H/S, T/K, A/F), 2HS (S/M, D/M), 3HL (A/S, H/S, T/K, A/F), 5HL (D/M, H/S), 6HL (C/H, A/S, T/K), 7HS (D/M, C/H, T/K); response to extended photoperiod: 1HL (S/M, D/M), 1HS (A/S), 2HL (A/S), 2HS (S/M, D/M, C/H, A/S, H/S, A/F), 3HL (S/M), 5HL (C/H; H/S, A/F), 5HS(S/M), 6HL (D/M), 7HS (D/M)

Q 94:   *Title:* A major QTL controlling seed dormancy and preharvest sprouting/grain alpha-amylase in two-rowed barley (*Hordeum vulgare* L.)
Reference: Li et al. 2003b
Population: Chebec × Harrington, 120 DH lines
Map: M 57 (Barr et al. 2003b)
Phenotyping: Field experiments with 2 locations × 1 year
Method: MapManager QTX
QTLs: Seed dormancy: 5HL; *α*-amylase activity: 5HL
Remark: The major QTL explained 70% of *α*-amylase activity

Q 95:   *Title:* Mapping genes for resistance to *Puccinia hordei* in barley
Reference: Park et al. 2003
Population: Alexis × Sloop (A/S), 111 DH lines; Chebec × Harrington (C/H), 120 DH lines; Galleon × Haruna Nijo (G/H), 112 DH lines; Tallon × Kaputar (T/K), 65 DH lines; Sloop × Halycon (S/H), 166 DH lines; Arapiles × Franklin (A/F), 168 lines; Tallon × Patty (T/P)
Map: M 55 (Barr et al. 2003a); M 57 (Barr et al. 2003b); M 58 (Karakousis et al. 2003a); M 62 (Cakir et al. 2003); M 61 (Read et al. 2003)
Phenotyping: Glasshouse experiments
Method: MapManager QTX
QTLs: Resistance to leaf rust: A/S: 5H(2), 7HL; P/T: 1H, 5H; T/K: 2H, 5H; S/H: 5H, 7H

Q 96:   *Title:* Molecular mapping as tool for preemptive breeding for resistance to exotic barley pathogen, *Puccinia striiformis* f. sp *hordei*
Reference: Choi et al. 1999
Population: Tallon × Kaputar (T/K), 65 DH lines; Arapiles × Franklin (A/F), 150 lines
Map: M 62 (Cakir et al. 2003)

Phenotyping: Field experiments with 1 year × 2 locations
Method: MapManager QTX, QGENE
QTLs: Barley leaf stripe resistance: T/K: 2H, 5H; A/F: 2H, 5H

Q 97:   *Title:* Mapping of genomic regions associated with net form of net blotch resistance in barley
Reference: Raman et al. 2003
Population: WI-2875-1 × Alexis (W/A), 153 RI lines; Alexis × Sloop (A/S), 111 DH lines; Arapiles × Franklin (A/F), 168 DH lines; Sloop × Halycon, 166 DH lines
Map: M 54, M 55 (Barr et al. 2003a); Moody et al. (unpublished); M 61 (Read et al. 2003).
Phenotyping: Glasshouse experiments (2 × 5 to 7 plants per line)
Method: MapManager QTX
QTLs: W/A: 2HS, 3HL; A/S: 2HL, 3HL; A/F: 2HS, 2HL, 3HL(2); S/H: 4H, 6H

Q 98:   *Title:* Mapping and validation of genes for resistance to *Pyrenophora teres* f. *teres* in barley (*Hordeum vulgare* L.)
Reference: Cakir et al. 2003
Population: Tallon × Kaputar, 65 DH lines; VB9524 × ND11231 (V/N), 189 DH lines
Map: M 62 (Cakir et al. 2003; Embibiri unpublished)
Phenotyping: Glasshouse experiments sand field experiments (1 location × 1 year)
Method: MapManager QTX
QTLs: T/K: Seedling net blotch resistance: T/K: 2H, 3H, 6H; adult plant net blotch resistance: T/K: 6H
Remark: Field experiments were performed for cross T/K only

Q 99:   *Title:* Comparison of genetics of seedling and adult plant resistance to spot form of net blotch (*Pyrenophora teres* f. *maculata*)
Reference: Williams et al. 2003
Population: Galleon × Haruna Nijo (G/H), 112 DH lines; Chebec × Harrington (C/H), 120 DH lines; CI9214 × Stirling (C/S); Keel × Gairdner 8k7G); Tilga × Tantangara (T/T); VB9104 × Dash (V/BF)
Map: M 58 (Karakousis et al. 2003a); M 57 (Barr et al. 2003b)
Phenotyping: Glasshouse experiment for seedling resistance and field experiment for adult plant resistance
Method: MapManager QTX
QTLs: Spot form net blotch adult plant

resistance: G/H: 4H, 5H, 7H(3); V/D: 4H, 5H, 7H; C/S: 7H; K/G: 7H; T/T: 7H
Remark: Also includes data from Williams et al. 1999 (G/H)

Q 100: *Title:* Analysis of quantitative trait loci in multienvironment trials using multiplicative mixed model
Reference: Verbyla et al. 2003
Population: Arapiles × Franklin (A/F), 168 DH lines
Map: Moody et al. (unpublished)
Phenotyping: Field experiments at 11 locations at 1 to 3 years
Method: Least-square interval mapping extended for multienvironment trials
QTLs: Kernel yield: 1H, 2H, 3H(2), 4H, 5H, 7H(2)
Remark: QTL analysis as demonstration for method presented

Q 101: *Title:* New molecular markers linked to qualitative and quantitative powdery mildew and scald resistance genes in barley for dry areas
Reference: Sayed et al. 2004
Population: Tadmor × WI2991, 71 DH lines
Map: M 66 (Sayed et al. 2004)
Phenotyping: Infections on detached leaves with 4 *Blumeria* and 7 *Rhynchosporium* isolates
Method: PLABQTL
QTLs: Scald resistance: 2H, 3H(2)

Q 102: *Title:* Genetic control over grain damage in a spring barley mapping population
Reference: Rajasekaran et al. 2004
Population: Tankard × Livet, 184 RI lines
Map: M 67 (Rajasekaran et al. 2004)
Phenotyping: Field trials at 1 location × 2 years
Method: PLABQTL
QTLs: Kernel splitting: 1H, 4H(2), 5H(2); gap between *lemma* and *palea*: 3H, 6H; skinning less than 25%: 5H; milling energy: 1H(2), 1H, 4H, 6H; sieve fraction > 2.5 mm: 6H; grain weight: 6H; grain length: 6H; grain width: 6H; grain shape: 4H; heading date: 6H; plant height: 4H(2), 6H

Q 103: *Title:* Two loci on chromosome 5H determine low-temperature tolerance in a Nure (winter) × Tremois (spring) barley map
Reference: Francia et al. 2004
Population: Nure × Tremois, 136 DH lines
Map: M 68 (Francia et al. 2004)
Phenotyping: Field experiments and growth chamber experiments
Method: PLABQTL, MAPQTL
QTLs: Winter survival: 5H(2); frost tolerance (field): 5H(2); frost tolerance (growth chamber): 5H(2); COR protein accumulation: 5H, 6H; TMC-Ap3-accumulation: 5H; heading date: 1H, 2H, 6H; vernalization requirement: 5H

Q 104: *Title:* Genetic basis of adaptive population differentiation: a quantitative trait locus analysis of fitness traits in two wild barley populations from contrasting habitats
Reference: Verhoeven et al. 2004a
Population: *H.v.* ssp *spontaneum* × *H.v.* ssp *spontaneum*, 140 $F_{2:3}$ families
Map: M 70 (Verhoeven et al. 2004a)
Phenotyping: Field experiments in 1 year and several locations and glasshouse experiments with low and high N supply
Method: MAPQTL
QTLs: Viability: 3H; heads per plant: 2H (2), 5H, 7H; kernel per head: 2H, 4H, 7H; kernel weight: 1H, 2H(3)3H, 4H, 6H; kernel number per area: 1H, 2H, 3H(2), 5H, 7H; kernel mass per area: 2H(3), 5H, 6H

Q 105: *Title:* Quantitative trait loci affecting germination traits and malt friability in a two-row by six-row barley cross
Reference: Edney and Mather 2004
Population: Harrington × Morex, 140 DH lines
Map: M 19 (Hayes et al. 1997)
Phenotyping: Field experiments in 2 locations × 1 year
Method: MQTL
QTLs: Germination in 4 ml water: 2H, 5H(2), 7H; germination in 8 ml water: 3H, 5H(2), 6H; malt friability: 1H(2), 2H, 3H, 4H, 6H

Q 106: *Title:* Mapping of QTL for malting quality attributes in barley based on a cross of parents with low grain protein concentration
Reference: Emebiri et al. 2004
Population: VB9524 × NB11231*12, 180 DH lines
Map: M 64 (Emebiri et al. 2003)
Phenotyping: Field experiments in 2 locations × 1 year
Method: R/qtl
QTLs: Grain protein content: 4H, 5H(2), 7H; malt extract: 2H(2), 7H; α-amlylase: 2H, 3H, 4H, 5H, 6H; β-glucanase: 1H, 3H, 5H, 7H; diastatic power: 2H, 4H(2), 5H; free α-amino ni-

trogen: 2H(2), 3H, 4H; wort viscosity: 2H(2), 3H, 4H; wort β-glucan content: 2H(2), 3H, 5H, 7H

Q 107: *Title:* QTL mapping for resistance against non-parasitic leaf spots in a spring barley doubled-haploid population
Reference: Behn et al. 2004
Population: IPZ24727 × Barke, 86 DH lines
Map: M 70 (Behn et al. 2004)
Phenotyping: Field experiments with 2 locations × 3 years
Method: PLABQTL v.1.1
QTLs: Nonparasitic leaf spots: 1H, 4H, 7HS

Q 108: *Title:* Advanced backcross QTL analysis in barley (*Hordeum vulgare* L.)
Reference: Pillen et al. 2003
Population: (Harry × ISR101-23) × Harry, 164 BC$_2$F$_2$ plants
Map: M 6 (Steptoe × Morex; Kleinhofs et al. 1993, 1994)
Phenotyping: Field experiments with 1 location × 2 years
Method: ANOVA
QTLs: Ears per area: 1H, 4H(2); heading date: 1H(2), 2H(3), 3H(3), 4H(4), 5H(6), 6H(2), 7H(7); plant height: 2H, 3H, 4H(2), 5H, 6H; harvest index: 2H(2), 3H, 5H; kernels per ear: 5H; lodging at flowering: 1H(2), 2H(2), 5H(2); lodging at harvest: 2H(2), 5H; aboveground biomass: 4H; malt tenderness: 3H; grain weight: 1H(2), 2H(2), 3(3), 4H, 5H(2); water absorption: 2H; grain yield: 2H(2), 3H, 4H(3), 5H(5)

Q 109: *Title:* Genetic control of dormancy in a Triumph/Morex cross in barley
Reference: Prada et al. 2004
Population: Triumph × Morex, 107 DH lines
Map: M 71 (Prada et al. 2004)
Phenotyping: Field experiments in 2 environments × 1 to 2 years and subsequent lab experiments
Method: MQTL
QTLs: Germinated seeds after 3 d of incubation at 7 d postharvest: 5H; germinated seeds after 7 d of incubation at 7 d postharvest: 3H, 5H; dormancy release through after-ripening: 2H, 5H

Q 110: *Title:* Molecular mapping of a gene responsible for *Al*-activated secretion of citrate in barley
Reference: Ma et al. 2004
Population: Murasakimochi × Morex, 100 F$_{2:3}$

families
Map: Partial map of chromosome 4H (Ma et al. 2004)
Phenotyping: Growth-chamber experiments with 12 pants per line
Method: MAPMAKER/QTL
QTLs: Citrate secretion: 4H

Q 111: *Title:* Identification of *Hordeum spontaneum* QTL alleles improving field performance of barley grown under rain-fed conditions
Reference: Talame et al. 2004
Population: 123 DH lines from a BC$_1$F$_2$: (HOR11508 × Barke) × Barke
Map: Positions relate to M 32 (Ramsay et al. 2000)
Phenotyping: Field experiments with 3 locations × 1 year
Method: ANOVA
QTLs: Growth habit: 1H, 3H, 6H; heading date: 1H($\geq$2), 2H($\geq$3), 3H($\geq$3), 4H($\geq$3), 5H($\geq$3), 7H($\geq$3); plant height: 1H(1), 2H(3), 3H($\geq$3), 4H(2), 5H($\geq$2), 7H($\geq$2); ear extrusion: 1H(2), 2H($\geq$3), 3H($\geq$3), 4H(4), 5H($\geq$2), 6H(2), 7H($\geq$3); ear length: 3H, 5H(2), 6H, 7H($\geq$3); kernel weight: 2H, 3H, 5H, 6H($\geq$2), 7H; grain yield: 1H(2), 2H(3), 3H(3), 4H, 5H($\geq$2), 6H($\geq$3), 7H($\geq$4)

Q 112: *Title:* Quantitative trait loci affecting growth-related traits in wild barley (*Hordeum spontaneum*) grown under different levels of nutrient supply
Reference: Elberse et al. 2004
Population: *H.v.* ssp *spontaneum* Ashquelon × *H.v.* ssp *spontaneum* Mehola, 140 F$_{2:3}$ families
Map: M 69 (Verhoeven et al. 2004a)
Phenotyping: Growth chamber experiments (7 replications) with high and low nutrient level (HN and LN)
Method: MAPQTL
QTLs: Relative growth rate: HN: 6H; LN: 6H; Leaf area/Plant mass ratio: :LN: 1H, 2H, HN: 4H; leaf area/leaf mass ratio: LN: 3H, 4H; HN: 3H; leaf mass/plant mass ration: LN: 1H, 2H, HN: 4H; tillers/plant: HN: 4H; leaves/plant: LN: 7H; HN: 2H, 4H, 5H, 6H; roots/plant: HN: 5H, 6H; leaf length: LN: 1H, 2H, 4H; HN: 2H, 5H, 7H; leaf width: LN: 2H, 6H; HN: 4H; seed mass: 1H, 6H

Q 113: *Title:* Can a genetic correlation with seed mass constrain adaptive evolution of seedling desiccation tolerance in wild barley?

Reference: Verhoeven et al. 2004b
Population: H.v. ssp *spontaneum* Ashquelon ×H.v. ssp *spontaneum* Mehola, 140 $F_{2:3}$ families
Map: M 69 (Verhoeven et al. 2004a)
Phenotyping: Germination and growth-chamber experiments (16 seeds per line)
Method: MAPQTL
QTLs: Survival probability after dessication: no QTL; mean seed mass: 4H

Q 114: *Title:* Host genetic effect on deoxynivalenol accumulation in fusarium head blight of barley
Reference: Smith et al. 2004
Population: Frederickson × Stander, 130 RI lines from F_4
Map: M 47 (Mesfin et al. 2003)
Phenotyping: Growth-chamber experiments
Method: PLABQTL
QTLs: DON accumulation: 3H, 4H

Q 115: *Title:* Ecogeographic and genetic determinants of kernel weight and color of wild barley (*Hordeum spontaneum*) populations in Israel
Reference: Chen et al. 2004
Population: 93 H.v. ssp. *spontaneum* genotypes, collected in Israel.
Map: Tentative consensus map based on different populations
Phenotyping: Analysis of grains of material propagated in one environment
Method: Kruskal-Wallis test
QTLs: Kernel weight: 1H(2), 2H, 6H, 7H; Kernel color: 4H

Q 116: *Title:* QTL mapping of net blotch resistance genes in a doubled-haploid population of six-row barley
Reference: Ma et al. 2004
Population: Chevron × Stander, 147 DH lines
Map: M 31 (Ma et al. 2000)
Phenotyping: QTL mapping of net blotch resistance genes in a doubled-haploid population of six-row barley
Method: MAPMAKER/QTL
QTLs: Net blotch resistance: 2H, 6H

Q 117: *Title:* Fine mapping of a malting-quality QTL complex near chromosome 4H S telomere in barley
Reference: Gao et al. 2004
Population: 13 $BC_3F_{1:3}$ families: ((Steptoe × Morex) × Morex) × Morex; 12 $BC_3F_{1:3}$ families: ((Steptoe × Morex) × Steptoe) × Steptoe

Map: M 6 (Kleinhofs et al. 1993; Kleinhofs et al. 1994)
Phenotyping: Field experiments with 3 locations × 1 to 2 years (5 environments)
Method: ANOVA
QTLs: Malt extract: 4H(3); diastatic power: 4H(4); α-amylase activity: 4H(6); β-glucan content: 4H

Q 118: *Title:* Linkage disequilibrium mapping of yield and yield stability in modern spring barley cultivars
Reference: Kraakman et al. 2004
Population: 146 European spring barley varieties
Map: Consensus map based on M 21 (Qi et al. 1998a), M 23 (Yin et al. 1999b)
Phenotyping: Data from official variety trials, treated (T) and untreated (U)
Method: LD mapping
QTLs: Yield: T: 3H, 4H, 5H(2); U: 2H(2), 3H, 4H, 5H (2); adaptability: T: 7H; yield stability: T: 2H(2), 4H(2), 6H

4.5
Marker-Assisted Breeding

Marker assisted selection (MAS) is an indirect selection method based on markers linked with the target gene affecting the desirable trait. Theoretically, MAS is more efficient than conventional phenotypic selection (CPS) when correlation between the marker genotype scores and the phenotypic values is greater than the square root of heritability of the trait, assuming that the heritability of the marker is 1 (Dudley 1993). Applying this technique saves time by the early selection based on single plant evaluation, simplifies selection of traits that are difficult to score in CPS, and improves the efficiency of capturing desirable characters in newly developed barley varieties. With marker-assisted backcrossing, genes, such as qualitative and quantitative resistance genes, can be transferred rapidly from wild progenitors to advanced breeding lines, and several resistance genes can be pyramided into a single line. Applying MAS requires, first, segregation for both the marker and the target gene and, second, close linkage between a marker and the target gene.

Despite the sparse use of molecular markers in barley breeding, MAS is widely used by European

breeders in breeding for barley yellow mosaic virus (BaYMV). European breeder use molecular markers that are linked with two resistance genes for BaYMV, *rym4* and *rym5*. Tuvesson et al. (1998) developed a procedure for the large-scale molecular breeding of *ym4*, allowing resistance to BaYMV to be fixed in early breeding generations of winter barley. This procedure theoretically allows one to extract DNA from 5,000 samples in a single day, and to examine the resistance gene *ym4,* which is linked with a codominant STS marker derived from the restriction fragment length polymorphism marker MWG838. In a cross between the resistance cultivar Franklin and the susceptible cultivar Kaputar, Raman and Barbara (1999) reported the implementation of MAS of the gene *Ryd2* conferring resistance against barley yellow dwarf luteoviruses (BYDV). The RFLP marker BCD828 cosegregated with the resistance gene *Ryd2*.

Malt is an important end use of barley. MAS for QTL for malting quality was applied for the use of larger populations. Igartua et al. (2000) confirmed the presence of QTL on chromosome 5H affecting grain weight and plumpness, grain protein, extract ß-glucan content, the difference between fine-grind and coarse-grind extract, soluble protein, diastatic power, α-amylase activity, and fine-grind extract. Different types of markers have been found to be linked with malting quality parameters (see Sect. 4 in this chapter); in particular, marker-based selection for two regions on chromosome 7 was effective in identifying phenotypically superior lines in the two-rowed barley cross between the cultivar Harrington and the breeding line TR306. The RFLP markers MWG502, ABG610, ABC622, and MWG632, which represent or flank the QTL regions underlying malting quality, were used.

Marker-based selection was applied for α-amylase activity in a DH population originating from the cross Morex × Labelle. The variety Morex provides the positive allele for α-amylase activity, which is located on chromosome 5H. Two RFLP markers, ABC302 and ABC717, represented the target region. Selection for the Morex allele with two PCR markers on chromosome 5H was effective in increasingα-amylase activity (Ayoub et al. 2003).

4.6
Map-Based Cloning of Resistance Genes in Barley

4.6.1
mlo and *Ror* Genes

The *mlo* gene is a recessive mutation in barley that confers durable, broad-spectrum resistance against the obligate biotrophic fungal pathogen *Blumeria graminis* f. sp. *hordei* (Bgh). The mutant *mlo* exhibits a spontaneous mesophyll cell death phenotype (Wolter et al. 1993; Peterhänsel et al. 1997). Furthermore, it has been suggested that the *Mlo* protein is involved in regulation of one or more early cell defense responses, and if this regulation is missing (in *mlo* mutants), the cell defense responses are activated earlier and/or more strongly than in susceptible barley (Jørgensen 1992). All suggested disease resistance mechanisms of *Mlo* are related to apposition/papilla formation and cell wall modifications and include effects on the timing and size of the host papilla response, callose deposition, production of phenolic compounds, and cell wall strengthening by cross linking (Lyngkjær et al. 2000).

The *mlo* was the first barley disease resistance gene isolated by map-based cloning (Büschges et al. 1997) (Table 4). The gene was identified through the use of a yeast artificial chromosome (YAC) library and the subsequent construction of a bacterial artificial chromosome (BAC) library from the YAC clone spanning the resistance locus. Scanning N-glycosylation mutagenesis and *Mlo*-Lep fusion proteins demonstrated that *Mlo* is membrane-anchored by 7 transmembrane (TM) helices. *MLO* is the only plant polytopic membrane protein experimentally shown to consist of seven membrane-spanning domains (Devoto et al. 1999).

The wild type *Mlo* allele was estimated to be 1,599 bp. The coded protein contains 533 amino acids, and the corresponding 60-kDa proteins are membrane-anchored and resistance is based on loss of function of this protein (Büschges et al. 1997).

The function of many resistance genes has been shown to work by activation by other genes, which are required for the full expression of the resistance genes. With the assistance of mutation studies two genes, *Ror1* and *Ror2,* were found not only to be required for full expression of the non-race-specific *mlo*-mediated

resistance to Bgh (Freialdenhoven et al. 1996) but also contributed to low-level basal penetration resistance expressed in "susceptible" wild-type *Mlo* backgrounds (Collins et al. 2003). *Ror2* was isolated using barley–rice synteny and map-based cloning. Another gene is required for basal penetration resistance, *PEN1*, which plays a role in nonhost resistance of *Arabidopsis* to barley powdery mildew (*Blumeria graminis*) (Collins et al. 2003). Both genes *Ror2* and *PEN1* encode functionally homologous syntaxins, demonstrating a mechanistic link between nonhost resistance and basal penetration resistance in monocotyledons and dicotyledons (Collins et al. 2003). Out of the 24 syntaxins in *Arabidopsis*, *PEN1* has the closest resemblance to *Ror2* (62% identity and 77% similarity in the cytosolic region). Also, it has been shown that resistance in barley requires a SNAP-25 (synaptosome-associated protein, molecular mass 25 kDa) homolog capable of forming a binary SNAP receptor (SNARE) complex with *Ror2* (Collins et al. 2003).

4.6.2
Mla and *Rar* Genes

The race-specific resistance genes are usually clustered in a chromosomal region. The *Mla* locus is believed to be a cluster of closely linked genes (Jahoor et al. 1993; Schwarz et al. 1999; Wei et al. 1999). At least four *Mla* alleles have been isolated so far. A high-resolution map of the *Mla* locus was constructed by using a cross between two near isogeneic lines P01 (*Mla1*) and P10 (*Mla12*) (Schwarz et al. 1999). The majority of plant disease resistance genes cloned to date include in their coding region domains either a Nucleotide Binding Site (NBS) motif followed by long C-terminal regions or well-organized Leucine Rich Repeats (LRRs) (Madsen et al. 2003). The complex 240-kb *Mla* locus encodes at least 32 characterized resistance specificities to barley powdery mildew (Weibull et al. 2003). This complex locus harbors multiple members of three distantly related gene families that encode proteins that contain an N-terminal coiled-coil (CC) structure, a central nucleotide binding (NB) site, a Leu-rich repeat (LRR) region, and a C-terminal non-LRR (CT) region (Shen et al. 2003). NBS-LRR proteins have been shown to provide plant recognition of fungal and bacterial pathogens in cereals, but they also recognize viral, nematode, and insect species that parasitize dicotyledonous plants (Ayliffe and Lagudah 2004).

In an attempt to proof *Mla1* within the complex 240-kb *Mla* locus, a single-cell transient expression assay was employed using entire cosmid DNAs. The *Mla1* cDNA encoded a 108-kD protein containing an N-terminal coiled-coil structure, a central nucleotide binding domain, and a C-terminal leucine-rich repeat region; it also contained a second short open reading frame at the 5′ end. The assumed protein sequence of *Mla1* revealed a modular domain architecture and significant sequence similarities to plant NB-LRR proteins (Zhou et al. 2001). A previous study provided evidence that the majority of tested *Mla* resistance specificities require for their function at least two additional genes, *Rar1* and *Rar2* (Jørgensen 1996). Previous genetic data conferred evidence that *Mla1*, unlike most other resistance specificities encoded at *Mla*, does not require *Rar1* for its function (Jørgensen 1996) and *Mla1* expressed full resistance in the presence of the severely defective *rar1-2* mutant allele (Zhou et al. 2001). Although *Mla1* and *Mla6* are closely related to each other, *Mla6* function, contrary to that of *Mla1*, is fully dependent on *Rar1*, although the two deduced proteins are 91% identical in sequence (Halterman et al. 2001). A COILS analysis of the *Mla6* protein sequence revealed with greater than 95% probability that a coiled-coil region is located between amino acids 24 and 50, indicating that *Mla6* belongs to the coiled-coil subset of NBS-LRR resistance proteins. The deduced protein sequence encoded by the *Mla6* open reading frame included 956 amino acids with an estimated molecular mass of 107.8 kDa. (Halterman et al. 2001).

Two *Mla* alleles, *Rar1*-independent *Mla7* and *Rar1*-dependent *Mla10*, were isolated and characterized by Halterman and Wise (2004a,b). Only two amino acids exclusively conserved in *RAR1*-independent *Mla6*, *Mla10*, *Mla12,* and *Mla10* were different at the corresponding position in *RAR1*-independent *Mla1* and *Mla7*. Site-directed mutagenesis of these residues showed that RAR1-independence requires the presence of an aspertate at position 721, as mutation of this residue to a structurally similar but uncharged asparagine did not alert *RAR1* dependence. These results demonstrated that a single-amino-acid substitution in the six *Mla* LRRs could alert host signaling but not resistance specificity to *Bgh* (Halterman and Wise 2004a,b).

The *Mla12* allele, which encoded a CC-NB-LRR-CT protein, shared 89% and 92% identical residues with the known proteins *Mla1* and *Mal6*, respectively (Shen et al. 2003). The allele has a slow trigger re-

sponse compared with the rapid *Mla1 / Mla6*-like resistance. The gene was isolated using a genomic cosmid library comprising five barley genome equivalents using DNA from cv. Sultan5-containing *Mla12*. Sixteen cosmid clones were isolated from this library with a DNA probe corresponding to the LRR region of *Mla1*. Low-pass DNA sequencing of the cosmid clones revealed that all of them contained NBLRR-type *RGH*s. Two clones, designated Sp14-1 and Sp14-4, contain identical *RGH*s, showing ca. 90% sequence identity to *Mla1* and *Mla6* in deduced exon and intron sequences (Shen et al. 2003).

The barley *Rar1* and *Rar2* genes are an essential component of the race-specific, *Mla12*-specified powdery mildew resistance reaction. The gene *Rar1* is shown to function upstream of H_2O_2 accumulation in attacked host cell, which precedes localized host cell death (Shirasu et al. 1999). The gene was isolated with the help of a map-based cloning strategy and yeast artificial chromosomes (YACs). Five barley yeast artificial chromosomes (YACs) have been identified, ranging in size from 300 to 1,100 kb spanning the *Rar* locus. PCR-based YAC end-specific markers have been established and were employed to construct YAC contigs. Four out of five YAC clones were found to be noncollinear with the source DNA. High-resolution genetic mapping of the YAC ends demonstrated that the set of five overlapping YAC clones encompasses the barley *Rar1* gene. The centromere of barley chromosome 2H is separated from the *Rar1* locus by about 22 cM. It covers a physical distance of 460 Mb and has been located in a 1.4-cM interval bordered by RFLP markers MWG2287 and cMWG658 (Lahaye et al. 1998). Shirasu et al. (1999) Isolated the gene *Rar1* by map-based cloning and found that the gene is 25.5 kDa. This protein reveals two copies of a 60-amino-acid domain. It encodes a novel protein containing two 60-amino-acid (aa) cysteine- and histidine-rich domains, designated CHORD. Biochemical analysis of *Rar1* protein reveals that CHORD is an autonomous Zn^{2+}-binding domain (Shirasu et al. 1999).

4.6.3
Rpg Genes

The construction of a BAC library from the barley cultivar Morex (Yu et al. 2000) has facilitated the map-based isolation of the powdery mildew resistance genes *Mla1* and *Mla6* (Halterman et al. 2001; Zhou et al. 2001) as well as the stem rust resistance gene *Rpg1*. Stem rust caused by *Puccinia graminis* f. sp. *tritici* was among the most serious diseases of barley in North America. The gene *Rpg1* has provided durable protection against most pathotypes of *P. graminis* f. sp. *tritici* in broadly grown barley cultivars. The first attempt to isolate this gene was done by using rice-barley gene microcollinearity (Han et al. 1999a). This attempt led to the generation of markers near *Rpg1*, but it did not ultimately lead to its isolation. The *Rpg1* gene was cloned by high-resolution genetic and physical mapping. The map was constructed with 8,518 gametes, and a 330 kb bacterial artificial chromosome (BAC) was isolated. The genomic and cDNA sequence comparisons predicted the gene to contain 14 exons in a total sequence of 4,466 bp coding for an 837-aa (94.5 kDa) protein. The *Rpg1* gene product was unique because it contains, in addition to a receptor kinaselike protein, two tandem protein kinase domains, but no recognizable receptor and membrane anchor domains (Brüeggeman et al. 2002). To determine whether the stem-rust-susceptible cultivar Golden Promise can be converted into a resistant cultivar, Horvath et al. (2003) transformed the resistance gene *Rpg1* genomic clone of cv. Morex, which contains a 520-bp 5′ promoter region, 4,919-bp gene region, and 547-bp 3′ nontranscribed sequence, into the susceptible cultivar Golden Promise. The transformations were done by *Agrobacterium*-mediated technique, then characterized for the infection response to the stem rust fungus. The results demonstrated that stem-rust-susceptible barley could be made resistant by transformation with the cloned *Rpg1* gene.

4.7
Future Scope of Works

The DNA markers linked with different traits provide a tool to identify genotypes with desirable gene combinations via marker-assisted selection. This chapter presents a comprehensive survey of the literature on qualitative and quantitative inherited genes as well as on mapped or resistance genes cloned so far. It appears that several important agronomic traits including malting quality and disease resistance against most important diseases have been mapped with molecular markers in barley. Therefore, DNA markers can be selected to conduct MAS for desirable genotypes. However, only few markers have been or are being

Table 4. Isolated resistance genes in barley

Disease	Gene	Method	Function	Reference
Powdery mildew	*mlo*	Map-based	Seven-transmembrane proteins	Büschges et al. 1997
Powdery mildew	Rar-1	Map-based	Zinc binding protein	Lahaye et al. 1998
Powdery mildew	*Ror-2*	Barley-rice synteny and map-based	Syntaxin	Collins et al. 2003
Powdery mildew	*Mla6*	Map-based	CC-NBS-LRR	Wei et al. 1999, Halterman et al. 2001
Powdery mildew	*Mla13*	Map-based	CC-NBS-LRR	Wei et al. 1999
Powdery mildew	*Mla1*	Map-based	CC-NBS-LRR	Halterman et al. 2001, Zhou et al. 2001
Powdery mildew	*Mla12*	Genomic cosmid library	CC-NBS-LRR	Shen et al. 2003
Stem rust	*Rpg1*	Map-based	Receptor kinaselike protein	Brüeggeman et al. 2002

CC = coiled-coil, NBC = nucleotide binding site, LRR = leucine-rich repeats

used in practical breeding. To employ DNA markers in plant breeding, they have to be breeder-friendly and they should be linked very closely. By simultaneous application of several DNA markers linked to different traits, breeding lines can be selected that will possess desirable gene combinations. DNA chip technology is developing fast. DNA chips will be available that contain specific primers for different genes underlying agronomic traits. These DNA chips will allow identification not only of different loci but also of different alleles of a locus. RGAs have been found to be linked with qualitative as well as quantitative disease resistance genes in barley (Madsen et al. 2003). A large number of ESTs are found in barley. Hence, genes can be searched in the EST database, and with the help of this database perhaps DNA-chips could be developed to conduct MAS. Further, linked DNA markers have also been employed to isolate disease resistance genes. The isolated resistance genes can now be transferred to susceptible genotypes in order to improve resistance. However, the acceptance of genetically modified organisms has not been universally very well adopted. Consequently, a technique has to be developed to overcome the GMO problem. Recently, an emerging technology called TILLING (Targeting Induced Local Lesions In Genomes; McCallum et al. 2000) might replace the GMO in the future. This technique has also been developed for barley (Mejlhede et al. 2004).

References

Abbott DC, Lagudah ES, Brown AHD (1995) Identification of RFLPs flanking a scald resistance gene on barley chromosome 6. J Hered 86: 152–154

Åberg E (1938) *Hordeum* a nova sp., a wild six-rowed barley. Ann Agri Colt Sweden 6:159–216

Ahokas H (1999) On the territorial distribution of chromosomal interchanges in wild barley, *Hordeum vulgare* spp. *Spontaneum.* Barley Genet Newslett 29:40–42

Arru L, Niks RE, Lindhout P, Vale G, Francia E, Pecchioni N (2002) Genomic regions determining resistance to leaf stripe (*Pyrenophora graminea*) in barley. Genome 45:460–466

Arru L, Francia E, Pecchioni N (2003) Isolate specific QTLs of resistance to leaf stripe (*Pyrenophora graminea*) in the 'Steptoe' × 'Morex' spring barley cross. Theor Appl Genet 106:668–675

Asfaw Z, von Bothmer R (1990) Hybridization between landrace varieties of Ethiopian barley (*Hordeum vulgare* ssp. *vulgare*) and the progenitor of barley (*H. vulgare* ssp. *spontaneum*). Hereditas 112:57–64

Ayliffe MA, Lagudah ES (2004) Molecular genetics of disease resistance in cereals. Ann Bot 94:765–773

Ayoub M, Symons SJ, Edney MJ, Mather DE (2002) QTLs affecting kernel size and shape in a two-rowed by six- rowed barley cross. Theor Appl Genet 105:237–247

Ayoub M, Armstrong E, Bridger G, Fortin MG, Mather DE (2003) Marker-based selection in barley for a QTL region affecting α-amylase activity of malt. Crop Sci 43:556–561

Backes G, Graner A, Foroughi-Wehr B, Fischbeck G, Wenzel G, Jahoor A (1995) Localization of quantitative trait loci (QTL) for agronomic important characters by the use of a RFLP map in barley (*Hordeum vulgare* L.). Theor Appl Genet 90:294–302

Backes G, Schwarz G, Wenzel G, Jahoor A (1996) Comparison between QTL analysis of powdery mildew resistance in barley based on detached primary leaves and on field data. Plant Breed 115:419–421

Backes G, Madsen LH, Jaiser H, Stougaard J, Herz M, Mohler V, Jahoor A (2003) Localisation of genes for resistance against *Blumeria graminis* f. sp. *hordei* and *Puccinia graminis* in a cross between a barley cultivar and a wild barley (*Hordeum vulgare* ssp. *spontaneum*) line. Theor Appl Genet 106:353–362

Badr A, Muller K, Schafer-Pregl R, El Rabey H, Effgen S, Ibrahim HH, Pozzi C, Rohde W, Salamini F (2000) On the origin and domestication history of barley (*Hordeum vulgare*). Mol Biol Evol 17(4):499–510

Baker RJ, Larter RN (1963) The inheritance of scald resistance in barley. Can J Genet Cytol 5:445–449

Barr AR, Chalmers KJ, Karakousis A, Kretschmer JM, Manning S, Lance RCM, Lewis J, Jefferies SP, Langridge P (1998) RFLP mapping of a new cereal cyst nematode resistance locus in barley. Plant Breed 117:185–187

Barr AR, Jefferies SP, Broughton S, Chalmers KJ, Kretschmer JM, Boyd JR, Collins HM, Roumeliotis S, Logue SJ, Coventry SJ, Moody DB, Read BJ, Poulsen D, Lance RCM, Platz GJ, Park RF, Panozzo JF, Karakousis A, Lim P, Verbyla AP, et al (2003a) Mapping and QTL analysis of the barley population Alexis × Sloop. Aust J Agri Res 54:1117–1123

Barr AR, Karakousis A, Lance RCM, Logue SJ, Manning S, Chalmers KJ, Kretschmer JM, Boyd JR, Collins HM, Roumeliotis S, Coventry SJ, Moody DB, Read BJ, Poulsen D, Li C-D, Platz GJ, Inkerman PA, Panozzo JF, Cullis BR, Smith DB et al (2003b) Mapping and QTL analysis of the barley population Chebec × Harrington. Aust J Agri Res 54:1125–1130

Barua UM, Chalmers KJ, Thomas WTB, Hackett CA (1993) Molecular mapping of genes determining height, time to heading, and growth habit in barley (*Hordeum vulgare*). Genome 36:1080–1087

Basten CJ, Weir BS, Zeng Z-B (1997) QTL Cartographer: a reference manual and tutorial for QTL mapping. Department of Statistics, North Carolina Univ, Raleigh, NC

Bauer E, Graner A (1995) Basic and applied aspects of the genetic analysis of the *ym4* virus resistance locus in barley. Agronomie 15(7-8):469–473

Baum M, Grando S, Backes G, Jahoor A, Sabbagh A, Ceccarelli S (2003) QTLs for agronomic traits in the Mediterranean environment identified in recombinant inbred lines of the cross 'Arta' × *H. spontaneum* 41-1. Theor Appl Genet 107:1215–1225

Beecher B, Bowman J, Martin JM, Bettge AD, Morris CF, Blake TK, Giroux MJ (2002) Hordoindolines are associated with a major endosperm-texture QTL in barley (*Hordeum vulgare*). Genome 45:584–591

Behn A, Hartl L, Schweizer G, Wenzel G (2004) QTL mapping for resistance against non-parasitic leaf spots in a spring barley doubled haploid population. Theor Appl Genet 108:1229–1235

Bekele E (1983) A differential rate of regional distribution of barley flavonoid patterns in Ethiopia, and a view on the center of origin of barley. Hereditas 98:269–280

Bezant J, Laurie D, Pratchett N, Chojecki J, Kearsey M (1996) Marker regression mapping of QTL controlling flowering time and plant height in a spring barley (*Hordeum vulgare* L.) cross. Heredity 77:64–73

Bezant JH, Laurie DA, Pratchett N, Chojecki J, Kearsey MJ (1997a) Mapping of QTL controlling NIR predicted hot water extract and grain nitrogen content in a spring barley cross using marker-regression. Plant Breed 116:141–145

Bezant J, Laurie DA, Pratchett N, Chojecki J, Kearsey M (1997b) Mapping QTL controlling yield and yield components in a spring barley (*Hordeum vulgare* L) cross using marker regression. Mol Breed 3:29–38

Bjørnstad Å, Patil V, Tekauz A, Marøy AG, Skinnes H, Jensen A, MacKey J (2002) Resistance to scald (*Rhynchosporium secalis*) in barley (*Hordeum vulgare*) studied by near-isogenic lines. I. Markers and differential isolates. Phytopathology 92:710–720

Blattner FR, Badani Méndez AG (2001) RAPD data do not support a second centre of barley domestication in Morocco. Genet Resource Crop Evol 48:13–19

Borém A, Mather DE, Rasmusson DC, Fulcher RG, Hayes PM (1999) Mapping quantitative trait loci for starch granule traits in barley. J Cereal Sci 29:153–160

Borner A, Korzun V, Malyshev S, Ivandic V (1999) Molecular mapping of two dwarfing genes differing in their GA response on chromosome 2H of barley. Theor Appl Genet 99(3–4):670–675

Borovkova LU, Steffenson BJ, Jin Y, Rasmussen LB, Kilian A, Kleinhofs A, Rossnagel BG, Kao K-N (1995) Identification of molecular markers linked to the stem rust resistance gene *rpg4* in barley. Phytopathology 85:181–185

Borovkova IG, Jin Y, Steffenson B, Kilian A, Blake TK, Kleinhofs A (1997) Identification and mapping of a leaf rust resistance gene in barley line Q21861. Genome 40:236–241

Borovkova IG, Jin Y, Steffenson BJ (1998) Chromosomal location and genetic relationship of leaf rust resistance genes *Rph9* and *Rphl2* in barley. Phytopathology 88:76–80

Boyd WJR, Li C-D, Grime CR, Cakir M, Potipibool S, Kaveeta, Men S, Kamali M, Barr AR, Moody DB, Lance RCM, Logue SJ, Raman H, Read BJ (2003) Conventional and molecular genetic analysis of factors contributing to variation in the timing of heading among spring barley (*Hordeum vulgare* L.) genotypes grown over a mild winter growing season. Aust J Agric Res 54:1277–1301

Bregitzer P, Campbell RD (2001) Genetic markers associated with green and albino plant regeneration from embryogenic barley callus. Crop Sci 41:173–179

Briggs FN, Stanford BH (1938) Linkage of factors for resistance to mildew in barley. J Genet 37:107–117

Briggs FN, Stanford BH (1943) Linkage relations of the Goldfoil factor for resistance to mildew in barley. J Agric Res 66:1–5

Broman KW, Wu H, Sen S, Churchill GA (2003) R/qtl: QTL mapping in experimental crosses. Bioinformatics 19:889–890

Brueggeman R, Rostoks N, Kudrna D, Kilian A, Han F, Chen J, Druka A, Steffenson B, Kleinhofs A (2002) The barley stern rust-resistance gene *Rpg1* is a novel disease-resistance gene with homology to receptor kinases. Proc Natl Acad Sci USA 99:9328–9333

Brunner A, Rdder MS, Unger O, Meinel A (2000) The detection and molecular mapping of a major gene for non-specific adult-plant resistance against stripe rust (*Puccinia striformis*) in wheat. Theor Appl Genet 100:1095–1099

Brown AHD, Zohary D, Nevo E (1978) Outcrossing rates and heterozygosity in natural populations of *Hordeum spontaneum* Koch in Israel. Heredity 41:49–62

Buck-Sorlin GH (2002) The search for QTL in barley (*Hordeum vulgare* L.) using a new mapping population. Cell Mol Biol Lett 7:523–535

Büschges R, Hollricher K, Panstruga R, Simons G, Wolter M, Frijters A, van Daalen R, van der Lee T, Diergarde P, Groenendijk J, Töpsch S, Vos P, Salamini F, Schulze-Lefert P (1997) The barley Mlo gene: A novel control element of plant pathogen resistance. Cell 88:695–705

Cakir M, Gupta S, Platz GJ, Ablett GA, Loughman R, Emebiri LC, Poulsen D, Li C-D, Lance RCM, Galwey NW, Jones MGK, Apples R (2003) Mapping and validation of the genes for resistance to *Pyrenophora teres* f. *teres* in barley (*Hordeum vulgare* L.). Aust J Agric Res 54:1369–1377

Canci PC, Nduulu LM, Dill-Macky R, Muehlbauer GM, Rasmusson DC, Smith KP (2003) Genetic relationship between kernel discoloration and grain protein concentration in barley. Crop Sci 43:1671–1679

Castro AJ, Chen X, Hayes PM, Knapp SJ, Line RF, Toojinda T, Vivar H (2002) Coincident QTL which determine seedling and adult plant resistance to stripe rust in barley. Crop Sci 42:1701–1708

Castro AJ, Capettini F, Corey AB, Filichkina T, Hayes PM, Kleinhofs A, Kudrna D, Richardson K, Sandoval-Islas S, Rossi C et al (2003) Mapping and pyramiding of qualitative and quantitative resistance to stripe rust in barley. Theor Appl Genet 107:922–930

Chalmers KJ, Barua UM, Hackett CA, Thomas WTB, Waugh R, Powell W (1993) Identification of RAPD markers linked to genetic factors controlling the milling energy requirement of barley. Theor Appl Genet 87:314–320

Chen FQ, Prehn D, Hayes PM, Mulrooney D, Corey A, Vivar H (1994) Mapping genes for resistance to barley stripe rust (*Puccinia striiformis* f. sp. *hordei*). Theor Appl Genet 88:215–219

Chen GX, Suprunova T, Krugman T, Fahima T, Nevo E (2004) Ecogeographic and genetic determinants of kernel weight and colour of wild barley (*Hordeum spontaneum*) populations in Israel. Seed Sci Res 14:137–146

Choi D-W, Close TJ (2000) A newly identified barley gene, *Dhn12*, encoding a YSK2 DHN, is located on chromosome

6H and has embryo-specific expression. Theor Appl Genet 100:1274–127

Choi D-W, Zhu B, Close TJ (1999) The barley (*Hordeum vulgare* L.) dehydrin multigene family: sequences, allele types, chromosome assignments, and expression characteristics of 11 *Dhn* genes of cv Dicktoo. Theor Appl Genet 98:1234–1247

Clancy JA, Han F, Ullrich SE (2003) Comparative mapping of beta-amylase activity QTLs among three barley crosses. Crop Sci 43:1043–1052

Collins NC, Paltridge NG, Ford CM, Symons RH (1996) The *Yd2* gene for barley yellow dwarf virus resistance maps close to the centromere on the long arm of barley chromosome 3. Theor Appl Genet 92(7):858–864

Collins N, Park R, Spielmeyer W, Ellis J, Pryor AJ (2001) Resistance gene analogs in barley and their relationship to rust resistance genes. Genome 44:375–381

Collins NC, Thordal-Christensen H, Lipka V, Bau S, Kombrink E, Qiu JL, Huckelhoven R, Stein M, Freialdenhoven A, Somerville SC, Schulze-Lefert P (2003) SNARE-protein-mediated disease resistance at the plant cell wall. Nature 425(6961):973–977

Costa JM, Corey A, Hayes PM, Jobet C, Kleinhofs A, Kopisch-Obusch A, Kramer SF, Kudrna D, Li M, Riera-Lizarazu O, Sato K, Szucs P, Toojinda T, Vales MI, Wolfe RI (2001) Molecular mapping of the Oregon Wolfe Barleys: a phenotypically polymorphic doubled-haploid population. Theor Appl Genet 103:415–424

Costantini L (1984) The beginning of agriculture in the Kachi plain: the evidence from Mehrgarh. In: Allchin B (ed) South Asian Archaeology 1981. Cambridge University Press, Cambridge, UK, pp 29–33

Dahleen LS, Horsley RD, Steffenson BJ, Schwarz PB, Mesfin A, Franckowiak JD (2003) Identification of QTLs associated with *Fusarium* head blight resistance in Zhedar 2 barley. Theor Appl Genet 108:95–104

Darby WJ, Ghalioungui P, Grivetti L (1977) Food: The Gift of Osiris. Academic, London

De la Peña RC, Smith KP, Capettini F, Muehlbauer GJ, Gallo-Meagher M, Dill-Macky R, Somers DA, Rasmusson DC (1999) Quantitative trait loci associated with resistance to *Fusarium* head blight and kernel discoloration in barley. Theor Appl Genet 99:561–569

Devoto A, Piffanelli P, Nilsson I, Wallin E, Panstruga R, von Heijne G, Schulze-Lefert P (1999) Topology, subcellular localization, and sequence diversity of the *Mlo* family in plants. J Biol Chem 274:34993–35004

Doll H, Jensen HP (1986) Localization of powdery mildew resistance gene *Ml-ra* on barley chromosome 5. Hereditas 105:61–65

Druka A, Kudran D, Han F, Kilian A, Steffensen B, Frisch D, Tomkins J, Wing R, Kleinhofs R (2000) Physical mapping of the barley stem rust resistance gene *rpg4*. Mol Gen Genet 264:283–290

Dudley JW (1993) Molecular markers in plant improvement: manipulation of genes affecting quantitative traits. Crop Sci 33:660–668.

Dyck PL, Schaller C (1961) Inheritance of resistance in barley to several physiologic races of the scald fungus. Can J Genet Cytol 3:153–164.

Edney MJ, Mather DE (2004) Quantitative trait loci affecting germination traits and malt friability in a two-rowed by six rowed barley cross. J Cereal Sci 39:283–290

Edwards MC, Steffenson BJ (1996) Genetics and mapping of barley stripe mosaic virus resistance in barley. Phytopathology 86:184–187

El Attari H, Rebai A, Hayes PM, Barrault G, Dechamp-Guillaume G, Sarrafi A (1998) Potential of doubled-haploid lines and localization of quantitative trait loci (QTL) for partial resistance to bacterial leaf streak (*Xanthomonas campestris* pv. *hordei*) in barley. Theor Appl Genet 96:95–100

Elberse IAM, Vanhala TK, Turin JHB, Stam P, van Damme JMM, van Tienderen PH (2004) Quantitative trait loci affecting growth-related traits in wild barley (*Hordeum spontaneum*) grown under different levels of nutrient supply. Heredity 93:22–33

Ellis RP, Forster BP, Gordon DC, Handley LL, Keith RP, Lawrence P, Meyer R, Powell W, Robinson D, Scrimgeour CM, Young G, Thomas WTB (2002) Phenotype/genotype associations for yield and salt tolerance in a barley mapping population segregating for two dwarfing genes. J Exp Bot 53:1163–1176

El Rabey H, Salamini F (2000) Domestication history of barley and phylogenetic relationships in the genus *Hordeum*. In: Barley Genetics VIII. Proc 8th Int Barley Genet Symp, Adelaide, Australia, pp 32–36

Emebiri L, Moody DB, Panozzo JF, Chalmers KJ, Kretschmer JM, Ablett GA (2003) Identification of QTLs associated with variations in grain protein concentration in two-row barley. Aust J Agric Res 54:1211–1221

Emebiri LC, Moody DB, Panozzo JF, Read BJ (2004) Mapping of QTL for malting quality attributes in barley based on a cross of parents with low grain protein concentration. Field Crops Res 87:195–205

Falak I, Falk DE, Tinker NA, Mather DE (1999) Resistance to powdery mildew in a doubled haploid barley population and its association with marker loci. Euphytica 107:185–192

Feuerstein U, Brown AHD, Burdon JJ (1990) Linkage of rust resistance genes from wild barley (*Hordeum sponraneum*) with isozyme markers. Plant Breed 104:318–324

Finlay KW, Wilkinson GN (1963) Analysis of adaptation in plant-breeding programme. Aust J Agric Res 14 (6):742–754

Forster BP (2001) Co-ordinators report: Chromosome 1H. Barley Genet Newslett 31:53–56

Francia E, Rizza F, Cattivelli L, Stanca AM, Galiba G, Tóth B, Hayes PM, Skinner JS, Pecchioni N (2004) Two loci on chromosome 5H determine low-temperature tolerance in a 'Nure' (winter) × 'Tremois' (spring) barley map. Theor Appl Genet 108:670–680

Franckowiak JD (1997) Revised linkage maps for morphological markers in barley, *Hordeum vulgare*. Barley Genet Newslett 26:9–21

Franckowiak J (2001) Coordinator's report: Chromosome 2H(2) Barley Genet Newslett 31:45–51

Freialdenhoven A, Peterhänsel C, Kurth J, Kreuzaler F, Schulze-Lefert P (1996) Identification of genes required for the function of non-race-specific *mlo* resistance to powdery mildew in barley. Plant Cell 8:5–14

Freisleben R, Lein A (1942) Ueber die Auffindung einer mehltauresistenten Mutante nach Roentgenbestrahlung einer anfälligen reinen Linie von Sommergerste. Naturwissenschaft 30:608

Frost S, Holm G, Asker S (1975) Flavonoid patterns and phylogeny of barley. Hereditas 79 (1):133–142

Gao W, Clancy JA, Han F, Jones BL, Budde A, Wesenberg DM, Kleinhofs A, Ullrich SE, North American Barley Genome Project (2004) Fine mapping of a malting-quality QTL complex near the chromosome 4H S telomere in barley. Theor Appl Genet 109:750–760

Garvin DF, Brown AHD, Burdon JJ (1997) Inheritance and chromosome locations of scald-resistance genes derived from Iranian and Turkish wild barleys. Theor Appl Genet 94:1086–1091

Garvin DF, Brown AHD, Raman H, Read BJ (2000) Genetic mapping of the barley *Rrsl4* scald resistance gene with RFLP, isozyme and seed storage protein markers. Plant Breed 119:193–196

Giese H (1981) Powdery mildew resistance genes in the *Ml-a* and *Ml-k* regions on barley chromosome 5. Hereditas 95:51–62

Giese H, Holm-Jensen G, Mathiasen H, Kjær B, Rasmussen SK, Bay H, Jensen J (1994) Distribution of RAPD markers on a linkage map of barley. Hereditas 120:267–273

Giles BE, Lefkovitch LP (1984) Differential germination in *Hordeum spontaneum* from Iran and Morocco. Z Pflanzenzucht 92:234–238

Giles BE, Lefkovitch LP (1985) Agronomic differences in *Hordeum spontaneum* from Iran and Morocco. Z Pflanzenzucht 94:25–40

Giles BE, von Bothmer R (1985) The progenitor of barley (*Hordeum vulgare* ssp. *spontaneum*) – its importance as a gene source. J Swedish Seed Assoc 95:53–61

Görg R, Holiricher K, Schuize-Lefert P (1993) Functional analysis and RFLP-mediated mapping of the *Mlg* resistance locus in barley. Plant J 3:857–866

Graner A, Tekauz A (1996) RFLP mapping in barley of a dominant gene conferring resistance to scald (Rhynchosporium secalis). Theor Appl Genet 93:421–425

Graner A, Jahoor A, Schondelmaier J, Siedler H, Pillen K, Fischbeck G, Wenzel G, Herrmann RG (1991) Construction of an RFLP map of barley. Theor Appl Genet 83:250–256

Graner A, Bauer E, Kirchner S, Muraya JK, Jahoor A, Wenzel G (1994) Progress of RFLP-map construction in winter barley. Barley Genet Newslett 23:53–59

Graner A, Foroughi-Wehr B, Tekauz A (1996) RFLP mapping of a gene in barley conferring resistance to net blotch (*Pyrenophora teres*). Euphytica 91:229–234

Graner A, Streng S, Drescher A, Jin Y, Borovkova IG, Steffenson B (2000) Molecular mapping of the leaf rust resistance gene *Rph7* in barley. Plant Breed 119:389–392

Grønnerød S, Marøy AG, MacKey J, Tekauz A, Penner GA, Bjørnstad Å (2002) Genetic analysis of resistance to barley scald (*Rhynchosporium secalis*) in the Ethiopian line 'Abyssinian' (CI668). Euphytica 126:235–250

Habgood RM, Hayes JD (1971) The inheritance of resistance to *Rhynchosporium secalis* in barley. Heredity 27:25–37

Hackett CA, Ellis RP, Forster BP, McNicol JW, Macaulay M (1992) Statistical analysis of a linkage experiment in barley involving quantitative trait loci for height and ear-emergence time and two genetic markers on chromosome 4. Theor Appl Genet 85:120–126

Halterman DA, Wise RP (2004a) Deciphering Rar1 signaling specificity in barley/powdery mildew reactions. Plant & Animal Genome XII Conf, San Diego, W199

Halterman DA, RP Wise RP (2004b) A single amino acid substitution in the sixth leucine-rich repeat of barley MLA6 and MLA13 alleviates dependence on RAR1 for disease resistance signaling. Plant J 38:215–226

Halterman D, Zhou FS, Wei FS, Wise RP, Schulze-Lefert P (2001) The *MLA6* coiled-coil, NBS-LRR protein confers Avr*Mla6*-dependent resistance specificity to *Blumeria graminis* f. sp. *hordei* in barley and wheat. Plant J 25 (3):335–348

Han F, Ullrich SE, Chirat S, Menteur S, Jestin L, Sarrafi A, Hayes PM, Jones BL, Blake TK, Wesenberg DM, Kleinhofs A, Kilian A (1995) Mapping of beta-glucan content and beta-glucanase activity loci in barley grain and malt. Theor Appl Genet 91:921–927

Han F, Ullrich SE, Kleinhofs A, Jones BL, Hayes PM, Wesenberg DM (1997) Fine structure mapping of the barley chromosome-1 centromere region containing malting-quality QTLs. Theor Appl Genet 95:903–910

Han F, Kilian A, Chen JP, Kudrna D, Steffenson B, Yamamoto K, Matsumoto T, Sasaki T, Kleinhofs A (1999a) Sequence analysis of a rice BAC covering the syntenous barley *Rpg1* region. Genome 42:1071–1076

Han F, Ullrich SE, Clancy JA, Romagosa I (1999b) Inheritance and fine mapping of a major barley seed dormancy QTL. Plant Sci 143:113–118

Han F, Ullrich SE, Romagosa I, Clancy JA, Froseth JA, Wesenberg DM (2003) Quantitative genetic analysis of acid detergent fibre content in barley grain. J Cereal Sci 38:167–172

Harlan JR (1992) Crops and Man, 2nd edn. American Society of Agronomy, Madison, WI

Harlan JR (1995) Barley. In: Smartt J, Simmonds NW (eds) Evolution of Crop Plants, 2nd edn. Longrnan Scientific and Technical, pp 140–147

Harlan JR, de Wet JMJ (1971) Toward a rational classification of cultivated plants. Taxon 20:509–517

Hayes PM, Blake TK, Chen THH, Tragoonrung S, Chen F, Pan A, Liu B (1993a) Quantitative trait loci on barley (*Hordeum vulgare* L.) chromosome 7 associated with components of winterhardiness. Genome 36:66–71

Hayes PM, Liu BH, Knapp SJ, Chen F, Jones B, Blake T, Franckowiak J, Rasmusson D, Sorrells M, Ullrich SE, Wesenberg D, Kleinhofs A (1993b) Quantitative trait locus effects and environmental interaction in a sample of North American barley germ plasm. Theor Appl Genet 87:392–401

Hayes P, Prehn D, Vivar H, Blake T, Comeau A, Henry I, Johnston M, Jones B, Steffenson B et al (1996) Multiple disease resistance loci and their relationship to agronomic and quality loci in a spring barley population, J Agri Genom 2: http://www.cabi-publishing.org/jag/papers96/paper296/jqtl22.html

Hayes PM, Cereno J, Witsenjboer H, Kuiper M, Zabeau M, Sato K, Kleinhofs A, Kudrna D, Saghai Maroof M, Hoffman D, North American Barley Genome Project (1997) Characterizing and exploiting genetic diversity and quantitative traits in barley (*Hordeum vulgare*) using AFLP markers. http://www.cabi-publishing.org/jag/papers97/paper297/jqtl1997–02.html

Hayes PM, Chen X, Corey A, Johnston M, Kleinhofs A, Korte J, Kudrna D, Toojinda T, Vivar H (1999) A summary of barley stripe rust mapping efforts. Plant & Animal Genome VII Conf, San Diego. http://www.intl-pag.org/

Helbaek H (1969) Plant-Collecting, Dry-Farming, and Irrigation Agriculture in Prehistoric Deh Luran. In: Hole F, Flannery KV, Neely JA (eds) Prehistory and Human Ecology of the Deh Luran Plain. University of Michigan Museum of Anthropology Memoir 1. Ann Arbor: University of Michigan Museum of Anthropology, pp 383–426

Hernàndez P, Dorado G, Prieto P, Giménez MJ, Ramírez MC, Laurie DA, Snape JW, Martín A (2001) A core genetic map of *Hordeum chilense* and comparisons with maps of barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*). Theor Appl Genet 102:1259–1264

Heun M (1992) Mapping quantitative powdery mildew resistance of barley using a restriction fragment length polymorphism map. Genome 35:1019–1025

Heun M, Kennedy AE, Anderson JA, Lapitan NLV, Sorrells ME, Tanksley SD (1991) Construction of a restriction fragment length polymorphism map for barley (*Hordeum vulgare*). Genome 34:437–447

Hilbers S, Fischbeck G, Jahoor A (1992) Localization of the *Laevigatum* resistance gene *MlLa* against powdery mildew in the barley genome by the use of RFLP markers. Plant Breed 109:335–338

Hillman GC (1975) The plant remains from Abu Hureyra: a preliminary report. Proc Prehistoric Soc 41:70–73

Hinze K, Thompson RD, Ritter E, Salamini F, Schulze-Lefert P (1991) Restriction fragment length polymorphism-

mediated targeting of the mlo resistance locus in barley (*Hordeum vulgare*). Proc Natl Acad Sci USA 88:3691–3695

Hiura U (1960) Studies on the disease resistance in barley. VI. Genetics of resistance to powdery mildew. Berichte des Ohara Instituts für Landwirtschaft Okayama 11:235–300

Ho PT (1977) Indigenous origins of Chinese agriculture. In: Reed CA (ed) Origin of Agriculture. Mouton, The Hague, pp 413–418

Honecker L (1931) Beiträge zum Mehltauproblem bei der Gerste mit besonderer Berücksichtigung der züchterischen Seite. Pflanzenbau 8:78–84

Hopf M (1991) South and Southwest Europe. In: van Zeist W, Wasilikowa K, Behre KE (eds) Progress in Old World Paleoethnobotany. Balkema, Rotterdam, The Netherlands, pp 241–277

Hori K, Kobayasi T, Sato K, Takeda K, Kawasaki S (2003) Efficient construction of high-density linkage map and its application to QTL analysis in barley. Theor Appl Genet 107:806–813

Horvath H, Rostoks N, Brueggeman R, Steffenson B, von Wettstein D, Kleinhofs A (2003) Genetically engineered stem rust resistance in barley using the *Rpg1* gene. Proc Natl Acad Sci USA 100(1):364–369

Hossain MA, Sparrow DHB (1991a) Resistance to powdery mildew (*Erysiphe gramiuis* f.sp. *hordei*) in the barley cultivar Galleon. I. Relationship with known genes for resistance. Euphytica 52:1–9

Hossain MA, Sparrow DHB (1991b) Resistance to powdery mildew (*Eryriphe graminis* f.sp. *hordei*) in the barley cultivar Galleon. II. Chromosomal location and linkage with hordein genes. Euphytica 52:11–17

Hunter H (1952) The Barley Crop. Crosby Lockwood and Sons, London

Igartua E, Edney M, Rossnagel BG, Spaner D, Legge WG, Scoles GJ, Eckstein PE, Penner GA, Tinker NA, Briggs KG, Falk DE, Mather ED (2000) Marker-based selection of QTL affecting grain and malt quality in two-row barley. Crop Sci 40:1426–1433

Ivandic V, Walther U, Graner A (1998) Molecular mapping of a new gene in wild barley conferring complete resistance to leaf rust (*Puccinia hordei* Otth). Theor Appl Genet 97:1235–1239

Jahoor A, Fischbeck G (1987) Genetical studies of resistance of powdery mildew in barley lines derived from *Hordeum spontaneum* collected from Israel. Plant Breed 99:265–273

Jahoor A, Jacobi A, Schüller CME, Fischbeck G (1993) Genetical and RFLP studies at the *Mla* locus conferring powdery mildew resistance in barley. Theor Appl Genet 85:713–718

Jensen J (2002) Coordinator's Report: Barley Chromosome 1H (5). Barley Genet Newslett 32:141–143

Jensen J, Jørgensen JH (1997) Location of the yellow rust resistance gene *Yr4* on barley chromosome 5. Barley Genet Newslett 7:37–40

Jensen J, Backes G, Skinnes H, Giese H (2002) Quantitative trait loci for scald resistance in barley localized by a non-interval mapping procedure. Plant Breed 121:124–128

Jin Y, Statler GD, Franckowiak JD, Steffenson BJ (1993) Linkage between leaf rust resistance genes and morphological markers in barley. Phytopathology 83:230–233

Jørgensen JH (1992) Discovery, characterization and exploitation of *Mlo* powdery mildew resistance in barley. Euphytica 63:141–152

Jørgensen JH (1996) Effect of three suppressors on the expression of powdery mildew resistance genes in barley. Genome 39:492–498

Kai H, Baba T, Tsukazaki M, Uchimura Y, Furusho M (2003) The QTL analysis of hull-cracked grain in Japanese malting barley. Breed Sci 53:225–230

Kandemir N, Kudrna D, Ullrich SE, Kleinhofs A (2000) Molecular marker assisted genetic analysis of head shattering in six-rowed barley. Theor Appl Genet 101:203–210

Kaneko T, Zhang W, Takahashi H, Ito K, Takeda K (2001) QTL mapping for enzyme activity and thermostability of beta-amylase in barley (*Hordeum vulgare* L.). Breed Sci 51:99–195

Karakousis A, Barr AR, Kretschmer JM, Manning S, Logue SJ, Logue SJ, Li C-D, Lance RCM, Langridge P (2003a) Mapping and QTL analysis of the barley population Galleon × Haruna Nijo. Aust J Agri Res 54:1131–1135

Karakousis A, Barr AR, Kretschmer JM, Manning S, Jefferies SP, Chalmers KJ, Islam AKM, Langridge P (2003b) Mapping and QTL analysis of the barley population Clipper × Sahara. Aust J Agri Res 54:1137–1140

Karakousis A, Gustafson JP, Chalmers KJ, Barr AR, Langridge P (2003c) A consensus map of barley integrating SSR, RFLP, and AFLP markers. Aust J Plant Physiol 54:1173–1185

Karsai I, Mészáros K, Bedö Z, Hayes PM, Pan A, Chen F (1997) Genetic analysis of the components of winterhardiness in barley (*Hordeum vulgare* L.). Acta Biol Hung 48:67–76

Kasha KJ, Kao KN (1970) High frequency of haploid production in barley (*Hordeum vulgare* L.). Nature 225:874–876

Kasha KJ, Kleinhofs A (1994) North American Barley Genome Project Mapping of the barley cross Harrington × TR306. Barley Genet Newslett 23:65–69

Kicherer S, Backes G, Walther U, Jahoor A (2000) Localising QTLs for leaf rust resistance and agronomic traits in barley (*Hordeum vulgare* L.). Theor Appl Genet 100:881–888

Kikuchi S, Taketa S, Ichii M, Kawasaki S (2003) Efficient fine mapping of the naked caryopsis gene (*nud*) by HEGS (High Efficiency Genome Scanning)/AFLP in barley. Theor Appl Genet 108 (1):73–78

Kislev ME, Nadel D, Carmi I (1992) Grain and fruit diet 19,000 years old at Ohalo II, Sea of Galilee. Israel Rev Paleobot Palynol 73:161–166

Kjær B, Jensen J (1996) Quantitative trait loci for grain yield and yield components in a cross between a six-rowed and a two-rowed barley. Euphytica 90:39–48

Kjær B, Haahr V, Jensen J (1991) Associations between 23 quantitative traits and 10 genetic markers in a barley cross. Plant Breed 106:261–274

Kjær BJ, Jensen J, Giese H (1995) Quantitative trait loci or heading date and straw characters in barley. Genome 38:1098–1104

Kleinhofs A, Kilian A, Maroof MAS, Biyashev RM, Hayes PM, Chen FQ, Lapitan N, Fenwick A, Blake TK, Kanazin V, Ananieve E, Dahleen L, Kudrna D, Bollinger J, Knapp SJ, Liu B, Sorrells ME, Heun M, Franckowiak JD, Hoffman D, et al (1993) A molecular, isozyme and morphological map of the barley (*Hordeum vulgare*) genome. Theor Appl Genet 86:705–712

Kleinhofs A, Kilian A, Kudrna D, North American Barley Genome Project (1994) The NABGMP Steptoe × Morex Mapping progress report. Barley Genet Newslett 23:79–83

Komatsuda T, Mano Y (2002) Molecular mapping of the intermedium spike-c (*int-c*) and non-brittle rachis 1 (*btr1*) loci in barley (*Hordeum vulgare* L.). Theor Appl Genet 105:85–90

Komatsuda T, Annaka T, Oka S (1993) Genetic mapping of a quantitative trait locus (QTL) that enhances the shoot differentiation rate in *Hordeum vulgare* L. Theor Appl Genet 86:713–720

Komatsuda T, Kawasaki S, Nakamura I, Takaiwa F, Taguchi-Shiobara F, Oka S (1997) Identification of random amplified polymorphic DNA (RAPD) markers linked to the *nu* locus in barley, *Hordeum vulgare* L. Theor Appl Genet 95:637–642

Körber-Grohne U (1987) Spargelerbse, Spargelschote (*Tetragonolobus purpureus* Moench = *Lotus tetragonolobus* L. = *Lotus edulis* L.). Nutzpflanzen in Deutschland, Kulturgeschichte und Biologie, Konrad Theiss, Stuttgart, pp 436–437

Korzun L, Künzel G (1996) Integration of translocation breakpoints of barley chromosomes 3 and 7 into the Igri/Franka derived RFLP maps. Barley Genet Newslett 25:13–16

Kraakman ATW, Niks RE, Van den Berg PMMM, Stam P, van Eeuwijk F (2004) Linkage disequilibrium mapping of yield and yield stability in modern spring barley cultivars. Genetics 168:435–446

Kretschmer JM, Chalmers KJ, Manning S, Karakousis A, Barr AR, Islam AKMR, Logue SJ, Choe YW, Barker SJ, Lance RCM, Langridge P (1997) RFLP mapping of the *Ha2* cereal cyst nematode resistance gene in barley. Theor Appl Genet 94(8):1060–1064

Ladizinsky G (1999) Plant Evolution under Domestication. Kluwer, Dordrecht

Lahaye T, Shirasu K, Schulze-Lefert P (1998) Chromosome landing at the barley *Rar1* locus. Mol Gen Genet 260(1):92–101

Lakev B, Semane Y, Alemayehu F, Gehre H, Grando S, van Leur AJ, Ceccarelli S (1997) Exploiting and diversity in barley landraces in Ethiopia. Genet Resource Crop Evol 44:2

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Langridge P, Karakousis A, Kretschmer J, Collins N, Manning S (1995) A consensus linkage map of barley. Mol Breed 1:389

Laurie DA, Pratchett N, Romero C, Simpson E, Snape JW (1993) Assignment of the denso dwarfing gene to the long arm of chromosome 3(3H) of barley by use of RFLP markers. Plant Breed 111:198–203

Laurie DA, Pratchett N, Bezant JH, Snape JW (1995) RFLP mapping of 5 major genes and 8 quantitative trait loci controlling flowering time in a winter × spring barley (*Hordeum vulgare* L.) cross. Genome 38:575–585

Le Gouis J, Devaux P, Werner K, Hariri D, Bahrman N, Beghin D, Ordon F (2004) *rym15* from the Japanese cultivar Chikurin Ibaraki 1 is a new barley mild mosaic virus (BaMMV) resistance gene mapped on chromosome 6H. Theor Appl Genet 108(8):1521–1525

Lehmann, LC, Jonsson R, Gustafsson M (1998) Identification of resistance genes to powdery mildew isolated from *Hordeum vulgare* ssp. *spontaneum* and land races of barley. J Swedish Seed Assoc 108:94–101

Lev-Yadun S, Gopher A, Abbo S (2000) The cradle of agriculture. Science 288:1602–1603

Li C-D, Lance RCM, Collins HM, Tarr A, Harasymow S, Cakir M, Fox GP, Young KJ, Raman H, Barr AR, Moody DB, Read BJ (2003a) Quantitative trait loci controlling kernel discoloration in barley (*Hordeum vulgare* L.). Aust J Agric Res 54:1251–1259

Li C-D, Tarr A, Lance RCM, Harasymow S, Uhlmann J, Westcot, Young KJ, Grime CR, Cakir M, Broughton S, Apples R (2003b) A major QTL controlling seed dormancy and pre-harvest sprouting/grain alpha-amylase in two-rowed barley (*Hordeum vulgare* L.). Aust J Agric Res 54:1303–1313

Lisitsina GN (1984) The Caucasus – a centre of ancient farming in Eurasia. In: van Zeist W, Casparie WA (eds) Plants and Ancient Man. Balkema, Rotterdam, The Netherlands, pp 285–292

Liu BH, Knapp SJ (1990) GMENDEL: A program for Mendelian segregation and linkage analysis of individual and multiple progeny populations using log-likelihood ratios. J Hered 81:407

Long NR, Jefferies SP, Karakousis A, Kretschmer JM, Hunt C, Lim P, Eckermann PJ, Barr AR (2003) Mapping and QTL analysis of the barley population Mundah x Keel. Aust J Plant Physiol 54:1163–1171

Löve A (1984) Conspectus of the Triticeae. Feddes Report 95:425–521

Lyngkjær MF, Newton AC, Atzema JL, Baker SJ (2000) The Barley *mlo*-gene: an important powdery mildew resistance source. Agronomie 20:745–756

Ma Z, Steffenson BJ, Prom LK, Lapitan NLV (2000) Mapping of quantitative trait loci for Fusarium head blight resistance in barley. Phytopathology 90:1079–1088

Ma ZQ, Lapitan NLV, Steffenson B (2004) QTL mapping of net blotch resistance genes in a doubled-haploid population of six-rowed barley. Euphytica 37:291–296

Madsen LH, Collins NC, Rakwalska M, Bakes G, Sandal N, Krusell L, Jensen J, Waterman EH, Jahoor A, Ayliffe M, Pryor AJ, Langridge P, Schulze-Lefert P, Stougaard J (2003) Barley disease resistance gene analogs of the NBS-LRR class: identification and mapping. Mol Genet Genom 269:150–161

Mammadow JA, Zwonitzer JC, Biyashev R, Griffey CA, Jul Y, Steffenson B, Maroof MAS (2003) Molecular mapping of leaf rust resistance gene *Rph5* in barley. Crop Sci 43:388–393

Manly KF (1993) RI Manager, a microcomputer program for analysis of data from recombinant strains. Mamm Genom 1:123–126

Manly KF, Olson JM (1999) Overview of QTL mapping software and introduction to map manager QT. Mamm Genom 10:327–334

Manly KF, Cudmore RH, Meer JM (2001) Map manager QTX, cross-platform software for genetic mapping. Mamm Genom 12:930–932

Manninen OM (2000) Associations between anther-culture response and molecular markers on chromosomes 2H, 3H and 4H of barley (*Hordeum vulgare* L.). Theor Appl Genet 100:57–62

Mano Y, Komatsuda T (2002) Identification of QTLs controlling tissue-culture traits in barley (*Hordeum vulgare* L.). Theor Appl Genet 105:708–715

Mano Y, Takahashi H, Sato K, Takeda K (1996) Mapping genes for callus growth and shoot regeneration in barley (*Hordeum vulgare* L.). Breed Sci 46:137–142

Mano Y, Takeda K (1997) Mapping quantitative trait loci for salt tolerance at germination and the seedling stage in barley (*Hordeum vulgare* L). Euphytica 94:263–272

Marquez-Cedillo LA, Hayes PM, Jones BL, Kleinhofs A, Legge WG, Rossnagel BG, Sato K, Ullrich E, Wesenberg DM (2000) QTL analysis of malting quality in barley based on the doubled-haploid progeny of two elite North American varieties representing different germplasm groups. Theor Appl Genet 101:173–184

Marthe F, Künzel G (1994) Localization of translocation breakpoimts im somatic metaphase churomosomes of barley. Theor Appl Genet 89

Mather DE, Tinker NA, LaBerge DE, Edney M, Jones BL, Rossnagel BG, Legge WG, Briggs KG, Irvine RB, Falk DE, Kasha KJ (1997) Regions of the genome that affect grain and malt quality in a North American two-row barley cross. Crop Sci 37:544–554

McDaniel MB, Hathcock BR (1969) Linkage of the Pa and Mia loci in barley. Plant Breed 104:318–324

McCallum CM, Comai L, Greene EA, Henikoff S (2000) Targeted screening for induced mutations. Nat Biotechnol 18:455–457

Mejlhede N, Kyjovska Z, Backes G, Jahoor A (2004) TILLING for Detection of Single Nucleotide Polymorphism in Resistance Genes in Barley. Proc 9th Int Barley Genet Symp, 20–26 June 2004, Brno, Czech Republic

Mesfin A, Smith KP, Dill-Macky R, Evans CK, Waugh R, Gustus CD, Muehlbauer GJ (2003) Quantitative trait loci for Fusarium head blight resistance in barley detected in a two-rowed by six-rowed population. Crop Sci 43:307–318

Mickelson S, See D, Meyer FD, Garner JP, Foster CR, Blake TK, Fischer AM (2003) Mapping of QTL associated with nitrogen storage and remobilization in barley (*Hordeum vulgare* L.) leaves. J Exp Bot 54:801–812

Miyazaki C, Osanai E, Saeki K, Hirota N, Ito K, Konishi T, Saito A (2000) A barley linkage map using an F_2 population compared with a map based on female recombination-derived doubled haploid lines. Breed Sci 50:241–250

Moharramipour S, Tsumuki H, Sato K, Yoshida H (1997) Mapping resistance to cereal aphids in barley. Theor Appl Genet 94:592–596

Molina-Cano JL, Gomez-Campo C, Conde L (1982) *Hordeum spontaneum* C. Koch as a weed of barley fields in southern Morocco. Z Pflanzenzuecht 88:161–167

Molina-Cano JL, Fra-Mon P, Salcedo G, Aragonicillo C, Roca de Togores F, Gardia-Olmedo F (1987) Morocco as a possible domestication center for barley: biochemical and agromorphological evidence. Theor Appl Genet 73:531–536

Molina-Cano JL, Moralejo E, Igartua E, Romagosa I (1999) Further evidence supporting Morocco as a centre of origin of barley. Theor AppI Genet 98:913–918

Moseman JG, Smith DH Jr. (1981) Purpose, development, utilization maintenance and status of the USDA barley collection. In: Whitehouse RNH (ed) Barley Genetics IV. University of Edinburgh Press, Edinburgh, UK, pp 67–70

Negassa M (1985) Genetics of resistance to powdery mildew in some Ethiopian barleys. Hereditas 102:123–138

Nelson JC (1997) QGENE: Software for marker-based genomic analysis and breeding. Mol Breed 3:239–245

Nevo H (1992) Origin, evolution, population genetics and resources for breeding of wild barley *Hordeum spontaneum* in the Fertile Crescent. In: Shewry PR (ed) Barley: Genetics, Biochemistry, Molecular Biology and Biotechnology. CABI, Wallingford, UK, pp 19–43

Oziel A, Hayes PM, Chen FQ, Jones B (1996) Application of quantitative trait locus mapping to the development of winter-habit malting barley. Plant Breed 115:43–51

Pallotta M, Asayama S, Reinheimer JM, Davies PA, Barr AR, Jefferies SP, Chalmers KJ, Lewis J, Collins HM, Roumeliotis S, Logue SJ, Coventry SJ, Lance RCM, Karakousis A, Lim P, Verbyla AP, Eckermann PJ (2003) Mapping and QTL analysis of the barley population Amagi Nijo × WI2585. Aust J Agric Res 54:1141–1144

Pan A, Hayes PM, Chen F, Chen THH, Blake T, Wright S, Karsai I, Bedö Z (1994) Genetic analysis of the components of winterhardiness in barley (*Hordeum vulgare* L.). Theor Appl Genet 89:900–910

Park R, Poulsen D, Barr AR, Cakir M, Moody DB, Raman H, Read BJ (2003) Mapping genes for resistance to *Puccinia hordei* in barley. Aust J Agri Res 54:1323–1333

Park RF, Karakousis A (2002) Characterization and mapping of gene *Rphl9* conferring resistance to *Puccinia hordei* in the cultivar 'Reka 1' and several Australian barleys. Plant Breed 121:232–236

Parlevliet IB (1976) The genetis of seedling resistance to leaf rust, *Puccinia hordci* Otth. in some spring barley cultivars. Euphytica 25:249–254

Paterson AH, Lander ES, Hewitt J D, Peterson S, Lincoln SE, Tanksley SD (1988) Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. Nature 335:721–726

Patil V (2001) Genetics of *Rhynchosporium secalis* (Oud.) JJ. Davis resistance in barley (*Hordeum vulgare* L.). PhD Thesis, Agricultural University of Norway, Ås, Norway

Patil V, Bjørnstad A, MacKey J (2003) Molecular mapping of a new gene *Rrs4c11549* for resistance to barley scald (*Rhynchosporium secalis*). Mol Breed 12:169–183

Pecchioni N, Faccioli P, Toubia-Rahme H, Vale G, Terzi V (1996) Quantitative resistance to barley leaf stripe (*Pyrenophora graminea*) is dominated by one major locus. Theor Appl Genet 93:97–101

Pecchioni N, Vale G, Toubia-Rahme H, Faccioli P, Terzi V, Delogu G (1999) Barley-*Pyrenophora graminea* interaction: QTL analysis and gene mapping. Plant Breed 118:29–35

Peterhänsel C, Freialdenhoven A, Kurth J, Kolsch R, Schulze-Lefert P (1997) Interaction analyses of genes required for resistance responses to powdery mildew in barley reveal distinct pathways leading to leaf cell death. Plant Cell 9:1397–1409

Pickering RA (1984) The influence of genotype and environment on chromosome elimination in crosses between *Hordeum vulgare* × *H. bulbosum*. Plant Sci 34:153–164

Pickering R (2000) Do the wild relatives of cultivated barley have a place in barley improvement? In: Barley Genetics VIII. Proc 8th Intl Barley Genet Symp, Adelaide, Australia, pp 223–230

Pillen K, Binder A, Kreuzkam B, Ramsay L, Förster J, Léon J (2000) Mapping new EMBL-derived barley microsatellites and their use in differentiating German barley cultivars. Theor Appl Genet 101:652–660

Pillen K, Zacharias A, Léon J (2003) Advanced backcross QTL analysis in barley (*Hordeum vulgare* L.). Theor Appl Genet 107:340–352

Poehlman JM (1959) Breeding Field Crops. Holl, New York, pp 22–50, 150–173

Prada D, Ullrich SE, Molina-Cano JL, Cistué L, Clancy JA, Romagosa I (2004) Genetic control of dormancy in a Triumph/Morex cross in barley. Theor Appl Genet 109:62–70

Qi X, Stam P, Lindhout P (1996) Comparison and integration of four barley genetic maps. Genome 39:379–394

Qi X, Stam P, Lindhout P (1998a) Use of locus-specific AFLP markers to construct a high density molecular map in barley. Theor Appl Genet 96:376–384

Qi X, Niks RE, Stam P, Lindhout P (1998b) Identification of QTLs for partial reistance to leaf rust (*Puccinia hordei*) in barley. Theor Appl Genet 96:1205–1215

Qi X, Jiang G, Chen W, Niks RE, Stam P, Lindhout P (1999) Isolate-specific QTLs for partial resistance to *Puccinia hordei* in barley. Theor Appl Genet 99:877–884

Qi X, Fufa F, Sijtsma D, Niks RE, Lindhout P, Stam P (2000) The evidence for abundance of QTLs for partial resistance to *Puccinia hordei* on the barley genome. Mol Breed 6:1–9

Rajasekaran P, Thomas WTB, Wilson A, Lawrence P, Young G, Ellis RP (2004) Genetic control over grain damage in a spring barley mapping population. Plant Breed 123:17–23

Raman H, Barbara JR (1999) Efficient marker assisted selection for resistance to barley yellow dwarf virus using leaf tissue and sap as templates in barley. Barley Genet Newslett 29:8–13

Raman H, Platz GJ, Chalmers KJ, Raman R, Read BJ, Barr AR, Moody DB (2003) Mapping of genomic regions associated with net form of net blotch resistance in barley. Aust J Agric Res 54:1359–1367

Ramsay L, Macaulay M, Ivanissevich SD, MacLean K, Cardle L, Fuller J, Edwards KJ, Tuvesson S, Morgante M, Massari A, Maestri E, Marmiroli N, Sjakste T, Ganal M, Powell W, Waugh R (2000) A simple sequence repeat-based linkage map of barley. Genetics 156:1997–2005

Read BJ, Raman H, McMichael G, Chalmers KJ, Ablett GA, Platz GJ, Raman R, Genger RK, Boyd JR, Li C-D, Grime CR, Park RF, Wallwork H, Prangnell R, Lance RCM (2003) Mapping and QTL analysis of the barley population Sloop × Halcyon. Aust J Plant Physiol 54:1145–1153

Roane CW, Starling TM (1967) Inheritance of reaction to *Puccinia hordei* in barley. II. Gene symbols for loci in differential cultivars. Phytopathology 57:66–68

Ruge B, Linz A, Pickering R, Proeseler G, Greif P, Wehling P (2003) Mapping of Rym14Hb, a gene introgressed from Hordeum bulbosum and conferring resistance to BaMMV and BaYMV in barley. Theor Appl Genet 107:965–971

Saeki K, Miyazaki C, Hirota N, Saito A, Ito K, Konishi T (1999) RFLP mapping of BaYMV resistance gene rym3 in barley (Hordeum vulgare). Theor Appl Genet 99 (3–4):727–732

Salvo-Garrido H, Laurie DA, Jaffe B, Snape JW (2001) An RFLP map of diploid *Hordeum bulbosum* L. and comparison with maps of barley (*H. vulgare* L.) and wheat (*Triticum aestivum* L.). Theor Appl Genet 103:869–880

Sanguineti MC, Tuberosa R, Stefanelli S, Noli E, Blake TK, Hayes PM (1994) Utilization of a recombinant inbred population to localize QTLs for abscisic-acid content in leaves of drought-stressed barley (*Hordeum vulgare* L.). Russ J Plant Physiol 41:572–576

Sayed H, Backes G, Kayyal H, Yahyaoui A, Ceccarelli S, Grando S, Jahoor A, Baum M (2004) New molecular markers linked

to qualitative and quantitative powdery mildew and scald resistance genes in barley for dry areas. Euphytica 135:225–228

Scheurer KS, Friedt W, Huth W, Waugh R (2001) QTL analysis of tolerance to a German strain of BYDV-PAV in barley (*Hordeum vulgare* L.). Theor Appl Genet 103:1074–1083

Schönfeld M, Ragni A, Fischbeck G, Jahoor A (1996) RFLP mapping of three new loci for resistance genes to powdery mildew (*Erysiphe graminis* f. sp. *hordei*) in barley. Theor Appl Genet 93:48–56

Schwarz G, Michalek W, Mohler V, Wenzel G, Jahoor A (1999) Chromosome landing at the *Mla* locus in barley (*Hordeum vulgare* L.) by means of high-resolution mapping with AFLP markers. Theor Appl Genet 98:521–530

Schüller C, Backes G, Fischbeck G, Jahoor A (1992) RFLP markers to identi the alleles on the *Mla* locus conferring powdery mildew resistance in barley. Theor Appl Genet 84:330–338

Schweizer GF, Baumer M, Daniel G, Rugel H, Röder MS (1995) RFLP markers linked to scald (*Rhynchosporium secalis*) resistance gene *Rh2* in barley. Theor Appl Genet 90:920–924

See D, Kanazin V, Kephart K, Blake T (2002) Mapping genes controlling variation in barley grain protein concentration. Crop Sci 42:680–685

Shen QH, Zhou FS, Bieri S, Haizel T, Shirasu K, Schulze-Lefert P (2003) Recognition specificity and *RAR1/SGT1* dependence in barley *Mla* disease resistance genes to the powdery mildew fungus. Plant Cell 15(3):732–744

Shirasu K, Lahaye T, Tan MW, Zhou FS, Azevedo C, Schulze-Lefert P (1999) A novel class of eukaryotic zinc-binding proteins is required for disease resistance signaling in barley and development in C-elegans. Cell 99(4):355–366

Smith BD (1995) The Emergence of Agriculture. Scientific American Library, New York

Smith KP, Evans CK, Dill-Macky R, Gustus C, Dong Y (2004) Host genetic effect on deoxynivalenol accumulation in fusarium head blight of barley. Phytopathology 94:766–771

Sorokin A, Marthe F, Künzel G (1995) Integration of 37 translocation breakpoints of barley chromosome 5 into the Igri/Franka derived RFLP map. Barley Genet Newslett 24:108–112

Stam P (1993) Construction of integrated genetic-linkage maps by means of a new computer package – JoinMap. Plant J 3:739–744

Steffenson BJ, Hayes PM, Kleinhofs A (1996) Genetics of seedling and adult plant resistance to net blotch (*Pyrenophora teres* f. *teres*) and spot blotch (*Cochliobolus sativus*) in barley. Theor Appl Genet 92:552–558

Straub PF, Shen Q, Ho TD (1994) Structure and promoter analysis of an ABA- and stress-regulated barley gene, *HVA1*. Plant Mol Biol 26(2):617–630

Subrahmanyam NC, von Bothmer R (1987) Interspecific hybridization with *Hordeum bulbosum* and development of hybrids and haploids. Hereditas 106:119–127

Tacconi G, Cattivelli L, Faccini N, Pecchioni N, Stanca AM, Vale G (2001) Identification and mapping of a new leaf stripe resistance gene in barley (*Hordeum vulgare* L.). Theor Appl Genet 102:1286–1291

Takahashi R (1955) The origin and evolution of cultivated barley. Advances in Genetics 7. Academic, New York, pp 227–266

Takahashi R (1987) Genetic features of East Asian barleys. In: Barley Genetics V. Proc 5th Int Barley Genet Symp, Okayama, Japan, pp 7–20

Takahashi R, Hayashi J (1964) Linkage study of two complementary genes for brittle rachis in barley. Ber Ohara Inst Landw Biol Okayama Univ 12:99–105

Takahashi R, Yamamoto J (1949) Studies on the classification and the geographic distribution of barley varieties, VIII. Nogaku Kenkyu 38:81–90 (in Japanese)

Takahashi R, Yasuda S (1956) Genetic studies of spring and winter habit of growth in barley. Ber Ohara Inst Landw Biol Okayama Univ 10:245–308

Takahashi R, Yasuda S (1958) Genetic studies on heading date in barley. In: Sakai K, Takahashi R, Akemine H (eds) Studies on the Bulk Method of Plant Breeding. Yokendo, Tokyo, pp 44–64 (in Japanese)

Takahashi R, Hayashi J, Yasuda S, Hiura U (1963) Characteristics of the wild and cultivated barleys from Afghanistan and its neighboring regions. Ber Ohara Inst Landw Biol Okayama Univ 12:1–23

Takahashi R, Hayashi J, Hiura U, Yasuda S (1968) A study of cultivated barleys from Nepal, Himalaya and North India with special reference to their phylogenetic differentiation. Ber Ohara Inst Landw Biol Okayama Univ 14:85–122

Takahashi R, Yasuda S, Hayashi J, Fukuyama T, Moriya I, Konishi T (1983) Catalogue of Barley Germplasm Preserved in Okayama University, Japan

Takahashi H, Sato K, Takeda K (2001) Mapping genes for deep-seeding tolerance in barley. Euphytica 122:37–43

Takeda K (1995) Varietal variation and inheritance of seed dormancy in barley. In: Nods K, Mares DJ (eds) Pre-harvest Sprouting in Cereals. Center for Academic Societies, Osaka, Japan, pp 205–212

Taketa S, Takahashi H, Takeda K (1998) Genetic variation in barley of crossability with wheat and its quantitative trait loci analysis. Euphytica 103:187–193

Talame V, Sanguineti MC, Chiapparino E, Bahri H, Ben Salem M, Forster BP, Ellis RP, Rhouma S, Zoumarou W, Waugh R, Tuberosa R (2004) Identification of *Hordeum spontaneum* QTL alleles improving field performance of barley grown under rainfed conditions. Ann Appl Biol 144:309–319

Tanno K, Takaiwa F, Ota S, Komatsuda T (1999) A nucleotide sequence linked to the *vrsl* locus for studies of differentiation in cultivated barley (*Hordeum vulgare* L.). Hereditas 130:77–82

Tanno K, Taketa S, Takeda K, Komatsuda T (2002) A DNA marker closely linked to the *vrsl* locus (row type gene)

indicates multiple origins of six-rowed cultivated barley (*Hordeum vulgare* L.). Theor Appl Genet 104:54–60

Teulat B, This D, Khairallah M, Borries C, Ragot C, Sourdille P, Leroy P, Monneveux P, Charrier A (1998) Several QTLs involved in osmotic adjustment trait variation in barley (*Hordeum vulgare* L.). Theor Appl Genet 96:688–698

Teulat B, Borries C, This D (2001a) New QTLs identified for plant water status, water-soluble carbohydrate and osmotic adjustment in a barley population grown in a growth-chamber under two water regimes. Theor Appl Genet 103:161–170

Teulat B, Merah O, Souyris I, This D (2001b) QTLs for agronomic traits from a Mediterranean barley progeny grown in several environments. Theor Appl Genet 103:774–787

Teulat B, Merah O, Sirault X, Borries C, Waugh R, This D (2002) QTLs for grain carbon isotope discrimination in field-grown barley. Theor Appl Genet 106:118–126

Teulat B, Zoumarou-Wallis N, Rotter B, Ben Salem M, This D (2003) QTL for relative water content in field-grown barley and their stability across Mediterranean environments. Theor Appl Genet 108:181–188

Thomas WTB, Powell W, Waugh R, Chalmers KJ, Barua UM, Jack P, Lea V, Forster BP, Swanston JS, Ellis RP, Hanson PR (1995) Detection of quantitative trait loci for agronomic, yield, grain and disease characters in spring barley (*Hordeum vulgare* L.). Theor Appl Genet 91:1037–1047

Thomas WTB, Powell W, Swanston JS, Ellis RP, Chalmers KJ, Barua UM, Jack P, Lea V, Forster BP, Waugh R, Smith DB (1996) Quantitative trait loci for germination and malting quality characters in a spring barley cross. Crop Sci 36:265–273

Thomas WTB, Baird E, Fuller JD, Lawrence P, Young GR, Russell J, Ramsay L, Waugh R, Powell W (1998) Identification of a QTL decreasing yield in barley linked to *Mlo* powdery mildew resistance. Theor Appl Genet 4:381–393

Thomsen SB, Jensen HP, Jensen J, Skou JP, Jørgensen JH (1997) Localization of a resistance gene and identification of sources of resistance to barley leaf stripe. Plant Breed 116:455–459

Tinker NA, Mather DE (1995) MQTL: software for simplified composite interval mapping of QTL in multiple environments. J Agric Gen 1. http://www.cabi-publishing.org/jag/index.html

Tinker NA, Mather DE, Rossnagel BG, Kasha KJ, Kleinhofs A, Hayes PM, Falk DE, Ferguson T, Shugar LP, Legge WG, Irvine RB, Choo TM, Briggs KG, Ullrich SE, Franckowiak JD, Blake TK, Graf RJ, Dofing SM, Maroof MAS, Scoles GJ, et al (1996) Regions of the genome that affect agronomic performance in two-row barley. Crop Sci 36:1053–1062

Toojinda T, Broers LH, Chen XM, Hayes PM, Kleinhofs A, Korte J, Kudrna D, Leung H, Line RF, Powell W, Ramsay L, Vivar H, Waugh R (2000) Mapping quantitative and qualitative disease resistance genes in a doubled haploid population of barley (*Hordeum vulgare*). Theor Appl Genet 101:580–589

Tuleen NA, McDaniel ME (1971) Location of genes *P* and *Pa5*. Barley Newslett 15:106–107

Tuvesson S, von Post L, Ohlund R, Hagberg P, Graner A, Svitashev S, Schehr M, Elovsson R (1998) Molecular breeding for the BaMMV/BaYMV resistance gene *ym4* in winter barley. Plant Breed 117(1):19–22

Ukai Y, Ohsawa A, Saito A, Hayashi T (1995) MAP – a package of computer programs for construction of DNA polymorphism linkage maps and analysis of QTL. Breed Sci 45:139–142

Utz HF, Melchinger AE (1996) PLABQTL: A program for composite interval mapping of QTL. J Agric Gen 2. http://www.cabi-publishing.org/jag/index.html

Van Oijen J, Maliepaard C (1996) MapQTL™ version 3.0: Software for the calculation of QTL position on genetic maps. CPRO-DLO, Wageningen, The Netherlands

Van Rijn CPE, Heersche I, Van Berkel YEM, Nevo E, Lambers H, Poorter H (2000) Growth characteristics in *Hordeum sportaneum* populations from different habitats. New Phytol 146:471–481

Vaz Patto M, Rubiales D, Martín A, Hernàndez P, Lindhout P, Niks RE, Stam P (2003) QTL mapping provides evidence for lack of association of the avoidance of leaf rust in *Hordeum chilense* with stomata density. Theor Appl Genet 106:1283–1292

Verbyla AP, Eckermann PJ, Thompson R, Cullis BR (2003) The analysis of quantitative trait loci in multi-environment trials using a multiplicative mixed model. Aust J Agric Res 54:1395–1408

Verhoeven KJF, Vanhala TK, Biere A, Nevo E, van Damme JMM (2004a) The genetic basis of adaptive population differentiation: A quantitative trait locus analysis of fitness traits in two wild barley populations from contrasting habitats. Evolution 58:270–283

Verhoeven KJF, Biere A, Nevo E, van Damme JMM (2004b) Can a genetic correlation with seed mass constrain adaptive evolution of seedling desiccation tolerance in wild barley? Int J Plant Sci 165:281–288

von Bothmer R, Flink J, Jacobsen N, Kotiniaki M, Landstrorn T (1983) Interspecific hybridization with cultivated barley. Hereditas 99:219–244

von Bothmer R, Jacobsen N, Baden C, Jørgensen RB, Linde-Laursen I (1995) An ecogeographical study of the genus *Hordeum*. Systematic and Ecogeographic Studies on Crop Genepools 7, 2nd edn. IPGRI, Rome

von Bothmer R, Linde-Laursen I (1989) Backcrosses to cultivated barley (*Hordeum vulgare* L.) and partial elimination of alien chromosomes. Hereditas 111:145–147

Wei FS, Gobelman-Werner K, Morroll SM, Kurth J, Mao L, Wing R, Leister D, Schulze-Lefert P, Wise RP (1999) The *Mla* (powdery mildew) resistance cluster is associated with three NBS-LRR gene families and suppressed recombination within a 240-kb DNA interval on chromosome 5S (1HS) of barley. Genetics 153(4):1929–1948

Weibull J, Walther U, Sato K (2003) Diversity in resistance to biotic stresses. In: von Bothmer R, Hintum T von, Knüpffer H, Sato K (eds) Diversity in barley (*Hordeum vulgare*). Elsevier, Amsterdam, pp 143–178

Werner K, Pellio B, Ordon F, Friedt W (2000) Development of an STS marker and SSRs suitable for marker-assisted selection for the BaMMV resistance gene *rym9* in barley. Plant Breed 119 (6):517–519

Werner K, Ronicke S, Le Gouis J, Friedt W, Ordon F (2003) Mapping of a new BaMMV-resistance gene derived from the variety 'Taihoku A'. J Plant Dis Protect 110 (3):304–311

Wiberg A (1974) Genetical studies of spontaneous sources of resistance to powdery mildew. Hereditas 77:89–148

Williams KJ, Lichon A, Gianquitto P, Kretschmer JM, Karakousis A, Manning S, Langridge P, Wallwork H (1999) Identification and mapping of a gene conferring resistance to the spot form of net blotch (*Pyrenophora teres* f. *maculata*) in barley. Theor Appl Genet 99:323–327

Williams KJ, Platz GJ, Barr AR, Cheong J, Willsmore K, Cakir M, Wallwork H (2003) A comparison of the genetics of seedling and adult plant resistance to the spot form of net blotch (*Pyrenophora teres* f. *maculata*). Aust J Agric Res 54:1387–1394

Wolter M, Hollricher K, Salamini F, Schulze-Lefert P (1993) The *mlo* resistance alleles to powdery mildew infection in barley trigger a developmentally controlled defense mimic phenotype. Mol Gen Genet 239:122–128

Yasuda S (1992) Differentiation and geographical distribution of spring genes in barley. Iden 46:26–30 (in Japanese)

Yasuda S, Hayashi J, Moriya I (1993) Genetic constitution for spring growth habit and some other characters in barley cultivars in the Mediterranean coastal regions. Euphytica 70:77–83

Yin X, Kropff MJ, Stam P (1999a) The role of ecophysiological models in QTL analysis: the example of specific leaf area in barley. Heredity 82:415–421

Yin XY, Stam P, Dourleijn CJ, Kropff MJ (1999b) AFLP mapping of quantitative trait loci for yield-determining physiological characters in spring barley. Theor Appl Genet 99:244–253

Yin X, Chasalow SD, Stam P, Kropff MJ, Dourleijn CJ, Bos I, Bindraban PS (2002) Use of component analysis in QTL mapping of complex crop traits: a case study on yield in barley. Plant Breed 121:314–319

Yu Y, Tomkins JP, Waugh R, Frisch DA, Kudrna D, Kleinhofs A, Brueggeman RS, Muehlbauer GJ, Wise RP, Wing RA (2000) A bacterial artificial chromosome library for barley (*Hordeum vulgare* L.) and the identification of clones containing putative resistance genes Theor Appl Genet 101:1093–1099

Zhong S, Effertz RJ, Jin Y, Franckowiak JD, Steffenson BJ (2003) Molecular mapping of the leaf rust resistance gene *Rph6* in barley and its linkage relationships with *Rph5* and *Rph7*. Phytopathology 93:604–609

Zhou FS, Kurth JC, Wei FS, Elliott C, Vale G, Yahiaoui N, Keller B, Somerville S, Wise R, Schulze-Lefert P (2001) Cell-autonomous expression of barley *Mla1* confers race-specific resistance to the powdery mildew fungus via a *Rar1*-independent signaling pathway. Plant Cell 13 (2):337–350

Zhu H, Briceño G, Dovel R, Hayes PM, Liu BH, Liu CT (1999) Molecular breeding for grain yield in barley: an evaluation of QTL effects in a spring barley cross. Theor Appl Genet 98:772–779

Zohary D, Hopf M (1993) Domestication of Plants in the Old World: the Origin and Spread of Cultivated Plants in West Asia, Europe, and the Nile Valley, 2nd edn. Clarendon, Oxford

Zwickert-Menteur S, Jestin L, Branlard G (1996) *Amy2* polymorphism as a possible marker of beta-glucanase activity in barley (*Hordeum vulgare* L). J Cereal Sci 24:55–63

5 Oat

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5.1 Introduction

The primary focus of this review is genome mapping and molecular breeding in oat. We begin with a brief history of the crop and summaries of the biology of the species and descriptions of the traits that guide oat breeding to provide context for these discussions. More thorough coverage of background areas can be found in oat monographs and review articles (e.g., Rajhathy and Thomas 1974; Baum 1977; Marshall and Sorrells 1992; Welch 1995; Holland 1997) as well as original reports cited in this review.

5.1.1 Brief History and Biology of Oat

Oat is a cereal grain of the family Gramineae (Poaceae) with unknown center of origin, but likely in the Mediterranean basin or the Middle East (Murphy and Hoffman 1992). It occurs at three ploidy levels, diploid, tetraploid, and hexaploid, with a base chromosome number of 7. Species numbers and names have varied depending on the classifier and the criteria used to delineate taxa (reviewed by Baum 1977). The primary cultivated oat (*A. sativa*) is a hexaploid of $2n = 6x = 42$ originating as an aggregation of three diploid genomes (AA, CC, DD). Two distinct genome types of diploid species occur, AA and CC. The D genome of the hexaploid is quite similar to the A genome and is probably only a recent variant of it. No extant DD genome diploids have been identified. There are 15 or 16 A genome diploid species, depending on the classifier (Leggett and Thomas 1995). The A genome diploids have been placed into subgroups such as A_s, A_l, and A_c based on interfertility and degree of pairing in F_1 hybrids made between them. The C genome diploids are a group of three species with two sub-groups, C_p comprised of two species, *A. clauda* and *A. eriantha*, and C_v comprised of one species *A. ventricosa*. The members of the C genome group are genetically isolated from the A genome group and distinct in their cytoplasmic ribulose diphosphate carboxylase form (Steer 1975), chloroplast DNA (Murai and Tsunewaki 1987), and high heterochromatic chromosome constitution (Fominaya et al. 1988). The diploids include one primary domesticated form, the A genome species *A. strigosa*, which is grown as a forage, primarily in northern Europe and in temperate regions of South America. Cultivated diploid oats are often referred to as "black oats" because the commonly grown type is a dark hulled variety "Saia", of which there are numerous versions. These diploid black oats cover several hundred thousand hectares, particularly in southern Brazil in the winter months.

There are three distinct types of tetraploid oat species. In the first, AABB, the B genome chromosomes are quite similar to the A genome chromosomes, suggestive of an autopolyploid origin. However, the species show strictly disomic inheritance (Leggett 1992) and a satellite DNA sequence has been reported to provide discrimination between two sets of constituent chromosomes (Irigoyen et al. 2001). The AABB species include *A. barbata*, a common weedy species in semitemperate regions, as well as the nonshattering *A. abyssinica*, which was domesticated from *A. vaviloviana* in the Ethiopian highlands and is grown only there, mainly as a contaminant in barley fields (Ladizinsky 1988). The AACC group, likely progenitors of the AACCDD hexaploids, include two species in the western Mediterranean region, *A. maroccana (magna)* and *A. murphyi*, and the fairly recently discovered *A. insularis*; the latter is thought to be closer to the actual progenitor of hexaploid oat because of higher interspecific chromo-

some pairing frequencies in F_1 hybrids with hexaploid oat (Ladizinsky 1998). The third type of oat tetraploid, *A. machrostachya*, is an outlier in that it is the only perennial *Avena* species. It is also cross-fertilizing, allotetraploid in nature, and shows only a distant relationship to AA and CC genome diploids (Leggett and Thomas 1995).

The major hexaploid species include the winter-habit weedy species *A. sterilis*, the spring habit weedy species *A. fatua*, and the cultivated species *A. sativa*. The generally winter habit cultivated oats originating in southwestern Europe have been placed as a separate red oat species, *A. byzantina* C. Koch, by some taxonomists; however, they are highly interfertile with other *A. sativa*, and intercrossing by breeders has made the two groupings less distinct. Ladizinsky and Zohary (1971) suggested all hexaploid oats should be considered a single species, *A. sativa*, because of their high interfertility. For practical purposes, though, oat researchers have retained the separation of the cultivated and weedy hexaploid oat species based on their important differences in seed disarticulation. While most oat "seeds" are characterized by the retention of the lemma and palea, termed the "hull", surrounding the caryopsis, termed the "groat", there is a variant or hulless oat also sometimes referred to as "naked" oat. Because of the distinct free-threshing nature of the oat caryopsis in the hulless oat, it was initially classified as a separate species, *A. nuda* L.; however, with the realization that the hulless trait is governed primarily by a single gene with a few modifiers, hexaploid hulless oat is now considered only a variant of *A. sativa*.

The domestication of oat occurred much later than for wheat and barley and likely outside its area of diversity (Murphy and Hoffman 1992). In northern Europe nonshattering grain was probably selected from the weedy oats present as a contaminant in wheat and barley as these species were introduced and grown as cereal crops. Some of the earliest records of oat grown as a grain crop date to the period of the Roman occupation of Europe. Writings in the 18th century describe oat as being primarily a feed for horses and livestock in England and other parts of Europe but an important part of the human diet in Scotland, Wales, and Ireland. The primarily spring-habit *A. sativa* oats were brought to the United States and southern Canada as an important grain crop by northern European colonists and immigrants, and also to Australia and New Zealand, where they became an important winter season crop. The winter-habit red oats extant in Spain and Portugal were carried from there to South America and

southern North America, where they were grown as a forage crop as well as grain for horses. Hulless or naked oats apparently arose in temperate Asia and became the endemic type in northern China and surrounding areas.

Currently, oat remains an important grain and forage crop in many parts of the world grown on 13.2 million hectares with a grain production of 26.2 million metric tons in 2003 (USDA, Foreign Agricultural Service. Commodity production, supply, and disposition database http://fas.usda.gov/psd). The Russian Federation is the largest producer followed by Canada and the USA. Land area devoted to oat has fallen substantially the past several decades with oat being displaced by higher value crops, such as soybean in the USA. Also, the role of oat as a major protein source in animal feed rations has been displaced by higher protein meals of soybean and other oilseed crops. The production of oat for grain has remained more competitive in northern latitudes including the western prairie area of Canada and in northern Europe, especially Scandinavia and Finland, where there are fewer alternative crop choices. Also, oat is best adapted to these cooler areas that allow full season growth and plump grain development. The sensitivity of cultivated oat to high temperatures necessitates the growing of short-season, often less productive, varieties in warmer temperate regions where grain fill can be reduced by high mid-summer temperatures producing grain of lower quality. Winter-habit oat lacks the winter-hardiness of rye, wheat, and even barley, limiting it to regions of moderate winters such as the southern states in the USA or coastal countries of Europe. Oat grown in mild temperate and subtropical regions including the southern USA, southern Europe, India, and east-central Australia is primarily as a winter forage crop.

5.1.2
Oat Grain Composition, Other Grain Quality Factors, and Agronomic Traits and Their Relation to Breeding Objectives

Oat grain has long been recognized as a high-quality food and feed. It has the highest protein level among the cereals with 12 to 20% protein in the dehulled kernel and 9 to 15% in the whole grain, depending on genotype and environmental growth conditions (Peterson 1992). Furthermore, it has a superior amino acid profile compared with wheat, barley, or maize, with higher levels of all the essential amino acids. This

amino acid profile appears to be maintained across protein levels, thus allowing breeding and selection for higher protein content as a breeding objective for both animal feed and human nutrition.

Oat is unique among the cereals in that the majority of its oil is found in the endosperm (Peterson 1992). The concentration of oil in oat cultivars can range between 4 and 11%. Levels of 18% have been obtained in an experimental line by recurrent selection for high oil concentration but grain yield was reduced (Holland et al. 2001a). The more dense energy content of oil compared to carbohydrates makes higher-oil oats more valued in a livestock ration. There is an active project in the United Kingdom to produce a high-oil hulless oat as a premium feed for poultry (Valentine and Cowan 2004). In contrast, a low oil concentration is now often desired for use in human food because of the high caloric content of oil. The composition of oat oil is favorable for nutrition with a high proportion of unsaturated fatty acids. However, high oil content with high proportions of unsaturated fatty acids can reduce the shelf life of oat products. Heat treatment during processing to inactivate lipases and lipoxygenases reduces the problem. Still there can remain the dichotomy in oat breeding objectives of high oil for animal feed purposes vs. low oil for oats destined for human consumption.

The beta-glucan level in oat grain can lead to a similar dichotomy in breeding objectives. Beta-glucan concentrations in oat groats usually range from 3 to 6% depending on genotype and environment, but an experimental line resulting from selection with 7.1% beta-glucan has been reported (Cervantes-Martinez et al. 2001). A high level of mixed-linked beta-glucan (soluble fiber) is desired in oats for human foods. Oat beta-glucan inclusion in diets has shown positive effects both in reducing serum cholesterol levels to decrease risk for heart disease and in slowing of blood sugar increases following a meal as an important characteristic of dietary control of type II diabetes (Peterson 2004). In contrast, beta-glucan in higher amounts can be detrimental to digestibility and utilization efficiency in poultry and livestock feeds. Thus, a high level of beta-glucan is desirable for oat for human consumption but a low level for livestock feed.

Tocols and avenanthramides are secondary metabolite compounds found in oat grain which are of interest for their possible healthful effects in a diet (Peterson 2004). They are considered primarily as antioxidants but they may also have other beneficial activi-

ties. Variation in their levels due to both genotype and environmental growth conditions of grain production has been documented, with the avenanthramides found to be highly induced in pathogen-stress conditions. However, no efforts have yet been reported to breed for altered levels of these compounds as their function and value are still under study.

The groat-to-hull ratio, usually expressed as percent dehulled grain (groat) weight of total grain weight, is a primary determining factor in grain quality, whether for feed or milling purposes. The groat contains almost all the nutritive value, and the hull is often considered of little value or even a negative component in feed or processing. Groat percent in field-harvested grain usually runs 70 to 75% depending on genotype and growth conditions, but it can be much lower in biotic or abiotic stress growth environments and up to 80% with certain genotypes in ideal crop growth conditions. Hulless or naked oat varieties can be and have been bred. The status of this commodity has been recently reviewed by Burrows (2004). However, because of the soft texture of the oat kernel or groat (e.g., compared to a wheat kernel) and the associated higher susceptibility to weathering and discoloration, saprophytic fungal invasion, and harvest damage in hulless oat, grain with the hull present is still often preferred for production and processing. The mechanical methodology used to determine groat percentage is tedious and can give somewhat variable results depending on the technique employed. Commercial oat milling is also a complex process, and some laboratory equipment attempts to mimic industrial-scale dehulling equipment to give groat yields that account for other "milling efficiency" factors such as breakage, hull adherence, and kernel size distribution. Near-infrared technology is being tested as a faster, more reliable way to measure groat percent (A. McElroy, pers. comm.) but is unlikely to address other factors affecting milling efficiency.

Although oat hulls normally contain high levels of indigestible fiber and thus are of low available energy content, a variant hull type with low acid detergent lignin hull in which hull digestibility is doubled was described by Thompson et al. (2000). This trait is being incorporated into selections in western Canada to improve the cattle feed value of oat grain with hulls (Rossnagel et al. 2004b).

Test weight or weight-per-volume is the traditional way to measure oat grain quality. Test weight is often a good predictor of groat percent within a vari-

ety grown in different environmental conditions but a much less reliable predictor among varieties with different groat and kernel morphological relationships and container-packing characteristics. Digital imaging of grain size and shape (Symons and Fulcher 1988) has been investigated as an alternative means to predict quality and milling efficiency of the oat grain but is not yet in common practice.

Yield gains in oat resulting from genetic improvement have been estimated in various studies to range from essentially no gain up to 0.8% per year depending on the location, the time period covered, and the materials chosen to represent different eras (Holland 1997). Most studies agree, though, that more recent oat cultivars have improved lodging resistance, higher harvest index, and reduced hull content. The more modest gains in genetic improvement in yield compared to that reported in other grains have been attributed to a focus by breeders on correcting negative aspects of elite cultivars, such as disease susceptibilities (Stuthman 1995). Strategies in breeding for yield improvement including breeding for specific "components of yield" and breeding methods employed have been reviewed by Holland (1997).

Disease resistance has been a major focus of oat genetic improvement efforts. Crown rust (caused by *Puccinia coronata* f.sp. *avenae*) has been the primary disease problem in the major oat-producing areas of the upper Midwest USA and central prairies of Canada for the past few decades as well as in oat-growing regions of South America and several other areas around the world. Since the 1960s breeders have obtained resistance primarily by incorporating a series of major resistance genes from the wild hexaploid oat *A. sterilis*. However, the resistance of each of these *Pc* race-specific genes has been overcome by rapid shifts in the crown rust virulence pattern after release of cultivars containing the genes. The ever-growing prominence in the landscape in the upper Midwest USA of the alternate host buckthorn, *Rhamnus cathartica* L., has probably sped the virulence pattern shift there. Use of combinations of *Pc* genes including *Pc*38 plus *Pc*39, and then with *Pc*68 added, appeared to prolong their period of effectiveness (Chong and Zegeye 2004), but that effectiveness is now almost lost. Because of a lack of new, effective major genes and attempts to obtain more long-lasting or durable resistance, breeders have turned to efforts to identify and incorporate partial resistance. Such resistance tends to be multigenic, environmentally variable, and difficult to measure, but hopefully race nonspecific and hence more durable,

albeit a challenge to genetically manipulate and select.

Oat stem rust, caused by *Puccinia graminis* f.sp.*avenae*, can cause serious oat loss in several oat-growing regions. Oats in the prairie provinces of Canada have had effective resistance for several years from a combination of genes *Pg2* and *Pg13*. However, stem rust races NA67 and NA74 with virulence to these *Pg* genes have recently arisen (McCallum et al. 2000). Effective resistance to these rust races has been identified in accessions of the diploid oat *A. strigosa* (Fetch and Dunsmore 2004); however, transfer of genes from diploid to hexaploid has proven a major challenge requiring much time and special manipulations, including in vitro embryo rescue, colchicine treatments for chromosomal doubling, and radiation treatments to induce chromosomal exchange.

Another widespread disease of oat is barley yellow dwarf virus (BYDV). Resistance (or tolerance) to this aphid-transmitted virus tends to be multigenic and difficult to reliably assess. Critical screening of germplasm for resistance or tolerance requires rearing and controlled inoculations with viruliferous aphids (Harder and Haber 1992).

Other diseases of oat important in at least some oat-growing regions worldwide and for which genetic resistance is sought include loose and covered smuts, powdery mildew, Septoria leaf blight, Victoria blight, bacterial blights, soil-borne viruses, and nematodes (Harder and Haber 1992). One disease rising in public prominence because of mycotoxin production is the *Fusarium* complex (Campbell et al. 2000).

5.1.3
Limitations of Conventional Genetics and Breeding Approaches and the Utility of Molecular Mapping

Because of the hexaploid nature of cultivated oat, few single genes in oat have been identified based on their phenotypic effect. These genes have been summarized most recently by Marshall and Shaner (1992). Several of the described genes are in the domesticated diploid oat, *A. strigosa*. In the hexaploid oat, most gene mutations involving loss of function, even in a homozygous state, would be masked by genes having the same function located on homoeologous chromosomes in the other component genomes. Many of the single genes identified in hexaploid oat are for disease resistance, especially crown or stem rust resistance; such genes often give a dominant effect that is not

duplicated by a gene in another component genome. Historically, while genetic linkage had been detected between some of these genes, it was far too little to construct a genetic linkage map for oat. The advent of molecular markers with nearly unlimited variation or polymorphism at the DNA sequence level and without dependence on phenotypic effects has provided the means to construct oat genetic linkage maps.

The major role of molecular mapping in genetic improvement is as a tool to provide an understanding of the genetic basis of a trait, allow manipulation of the trait through genetic rather than phenotypic selection, and possibly even the isolation or cloning of the specific genes involved. Although ideally one can most readily manipulate a trait on a molecular marker basis if variants in the DNA sequence of the actual gene for the trait are available, molecular markers tightly linked to or even flanking the trait locus of interest allow genetic selection on seedlings or even half-endosperm samples. Such genetic selection can enable high-throughput screening for traits that are difficult or expensive to measure or are highly sensitive to environmental conditions. Because only a few economically important traits in cultivated oat are governed by single genes, the marker-based characterization of quantitative trait loci (QTLs) for most traits is key to their genetic manipulation.

5.2
Development of Molecular Linkage Maps in Oat

5.2.1
Mapping in Diploid Oats

Molecular mapping in oats began early relative to many other crops of similar economic importance. Mapping commenced among collaborating laboratories in North America with funding to several laboratories by The Quaker Oats Company, Chicago, IL, being an important source of support. A summary of oat mapping crosses and associated molecular mapping studies is provided in Table 1. Our discussion parallels the summary given in this table.

Although hexaploid *A. sativa* is the primary cultivated oat species, the first molecular-marker maps were constructed in crosses among related diploid species. This approach was intended to reduce the complexity of map construction relative to the hexaploid and to simplify the future construction of hexaploid maps. Advantages of mapping in a diploid species include the reduced complexity of identifying alleles that belong to homologous loci, avoidance of possible homoeologous pairing, and a smaller map with fewer linkage groups.

Two diploid maps were constructed in the early 1990s. One was based on F_2 families derived from a cross between two nondomesticated species, *A. atlantica* × *A. hirtula* (O'Donoughue et al. 1992), and the other was constructed from F_2 families derived from a cross between accessions of the domesticated diploid oat *A. strigosa* and nondomesticated *A. wiestii* (Rayapati et al. 1994). All four of these diploid oat taxa belong to the *strigosa* group and are considered morphological variants of the same biological species (Leggett and Thomas 1995). Hybrids between them exhibit normal chromosome pairing with no evidence of chromosomal rearrangements.

The *A. atlantica* × *A. hirtula* map was based on restriction fragment length polymorphism (RFLP) loci detected using cDNA clones derived primarily from oat and barley. Because the same RFLP clones were mapped in several other grass species, the *A. atlantica* × *A. hirtula* map became a cornerstone for oat in comparative mapping among grasses (e.g., Moore et al. 1995; Devos and Gale 2000). Although based on a relatively small population (44 F_2 families), the *A. atlantica* × *A. hirtula* map contains seven linkage groups that appear to coincide with seven diploid oat chromosomes. It is possible that in a small population, the ordering of loci and even the placement of loci into linkage groups can contain statistical artifacts. Therefore, this should always be considered as a potential cause of differences when comparing with other oat maps (see later) or with maps in other grass species.

Probes used to map loci in *A. strigosa* × *A. wiestii* by Rayapati et al. (1994) were not widely available, and later research indicated some potential problems with the ordering of loci in this map (Yu and Wise 2000; Kremer et al. 2001). A new population based on independent progeny from the same cross was used to construct additional diploid maps, including one based on amplified fragment length polymorphism (AFLP) loci in an $F_{8:9}$ population (Yu and Wise 2000) and one based on RFLP loci in an earlier $F_{6:8}$ generation derived from the same set of recombinant inbred line (RIL) families (Kremer et al. 2001; Portyanko et al. 2001). Because of problems with the original map, and because the new maps contain additional loci, these new diploid maps are more useful for compara-

Table 1. Summary of molecular mapping studies in oat

Cross	Population	Reference	Marker types	No. of loci	No. of linkage groups	Map length (cM)	Comments
A. atlantica × A. hirtula	44 F$_2$ families	O'Donoughue et al. 1992	RFLP	354	7	737	Diploid, interspecific cross
A. strigosa × A. wiestii	88 F$_2$ families	Rayapati et al. 1994	RFLP	203	10	2416	Diploid, interspecific cross
	100 F$_{8:9}$ RILs	Yu and Wise 2000	AFLP etc.	513	7	3513	Different sample of progeny used in first study vs. later studies
	100 F$_{6:8}$ RILs	Kremer et al. 2001	RFLP	181	9	880	
Kanota × Ogle	71 F$_6$ RILs	O'Donoughue et al. 1995	RFLP etc.	561	34	1482	Winter × spring type
	133 F$_6$ + F$_9$ RILs	Wight et al. 2003	RFLP, RAPD, AFLP, etc.	1166	45	1890	Expands and improves previous map
Kanota × Marion	137 F$_6$ RILs	Groh et al. 2001b	AFLP	121	27	736	Winter × spring type; Comparison to Kanota × Ogle using AFLP markers
Ogle × TAMO-301	136 F$_{6:7}$ RILs	Portyanko et al. 2001	RFLP, RAPD, AFLP, etc.	441	34	2049	Spring × winter type
Clintland64 × IL86-5698	126 RILs	Jin et al. 2000	AFLP, RFLP	265	30	1363	Comparison to Kanota × Ogle using AFLP markers
Ogle × MAM17-5	152 F$_{5:6}$ RILs	Zhu and Kaeppler 2003a	AFLP, RFLP, SSR etc.	510	28	1396	Spring × winter type
Terra × Marion	101 F$_{5:6}$ RILs	De Koeyer et al. 2004	AFLP, RFLP, RAPD, etc.	430	35	727	Hulless × covered cross
Potoroo × Mortlock	170 F$_8$ RILs	Williams et al. 2004	AFLP, RFLP, SSR	440	24	2145	Further mapping is in progress
MN841801-1 × Noble-2	158 F$_{6:8}$ RILs	Portyanko et al. 2005	RFLP, AFLP	231	30	1509	Further mapping is in progress

tive mapping, and they largely supercede the former map.

Although the most recent maps in *A. strigosa* × *A. wiestii* are apparently based on the same set of RIL lines, they were developed independently for different purposes, and unfortunately have not been merged. The maps currently contain few markers in common, although each contains a small subset of loci that allows some comparison to *A. atlantica* × *A. hirtula* and/or hexaploid maps. A large difference in cumulative map length is apparent between these two maps. This difference is partly due to the larger number of loci in the map presented by Yu and Wise (2000), but it may also be a result of the large number of AFLP loci included in this map. Because AFLP loci mapping involves polymerase chain reaction (PCR) techniques, some fragments may not have been amplified, and this can cause expansion of map distances when such markers are included in the map.

5.2.2
Hexaploid Mapping: Kanota × Ogle

The first attempts at mapping molecular markers in hexaploid oat were performed in a RIL population derived from a cross between cv. Kanota, a facultative winter oat of the *A. byzantina* or red oat type, and cv. Ogle, a widely adapted spring oat of the *A. sativa* or white oat type. The wide diversity between these two cultivated oat types was considered advantageous for detecting and mapping molecular polymorphisms. Also, a putative complete set of 21 monosomics (lines missing one chromosome) had been reported in Kanota (Morikawa 1975). Such aneuploid cytogenetic stocks were thought to enable mapping or assigning molecular markers and linkage groups to chromosomes to provide an integrated chromosomal/genetic map.

The Kanota × Ogle population has been widely distributed, and mapping in this population has involved collaborations among many laboratories. The first map from Kanota × Ogle was described by O'-Donoughue et al. (1995). Progress in the development of this and other oat maps was summarized by O'-Donoughue et al. (1994) and Kianian et al. (2001). The Kanota × Ogle map is still considered the primary base map in cultivated oat with a more recent update by Wight et al. (2003). Future updates to this map will be made available in the Graingenes database (http://graingenes.org).

The primary molecular markers used in the first oat maps were RFLPs detected by Southern hybridization gel blots. In a hexaploid, the presence of three independent loci revealed by one probe is expected. In oat, the presence of more than three bands revealed by the same probe is not unusual, especially if a locus is heterozygous or if a probe reveals multiple loci in the diploid. This factor adds considerable complexity to the collection and interpretation of data in hexaploid oat. Unless alleles are known from previous mapping efforts, it is often not possible to identify allelic relationships until data are collected in segregating lines and cosegregating bands are determined. Even then, tightly linked loci revealed by the same probe have been found, and the apparent presence of duplications and deletions can further complicate the assignment of bands (alleles) to loci (O'Donoughue et al. 1995).

The first map in Kanota × Ogle contained 38 linkage groups and many unlinked markers. Several linkage groups were very short and/or consisted of few markers. The inability to assign all loci to linkage groups associated with 21 chromosome pairs expected in hexaploid oat has been attributed to statistical weakness of mapping a large genome using a relatively small (71 RILs) population. However, further attempts to map in an expanded population, and to join linkage groups through comparative mapping or other means, have failed to reduce the number of linkage groups. While some groups have been joined, other small groups have appeared, bringing the total to 44 linkage groups (Wight et al. 2003). Most of the larger groups have been assigned to oat chromosomes through the use of aneuploid stocks (Fox et al. 2001; see later discussion), and these assignments have been used to join linkage groups when possible (Wight et al. 2003).

The fact that many laboratories provided data to the Kanota × Ogle mapping effort contributed to the construction of a map with a wide diversity of loci. The map includes several types of DNA marker loci as well as markers based on isoenzymes. However, the distribution and subsequent maintenance of this population at several locations may have resulted in population drift, intermating, and potential mixups or contamination of seed sources. The advanced F_9 version of the mapping population seems to contain an overabundance of heterozygous loci, and the presence of some data based on these lines has caused difficulties in the mapping effort (Wight et al. 2003). The presence of partial data for an extended set of RILs has also caused difficulties, as many loci have

not been scored on the extended set. As a result, use of the complete data can cause problems in map generation. Wight et al. (2003) recommended the use of a framework set of markers and a reduced population for the majority of further map development. However, the complete marker set and the extended population may be useful for detailed studies.

A further difficulty with mapping in Kanota × Ogle, and many other oat crosses, is the presence of translocations. Some of these have been confirmed by cytological means (Jellen et al. 1993b) and others are suspected based on the presence of duplicated or deficient loci. These translocations can cause nonbinary pairing at meiosis, resulting in segregation distortions and nonlinear linkage relationships among markers. Such anomalies may be one cause of large groups of markers that map to tight clusters and the difficulty in ordering markers within those groups (Wight et al. 2004). The apparent plasticity of the oat genome and tolerance of translocations and other rearrangements seems to be one cause of ongoing difficulties in oat genome analysis.

Despite difficulties encountered in this population, the Kanota × Ogle map remains the largest and most complete set of mapped molecular markers in oat and is useful as a point of reference for further mapping. Having the most complete set of markers, it seems also to be the most likely map to give an estimate of genome size. O'Donoughue et al. (1995) estimated the complete hexaploid oat genome at 2,932 cM. This estimate was based on a total map distance of 1,482 cM and an assumption about average gaps between unjoined linkage groups. The map described by Wight et al. (2003) contained 1,890 cM and a larger number of linkage groups. Therefore, large variations using the same method of estimating genome size does not seem reasonable. Furthermore, different mapping strategies can cause large variations in map length. For example, Jin et al. (2000) estimated the Kanota × Ogle map to be 2,351 cM based on a subset of markers that were included in the map described by Wight et al. (2003). The difference is caused by the exclusion of loci that tended to expand short map intervals, possibly due to scoring errors or misamplification of PCR fragments. These differences emphasize the need for caution when extrapolating map length to meaningful estimates of genome size. Although most authors estimate cumulative map length (Table 1), maps have varying degrees of coverage, and some of these estimates may be inflated due to misscored markers. Therefore, these estimates should be used only as ap-

proximate guidelines in discussion of map coverage or genome size.

5.2.3
Other Hexaploid Oat Maps

To take advantage of allelic relationships established in Kanota × Ogle, and to provide potential information about common alleles at QTLs, many second-generation maps have included common parents. A series of hexaploid oat maps derived from common parents include Kanota × Ogle (O'Donoughue et al. 1995; Wight et al. 2003), Kanota × Marion (Groh et al. 2001b), Ogle × TAMO-301 (Portyanko et al. 2001), Ogle × MAM17-5 (Zhu and Kaeppler 2003a), and Terra × Marion (De Koeyer et al. 2004). The later maps were developed in populations that were more adapted than Kanota × Ogle and/or more likely to show specific types of quantitative trait segregation. Other maps that we are aware of include Clintland64 × IL86-5698 (Jin et al. 2000), Potoroo × Mortlock (Williams et al. 2004), MN841801-1 × Noble-2 (Portyanko et al. 2005), and several maps in progress that are being used primarily for QTL analysis (Table 1). Although these maps are less complete than the Kanota × Ogle map, they benefit from comparison to and integration with other maps with common loci.

Two hexaploid maps were developed primarily using AFLP loci. These include Clintland64 × IL86-5698 (Jin et al. 2000) and Kanota × Marion (Groh et al. 2001a). Although AFLP loci present some difficulties for comparative mapping (Groh et al. 2001b), both of the studies concluded that a reasonable number of loci could be comapped in Kanota × Ogle based on cosegregating bands. The maps in Ogle × TAMO-301 (Portyanko et al. 2001), Ogle × MAM17-5 (Zhu and Kaeppler 2003a), Terra × Marion (De Koeyer et al. 2004), and MN841801-1 × Noble-2 (Portyanko et al. 2005) all contain significant numbers of RFLP loci, which enable easier comparison to other maps. The Potoroo × Mortlock map (Williams et al. 2004) also contains many RFLP markers; however, few of these are currently common to other maps (K. Williams, pers. comm.).

5.2.4
Comparative and Integrative Mapping

Originally, it was hoped that strong colinearity would be found between diploid and hexaploid oat, and that this would facilitate mapping and molecular studies in oat. Unfortunately, this was not the case, and O'Donoughue et al. (1995) reported only segmental colinearity between a diploid and hexaploid map. Portyanko et al. (2001) and Wight et al. (2003) presented comprehensive diagrams illustrating correspondence and colinearity between two diploid and two hexaploid maps. Both analyses illustrated that many diploid groups have become fragmented in the hexaploid species, and that colinearity is often restricted to relatively short fragments. Nevertheless, the diploid maps continue to provide an alternate point of reference for comparison to other grass species, and this may provide the required information that is needed for detailed comparative studies and/or map-based cloning.

Colinearity among genotypes of the hexaploid species is expected to be stronger. However, despite several attempts to develop integrated maps in hexaploid oat, all such attempts at combining two or more maps have been limited to segmental comparisons. Most comparative mapping efforts have used the Kanota × Ogle map as a point of reference (Jin et al. 2000; Groh et al. 2001b; Portyanko et al. 2001; Zhu and Kaeppler 2003a; Wight et al. 2003); however, a complete set of comparisons across all maps is currently not available. To illustrate the difficulty of making a complete comparison, we have developed a diagram showing allelic relationships among four maps in regions represented by linkage groups 11 and 22 of Kanota × Ogle (Fig. 1). Since this diagram represents a fraction of the complete Kanota × Ogle map, and many linkage groups in other maps do not have known homologies to Kanota × Ogle, a complete set of comparative diagrams would occupy 15 to 20 times as much space as this figure.

The map regions shown in Fig. 1 were chosen because of the relatively good information about allelic loci and because they illustrate a comparative QTL analysis presented later. This figure illustrates that even in a region where good information is available there are still irresolvable issues that prevent the construction of an integrated consensus map. For example, the region of Ogle × MAM17-5 at the bottom of the figure appears to line up in the orientation shown. If so, the combined linkage group is extended by approximately 64 cM relative to the KxO linkage group. However, there is a reasonable chance that the three markers on which this alignment is based are incorrectly ordered on one map and that the opposite orientation exists for Ogle × MAM17-5. Similar situations exist in many regions of the mapped oat genome. Even when relatively strong evidence exists for the correct orientation, the large number of markers that are not in common would necessitate dangerous assumptions about the order of merged markers from two or more maps.

Reasons for the difficulties in developing integrated oat maps can be summarized as follows: (1) Inadequate number of common loci. For example, virtually all RFLP markers mapped in Kanota × Ogle were tested in Terra × Marion, and only a subset could be mapped in Terra × Marion. Few microsatelite markers have been developed in oat at this time. (2) When common RFLP probes have been used, or when PCR primers reveal multiple loci, allelic relationships among loci may not be available, or may have been established incorrectly. (3) There may be physical rearrangements causing differences in segregation among different populations. (4) The large genome size in oat and relatively small mapping populations may not give enough statistical power to consistently order markers in a correct orientation. (5) Marker coverage may not be adequate, partly due to lack of polymorphism and/or biased marker coverage.

5.2.5
Integration of Genetic and Chromosomal Maps

Construction of the first molecular-marker linkage maps in hexaploid oat revealed complex patterns of linkage groups, rather than three colinear sets reflective of three component sets of homoeologous chromosomes as was found in linkage maps of hexaploid wheat (O'Donoughue et al. 1995). Furthermore, none of the patterns aligned with those of diploid maps. As discussed in the preceding sections, only short fragments of linkage groups showed colinear patterns. These complex linkage patterns served to confirm earlier postulations based on cytological and species pairing relationships (Rajhathy and Thomas 1974) that oat speciation evolved through chromosomal segmental rearrangements. Evidence of chromosomal rearrangements among genomes and species has also been revealed by patterns of C-banding (Jellen
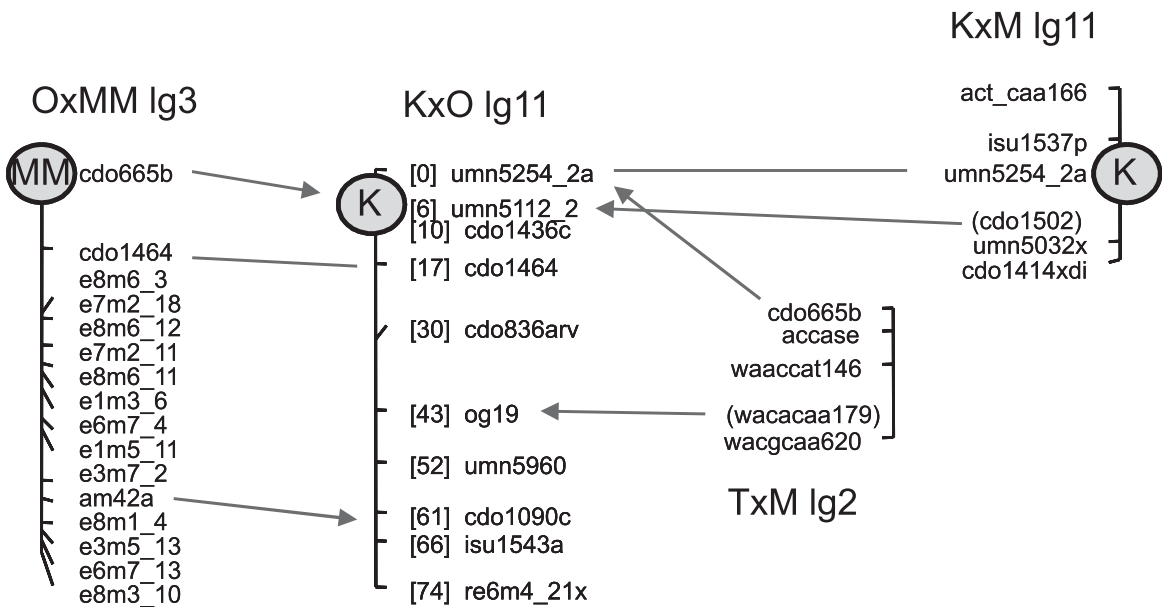
Fig. 1. Examples of comparative oat linkage groups mapped in Kanota × Ogle (KxO), Kanota × Marion (KxM), Terra × Marion (TxM), and Ogle × MAM17-5 (OxMM). Maps shown here are framework maps. Markers placed relative to the framework are not shown except those indicated by parentheses, which were used to establish allelic relationships. *Solid lines* connect loci known to be allelic. *Dashed lines* connect loci that might be allelic. *Arrow tips* indicate the position of a marker not shown on the diagram. Locations of QTLs that affect groat oil concentration are shown by *circles*. *Letters inside each circle* indicate the parent that contributed alleles that caused an increase in oil concentration

et al. 1993a,b) and mitotic cell in situ hybridization using as fluorescent-labeled probe either genomic DNA of a diploid *Avena* species (Chen and Armstrong 1994; Jellen et al. 1994; Leggett and Markhand 1995; Hayasaki et al. 2000) or genome-specific repetitive DNA sequences (Fominaya et al. 1995; Katsiotis et al. 2000; Linares et al. 2000; Ananiev et al. 2002; Irigoyen et al. 2002). The use of molecular markers now provides information on the specific segments involved. Relating the genetic linkage map to the chromosomal organization of oat is being accomplished through the assignment of markers to chromosomes using oat aneuploid stocks missing a specific chromosome or chromosome pair (Jellen et al. 1993b; Rooney et al. 1994a; Kianian et al. 1997; Start 2000; Fox et al. 2001). These assignments serve to link molecular-marker linkage groups that may be located on the same chromosome but are not joined in a linkage map due to the absence of a key joining marker. Also, the assignments serve to confirm the identity of chromosomes tentatively identified by chromosome morphology or C-banding. In the most recent published reports, 26 of 35 linkage groups identified in a hexaploid cross were assigned to 18 of the 21 oat chromosomes (Start 2000; Fox et al. 2001).

5.3
Gene Mapping

5.3.1
Gene mapping in Segregating Populations

The construction of molecular genetic recombination maps (discussed in the previous section) provides a molecular-marker framework for each oat chromosome that facilitates mapping many more genes or genetic loci, and with a higher degree of accuracy, than previously possible by classical mapping approaches (discussed in the introduction). Gene mapping in well-characterized mapping populations is direct and straightforward for any single gene locus whose phenotype can be scored in the segregating population. Of particular interest to oat breeders is tagging and mapping of genes for disease resistance (Table 2) and for morphological traits (Table 3).

The first oat map was created in the diploid cross of *A. atlantica* × *A. hirtula* and was constructed entirely from anonymous DNA molecular markers

Fig. 1. (continued)
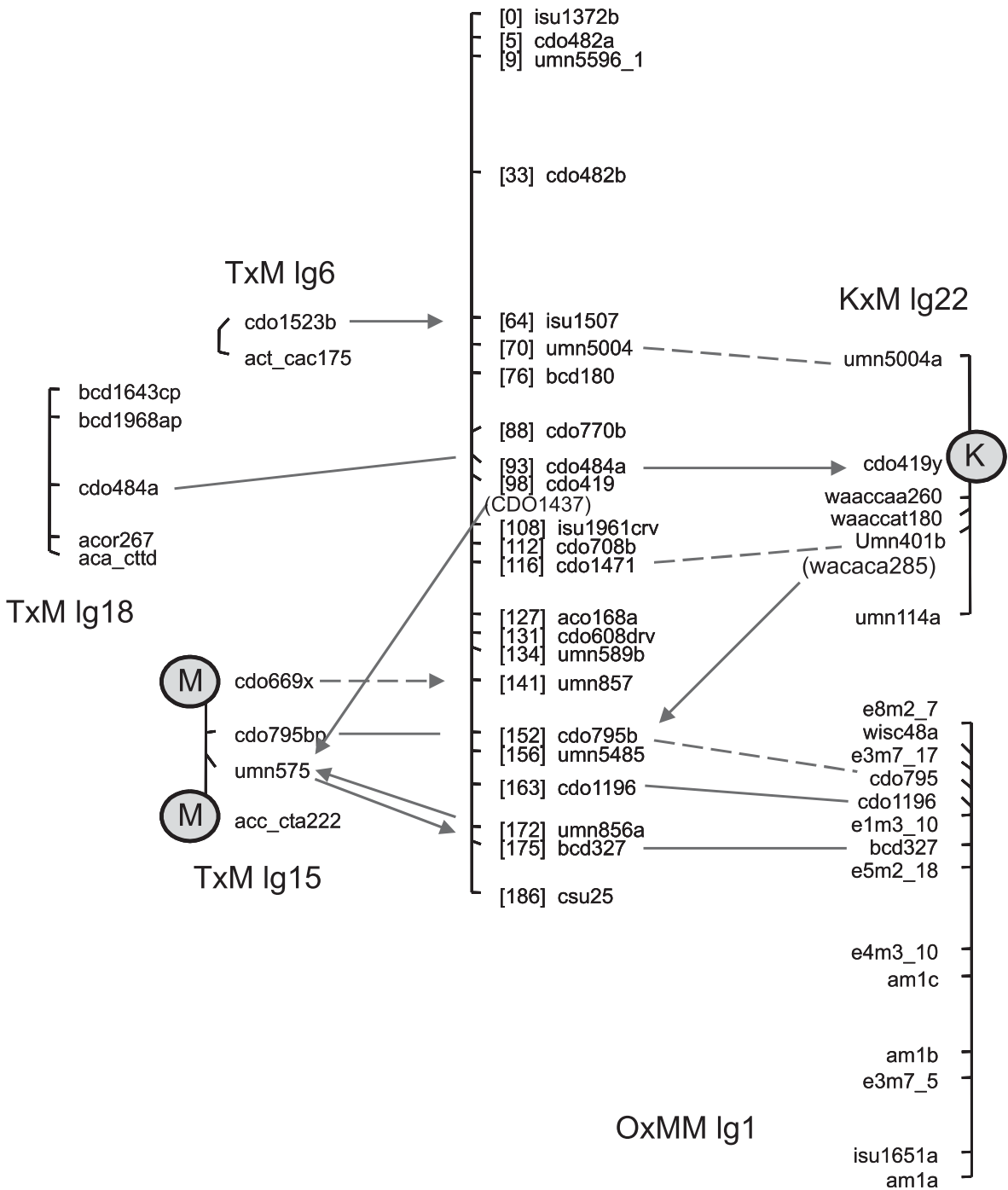
Table 2. Gene tagging and mapping: Disease resistance traits

Gene mapped (and trait)	Population(s) used	Strategy	Marker name (and type)	Linkage to trait	Linkage group	Comments	Reference
Pc38 (crown rust)	Pendek-48 × Pendek-38; OT328 × Dumont	BSA	Several (RFLP)	2 cM; 7 cM	Pendek4838_1 (KO_17); OT328Du_4 (KO_7_10_28)	Pc38 in tight cluster with Pc62 and Pc63; RFLPs were sequenced	Wight et al. 2004
Pc39 (crown rust)	Pendek-39 × Pendek-48; OT328 × Dumont	BSA	Several (RFLP)	0 cM; 6 cM	Pendek3948_1 (KO_37); OT328Du_3 (KO_37)	Pc39 in tight cluster with Pc55; RFLPs were sequenced	Wight et al. 2004
Pc48 (crown rust)	Pendek 39 × Pendek 48	BSA	Several (RFLP)	6 cM	Pendek_3948_2 (KO_22_44+18)	RFLPs were sequenced	Wight et al. 2004
Pc68 (crown rust)	F3 of a line with Pc68 × Rodney 0	BSA and cosegregation	UBC269 (RAPD)	~4.9 cM		Pc68 in tight cluster with Pc44, Pc46, Pc50, Pc95, PcX, Pg3, and Pg9	Penner et al. 1993c
Pc71 (crown rust)	NILs D526 and Y345; BC1F2 of NIL D526 with recurrent parent Lang	BSA	CDO1502, CDO783 (RFLP)	0.2 cM	KO 11	Pc71 was previously named R345	Bush et al. 1994; Bush and Wise 1998
R264B (crown rust)	NILs D486 and X434-II; BC1F2 to C237-89 or Lang	NILs and cosegregation	OG41b (RFLP)	8.1 cM; 10.2 cM			Bush et al. 1994
R203 (crown rust)	NILs D494 and X466-I; BC1F2 to C237-89 or Lang	NILs and cosegregation	ISU1719d (RFLP)	1.9 cM; 3.8 cM			Bush et al. 1994
Pc91 (crown rust)	F2 and BC1F6 of donor Amagalon and recurrent parent Ogle or Starter	NILs (BC derived) and cosegregation and aneuploids	UMN 145 (RFLP)	4.5 cM	Chromosome 18	Chromosome 18 is Kanota nullisomic 21. UMN145 also on chromosome arm present in Sun II ditelosomic stock XIII	Rooney et al. 1994b
Pc92 (crown rust)	F2 and BC1F6 of donor Obee/Midsouth and recurrent parent Ogle or Starter	NILs (BC derived) and cosegregation and aneuploids	OG 176 (RFLP)	13.5 cM	Could not be assigned by aneuploid analysis		Rooney et al. 1994b
Pc94 (crown rust)	F2 of Calibre × S42 for BSA; ibid and Makura-SunII-Pc68 × S42 for co-segregation	BSA and cosegregation	Unnamed (AFLP)	0.9 and 3.4 cM		Two derived SCARs co-map and at 0.9 and 3.4 cM from Pc94 in 2 populations	Chong et al. 2004

Table 2. (continued)

Gene mapped (and trait)	Population(s) used	Strategy	Marker name (and type)	Linkage to trait	Linkage group	Comments	Reference
Pg3 (stem rust)	NILs and F2 of Rodney 0 × Rodney 0-Pg3	NILs and cosegregation	ACOpR-2 (RAPD)	~0 cM		Pg3 in tight cluster with Pc68, Pc44, Pc46, Pc50, Pc95, PcX, and Pg9	Penner et al. 1993b
Pg9 (stem rust)	NILs and F3 of Rodney 0 × Rodney 0-Pg9; F3 of OT328 × Dumont	NILs and BSA and mapping and comparative mapping	Several (RFLP, RAPD, proteins)	2.7 cM; 1.1 cM	KO_4	Pg9 in tight cluster with Pc68, Pc44, Pc46, Pc50, Pc95, PcX, and Pg3; comparative mapping to hexaploid (KO) and diploid (AH) maps identified additional markers	O'Donoughue et al. 1996
Pg9 (stem rust)			Lrk10 Receptor Kinase RGA			Pg9 in tight cluster with Pc68, Pc44, Pc46, Pc50, Pc95, PcX, and Pg3	Cheng et al. 2002a
Pg13 (stem rust)	NILs and F3 of Rodney 0-Pg13; F3 of OT328 × Dumont	NILs and BSA and mapping and comparative mapping	Several (RFLP, RAPD, proteins)	0.0 cM; 0.7 cM	KO_3	Pg13 in tight cluster with Pg2; comparative mapping to hexaploid (KO) and diploid (AH) maps identified additional markers	O'Donoughue et al. 1996

Table 3. Gene tagging and mapping: Morphological traits

Gene mapped (and trait)	Population(s) used	Strategy	Marker name (and type)	Linkage to trait	Linkage group	Comments	Reference
Di1 (day-length insensitive)	F2 of Premier × OA906-1-16	BSA	UBC221; UBC136 (RAPD)	9.8 cM; 13.9 cM			Wight et al. 1994
Dw6 (dwarf)	F2 of Kanota × OT207	BSA	UMN145B (RFLP)	3.3 cM	Kanota nullisomic stock K21	UMN145 on chromosome missing in Kanota nullisomic stock K21	Milach et al. 1997
Dw6 (dwarf)	Aslak × Kontant	BSA	(RAPD)	~9 cM			Kiviharju et al. 2004
Dw7 (dwarf)	F2 of Kanota × NC2469-3	BSA and co-segregation	CDO1437N (RFLP)	4.3 cM	KO22		Milach et al. 1997
Dw8 (dwarf)	F2 of AV17/3/10 × Kanota; F2 of AV18/2/4 × Kanota	BSA and co-segregation	CDO1319A (RFLP)	4.9 cM; 6.6 cM	KO3		Milach et al. 1997
(Low-acid detergent lignin hull)	AC Assiniboia × OT775	BSA	OPD15 (RAPD)	~24 cM	KO 24_26_34	CDO373 and markers from this KO region are being tested. Flanking markers are CDO353a and re2M2_12x	Rossnagel et al. 2004a
(Loose smut resistance)	F5 RILs of OT281 × OT789 and F5 RILs of OT286 × OT789 for BSA;	BSA	UBC041 (RAPD)		Derived SCARs map to KO14	SCAR marker Ua300co linked at 5, 8, and 18 cM to 3 smut resistance genes in an unrelated pop	Eckstein et al. 2002; Kibite et al. 2000; Kibite et al 2004
N-1 (naked or hulless)	F10 derived NILs of NO 141-1	NILs	(AFLP)	5 cM			Burrows et al. 2001
(Percent hull)	F7 of Cascade × AC Marie	BSA and QTL analysis	12 markers in 3 clusters (RAPD)			3 unlinked marker clusters correspond to 3 QTL and explain 40% of variation	Ronald et al. 1997
(Panicle weight)	F3 of UFRGS 14 × OR2	BSA	3 putative markers (AFLP)				Malone et al. 2004

(O'Donoughue et al. 1992). This map was extended by Van Deynze et al. (1995), but there are no reports of it having been used for mapping of genes for specific traits. A second diploid oat map was developed by Rayapati et al. (1994) in an F_2 cross of *A. strigosa* × *A. wiestii*, and this was successfully used to map the *Pca* locus, which confers resistance against nine isolates of *Puccinia coronata*, the causal agent of crown rust. Subsequently, the complex *Pca* locus was mapped in greater detail in F_6 lines by first using bulked segregant analysis to identify two additional tightly linked molecular markers and then doing segregation analysis with specific rust isolates to define a cluster of five resistance loci (*R54*, *R263*, *R290*, *R62*, and *R202*) at the *Pca* region (Wise et al. 1996). These five were subsequently renamed as *Pc81*, *Pc82*, *Pc83*, *Pc84*, and *Pc85* (Yu and Wise 2000). The F_6-derived RIL population was used to develop two complete maps. An AFLP and retrotransposon based map includes gene mapping of six crown rust resistance loci, eight soybean-based resistance-gene analogs (RGAs), and a morphological marker (Yu and Wise 2000). An RFLP-based map includes gene mapping of 12S globulin seed storage protein, the *Pca* cluster, and five cereal-based RGAs (Kremer et al. 2001). The location of genes mapped on diploid maps can be used to infer the map location of homologous loci on hexaploid maps since the use of common anchor markers permits identification of homologous regions.

The first hexaploid oat mapping population, and still the most detailed, derives from a cross of Kanota × Ogle. In this population, esterases (*Est-A*, *Est-B*, *Est-C*, *Est-D*), isocitrate dehydrogenase (*Idh*), peroxidase (*Px-5*), malate dehydrogenase (*Mdhf*), 6-phosphogluconate dehydrogenase (*6-Pgd*), phosphoglucomutase (*Pgm*), and shikimate dehydrogenase (*Skdh*) were assayed on the RILs by standard isozyme procedures and mapped relative to the large set of molecular markers that define the map (O'Donoughue et al. 1995). Three avenin (alcohol-soluble seed storage proteins), the nucleolus organizer region (NOR) 18S-25S rDNA repeat, and one coleoptile color loci were also mapped in the same study. This major reference map has continued to evolve with the addition of more molecular and gene loci. The recent paper of Wight et al. (2003) reports the map location of acetyl co-A carboxylase (*Accase1*), acid phosphatases (*Acp1*, *Acp2*), 1-3,1-4-B-glucanase (*Bglucanase*), high-affinity K$^+$ transporter (*HKT1a*, *HKT1b*, *HKT1c*, *HKT1d*), a portion of the lipoxygenase gene (*LOX11*), malate dehydrogenase

(*MDHS*), phytoene desaturase (*PDS2*), phosphokexose isomerase (*PGI*), phytochrome A and B (*phyto A*, *phyto B*), ABA responsive protein kinase (*pKABA1*), prolamin storage protein (*POP6*), and starch synthase (*waxy*).

Additional gene mapping has been done in other hexaploid oat mapping populations. The cloned oat acetyl co-A carboxylase (ACCase) gene that had been used to map one locus (*Accase A*) in the Kanota × Ogle map also mapped two loci (*Accase A*, *Accase B*) in the Kanota × Marion hexaploid map, with *Accase A* associated with high oil content in both populations (Kianian et al. 1999; Groh et al. 2001a). No other genes have been mapped in Kanota × Marion. In a third hexaploid mapping population, Ogle × TAMO-301, the morphological traits plumule color (*Plc*), growth habit (*gh*), leaf sheath pubescence (*Lsp*), and seed pigmentation (*Spg*) were mapped (Portyanko et al. 2001). Also mapped were 12 isozyme loci as well as sequence tagged sites (STSs) for beta-1-hordein, 11S globulin seed storage protein (*Glav3*), and two alpha amylase genes (*Amy2A*, *Amy2D*). In the Terra × Marion hexaploid mapping population, the hulless character controlled by the naked (*N1*) locus was scored as a phenotypic marker and mapped relative to a full set of molecular markers (De Koeyer et al. 2004). A number of morphological characters (naked, dwarf), which are likely due to single genes, are segregating in an F2 mapping population, N327-6 × Hendon, being developed by Howarth et al. (2004). Similarly, dwarfing, nematode resistance, and other single genes are being mapped in the Potoroo × Mortlock (Williams et al. 2004) hexaploid mapping population. To date, there are no reports of single-gene mapping in the Clintland 64 × IL86-5698 (Jin et al. 2000), Ogle × MAM17-5 (Zhu and Kaeppler 2003a), or Cascade × Marie (Ronald et al. 1997) hexaploid mapping populations.

A standardized system of nomenclature has been established for classical genes and chromosomes in oats (Simons et al. 1978). Unfortunately, there is neither a consensus molecular map in oats nor the systematic naming of molecular linkage groups in the various maps. Homologous linkage groups are inferred based on common anchor loci, but many maps are incomplete, and homoeologous relationships remain problematic. Therefore comparing map locations of genes among different maps must be done with care.

5.3.2
Gene Tagging

As discussed above, gene mapping in a well-characterized mapping population is a powerful approach. However, suitable well-characterized mapping populations are not always available for mapping a gene of interest. In oats, the earliest efforts at gene mapping predate the availability of molecular maps and therefore were focused on "gene tagging", or simply the discovery of molecular markers closely linked to the gene of interest, whether those molecular markers were mapped or not. In some cases gene tagging studies made use of existing pairs of NILs (near isogenic lines), which are largely genetically identical except in the region of the genetic locus of interest and in a few other genomic regions. Any molecular marker that showed polymorphism between the pair of NILs had a high probability of being in the genomic region of the target gene. This putative association could be confirmed by cosegregation analysis for the marker tag and the trait of interest. In other cases, two bulks, composed of lines from the contrasting tails of a segregating population, were constructed to mimic NILs and the two bulks screened to discover polymorphic molecular markers, in phase with the parental alleles, according to the strategy of BSA (bulked segregant analysis) (Michelmore et al. 1991). Both approaches are designed to study single gene traits, although occasionally they have been successful at tagging two or three genes simultaneously. Both approaches are also very successful at reducing the number of probes or markers that have to be tested across all the lines of the mapping population to study segregation (Rooney et al. 1994b). In oats, these approaches have been particularly successful for tagging crown rust, stem rust, and other disease resistance genes (Table 2), all of which are largely controlled by single genes. Since rust resistance genes tend to be clustered, mapping one member of the cluster defines the map location of all other members of the same cluster. In a few studies, it was possible to simultaneously test putative markers on independently derived NILs (Bush et al. 1994), or on NILs and BSA bulks (O'Donoughue et al. 1996), or other combinations, thereby increasing the confidence of putative marker-trait linkage relationships. However, for true quantitative traits controlled by multiple genes, such as most agronomic and quality traits, rigorous QTL analysis (discussed in the next section) is the preferred approach. Despite this limitation, NIL and BSA continue to be used widely since, unlike QTL approaches, they do not require highly characterized mapping populations and are therefore broadly applicable for focused studies in any germplasm contrasting for the trait or gene of interest.

The emergence of oat maps extended the utility and potential of NIL and BSA approaches. Markers found linked to a gene of interest in a trait-specific population can often be subsequently mapped in a well-characterized mapping population to locate the linked target gene on a molecular-marker linkage group. Subsequently, flanking markers from the putative linkage group can be tested on the NILs or BSA bulks to confirm the map assignment and perhaps identify more tightly linked marker tags or linked markers with preferred technical features (illustrated by O'Donoughue et al. 1996; Wight et al. 2004). Comparative mapping between well-characterized oat mapping populations can both increase the number of flanking markers available for such a test and locate the target locus on multiple maps. Comparative mapping between oat populations in which QTLs have been identified for the trait can target the search for linked markers to markers known to map to those QTL regions. Finally, comparative mapping between oat and other grass species in which putative homologous genes or homologous QTLs have been mapped can also focus the initial search for linked markers to markers mapping to those genomic regions.

5.3.3
DNA Sequence-Based Mapping

Much of the gene mapping in oats has been of disease resistance genes, both because disease resistance is often a single gene trait and because of the importance of disease resistance in oat-breeding programs. A complementary approach is the mapping of resistance gene analogs (RGAs). These are PCR products generated using primers that are designed from the conserved regions of known disease resistance genes. The process involves the alignment of independent DNA sequences, mined from the public databases, to identify regions of low nucleotide variation. The expectation is that such primers will amplify sequences from related resistance genes in the original or a related species or variety. However, as resistance genes are often clustered, members of multigene families, and capable of rapid evolution, the functionality

of each cloned RGA must be determined. As noted above, eight soybean-based RGAs (Yu and Wise 2000) and five cereal-based RGAs (Kremer et al. 2001) have been mapped in the *A. strigosa* × *A. wiestii* (*Asw*) diploid oat mapping population. RGAs have also been isolated directly from the *A. strigosa* mapping parent using primers targeting several different classes of resistance genes (Irigoyen et al. 2004). Two RGAs were mapped to diploid linkage group AswBF and ten RGAs were mapped to 15 loci in the Kanota × Ogle (KO) hexaploid map. Comparative mapping identified RGAs tightly linked: to a BYDV locus (*Bydq-Pav 129*) on linkage group KO6; to a crown rust resistance QTL (*Pcq2*) on KO7_10_28; to the *Pca* region on KO7_10_28; to a slow rusting QTL (*PrPcq1*) on KO17; to a resistance gene cluster (*Pc71, Pc38, PrPcq4*) on KO11_41+20; and to a BYDV QTL (*Bydq Pav-129*) on KO24_26-34. Additional cereal RGAs have been mapped in the Ogle × Tam O-301 (OT) (Portyanko et al. 2001), KO (Cheng et al. 2002a), and MN841801-1 × Noble-2 (MN) (Portyanko et al. 2005) hexaploid oat mapping populations. The most completely studied are RGAs based on the sequence of the Lrk10 clone of wheat, which itself is a serine/threonine kinase class RGA that cosegregates with the *Lr10* leaf rust resistance gene in wheat. Two tightly linked loci (*plrk10c* and *plrk10a*) were mapped to linkage group OT29, which is homologous to KO4, and a third (*plrk10b*) to OT4. Both the OT29 and OT4 loci are homologous to two tightly linked loci (*plrk10a* and *plrk10b*) on diploid linkage group AswD (Portyanko et al. 2001). Using independently derived Lrk10-related primers, Cheng et al. (2002a) were able to map five polymorphisms to linkage groups KO4_12, KO5, KO6, KO13, and an unlinked locus, and two polymorphisms in Dumont × OT328, one of which was tightly linked to the *Pg9* stem rust resistance gene. Comparative mapping and aneuploid analysis was consistent with known homologies between the oat loci and wheat for *Triticeae* chromosomes 1 and 3. Primers designed to the extracellular domain of the oat Lrk10 RGA have been used to recover homologous RGAs from 15 species of *Avena* to study species relationships and the evolution of resistance genes (Cheng et al. 2003). Such RGAs become useful markers for resistance gene clusters and their member loci, and are thus of particular value for marker-assisted breeding for disease resistance. Furthermore, RGAs facilitate cloning of resistance genes themselves and provide an entry into the study of allele diversity and race specificity of resistance genes. In this context, the extracellular domain

fragment of the oat Lrk10 RGA was used to isolate a lambda clone carrying two head-to-head receptor kinase genes (Cheng et al. 2002b). The latter showed modified gene expression in hexaploid oats following inoculation of seedlings with the crown rust pathogen.

Not unlike the research into RGAs, public DNA sequence databases are increasingly being mined to develop and map PCR-based markers corresponding to known genes. Holland et al. (2001b) developed PCR-based markers in oat by targeting simple sequence repeats (SSRs) or introns found in oat sequences deposited in GenBank. Mapping these derived markers established putative map locations for the original genes, including ones for seed-storage protein 12 (*SSP12-3 locus*) in *A. strigosa* × *A. wiestii* (Kremer et al. 2001) and thaumatinlike pathogenesis-related protein (*Rast1-4*), 11S globulin (*GLAV 3.1, GLAV 3.2*), alpha-amylase (*Amy2A*), and alpha-amylase (*Amy2D*) in Ogle × TAM O-301 (Portyanko et al. 2001). The number of oat DNA sequences in public databases is rapidly increasing due primarily to large EST projects (Rines et al. 2004). As a result, there should be a rapid increase in the number of defined genes that are mapped in oats.

In parallel, the sequencing of already mapped oat cDNA clones is rapidly identifying the map location of many more genes (Van Deynze et al. 1998). In some cases, this sequencing is occurring a few clones at a time, as for example was the case with cDNA clone-derived RFLP markers flanking the *Pc38*, *Pc39*, and *Pc48* rust resistance gene loci (Wight et al. 2004). In other cases, it involves the systematic sequencing of large sets of mapped cDNA clones such as the barley-derived BCD clones (Van Deynze et al. 1998) or the oat-derived CDO (Van Deynze et al. 1998), UMN (Rines et al., unpubl.) and ACO clones (Molnar and Tinker, unpubl.), which populate many of the oat maps. Since many of the BCD and CDO clones are part of the anchor set for comparative mapping in grass genera (Van Deynze et al. 1998), they have been mapped and studied in other grass species as well. Thus, researchers in other grasses are identifying genes that were mapped anonymously in oat. Furthermore, the map location of many genes in oat can be inferred from their known location in other grass species by comparative mapping using the common anchor probes to define homologous relationships.

5.4
QTLs in Oat

5.4.1
Detection of QTLs

Most molecular mapping that has been performed in hexaploid oat was done primarily for the purpose of QTL discovery. Table 4 summarizes most QTL investigations of which we are aware, including some that are currently in progress. We will first discuss several of these investigations from the perspective of their general objectives, strategies, and unique aspects. Then we will discuss attempts to combine and integrate QTL information by target trait.

The Kanota × Ogle population represents a diverse set of alleles from both facultative winter and spring germplasm. These germplasm pools are seldom intercrossed for breeding purposes, and the only known common ancestor of these two parents is an old winter cultivar, Fulghum, which contributes less than 1% of common alleles to both parents. Because of the diversity of this cross, a high level of polymorphism is expected for QTLs as well as for molecular markers, and several studies have been conducted to investigate the locations of these QTLs. However, the facultative vernalization requirement in Kanota means that this population contains strong effects and environmental interactions for flowering time. The substantial differences in flowering time among progeny of Kanota × Ogle, as well as those of other winter × spring oat populations, can cause pronounced differences in the expression of many other traits. Thus, QTL studies that have been conducted in Kanota × Ogle, Kanota × Marion, Ogle × Tam0-301, and Ogle × MAM17-5 (Table 4) must consider potential pleiotropic effects of heading date on many other traits that appear to segregate in these populations. Most authors are aware of this and have discussed results accordingly.

Another factor that must be considered when interpreting all QTL studies is the issue of statistical error control. It is well known that an appropriate balance needs to be achieved between statistical power (the probability of declaring a real QTL effect) and type I error (the probability of declaring a false effect), and that this balance is controlled based on a significance threshold for one of several possible test statistics. There is, of course, no universal rule to dictate the appropriate level of error control. Further-more, since QTL analysis usually requires a search through many statistical tests (e.g., one test at each of 200 molecular markers), the number of false positives that are declared in an entire QTL experiment can be far greater than the small chance of declaring a false positive in just one of these tests. While it is possible to control type I error in an entire experiment, the power of finding true QTLs can become much smaller.

Investigations of QTLs have followed many different strategies and philosophies regarding error control, and it is impossible to compare or combine QTL studies without considering this factor. For example, Siripoonwiwat et al. (1996) investigated QTLs for agronomic traits in Kanota × Ogle using single-marker ANOVA, a method that achieved a type I error control of 5% per marker, per trait. They reported approximately 180 QTLs for seven agronomic traits, representing an estimated 106 genomic regions. A re-analysis of the same data (Wight and Tinker, unpubl.) using an experimentwide type I error control of 5% derived by permutation did not reveal any QTLs. Neither approach is "correct". Assuming that QTLs do segregate in this cross, the initial analysis is more likely to have found them. However, the reanalysis is "safer", and QTLs detected using this strategy are far more likely to be the right ones. Provided that caution is used in the interpretation of error rate, a large inventory of QTLs detected by the first strategy can be useful in validating other QTL studies. Unfortunately, due to heterogeneity in approaches to error control, integrative summaries of QTL locations will tend to be biased toward those detected in studies with higher levels of type I error.

5.4.2
Integrative and Comparative QTL Investigations

Investigations of QTL are often performed with the intention of using the results in molecular breeding. As in most crops, molecular breeding in oat when directed toward quantitative traits has not been adopted as quickly as many people hoped. The primary reason for this may be that plant breeders understand that QTLs detected in one cross may not be the same ones that are important in another cross. It is therefore very important that QTL studies be integrated and validated over several populations so that a clearer understanding of segregating QTL alleles can be developed. Once the locations of im-

portant QTL are well characterized, this information can be integrated with allele-trait association studies within the parental germplasm of ongoing breeding efforts.

In an attempt to integrate QTL studies with breeding populations, Beer et al. (1997) investigated marker-trait associations in a sample of 64 oat varieties and compared these associations with QTLs detected in Kanota × Ogle in an earlier study (Siripoonwiwat et al. 1996). The lack of substantial similarity between QTLs detected through these two studies is probably related to three factors: (1) the high level of type I error tolerated in both studies, (2) the fact that QTLs segregating in Kanota × Ogle were not necessarily polymorphic in a substantial subset of the varietal survey (and vice versa), and (3) the presence of strong QTLxE interactions that caused different QTLs to be important in each study. Future studies of allele-trait association (i.e., linkage disequilibrium) will probably benefit from the ability to restrict the search space to loci that have previously been validated through comparative QTL mapping in multiple populations.

A thorough comparative mapping effort has not yet been conducted among QTL studies now available in oat. The available information is limited to the most recent QTL reports that have compared newly identified QTL locations with those in previous reports. Examples include heading date (Holland et al. 2002; Zhu and Kaeppler 2003b; Portyanko et al. 2005); groat oil, protein, and beta-glucan content (Kianian et al. 1999, 2000; Zhu et al. 2004); BYDV tolerance (Zhu et al. 2003a); and several other agronomic and quality traits (De Koeyer et al. 2004).

Part of the difficulty in conducting comparative QTL analysis is related to the lack of good consensus or comparative linkage maps. Few QTL reports in any species have been integrated into electronic databases such as Graingenes, and there are many technical difficulties related to electronic interpretation of QTL results. A curated oat QTL database is being developed (Tinker et al., unpubl.) that will provide the ability to electronically search for QTLs based on trait, location, and approximate statistical significance. These data will be made available within the Graingenes database (http://graingenes.org).

The locations of groat oil QTLs shown in Fig. 1 give an example of how comparative mapping can be used to validate the importance of QTLs in multiple populations. This example is a partial analysis, since other groat oil QTLs are located on different linkage groups. However, it illustrates some of the difficulties that are encountered in comparative mapping, as well as some of the interpretations that need to be made. A QTL with a major effect on groat oil was reported on linkage group 11 of Kanota × Ogle, and in the homologous location in Kanota × Marion by Kianian et al. (1999). The location of this QTL was later confirmed in a third cross, Ogle × MAM17-5, by Zhu et al. (2004). It is clear from these two studies that the allele conferring high oil concentration originates from the two non-Ogle parents. A fourth study in Terra × Marion by De Koeyer et al. (2004) did not detect any QTL affecting oil at this location, nor was there any QTL affecting oil at homoeologous locations. From this we tentatively conclude that both Terra and Marion have similar alleles at this location, and that this QTL may not be useful for marker-assisted selection within much of the spring oat germplasm. However, the importance of this QTL in some populations, as well as its association with a candidate locus, ACCase (Kianian et al. 1999), means that it will be important to follow this locus in future studies involving groat oil.

A second region, homologous to Kanota × Ogle linkage group 22, affected groat oil in two other populations but not in Kanota × Ogle (Fig. 1). In this case, the QTL in Kanota × Marion was smaller and less significant than the QTL(s) in Terra × Marion, but the combined studies do add support for the presence of QTLs in this region. Because of statistical uncertainty about the number of QTLs involved in Terra × Marion as well as the exact comparative locations in Kanota × Ogle, this region may involve one, two, or even three or more discrete QTLs. Furthermore, although Marion alleles contributed toward higher oil in Terra × Marion, Marion alleles contributed toward lower oil in Kanota × Marion. This situation is easily explained if three different QTL alleles are present, or if different loci are segregating in each cross. However, it draws attention to the danger of conducting marker-assisted selection in germplasm where QTL associations have not been adequately predicted.

5.5
Marker-Assisted Breeding

The limiting factor in most breeding programs is the assessment of progeny lines to identify the "winners", i.e., those carrying the optimal combination of alleles

Table 4. Summary of QTL studies in oat

Cross	Population	Reference	Traits	Environments	No. of QTL	No. of regions	Methods	Comments
Kanota × Ogle	71 F$_6$ RILs	Bush and Wise 1996	Crown rust (quantitative)	Inoculated	2	2	Single marker ANOVA	
		Siripoonwiwat et al. 1996	Agronomic traits	7 site-years	178	106	Interval mapping (PlabQTL) and epistasis tests	
		Holland et al. 1997	Heading, height, vernalization, etc.		84	51		
		Beer et al. 1997	Agronomic traits	4 site-years	47	16	Trait associations combined with ANOVA	Validation of KxO QTL using allele-trait associations
		Barbosa-Neto et al. 2000	BYDV resistance	5 site-years	21	21	Single marker ANOVA	Validation in backcross populations
	71–133 F$_6$ and F$_9$ RILs	Kianian et al. 1999	Groat oil	5 site-years	4	4	Composite interval mapping (PlabQTL and MQTL)	Combined analyses in KxO and KxM
		Kianian et al. 2000	Beta-glucan	6 site-years	7	7		
		Groh et al. 2001a	Milling quality	5 site-years	15	10		
Kanota × Marion	137 F$_6$ RILs	Kianian et al. 1999	Groat oil	3 site-years	4	4		
		Kianian et al. 2000	Beta-glucan	4 site-years	4	4		
		Groh et al. 2001a	Milling quality	3 site-years	8	6		
Clintland64 × IL86-5698	126 RILs	Jin et al. 1998	BYDV resistance	Field (2 years)	6	6	Interval mapping (QGene)	
Recurrent Selection		De Koeyer and Stuthman 2001	Grain yield, height	2 years × 2 sites	7	7	Allelic shift caused by recurrent selection	Other minor QTLs were detected
Ogle × TAMO-301	136 F$_{6:7}$ RILs	Holland et al. 2002	Heading, vernalization, etc.	Field (2 years) and artificial	62	43	Interval mapping (PlabQTL) and epistasis tests	
Ogle × MAM17-5	152 F$_{5:6}$ RILs	Zhu and Kaeppler 2003b	Crown rust, height, heading	Field (2 years)	14	10	Composite Interval (PlabQTL) and epistasis tests	
		Zhu et al. 2003a	BYDV resistance	Field (2 years)	4	4		
		Zhu et al. 2003b	Crown rust (seedling)	Green-house (2 years)	2	2		
		Zhu et al. 2004	Groat protein and oil	Field (2 years)	23	22		

Table 4. (continued)

Cross	Population	Reference	Traits	Environments	No. of QTL	No. of regions	Methods	Comments
Terra × Marion	F6 RILs	De Koeyer et al. 2004	Agronomic and quality (18 traits)	13 site-years	49	23	Interval mapping (MQTL)	
Potoroo × Mortlock	170 F_8 RILs	Williams et al. 2004	Agronomic, quality, disease					In progress
Iltis × IA H611–447	3 advanced backcross populations	Yu et al. 2004	Agronomic and quality					In progress
UFRGS 14 × OR2	F_3 bulked to F_6	Malone et al. 2004	Panicle weight				Bulked Segregant Analysis	In progress
MN841801-1 × Noble-2	158 $F_{6:8}$ RILs	Portyanko et al. 2005	Crown rust resistance and flowering time	Field (3 site-years), Green-house (2 years)	14	10	Composite Interval (PlabQTL) and multiple regression	
N327-6 × Hendon		Howarth et al. 2004	Groat oil content					

at key genetic loci. Any diagnostic test that is specific, that facilitates selection earlier in the breeding cycle, or that reduces cost or complexity can be of major benefit to the breeding program, even if the test is not 100% accurate. Molecular markers closely linked to key genetic loci or QTLs have such potential, especially for disease resistance or quality traits that may be difficult or expensive to assess by other means. Molecular-marker-assisted selection (MAS) breeding is not well established yet in oats. However, MAS is a major rationale for many oat genomic research studies.

5.5.1
PCR-Based Markers

A common prerequisite to MAS in any crop is the availability of convenient molecular markers, which generally means robust PCR-based markers. Ironically, while RFLPs were the earliest markers developed in oats, are the most robust markers for recombination mapping and comparative mapping, and have been linked to many genes and QTLs, they are too labor intensive and costly to be used for the high-throughput genotyping required for most MAS. Many gene-tagging studies in oats use the more convenient PCR-based RAPDs (Table 2); however, these are not reproducible enough (Penner et al. 1993a) to be used for MAS. AFLPs have a complex multiband pattern, which is rich in genetic information because it samples many loci simultaneously. This attribute is an advantage for rapid recombination mapping or gene tagging in well-controlled research situations, but a challenge in MAS for single loci. Microsatellite markers (or SSRs, simple sequence repeats) have attractive characteristics for MAS but to date have limited utility in oat due to unexpectedly low polymorphism and the limited number currently mapped in oat (Li et al. 2000a; Holland et al. 2001b; Pal et al. 2002; Zhu and Kaeppler 2003a). Single nucleotide polymorphisms (SNPs) are emerging as the marker of choice in many crops but require a significant up-front development effort and so few are available yet in oat. To address some of the issues discussed above, oat researchers have converted a number of first-generation RFLP, RAPD, or AFLP markers into more robust PCR-based sequence characterized amplified region (SCAR) markers (Table 5). Those SCAR markers that require postamplification restriction enzyme digestion to reveal polymorphism are referred to as cleaved amplified polymorphic se-

quences (CAPS). The marker conversion process can be problematic. A review of the first 41 SCARs developed by Agriculture and Agri-Food Canada, Ottawa, suggested that ca. 15% were polymorphic on intraspecific crosses and that this value could be raised to approximately 30% with postamplification digestion (Molnar et al. 2000). This review also showed that approximately 40% of SCARs mapped to their target locus, and of those that do not, ca. 20% map to genomic regions that are clearly homoeologous to the intended target region. Thus, it is desirable to develop several SCARs per locus to insure useable polymorphism with the germplasm of interest. Fortunately, in a growing number of cases, such marker conversion is not required because PCR-based markers, suitable for MAS, have already been incorporated into the hexaploid oat map and are linked closely enough to the genetic locus or QTL of interest to be used directly for MAS. PCR-based markers developed from analysis of cereal DNA sequences obtained from large DNA and EST databases are an example (Holland et al. 2001b).

5.5.2
Pc68 and *Pc94* Case Studies

The most extensive development has been PCR-based markers for the *Pc68* crown rust resistance locus (Table 5). The *Pc68* locus was originally tagged in repulsion with the linked RAPD marker UBC269 (Penner et al. 1993c) (Table 2). Classical genetic analysis had shown previously that the *Pc68* locus was clustered with *Pg3* and *Pg9* stem rust resistance loci, as well as with other crown rust resistance loci. *Pg3* was independently tagged with RAPD marker ACOpR-2 (also known as acor195 or UBC195) (Penner et al. 1993b) and *Pg9* by the same UBC195 RAPD marker as well as by the UBC458 RAPD marker and several RFLP and storage protein markers (O'Donoughue et al. 1996) (Table 2). These multiple linked markers gave several candidates for marker conversion. Conversion of the RAPD markers was the preferred strategy since by definition the RAPD polymorphism that had been mapped originally must be associated with the 10-base-pair-long RAPD primers, and therefore contained within the DNA sequence of the RAPD PCR product. The latter is relatively easily isolated from the gel, purified through cloning, and sequenced, so that longer SCAR primers can be designed. Initial attempts to convert the UBC269 RAPD marker linked

Table 5. Marker conversion for marker assisted selection

Trait	Gene	Original marker name	Original marker type	Derived marker name	Derived marker type	Reference
Crown rust	*Pc48*				PCR-based	Chong et al. 2004
	Pc68 (in tight cluster with *Pg3* and *Pg9*)	UBC195	RAPD		SCAR and CAPS (RsaI) (based on Dumont sequence, carries *Pg9*) SCAR and CAPS (RsaI) (based on Rodney 0 sequence, relative to Pg3)	Orr et al. 1998, 1999; De Koeyer et al. 2000
			RFLP	Pc68-300	ASA	Scoles and Eckstein 2004
					SNP	Chen et al. 2004
	Pc38 (tight cluster with *Pc62* and *Pc63*)	CDO113	SCAR	CDO113s	SCAR	Wight et al. 2004
	Pc94		AFLP	SCAR94-1; SCAR94-2	SCAR	Chong et al. 2004
Loose smut	Unnamed (pathotype A13)	UBC041	RAPD	Ua300Co or Ua750	SCAR (co-dominant); SCAR (dominant)	Eckstein et al. 2002; Kibite et al. 2004
Dwarf	*Dw6*		RAPD		SNP	Kiviharju et al. 2004
BYDV		BCD1882; CDO270	RFLP	p1882; p270	SCAR	Pal et al. 2002
Cereal cyst nematode resistance					SNP	Williams et al. 2004
Low-acid detergent lignin hull		CDO373	RFLP		Conversion to PCR-based markers in progress	Rossnagel et al. 2004a

in repulsion to *Pc68* were unsuccessful; however, conversion was successful for the UBC195 markers linked to *Pg3* and *Pg9* (Orr and Molnar, unpubl. data; Orr et al. 1998, 1999) (Table 4). A dominant SCAR marker was developed based on the 0.4 kb band produced by UBC195 with Rodney0 DNA, a marker for *Pg3*. Postamplification restriction digestion with *Rsa*I produced a derived codominant CAPS marker that was applicable across broader germplasm (Scoles and Eckstein 2004). A very similar SCAR marker was developed from an alternate allele, at the UBC195 locus, found in Dumont, a variety carrying *Pg9*. This SCAR can also be used as a CAPS markers following *Rsa*I digestion. The two SCAR/CAPS markers were tested on one F$_2$, two F$_3$, and one F$_6$ breeding populations and found to predict *Pc68* resistance with 0.92 to 0.97 accuracy and susceptibility with 0.9 to 1.0 accuracy (De Koeyer et al. 2000). Because either forward primer works with either reverse primer, four primer combinations are possible. Hybrid combinations performed best in some laboratories (G. Scoles, pers. comm.). The SCAR or CAPS markers have been used extensively for MAS in the oat breeding programs at Agriculture and Agri-Food Canada, Ottawa (DeKoeyer et al. 2000). Improved second-generation markers have been developed from these SCARs/CAPS. An allele specific amplicon (ASA) marker (Pc68-300) was developed by designing a new primer that annealed to the *Rsa*I restriction site and differentiated the two alleles without the need for postamplification restriction digestion (Scoles 2004); it has been used extensively in the University of Saskatchewan oat-breeding program. Since one allele produced a single PCR product and the other produced no PCR product, and since this could be detected by UV light after addition of ethidium bromide to the PCR tube, genotypes could be inferred without the need for gel electrophoresis, a major improvement in efficiency (G. Scoles, pers. comm.). A SNP marker has also been designed that targets the same *Rsa*I site and gives a marker that can be screened on a DNA sequencer, rather than on a manual gel system, thereby increasing throughput (Chen et al. 2004).

The preceding examples illustrate that there is value in developing a variety of different PCR-based markers for the same locus for MAS purposes. Codominant markers are preferred for many MAS applications since they can identify the heterozygotes, allowing for strong selection for the desired class of homozygotes. Codominant markers also reveal failures in the PCR amplification and therefore limit false negative errors. A pair of dominant markers that

are out of phase with each other (in repulsion) can achieve the same discrimination of heterozygotes. If such a pair can be multiplexed, then it can achieve the same efficiency as a single codominant marker. Balanced against these genetic considerations are a number of technical ones. It has already been mentioned that multiplexing can increase efficiency. There are at least three multiplexing strategies: by having more than one primer pair in the PCR reaction simultaneously, by loading product from more than one PCR reaction into the same well of a gel, or by a second or third loading of a gel ca. 1 h after the previous loading and proceeding with the electrophoresis. These strategies require preplanning in the design of primers so that they share common primer annealing temperatures and PCR conditions and possibly that they produce size-distinguishable PCR products. Gel electrophoresis can be avoided completely with some SCARs since genotype is predicted on whether or not a product is formed and is detectable simply by ethidium bromide staining or fluorescent staining in the PCR tube.

Pc94 is currently the most effective crown rust resistance gene available in North America (Chong and Zegeye 2004). Two SCARs have been developed for *Pc94* (Chong et al. 2004) for MAS purposes, particularly for pyramiding *Pc94* with other rust resistance genes (Table 4). In a complementary initiative, a SCAR has been linked to the *Pc38* locus (Wight et al. 2004). This facilitates MAS to remove the gene *Pc38* from new varieties being developed, not simply because the gene has been defeated, but because *Pc38,* or a factor tightly linked to it, is known to suppress the action of the genes *Pc62* (Wilson and McMmullen 1997) and *Pc94* (Chong and Aung 1998). In a similar initiative, markers have been identified that tag the *Pc59* crown rust resistance locus (Satheeskumar et al. 2002) and converted to SCARs (Molnar et al., unpubl.). These markers and SCARs are being used at Agriculture Canada in Ottawa to pyramid *Pc59* and *Pc61*, since no differentials exist to detect *Pc59* in the presence of *Pc61*. It is anticipated that pyramiding *Pc59* with *Pc61* will provide more durable crown rust resistance.

5.5.3
Advances Toward MAS for Other Traits

PCR-based markers have been or are being developed for a whole range of traits. Three AFLP markers linked to BYDV disease resistance QTLs have been converted

to PCR-based markers for MAS (Jin et al. 1998, 1999). In addition, SCARs and CAPS have been designed from the sequences of six cDNA clones that are linked to the same BYDV resistance QTL (Pal et al. 2002). A third disease of oats, loose smut, is the target of two SCARs, the dominant Ua750 and the codominant Ua300Co (Kibite et al. 2000, 2004; Eckstein et al. 2002). Twelve genome- and/or taxon-specific RAPD markers have been converted to SCAR markers for taxonomic studies, but which could have utility for MAS (Drossou et al. 2004). PCR-based markers are under development for low acid detergent lignin hull (Rossnagel et al. 2004a).

The SNP marker developed for *Pc68* by Chen et al. (2004) was mentioned earlier. In addition, a SNP marker has been developed for the *Dw6* dwarfing gene (Kiviharju et al. 2004) and another SNP for cereal cyst nematode resistance (Williams et al. 2004). These should facilitate higher-throughput MAS.

Oat quality traits are another target for MAS. SCARs have been developed that are linked to oil and beta-glucan QTL (Orr and Molnar 2002; Molnar et al. 2004). Bioinformatic approaches are being used to develop SCARs and SNPs to candidate genes in the protein and oil biosynthetic pathways (Lybaert et al. cited in Molnar et al. 2004). Through alignment of DNA sequences for candidate genes, SNPs are being developed for ACCase, KAS, and other biosynthetic enzymes within the oil biosynthetic pathway (Howarth et al. 2004). Advanced Backcross QTL Analysis, as proposed by Tanksley and Nelson (1996), has been initiated in three breeding populations simultaneously to identify beta-glucan QTL responsible for high beta-glucan in line IA H611-447 and to introgress them into elite breeding lines (Yu et al. 2004).

Another practical example of the development of MAS in oats is that Svalof Weibull is collaborating with Goteborg University and the Swedish Farmers Supply and Crop Marketing Cooperative to initiate MAS for cold tolerance. Global gene expression studies, using RNA collected in winter from field plants, is being used to identify candidate genes that can be used as molecular markers for efficient selection in segregating breeding populations. The 2,866 different genes under analysis are also expected to yield markers for MAS for additional major traits in oat (Jonsson et al. 2004; Brautigam et al. 2004a,b).

5.6
Future Scope and Related Oat Genomic Research

Molecular-marker-based linkage maps have been developed for several oat populations, marker-trait associations have been identified for several oat genes and QTLs, and marker-assisted selection has been applied or is being evaluated for some economic traits in oat, although not to the same extent or marker density as in many of the major crops. This work will continue as oat researchers take advantage of advances in technology toward more rapid, less expensive, and higher-throughput marker development, DNA sequencing, and bioinformatic analyses. The complete sequencing of an oat genome or even the gene-rich regions is unlikely in the near future, nor is the development of huge databases of gene and genome sequences, as in several major crops and model plant species, likely; however, oat researchers will be able to draw upon the structural and functional genomic information generated in those other species to design markers or identify gene-trait relationships for molecular applications in oat. The identification of genes underlying economically important traits common to many plant species will enable the identification of "perfect markers" such as SNPs within the key genes enabling efficient, high-throughput selection at the genic rather than the phenotypic level, or eventually even the design and substitution of improved alleles for these genes by some form of gene-specific transformation.

Gene identification, mapping, and analyses will need to be continued in oat, though, because several of the valued traits as well as certain biological features are unique to oat. For example, oat is unique among the cereals in producing triterpenoids (avenacins) as active agents for combating certain root-infecting microbes (Crombie and Crombie 1986). Genes for enzymes in the pathway for avenacin biosynthesis have been cloned in the diploid oat, *A. strigosa*, and found to map as a cluster, even though the differing enzyme structures imply the genes arose other than through a duplication process from a single gene, thus raising an important evolutionary question regarding gene origin and regulation (Qi et al. 2004). Another trait unique to oat among the cereals is the presence of a large proportion of the grain oil in the endosperm, providing impetus to analyze it genetically not only to capitalize on the practical value of this trait but to

shed knowledge on regulation of tissue distribution of gene expression and product distribution (Kianian et al. 1999; Molnar et al. 2004; Howarth et al. 2004). The association of high oil with a gene for a key enzyme, ACCase, in oil biosynthesis in at least certain oat crosses illustrates how the knowledge of genes contributing to a trait may provide opportunities to manipulate or modify the trait. Oat also possesses several unique or elevated levels of secondary metabolites with food-healthy benefits such as the tocols and avenanthramides for which knowledge of their genetic basis will provide both new understanding and economic opportunities (Peterson 2004).

Continued sequencing of cDNAs of oat, particularly from subtraction and tissue-specific libraries, will provide identification of expressed genes which may be associated with particular traits. The genes may be ones novel to oat or they may be identified by their homology to ones identified in other species. The availability of a large fragment (e.g., BAC) DNA library of several genome equivalents of oat would allow the use of oat cDNA sequences, or even ones from other species, to select clones with specific oat genic regions including flanking sequences with elements involved in regulating expression of the genes of interest. The construction of a 4.1 haploid genome equivalent BAC library from diploid oat, *A. strigosa*, has been reported for further characterization and cloning of genes involved in saponin biosynthesis (Bahkt et al. 2003). The oat cDNA sequences could also be used to construct gene chips to study specific gene expression, as has been done recently in barley (Close et al. 2004).

New knowledge of the biology of oat gained from structural and functional genomic analyses is providing a better understanding of oat and how to genetically manipulate and improve it. Comparisons of DNA sequences including RFLPs, SSRs, RAPDs, AFLPs, and repetitive element families are continually shedding new light on phylogenetic relationships among *Avena* species as well as genomic relationships within the cultivated hexaploid species (Linares et al. 1998; Nocelli et al. 1999; Katsiotis et al. 2000; Li et al. 2000b; Ananiev et al. 2002; Drossou et al. 2004). Such tools and information facilitate identifying and manipulating homologous genes or alleles in related wild species or homoeologous loci within the hexaploid for trait improvement.

Also, the general conserved nature of genome organization, at least in segments, among grass species allows comparative mapping between cereals including oat that often identifies homologous genes and linked marker loci (Van Deynze et al. 1995). Comparative mapping of oat to other cereals as well as among genomes of oat has been limited, however, by the complex segmental polymorphism among oat genomes. Continued efforts to assign markers and linkage groups to chromosome to develop an integrated genic/chromosomal map and to reduce the number of genetic linkage groups to 21 are needed to facilitate these comparative mapping efforts.

Another biological feature unique to oat among the small-grained cereals is the capability to retain whole or, with manipulation, segments of individual chromosomes of maize in wide hybrid crosses (Kynast et al. 2004). The resulting oat lines each containing a segment of the maize genome provide novel opportunities to look at the genetic basis of such traits as specific disease resistances and C_3 vs. C_4 photosynthesis, thus being of both biological and potentially economic interest.

Methods for the transfer of genes from an organism into oat through genetic engineering technologies have been developed (reviewed by Somers 1999); however, no oat cultivars with such introduced genes have been commercially released. Genetic transformation technology and research in oat has lagged behind that of several major crops, partly because of reluctance of industry to fund such research based on unknown public acceptance of possible products. Also, extra caution is needed in oat in introducing genes for particular traits such as herbicide resistance because cultivated oat can readily outcross with its weedy wild oat relatives. However, limited oat transformation research is being done (e.g. Cho et al. 2003; Perrer et al. 2003; Nuutila et al. 2004) because of continuing technological improvements that will make such gene transfers more precise and presumably more publicly accepted. Also, such gene transfers or introductions are often an essential component of identifying or confirming a specific gene function to complement research on more conventional genetic manipulations.

In summary, while genomic mapping, molecular breeding, and related genomic research in oat are lagging to a certain extent compared to that in several major crops, oat's unique biological and commercial properties, together with a capability for oat researchers to draw upon the structural and functional genomics and knowledge and discoveries in other cereals, make it likely that development and application of molecular breeding will play a major role in oat genetic improvement in coming years.

References

Ananiev EV, Vales MI, Phillips RL, Rines HW (2002) Isolation of A/D and C genome specific dispersed and clustered repetitive DNA sequences from *Avena sativa*. Genome 45:431–441

Bahkt S, Qi X, Osbourn A (2003) Construction of a BAC library from diploid oat (*Avena strigosa*). In: Plant and Animal Genome XI Conf, San Diego, p 82. www.intl-pag.org

Barbosa-Neto JF, Siripoonwiwat W, O'Donoughue LS, Gray SM, Smith DM, Kolb FL, Gourmet C, Brown CM, Sorrells ME (2000) Chromosomal regions associated with barley yellow dwarf resistance in oat. Euphytica 114:67–76

Baum BR (1977) Oats: wild and cultivated. A monograph of the genus *Avena* L. (Poaceae). Monograph No. 14. Biosystematics Research Institute (currently ECORC), Agriculture and Agri-Food Canada, Ottawa, Canada

Beer SC, Siripoonwiwat W, O'Donoughue LS, Souza E, Matthews D, Sorrells ME (1997) Associations between molecular markers and quantitative traits in an oat germplasm pool: Can we infer linkages? J Agri Genom 3. http://www.cabi-publishing.org/jag/index.html

Brautigam M, Gustavsson A, Zakhrabekova S, Olsson B, Olsson O (2004a) Molecular characterization of *CBF* transcription factor genes in oat. In: Peltonen-Saino P, Topi-Hulmi M (eds) Proc 7th Int Oat Conf, Helsinki, Finland, p 192. www.mtt.fi/met/pdf/met51.pdf

Brautigam M, Lindlof A, Gharti-Chhetri G, Zakhrabekova S, Jonsson A, Olsson B, Olsson O (2004b) Analysis of 9703 expressed sequence tags in cold acclimated oat. In: Peltonen-Saino P, Topi-Hulmi M (eds) Proc 7th Int Oat Conf, Helsinki, Finland, p 193. www.mtt.fi/met/pdf/met51.pdf

Burrows VD (2005) Hulless oats. In: Abdel-Aal E-SM, Wood P (eds) Specialty Grains for Food and Feed. American Association of Cereal Chemists, St. Paul, MN, USA, pp 223–252

Burrows VD, Molnar SJ, Tinker NA, Marder T, Butler G, Lybaert A (2001) Groat yield of naked and covered oat. Can J Plant Sci 81:727–729

Bush AL, Wise RP (1996) Crown rust resistance loci on linkage groups 4 and 13 in cultivated oat. J Hered 87:427–432

Bush AL, Wise RP (1998) High-resolution mapping adjacent to the *Pc71* crown-rust resistance locus in hexaploid oat. Mol Breed 4:13–21

Bush AL, Wise RP, Rayapati PJ, Lee M (1994) Restriction fragment length polymorphisms linked to genes for resistance to crown rust (*Puccinia coronata*) in near isogenic lines of hexaploid oat (*Avena sativa*). Genome 37:823–831

Campbell H, Choo TM, Vigier B, Underhill L (2000) Mycotoxins in barley and oat samples from eastern Canada. Can J Plant Sci 80:977–980

Cervantes-Martinez CT, Frey KJ, White PJ, Wesenberg DM, Holland JB (2001) Selection for greater β-glucan content in oat grain. Crop Sci 41:1085–1091

Chen Q, Armstrong K (1994) Genomic *in situ* hybridization in *Avena sativa*. Genome 37:607–612

Chen G, Chong J, Gray M, Prashar S, Procunier JD (2004) Single nucleotide polymorphisms as next generation markers for high throughput screening for crown rust resistance in oat. In: Peltonen-Saino P, Topi-Hulmi M (eds) Proc 7th Int Oat Conf, Helsinki, Finland, p 86. www.mtt.fi/met/pdf/met51.pdf

Cheng DW, Armstrong KC, Tinker N, Wight CP, He S, Lybaert A, Fedak G, Molnar SJ (2002a) Genetic and physical mapping of *Lrk10*-like receptor kinase sequences in hexaploid oat (*Avena sativa* L.). Genome 45:100–109

Cheng DW, He S, Armstrong KC (2002b) Modified expression of two receptor kinase genes in hexaploid oat (*Avena sativa* L.) on inoculation with crown rust. Physiol Mol Plant Pathol 61:281–288

Cheng CW, Armstrong KC, Drouin G, McElroy A, Fedak G, Molnar SJ (2003) Isolation and identification of Triticeae chromosome 1 receptor-like kinase genes (*Lrk10*) from diploid, tetraploid, and hexaploid species of the genus *Avena*. Genome 46:119–127

Cho M-J, Choi H, Okamoto D, Zhang S, Lemaux P (2003) Expression of green fluorescent protein and its inheritance in transgenic oat plants generated from shoot meristematic cultures. Plant Cell Rep 21:467–474

Chong J, Aung T (1998) Interaction of the crown rust resistance gene *Pc94* with several *Pc* genes. In: Proc 9th European and Mediterranean Cereal Rusts and Powdery Mildews Conf, 2–6 September 1996, Lunteren, The Netherlands. European and Mediterranean Cereal Rust Foundation, Wageningen, pp 172–175

Chong J, Zegeye T (2004) Physiologic specialization of *Puccinia coronata* f. sp. *avenae*, the cause of oat crown rust, in Canada from 1999 to 2001. Can J Plant Pathol 26:97–108

Chong J, Reimer E, Somers D, Aung T, Penner GA (2004) Development of sequence-characterized amplified region (SCAR) markers for resistance gene *Pc94* to crown rust in oat. Can J Plant Pathol 26:89–96

Close TJ, Wanamaker SI, Caldo RA, Turner SM, Ashlock DA, Dickerson JA, Wing RA, Muehlbauer GJ, Kleinhofs A, Wise RP (2004) A new resource for cereal genomics: 22K barley GeneChip comes of age. Plant Physiol 134:960–968

Crombie WML, Crombie L (1986) Distribution of avenacins A-1, A-2, B-1 and B-2 in oat *Avena sativa* roots: their fungicidal activity towards take-all fungus *Gaeumannomyces graminis*. Phytochemistry 25:2069–2074

De Koeyer DL, Stuthman DD (2001) Allelic shifts and quantitative trait loci in a recurrent selection population of oat. Crop Sci 41:1228–1234

De Koeyer D, Orr W, Lybaert A, Deyl J, Chenier C, Tinker N, McElroy A, Chong J, Molnar S (2000) SCAR markers linked to the *Pc68* resistance allele are an effective tool for selection. In: Cross RJ (ed) Proc. 6th Int Oat Conf, Lincoln University, Lincoln, NZ, 13–16 Nov 2000, New Zealand Institute for Crop and Food Research, Christchurch, New Zealand. ISBN 0-478-10820-6: http://wheat.pw.usda.gov/ggpages/Oats/IOC6.html

De Koeyer DL, Tinker NA, Wight CP, Deyl J, Burrows VD, O'Donoughue LS, Lybaert A, Molnar SJ, Armstrong KC, Fedak G, Wesenberg DM, Rossnagel BG, McElroy AR (2004) A molecular linkage map with associated QTL from a hulless x covered spring oat population. Theor Appl Genet 108:1285–1298

Devos KM, Gale MD (2000) Genome relationships: the grass model in current research. Plant Cell 12:637–646

Drossou A, Katsiotis A, Leggett JM, Loukas M, Tsakas S (2004) Genome and species relationships in genus *Avena* based on RAPD and AFLP molecular markers. Theor Appl Genet 109:48–54

Eckstein P, Kibite S, Menzies J, Hay D, Rossnagel BG, Scoles GJ (2002) Dominant and co-dominant SCAR markers for a gene conferring loose smut resistance in oat. Poster Abstr, Am Oat Workers Conf, 5–7 May 2002, Wilmington, NC, p 33. In: Hoffman D (ed) Oat Newslett 48 (2002). http://wheat/pw.usda.gov/ggpages/oatnewsletter/v48/AOWC_Abstracts.htm

Fetch TG, Jr, Dunsmore KM (2004) Physiologic specialization of *Puccinia graminis* on wheat, barley, and oat in Canada in 2001. Can J Plant Pathol 26:148–155

Fominaya A, Vega C, Ferrer E (1988) Giemsa C-banded karyotypes of *Avena* species. Genome 30:627–632

Fominaya A, Hueros G, Loarce Y, Ferrer E (1995) Chromosomal distribution of a repeated DNA sequence from C-genome heterochromatin and the identification of a new ribosomal DNA locus in the *Avena* genus. Genome 38:548–557

Fox SL, Jellen EN, Kianian SF, Rines HW, Phillips RL (2001) Assignment of RFLP linkage groups to chromosomes using monosomic F_1 analysis in hexaploid oat. Theor Appl Genet 102:320–326

Groh S, Kianian SF, Phillips RL, Rines HW, Stuthman DD, Wesenberg DM, Fulcher RG (2001a) Analysis of factors influencing milling yield and their association to other traits by QTL analysis in two hexaploid oat populations. Theor Appl Genet 103:9–18

Groh S, Zacharias A, Kianian SF, Penner GA, Chong J, Rines HW, Phillips RL (2001b) Comparative AFLP mapping in two hexaploid oat populations. Theor Appl Genet 102:876–884

Harder DE, Haber S (1992) Oat diseases and pathologic techniques. In: Marshall HG, Sorrells ME (eds) Oat Science and Technology, Agron Monogr 33. ASA and CSSA, Madison, WI, pp 307–425

Hayasaki M, Morikawa T, Tarumoto I (2000) Intergenomic translocations of polyploid oats (genus *Avena*) revealed by genomic in situ hybridization. Genes Genet Syst 75:167–171

Holland JB (1997) Oat improvement. In: Kang MS (ed) Crop Improvement for the 21st Century. Research Signpost, Trivandrum, India, pp 57–98

Holland JB, Moser HS, O'Donoughue LS, Lee M (1997) QTLs and epistasis associated with vernalization responses in oat. Crop Sci 37:1306–1316

Holland JB, Frey KJ, Hammond EG (2001a) Correlated responses of fatty acid composition, grain quality, and agronomic traits to nine cycles of recurrent selection for increased oil content in oat. Euphytica 122:69–79

Holland JB, Helland SJ, Sharopova N, Rhyne DC (2001b) Polymorphism of PCR-based markers targeting exons, introns, promoter regions, and SSRs in maize and introns and repeat sequences in oat. Genome 44:1065–1076

Holland JB, Portyanko VA, Hoffman DA, Lee M (2002) Genomic regions controlling vernalization and photoperiod responses in oat. Theor Appl Genet 105:113–126

Howarth C, Langdon T, Cowan A, Leggett M, Valentine J (2004) Development and use of markers for oil in oats. In: Peltonen-Saino P, Topi-Hulmi M (eds) Proc 7th Int Oat Conf, Helsinki, Finland, p 71. http://www.mtt.fi/met/pdf/met51.pdf

Irigoyen ML, Loarce Y, Linares C, Ferrer E, Leggett M, Fominaya A (2001) Discrimination of the closely related A and B genomes in AABB tetraploid species of *Avena*. Theor Appl Genet 103:1160–1166

Irigoyen ML, Linares C, Ferrer E, Fominaya A (2002) Fluorescence *in situ* hybridization of *Avena sativa* L. cv. SunII and its monosomic lines using cloned repetitive DNA sequences. Genome 45:1230–1237

Irigoyen ML, Loarce Y, Fominaya A, Ferrer E (2004) Isolation and mapping of resistance gene analogs from the *Avena strigosa* genome. Theor Appl Genet 109:713–724

Jellen EN, Phillips RL, Rines HW (1993a) C-banded karyotypes and polymorphisms in hexaploid oat accessions (*Avena* spp.) using Wright's stain. Genome 37:1129–1137

Jellen EN, Rooney WL, Phillips RL, Rines HW (1993b) Characterization of the hexaploid oat *Avena byzantina* cv. Kanota monosomic series using C-banding and RFLPs. Genome 36:962–970

Jellen EN, Gill BS, Cox TS (1994) Genomic in situ hybridization differentiates between A/D- and C-genome chromatin and detects intergenomic translocations in polyploidy oat species (genus *Avena*). Genome 37:613–618

Jin H, Domier LL, Kolb FL, Brown CM (1998) Identification of quantitative loci for tolerance to barley yellow dwarf virus in oat. Phytopathology 88:410–415

Jin H, Domier LL, Kolb FL, Brown CM (1999) Conversion of AFLP markers associated with BYDV tolerance in oats to non-radioactive PCR markers. In: Plant and Animal Genome VII Conf, San Diego, p 396. www.intl-pag.org

Jin H, Domier LL, Shen X, Kolb FL (2000) Combined AFLP and RFLP mapping in two hexaploid oat recombinant inbred populations. Genome 43:94–101

Jonsson R, Gharti-Chhetri G, Brautigam M, Jonsson A, Olsson O (2004) Development of a Scandinavian winter oat by molecular breeding and tissue culture techniques. In: Peltonen-Saino P, Topi-Hulmi M (eds) Proc 7th Int Oat Conf, Helsinki, Finland, p 190. www.mtt.fi/met/pdf/met51.pdf

Katsiotis A, Loukas M, Heslop-Harrison JS (2000) Repetitive DNA, genome and species relationships in *Avena* and *Arrhenatherum* (Poaceae). Ann Bot 86:1135–1142

Kianian SF, Wu B-C, Fox SL, Rines HW, Phillips RL (1997) Aneuploid marker assignment in hexaploid oat with the C genome as a reference for determining remnant homoeology. Genome 40:386–396

Kianian SF, Egli MA, Phillips RL, Rines HW, Somers DA, Gengenbach BG, Webster FH, Livingston SM, Groh S, O'-Donoughue LS, Sorrells ME, Wesenberg DM, Stuthman DD, Fulcher RG (1999) Association of a major groat oil content QTL and an acetyl-CoA carboxylase gene in oat. Theor Appl Genet 98:884–894

Kianian SF, Phillips RL, Rines HW, Fulcher RG, Webster FH, Stuthman DD (2000) Quantitative trait loci influencing β-glucan content in oat (*Avena sativa*, 2n = 6x = 42). Theor Appl Genet 101:1049–1055

Kianian SF, Fox SL, Groh S, Tinker N, O'Donoughue LS, Rayapati PJ, Wise RP, Lee M, Sorrells ME, Fedak G, Molnar SJ, Rines HW, Phillips RL (2001) Molecular marker linkage maps in diploid and hexaploid oat (*Avena sp.*). In: Phillips RL, Vasil IK (eds) DNA–Based Markers in Plants. Kluwer, Dordrecht, pp 443–462

Kibite S, Menzies J, Thomas PL (2000) Inheritance of resistance to three pathotypes of loose and covered smut of oats. In: Cross RJ (ed) Proc 6th Int Oat Conf, Lincoln, NZ, pp 298–301. http://wheat.pw.usda.gov/ggpages/Oats/IOC6.html

Kibite S, Rossnagel B, Eckstein P, Hay D, Menzies J, Dill-Mackay R, Scoles G (2004) A molecular marker for, and the organization of, a cluster of loose smut resistance genes in oat. In: Peltonen-Saino P, Topi-Hulmi M (eds) Proc 7th Int Oat Conf, Helsinki, Finland, p 183. www.mtt.fi/met/pdf/met51.pdf

Kiviharju E, Manninen O, Pietila L, Tanhuanpaa P (2004) DNA marker for oat dwarfing gene. In: Peltonen-Saino P, Topi-Hulmi M (eds) Proc 7th Int Oat Conf, Helsinki, Finland, p 171. www.mtt.fi/met/pdf/met51.pdf

Kremer CA, Lee M, Holland JB (2001) A restriction fragment length polymorphism based linkage map of a diploid *Avena* recombinant inbred line population. Genome 44:192–204

Kynast RG, Okagaki RJ, Galatowitsch MW, Granath SR, Jacobs MS, Stec AO, Rines HW, Phillips RL (2004) Dissecting the maize genome by using chromosome addition and radiation hybrid lines. Proc Natl Acad Sci USA 101:9921–9926

Ladizinsky G (1988) The domestication and history of oats. In: Mattson B, Lyhagen R (eds) Proc 3rd Int Oat Conf, Svalof AB, Lund, Sweden, pp 7–12

Ladizinsky G (1998) A new species of *Avena* from Sicily, possibly the tetraploid progenitor of hexaploid oats. Genet Resource Crop Evol 45:263–269

Ladizinsky G, Zohary D (1971) Notes on species delineation, species relationships and polyploidy in *Avena* L. Euphytica 20:380–395

Leggett JM (1992) Classification and speciation in *Avena*. In: Marshall HG, Sorrells ME (eds) Oat Science and Technology, Agron Monogr 33. ASA and CSSA, Madison, WI, pp 29–52

Leggett JM, Markhand GS (1995) The genomic identification of some monosomics of *Avena sativa* L. cv. Sun II using genomic in situ hybridization. Genome 38:747–751

Leggett JM, Thomas H (1995) Oat evolution and cytogenetics. In: Welch RW (ed) The Oat Crop: Production and Utilization. Chapman and Hall, London, pp 120–149

Li C-D, Rossnagel BG, Scoles GJ (2000a) The development of oat microsatellite markers and their use in identifying relationships among *Avena* species and oat cultivars. Theor Appl Genet 101:1259–1268

Li C-D, Rossnagel BG, Scoles GJ (2000b) Tracing the phylogeny of the hexaploid oat *Avena sativa* with satellite DNAs. Crop Sci 40:1755–1763

Linares C, Ferrer E, Fominaya A (1998) Discrimination of the closely related A and D genomes of the hexaploid oat *Avena sativa* L. Proc Natl Acad Sci USA 95:12450–12455

Linares C, Irigoyan ML, Fominaya A (2000) Identification of C-genome chromosomes involved in intergenomic translocations in *Avena sativa* L., using cloned repetitive DNA sequences. Theor Appl Genet 100:353–360

Malone G, Marchioro V, Kopp MM, Malone E, Maia LC, Zimmer PD, de Carvalho IF, de Oliveira AC (2004) Finding AFLP markers associated to panicle weight through bulk segregant analysis. In: Peltonen-Saino P, Topi-Hulmi M (eds) Proc 7th Int Oat Conf, Helsinki, Finland, p 163. www.mtt.fi/met/pdf/met51.pdf

Marshall HG, Shaner GE (1992) Genetics and inheritance in oat. In: Marshall HG, Sorrells ME (eds) Oat Science and Technology, Agron Monogr 33. ASA and CSSA, Madison, WI, pp 510–571

Marshall HG, Sorrells ME (1992) Oat Science and Technology, Agron Monogr 33. ASA and CSSA, Madison, WI

McCallum BD, Harder DE, Dunsmore KM (2000) Stem rusts on wheat, barley, and oat in Canada in 1999. Can J Plant Pathol 22:23–28

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

Milach SCK, Rines HW, Phillips RL (1997) Molecular genetic mapping of dwarfing genes in oat. Theor Appl Genet 95:783–790

Molnar S, Orr W, Lybaert A, Tinker N, Cheng D, Smith A, Armstrong K, De Koeyer D (2000) Development of PCR

based markers for molecular marker assisted breeding. In: 6th Int Oat Conf, Lincoln University, Lincoln, NZ, pp 153–156. http://wheat.pw.usda.gov/ggpages/Oats/IOC6.html

Molnar SJ, Tinker N, De Koeyer D, Orr W, Lybaert A, Yan W, Mather D, Wight C, McElroy A (2004) Genomic investigation of oat quality. In: Peltonen-Saino P, Topi-Hulmi M (eds) Proc 7th Int Oat Conf, Helsinki, Finland, p 51. www.mtt.fi/met/pdf/met51.pdf

Moore G, Devos KM, Wang Z, Gale MD (1995) Cereal genome evolution: Grasses, line up and form a circle. Curr Biol 5:737–739

Morikawa T (1975) Identification of the 21 monosomic lines in *Avena byzantina* C. Koch cv. 'Kanota.' Theor Appl Genet 70:271–278

Murai H, Tsunewaki K (1987) Chloroplast genome evolution in the genus *Avena*. Genetics 116:613–621

Murphy JP, Hoffman LA (1992) The origin, history, and production of oat. In: Marshall HG, Sorrells ME (eds) Oat Science and Technology, Agron Monogr 33. ASA and CSSA, Madison, WI, pp 1–28

Nocelli E, Giovannini T, Bioni M, Alicchio R (1999) RFLP- and RAPD- based genetic relationships of seven diploid species of *Avena* with the A genome. Genome 42:950–959

Nuutila AM, Lehto K, Oksman-Caldentey K-M (2004) Transgenic oat for improved BYDV resistance. In: Peltonen-Saino P, Topi-Hulmi M (eds) Proc 7th Int Oat Conf, Helsinki, Finland, p 88. www.mtt.fi/met/pdf/met51.pdf

O'Donoughue LS, Wang Z, Röder M, Kneen B, Leggett M, Sorrells ME, Tanksley SD (1992) An RFLP-based linkage map of oats based on a cross between two diploid taxa (*Avena atlantica* x *A. hirtula*). Genome 35:765–771

O'Donoughue LS, Rayapati PJ, Kianian SF, Sorrells ME, Tanksley SD, Lee M, Rines HW, Phillips RL (1994) Development of RFLP-based linkage maps in diploid and hexaploid oat (*Avena* sp.) In: Phillips RL, Vasil IK (eds) DNA-Based Markers in Plants. Kluwer, Dordrecht, pp 359–374

O'Donoughue LS, Kianian SF, Rayapati PJ, Penner GA, Sorrells ME, Tanksley SD, Phillips RL, Rines HW, Lee M, Fedak G, Molnar SJ, Hoffman D, Salas CA, Wu B, Autrique E, Van Deynze A (1995) A molecular linkage map of cultivated oat. Genome 38:368–380

O'Donoughue LS, Chong J, Wight CP, Fedak G, Molnar SJ (1996) Localization of stem rust resistance genes and associated molecular markers in cultivated oat. Phytopathology 86:719–727

Orr W, Molnar SJ (2002) Development and mapping of PCR based SCAR markers linked to oil QTL in oat (*Avena sativa* L.). Poster Abstr Am Oat Workers Conf, 5–7 May 2002, Wilmington, NC, p 41. In: Hoffman D (ed) Oat Newslett 48 (2002). http://wheat.pw.usda.gov/ggpages/oatnewsletter/v48/AOWC_Abstracts.htm

Orr W, De Koeyer D, Chenier C, Tinker N, Molnar SJ (1998) SCAR markers for rust resistance genes *Pc68*, *Pg3* and *Pg9* designed for marker assisted selection in oats. Poster Abstr Am Oat Workers Conf. In: Chong J (ed) Oat

Newslett 44(1998):32. http://wheat.pw.usda.gov/ggpages/oatnewsletter/Poster_abstracts.html

Orr WM, De Koeyer D, Chenier C, Tinker NA, Molnar SJ (1999) SCAR markers for oat rust resistance genes (*Pc68*, *Pg3*, *Pg9*) designed for marker assisted selection. In: Plant and Animal Genome VII Conf, San Diego, p 395. www.intl-pag.org

Pal N, Sandhu JS, Domier LL, Kolb FL (2002) Development and characterization of microsatellite and RFLP-derived PCR markers in oat. Crop Sci 42:912–918

Penner GA, Bush A, Wise R, Kim W, Dormier L, Kasha K, Laroche A, Scoles G, Molnar SJ, Fedak G (1993a) Reproducibility of random amplified polymorphic DNA (RAPD) analysis among laboratories. PCR Methods Appl 2:341–345

Penner GA, Chong J, Levesque-Lemay M, Molnar SJ, Fedak G (1993b) Identification of a RAPD marker linked to the oat stem rust gene *Pg3*. Theor Appl Genet 85:702–705

Penner GA, Chong J, Wight CP, Molnar SJ, Fedak G (1993c) Identification of an RAPD marker for the crown rust resistance gene *Pc68* in oats. Genome 36:818–820

Perrer SP, Valentine J, Leggett MJ, Morris P (2003) Integration, expression and inheritance of transgenes in hexaploid oat (*Avena sativa* L.). J Plant Physiol 160:931–943

Peterson DM (1992) Composition and nutritional characteristics of oat grain and products. In: Marshall HG, Sorrells ME (eds) Oat Science and Technology, Agron Monogr 33. ASA and CSSA, Madison, WI, USA, pp 265–292

Peterson DM (2004) Oat – a multifunctional grain. In: Peltonen-Saino P, Topi-Hulmi M (eds) Proc 7th Intl Oat Conf, Helsinki, Finland, pp 21–26. www.mtt.fi/met/pdf/met51.pdf

Portyanko VA, Hoffman DL, Lee M, Holland JB (2001) A linkage map of hexaploid oat based on grass anchor DNA clones and its relationship to other oat maps. Genome 44:249–265

Portyanko VA, Chen G, Rines HW, Phillips RL, Leonard KJ, Ochocki GE, Stuthman DD (2005) Quantitative trait loci for partial resistance to crown rust, *Puccinia coronata*, in cultivated oat, *Avena sativa* L. Theor Appl Genet 111:313–324

Qi X, Bakht S, Leggett M, Maxwell C, Melton R, Osbourn A (2004) A gene cluster for secondary metabolism in oat: implications for the evolution of metabolic diversity in plants. Proc Natl Acad Sci USA 101:8233–8238

Rajhathy T, Thomas H (1974) Cytogenetics of oats (Avena L.). Miscellaneous publication of the Genetics Society of Canada No.2. Genetics Society of Canada, Ottawa, Ontario

Rayapati PJ, Gregory JW, Lee M, Wise RP (1994) A linkage map of diploid *Avena* based on RFLP loci and a locus conferring resistance to nine isolates of *Puccinia coronata* var. *avenae*. Theor Appl Genet 89:831–837

Rines HW, Phillips RL, Anderson OD, Vance CP, Crossman CC, Lazo GR, Miller SS, Taller JM (2004) ESTs, cytogenetic stocks, and other tools for oat genomics. In: Peltonen-Saino P, Topi-Hulmi M (eds) Proc 7th Int Oat Conf, Helsinki, Finland, p 69. www.mtt.fi/met/pdf/met51.pdf

Ronald, PS, Penner GA, Brown PD, Brule-Babel A (1997) Identification of RAPD markers for percent hull in oat. Genome 40:873–878

Rooney WL, Jellen EN, Phillips RL, Rines HW, Kianian SF (1994a) Identification of homoeologous chromosomes in hexaploid oat (*A. byzantina* cv. Kanota) using monosomics and RFLP analysis. Theor Appl Genet 89:329–335

Rooney WL, Rines HW, Phillips RL (1994b) Identification of RFLP markers linked to crown rust resistance genes *Pc91* and *Pc92* in oat. Crop Sci 34:940–944

Rossnagel B, Eckstein P, Williams S, Arganosa G, Kibite S, Scoles G (2004a) Low acid detergent lignin oat hull: molecular marker development and chromosome location. In: Peltonen-Saino P, Topi-Hulmi M (eds) Proc 7th Int Oat Conf, Helsinki, Finland, p 153. www.mtt.fi/met/pdf/met51.pdf

Rossnagel B, Zatorski T, Racz V, McKinnon J, Christensen D (2004b) Better feed for cattle. In: Peltonen-Saino P, Topi-Hulmi M (eds) Proc 7th Int Oat Conf, Helsinki, Finland, p 54. www.mtt.fi/met/pdf/met51.pdf

Satheeskumar S, Chapados J, Deyl J, Molnar S, McElroy A (2002) Genetic association of crown rust gene *Pc59* and AFLP markers in oats. Poster Abstr Am Oat Workers Conf, 5–7 May 2002, Wilmington, NC, p 44. In: Hoffman D (ed) Oat Newslett 48(2002). http://wheat.pw.usda.gov/ggpages/oatnewsletter/v48/AOWC_Abstracts.htm

Scoles G, Eckstein P (2004) The applications of biotechnology to disease resistance breeding in oat. In: Peltonen-Saino P, Topi-Hulmi M (eds) Proc 7th Int Oat Conf, Helsinki, Finland, pp 77–84. www.mtt.fi/met/pdf/met51.pdf

Simons MD, Martens JW, McKenzie RIH, Nishiyama I, Sadanaga K, Sebesta J, Thomas H (1978) Oats: a standardized system of nomenclature for genes and chromosomes and catalog of genes covering characters. Agriculture Handbook Number 509, issued April 1978 by United States Department of Agriculture (in cooperation with Iowa Agriculture and Home Economics Experiment Station), prepared by Science and Education Administration, for sale by Superintendent of Documents, US Government Printing Office, Washington, DC

Siripoonwiwat W, O'Donoughue LS, Wesenberg D, Hoffman DL, Barbosa-Neto JF, Sorrells ME (1996) Chromosomal regions associated with quantitative traits in oat. J Agric Genom 2. http://www.cabi-publishing.org/gateways/jag/index.html

Somers DA (1999) Transgenic cereals: *Avena sativa* (oat). In: Vasil IK (ed) Advances in Cellular and Molecular Biology of Plants, vol. 5. Molecular Improvement of Cereal Crops. Kluwer, Dordrecht, pp 317–339

Start MA (2000) RFLP association to chromosome using an oat aneuploid series. MS thesis, University of Minnesota, St Paul, MN

Steer MW (1975) Evolution in the genus *Avena*: Inheritance of different forms of ribulose diphosphate carboxylase. Can J Genet Cytol 17:337–344

Stuthman DD (1995) Oat breeding and genetics. In: Welch RW (ed) The Oat Crop: Production and Utilization. Chapman and Hall, London, pp 150–176

Symons SJ, Fulcher RG (1988) Determination of variation in oat kernel morphology by digital image analysis. J Cereal Sci 7:219–228

Tanksley SD, Nelson JC (1996) Advanced backcross QTL analysis: a method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. Theor Appl Genet 92:191–203

Thompson RK, Mustafa AF, McKinnon JJ, Maenz D, Rossnagel B (2000) Genotypic differences in chemical composition and ruminal digestability of oat hulls. Can J Animal Sci 80:377–379

Valentine J, Cowan S (2004) New directions in breeding for high quality oats. In: Peltonen-Saino P, Topi-Hulmi M (eds) Proc 7th Intl Oat Conf, Helsinki, Finland, pp 45–50: www.mtt.fi/met/pdf/met51.pdf

Van Deynze AE, Nelson JC, O'Donoughue LS, Ahn SN, Siripoonwiwat W, Harrington SE, Yglesias ES, Braga DP, McCouch SR, Sorrells ME (1995) Comparative mapping in grasses. Oat relationships. Mol Gen Genet 249:349–356

Van Deynze A, Sorrells ME, Park WD, Ayres NM, Fu H, Catinhour SW, Paul E, McCouch SR (1998) Anchor probes for comparative mapping of grass genera. Theor Appl Genet 97:356–369

Welch RW (1995) The Oat Crop: Production and Utilization. Chapman and Hall, London

Wight CP, Penner GA, O'Donoughue LS, Burrows VD, Molnar SJ, Fedak G (1994) The identification of random amplified polymorphic DNA (RAPD) markers for daylength sensitivity in oat. Genome 37:910–914

Wight CP, Tinker NA, Kianian SF, Sorrells ME, O'Donoughue LS, Hoffman DL, Groh S, Scoles GJ, Li CD, Webster FH, Phillips RL, Rines HW, Livingston SM, Armstrong KC, Fedak G, Molnar SJ (2003) A molecular marker map in Kanota × Ogle hexaploid oat (*Avena* spp.) enhanced by additional markers and a robust framework. Genome 46:28–47

Wight CP, O'Donoughue LS, Chong J, Tinker NA, Molnar SJ (2004) Discovery, localization, and sequence characterization of molecular markers for the crown rust resistance gene *Pc38*, *Pc39*, and *Pc48* in cultivated oat (*Avena sativa* L.). Mol Breed 14:349–361

Williams K, Willsmore K, Hoppo S, Eckermann P, Zwer P (2004) Mapping of quantitative trait loci for yield, quality and disease resistance. In: Peltonen-Saino P, Topi-Hulmi M (eds) Proc 7th Int Oat Conf, Helsinki, Finland, p 71. www.mtt.fi/met/pdf/met51.pdf

Wilson WF, McMullen MS (1997) Dosage dependent genetic suppression of oat crown rust resistance gene *Pc62*. Crop Sci 37:1699–1705

Wise RP, Lee M, Rayapati PJ (1996) Recombination within a 5-centimorgan region in diploid *Avena* reveals multiple specificities conferring resistance to *Puccinia coronata*. Phytopathology 86:340–346

Yu G-X, Wise RP (2000) An anchored AFLP- and retrotransposon-based map of diploid *Avena*. Genome 43:736–749

Yu J, Beuch S, Herrmann M, Hackauf B (2004) AB-QTL analysis for β-glucan content in oats. In: Peltonen-Saino P, Topi-Hulmi M (eds) Proc 7th Int Oat Conf, Helsinki, Finland, p 71. www.mtt.fi/met/pdf/met51.pdf

Zhu S, Kaeppler HF (2003a) A genetic linkage map for hexaploid, cultivated oat (*Avena sativa* L.) based on an intraspecific cross 'Ogle/MAM17-5'. Theor Appl Genet 107:26–35

Zhu S, Kaeppler HF (2003b) Identification of quantitative trait loci for resistance to crown rust in oat line MAM17-5. Crop Sci 43:358–366

Zhu S, Kolb FL, Kaeppler HF (2003a) Molecular mapping of genomic regions underlying barley yellow dwarf tolerance in cultivated oat (*Avena sativa* L.). Theor Appl Genet 106:1300–1306

Zhu S, Leonard KJ, Kaeppler HF (2003b) Quantitative trait loci associated with seedling resistance to isolates of *Puccinia coronata* in oat. Phytopathology 93:860–866

Zhu S, Rossnagel BG, Kaeppler HF (2004) Genetic analysis of quantitative trait loci for groat protein and oil content in oat. Crop Sci 44:254–260

6 Secale

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6.1
Introduction

6.1.1
Morphology

The genus *Secale* includes annual and perennial taxa, which generally contain two hermaphroditic florets in each spikelet. The lower leaf sheaths and blades are generally somewhat hairy, and a thin layer of wax is often seen, especially around the nodes. Most taxa are allogamous with rather long anthers (5 to 14 mm long). Only *S. sylvestre* is morphologically distinct from the other taxa, with the glume awn being several times longer in *S. sylvestre* than in any other taxa of *Secale* (Frederiksen and Petersen 1997). In addition, pollen of *S. sylvestre* is nearly spherical, whereas all other taxa have ovoid pollen (Frederiksen and Petersen 1997).

6.1.2
Cytology

The genus *Secale* is composed of diploid species with $2n = 2x = 14$ (Jain 1960; Petersen 1991). The genome has been designated R (Wang et al. 1996). However, some tetraploid strains have been created in cultivated rye (*S. cereale* L.). Giemsa banding or C-banding of chromosomes has shown the genus *Secale* to be characterized by large telomeric heterochromatic bands and a number of weak interstitial bands. The chromosomes of *S. sylvestre* differ from those of other taxa in the genus by their low number of interstitial bands and the small size of the telomeric bands (Singh and Röbbelen 1977). *Secale cereale* exhibits great variability in giemsa banding pattern, which is consistent with observations of such patterns in other allogamous species (Linde-Laursen et al. 1980). The distribution of some highly repeated DNA sequences detected by *in situ* hybridization (ISH) has shown that the self-pollinated annuals *S. sylvestre* and *S. vavilovii* contain considerably fewer repeated DNA sequences than the remaining taxa (Cuadrado and Jouve 1997). Karyotypes of some taxa are also known to differ from each other by a number of translocations (Riley 1955; Khush and Stebbins 1961; Khush 1962).

6.1.3
Origin of Cultivated Rye

There is general agreement that the weedy ryes of Central and Southeast Asia are direct progenitors of cultivated rye (Vavilov 1917, 1926). The weedy ryes taxonomically belong to *S. cereale*, occur only in connection with agriculture, and are much younger than wild species of the genus. However, there has been extensive disagreement about the ancestry of *S. cereale*. Vavilov (1926) and Roshevitz (1947) considered *S. vavilovii* to be the ancestor of *S. cereale*, and *S. vavilovii* to have evolved from *S. montanum*. Zhukovsky (1933) and Schiemann (1948) showed that *S. cereale* descended from *S. ancestrale,* and that *S. ancestrale* and *S. montanum* diverged from a common ancestor. Riley (1955) concluded that *S. cereale* originated from *S. montanum* due to the chance fixation of two translocations. Stutz (1957) regarded *S. cereale* as a product of a hybridization involving *S. montanum* and *S. sylvestre* because *S. sylvestre* and *S. cereale* have the same chromosome arrangement. After considering ecological preferences, breeding habits, geographical distribution, and morphological and cytological affinities of wild species and cultivated rye, Khush and Stebbins (1961) concluded that *S. cereale* evolved from *S. montanum* as a result of progressive cytological and morphological differentiation and that this differentiation was probably facilitated by adaptive superiority of translocation heterozygotes and rearrangement ho-

mozygotes. Furthermore, Khush (1962) showed that on the basis of geographical distribution, breeding system, growth habit, morphology, crossability, cytological, and genetic affinities, *S. sylvestre* differed from all of the other *Secale* species rather strikingly, while the other species seemed to be more closely related. It was suggested that *S. sylvestre* differentiated from *S. montanum* much earlier than the other species. In addition, Khush (1962) demonstrated *S. cereale* ssp. *segetale* was the immediate ancestor of cultivated ryes.

Based on extensive cytological, ecological, and morphological studies, Stutz (1972) concluded that cultivated rye originated from weedy products, which were derived from the introgression of *S. montanum* into *S. vavilovii*. *Secale vavilovii* appears to have been derived from *S. sylvestre* as a consequence of chromosomal translocations. *Secale sylvestre* was derived from *S. montanum* or a common ancestor. *Secale africanum, S. dalmaticum, S. ciliatoglume,* and *S. kuprijanovii* appeared to be only slightly modified, isolated populations of *S. montanum* (Fig. 1).

Isozyme data showed that *S. cereale* and *S. montanum* were closely related and genetically similar. These data supported the opinion that *S. vavilovii* and *S. sylvestre* originated from *S. montanum*, the oldest species of the genus (Vences et al. 1987). In contrast, most molecular data showed that *S. sylvestre* was the most ancient species (Reddy et al. 1990; Petersen and Doebley 1993; Pozo et al. 1995). A recent study by Cuadrado and Jouve (1997) was in accordance with prior molecular data and showed that the lack of a 480-bp repeated sequence in all telomeres of *S. sylvestre* supported the early separation and clear distinction of this species from the rest of *Secale* species. *S. vavilovii*, which possesses the 480-bp repeat family in the telomere, was considered to be more evolved than *S. sylvestre*. *S. cereale, S. montanum*, and *S. kuprijanovii*, which showed amplification and complex organization of repeated sequence families in the telomeres, interstitial formation, and a tendency toward the doubling of loci for the 120- and 480-bp sequences, were considered the most evolved species. The appearance of a new locus or 5S rRNA in *S. cereale* and *S. ancestrale* suggested that cultivated ryes evolved from this weedy species.

Phylogenetic relationships among the *Secale* species based on the presence and distribution of two simple sequence repeats (SSRs), three highly repeated sequences from rye, and 5S rDNA supported the notion that *S. sylvestre* had split off from *S. strictum* in the Miocene Period (Cuadrado and

Jouve 2002). The second stage in the evolution of the genus occurred in the Pleistocene Period, after the geographical separation of the perennial species *S. africanum*. A similar pattern of distribution of the clusters (AAC)n, (AAG)n, and the wild rye species demonstrated that *S. sylvestre* was the species that showed the greatest number of comparative sequence differences and therefore was the most distant of all the taxonomic units analyzed. *S. strictum* (Presh) ssp. *strictum* was most closely related to *S. strictum* ssp. *africanum* (Stapf) and *S. strictum* ssp. The presence of the 5S rDNA locus in chromosome arm 3RS of *S. cereale* and *S. vavilovii* supported the close relationship and common origin of both species. After an indefinite time, they became disjoined and evolved separately.

The internal transcribed spacer sequences of the 18S-5.8S rDNA (ITS-1) region of cultivated rye and *prijanovii* (Grossh) were compared to *S. strictum* ssp. *anatolicum* (Boiss.) Hammer. No significant differences were found between the weedy forms of *S. cereale* and cultivated rye (de Bustos and Jouve 2002).

6.1.4
Distribution of the Genus *Secale*

The genus *Secale* is a typical representative of Mediterranean flora and has a wide distribution from central Europe and the western Mediterranean through the Balkans, Anatolia, Israel, and the Caucasus to Central Asia (Fig. 2). An isolated population also appears in South Africa (Sencer and Hawkes 1980).

Perennial wild rye taxa grow mainly in primary habitats (meadows, rangeland, among bushes and rocks on calcareous slope, and forests) in the subalpine and alpine regions but may be found in segetal habitats (roadside, field borders, and cultivated land) in the Mediterranean basin, Southwest Asia, Transcaucasia, and South Africa (Roshevitz 1947). Among the annual wild species, *S. sylvestre* has been reported living in sandy pastures, sand dunes in river deltas, and seashores. Its range of distribution embraces an area that includes Eastern Europe, the Caucasus, and Central Asia. *S. vavilovii* has been reported from sandy ground by the Aras river, to mountain ranges, cultivated fields, and field borders in eastern Turkey. Annual weedy rye is always found as a weed in cultivated fields and field borders in Anatolia, the Caucasus, and Central Asia (Roshevitz 1947). Cultivated rye is commonly found in fields and open spaces from
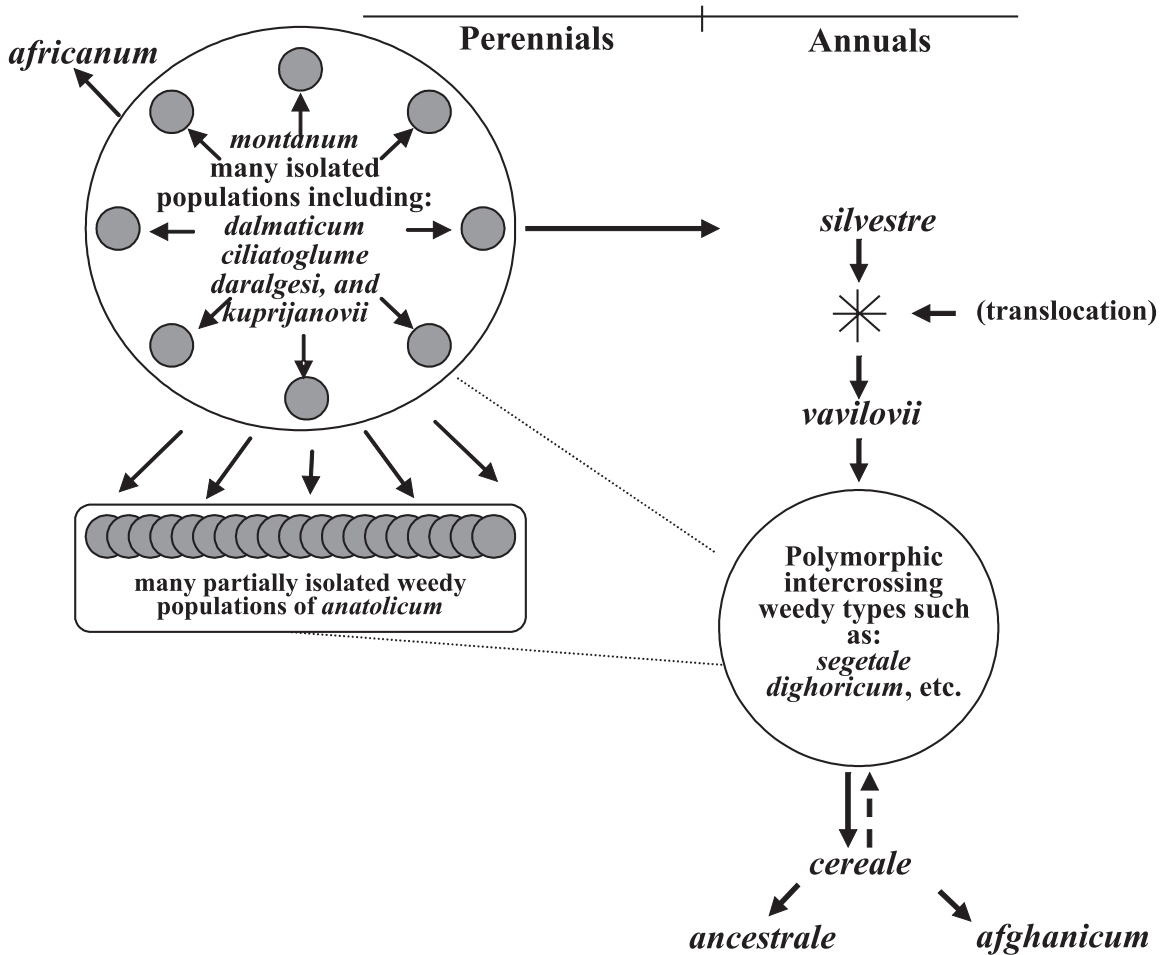
Fig. 1. Phylogenetic relationship in the genus *Secale* by Stutz (1972)

Europe to Southwest Asia. The geographical origin of cultivated rye was defined by de Candolle (1886) as the area between the Austrian Alps and the Caspian Sea.

6.1.5
Classification of the Genus *Secale*

Systematic classifications have recognized from three to 14 species within the genus *Secale* depending on the identification criteria used. Early studies involved the systematic classifications of the genus *Secale* based on morphological characteristics, life cycle, and geographical distribution. Vavilov (1917, 1926) accepted four species in the genus *Secale*: *S. africanum* Stapf., *S. cereale* L., *S. fragile* Marsch, and *S. montanum* Guss. Zhukovsky (1928) proposed three subspecies, *S. cereale* subsp. *cereale* Zhuk. for cultivated

rye, *S. cereale* subsp. *ancestrale* Zhuk., and *S. cereale* subsp. *segetale* for weedy rye. However, Zhukovsky (1933) subsequently raised subsp. *ancestrale* to species status. Roshevitz (1947) distinguished as many as 14 species based on crossability, which were grouped into three major series. The first series was composed of *S. montanum* and all perennial forms (*S. kuprijanovii, S. dalmaticum, S. ciliatoglume, S. daralagesi, S. anatolicum,* and *S. africanum*) constituting the series Kuprijanovia Rashev. The second group consists of *S. cereale* and all weedy annual relatives (*S. vavilovii, S. dighoricum, S. afghanicum, S. ancestrale,* and *S. segetale*), which constituted the series Cerealia Roshev. in which all members contained three translocated chromosomes (with respect to the *S. montanum* group chromosomes). Finally, the third group consisted of *S. sylvestre*, which stood alone as an annual species with the same chromosomal arrangement as *S. montanum* and constituted the series silvestria Ro-
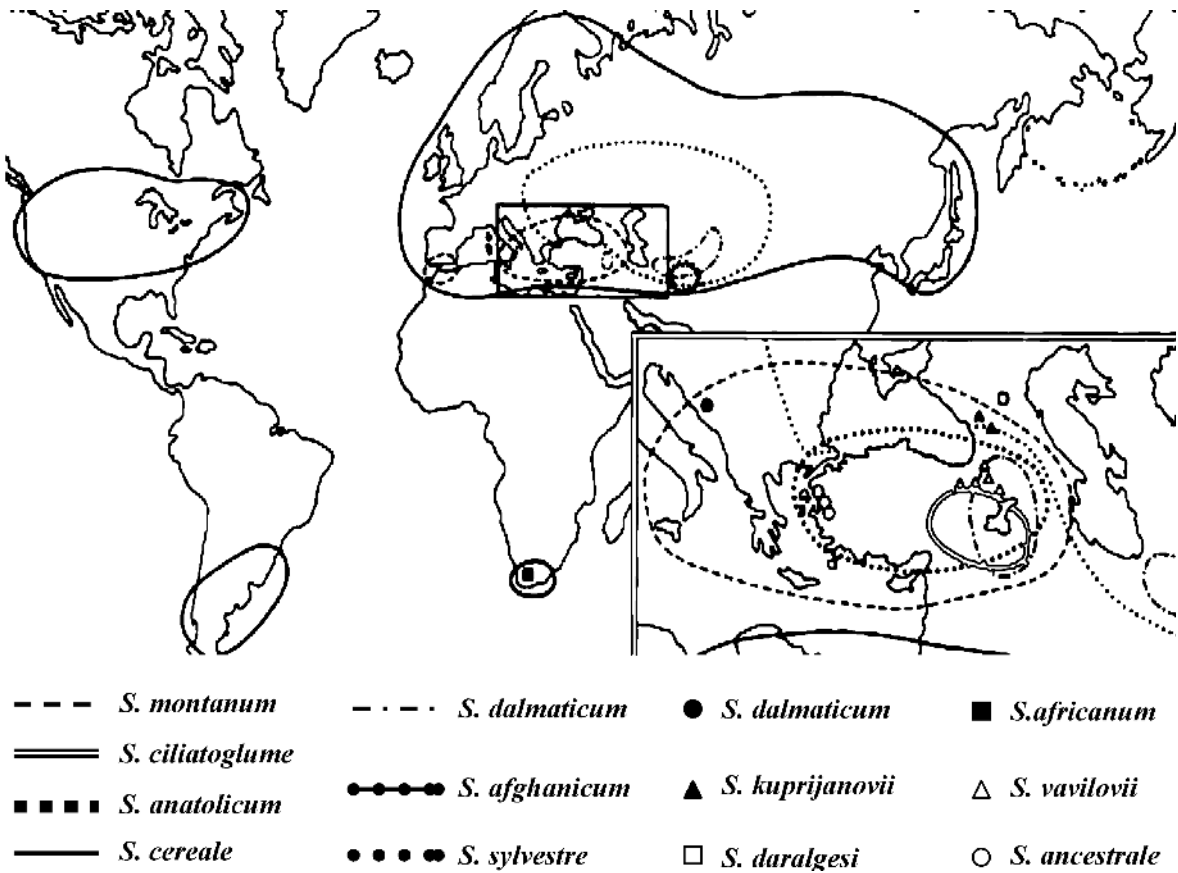
Fig. 2. Geographic distribution of the genus *Secale* (Vence 1987)

shev. Series Cerealia, which included cultivated rye, was the youngest series derived from the series Kuprijanovia. *S. cereale* arose under cultivation relatively recently, having been selected by humans for a nonbrittle rachis.

Khush and Stebbins (1961) conducted a series of studies using cytogenetic characteristics as criteria, which showed *S. sylvestre* and *S. cereale* to differ in three reciprocal translocations, of which two were the same as those found between *S. cereale* and *S. montanum*, with the third involving a short chromosome segment. *S. sylvestre* and *S. montanum* were found to differ by a small, single translocation. Khush (1962) did not find cytogenetic support to classify perennial ryes (*S. montanum*, *S. africanum*, *S. kuprijanovii* Grossh.) as separate species and proposed that they should be regarded as subspecies of *S. montanum*, while the weedy ryes (*S. ancestrale*, *S. afghanicum* Vav., *S. dighoricum* Vav., and *S. segetale* Zhuk.) should be considered as subspecies of *S. cereale*. Based on morphological characters, as well as cytogenetic and

other studies, Sencer (1975) proposed three biological species within the genus *Secale* L.; first, *S. montanum* including all the wild perennial taxa with high morphological resemblance and cytogenetic affinity to each other; second, *S. sylvestre*, a wild, annual species, which is isolated from *S. montanum* geographically, ecologically, and reproductively; and third, *S. cereale* containing the annual wild, weedy, and cultivated ryes.

On the basis of numerical taxonomy of phenolic compounds, Dedio et al. (1969) showed that *S. sylvestre* had a distinct chromatogram of its own, whereas *S. cereale*, *S. dighoricum*, and *S. segetale* were grouped together, and *S. montanum*, *S. africanum*, *S. dalmaticum*, and *S. kuprijanovii* appeared more closely related to each other than to the rest of the taxa. Using isozymes, Vences et al. (1987) supported Vavilov (1926) by accepting four species in the genus *Secale*. *S. cereale* and *S. montanum* appeared closely related and genetically similar and were almost equally related to *S. vavilovii* and *S. sylvestre* on the basis of genetic distance. *S. sylvestre* was easily distinguished

from *S. vavilovii*, and both were distinguished from the two open-pollinated species. However, there was no clear differentiation between *S. cereale* and *S. montanum*.

Recently, molecular data have been used to analyze phylogenetic relationships among species within *Secale*. Reddy et al. (1990), using rDNA spacer length variation, and Petersen and Doebley (1993), using RFLPs from the plastid genome, showed that DNA provided a useful character to supplement the conventional methods used for studying relationships between *Secale* species. They showed that only the annual species *S. sylvestre* was really distinct from the rest of the taxa, and that cultivated rye together with both the wild annual and perennial accessions were mixed. Using a polymerase chain reaction (PCR) technique, Pozo et al. (1995) agreed with the *Secale* group phylogeny as proposed by Khush (1962). However, *S. cereale* subsp. *ancestrale* was not included in *S. cereale*. *S. cereale* subsp. *anatolicum* was closer to *S. cereale* than to *S. montanum*, while *S. montanum* subsp. *kuprijanovii* was closer to *S. sylvestre*.

Finally, Frederiksen and Petersen (1998) made a taxonomic revision of *Secale* based on an examination of material in several herbaria and recognized only three species: *S. sylvestre, S. strictum*, and *S. cereale*. *S. strictum* contained two subspecies, ssp. *strictum* and ssp. *africanum*, and two varieties within ssp. *strictum,* var. *strictum* and var. *ciliatoglume* comb. nov. *S. cereale* also contained two subspecies. The cultivated taxa, marked by their tough rachises, were placed in ssp. *cereale* and the wild or weedy taxa that have a more or less fragile rachis were placed in ssp. *ancestrale*.

6.2
Phylogenetic Relationships Among *Secale* Species Utilizing AFLP Analysis

Cluster analysis based on AFLP-based analyses grouped together the annual taxa except for *S. sylvestre* and grouped the perennial taxa close to each other (Chikmawati 2003). This result indicated that life cycle (perennial vs. annual) played an important role in determining the relationships among *Secale* species. Further analysis using Fisher's exact test showed that 24% of the AFLPs detected were associated with the life cycle character.

AFLP analysis clearly resolved all accessions into three major groups, group 1 consisting of perennial taxa, group 2 consisting of annual taxa, and group 3 consisting of *S. sylvestre* (Chikmawati 2003), strongly supporting the validity of the three major series within *Secale* recognized by Roshevitz (1947), series *Kuprijanovia* Roshev. (*S. montanum* and all perennial), series *Cerealia* Roshev. (*S. cereale* and all weedy annuals), and series *Silvestria* Roshev. (the annual, *S. sylvestre*).

AFLP analyses showed that among the annual taxa (*Cerealia* Roshev.), *S. cereale* was more closely related to *S. ancestral, S. afghanicum, S. dighoricum,* and *S. segetale* than to *S. vavilovii* (Chikmawati 2003). Although *S. cereale* and *S. turkestanicum* are both cultivated species, they exhibited the most distant relationship to each other. The differences in breeding systems between two taxa (*S. turkestanicum* is self-pollinated and *S. cereale* is cross-pollinated) may explain this observation.

Among perennial taxa (*Kuprijanovia* Roshev.), *S. ciliatoglume* showed the most distant relationship to others (Chikmawati 2003). *S. ciliatoglume* is an isolated weedy population with pubescent culms endemic to orchards and vineyards near Mardin, Turkey. It is possible that this taxon maintained its distinct identity from the others because of its very limited distribution. Among the perennials, *S. africanum* was most distantly related to *S. montanum*, while *S. anatolicum* and *S. kuprijanovii*, which are close to each other, showed the closest relationship to *S. montanum*.

Somewhat surprisingly, the annual species *S. sylvestre* (*Silvestria* Roshev.) was closer to the perennial taxa than to the annual taxa. Since this species had the closest relationship to the outgroups, it can be considered as the most ancient among all the *Secale* species. Cluster analysis showed that *S. sylvestre* was the oldest while *S. cereale* was the youngest of the *Secale* species.

Based on cytological, ecological, and morphological studies, Stutz (1972) demonstrated that cultivated rye (*S. cereale* L.) originated from a weedy progenitor, which in turn was derived from the introgression of *S. montanum* (syn. *S. strictum*) into *S. vavilovii. S. africanum, S. dalmaticum, S. ciliatoglume*, and *S. kuprijanovii* appeared to be only a slightly modified isolated population of *S. montanum*. Populations of *S. anatolicum* were thought to be weedy forms of *S. montanum*, genetically and chromosomally distinct from the weedy annual forms. The species relationships within genus *Secale* based on AFLP data were

consistent with Stutz (1972) (Chikmawati 2003). However, Stutz (1972) also suggested that *S. montanum* was the common ancestor of all the *Secale* species, which conflicts with the AFLP data of Chikmawati (2003), and demonstrated that *S. sylvestre* was the most ancient species and the first to diverge from the common ancestor, while *S. montanum* diverged later.

6.3
Molecular Taxonomy of *Secale*

6.3.1
Distinction Among Annual Species

S. sylvestre, a low growing plant with fragile rachis, is widely distributed from Central Hungary eastward throughout the sandy steppes of Southern Russia and can be easily distinguished from other taxa by its long awned glumes (Stutz 1972). Khush and Stebbins (1961) showed that *S. sylvestre* is cytogenetically very distant from *S. cereale* and is geographically, ecologically, and reproductively isolated from *S. montanum* (Sencer and Hawkes 1980). In addition, *S. sylvestre* exhibits other unique characteristics, such as distinctive chloroplast DNA (Petersen and Doebley 1993), a spacer length variant of the ribosomal DNA (Reddy et al. 1990), and the internal transcribed spacer of the 18S-5.8S-26S rDNA (ITS-1) region. Given the strong distinction of *S. sylvestre* from other taxa for a wide assortment of characteristics, *S. sylvestre* has been considered a distinct species. In AFLP analysis, *S. sylvestre* demonstrated a distinct profile in all primer combinations (Chikmawati 2003). This taxon was well separated from others in all studies, and AFLP analyses confirmed that *S. sylvestre* was a distinct species.

The presence of a high degree of similarity among wild, weedy annual forms and cultivated rye was demonstrated by Khush (1963), who showed that there was no evidence of structural differences between the genome of cultivated rye and several weedy ryes (*S. cereale, S. vavilovii, S. ancestrale, S. afghanicum, S. dighoricum,* and *S. segetale*), which had previously been recognized as varieties, subspecies, or even species. These all readily crossed producing vigorous F_1s, which had similar chromosome arrangements, breeding habit, and periodicity, and also demonstrated geographical continuity. Khush (1963) proposed all annual forms to be subspecies of *S. cereale*. Morphometrical analyses concluded that it was im-

possible to recognize each annual taxon based on their morphology (Frederiksen and Petersen 1997). They proposed two intraspecific taxa within a single species (*S. cereale*), which are *S. cereale* subsp. *cereale* for cultivated rye and *S. cereale* subsp. *ancestrale* for weedy and wild taxa. Recently, de Bustos and Jouve (2002) found no differences between the weedy forms and cultivated rye in the ITS-1 region. Thus, previous studies have demonstrated that morphologically and genetically the annual taxa were too similar to be distinguished as separate species.

An AFLP analysis by Chikmawati (2003) showed that six accessions of *S. cereale* originating from different locations made a monophyletic group. *S. dighoricum* accessions also clustered together; however, those accessions originated from the same location. Other annual taxa represented by more than one accession did not make monophyletic clusters but intermingled with each other. Cluster analysis demonstrated that the inclusion of each annual taxon was not well supported. Except for cultivated rye, it is still difficult to discriminate wild and weedy rye using AFLP markers. Therefore, Chikmawati's (2003) AFLP analysis supports the results of Frederiksen and Petersen (1997).

6.3.2
Distinction Among Perennial Species

Among perennial species, *S. ciliatoglume* does not cluster together with the others. It stands alone between annual and perennial taxa according to cluster analyses (Chikmawati 2003), but the separation was intermediate (58% in phylogenetic and 74% in phenetic analysis). Principal coordinate analysis (Fig. 3) placed this accession in the same quadrant with the other perennial taxa. Information about *S. ciliatoglume* from the previous studies was very limited to morphological data, which showed that this species is morphologically similar to *S. montanum*, deviating only by having a dense cover of hairs over the internodes, leaf sheaths, and blades (Frederiksen and Petersen 1998). Frederiksen and Petersen (1997) suggested that *S. ciliatoglume* should be given an intraspecific rank.

Previous studies showed that several perennial forms (*S. anatolicum*, *S. africanum*, *S. dalmaticum*, and *S. montanum*) readily crossed to each other, and that crossing among them yielded normal chromosome configurations (Stutz 1972), indicating no re-
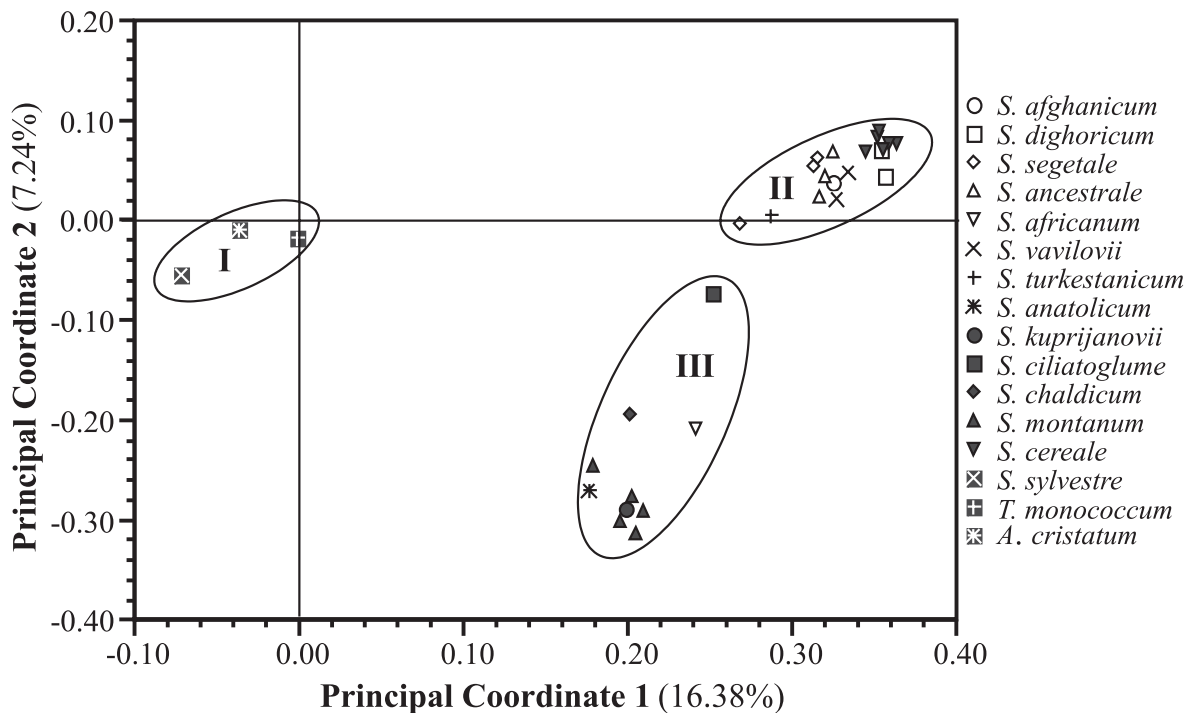
Fig. 3. Principal coordinate analysis of *Secale* species based on AFLPs. *I = S. sylvestre* and two outgroups. *II = annual forms*. *III = perennial forms*

6.4
Genetic Diversity Among Cultivated Rye Genotypes

productive barrier. Khush (1962) proposed all perennial taxa to be subspecies of *S. montanum*. Furthermore, Sencer and Hawkes (1980) showed that all the wild perennial forms had high morphological resemblance. The phylogenetic analysis based on AFLPs clustered six accessions of *S. montanum* together with low bootstrap support (36%), with the separation of the other perennial taxa showing only intermediate support (44 to 69%) (Chikmawati 2003). However, the genetic differentiation level among perennial taxa was very high (Gst = 0.90). This result suggests that the polymorphism of AFLP markers within perennial taxa were sufficient to discriminate and place them in an intraspecific rank, instead of a specific rank.

6.4
Genetic Diversity Among Cultivated Rye Genotypes

Morphologically, cultivated rye shows variation in a number of characters especially related to the color and hairiness of the bracts and color of the caryopsis; however, these characters also demonstrate overlapping and continuous variation (Tumania 1929). In

addition to morphological variation, *S. cereale* was thought to be extensively variable; however, a comparative study of four populations of *S. cereale* based on allelic frequencies and heterozygosity of allozymes revealed no significant differences among rye populations with different geographical origins (Peres de la Vega and Allard 1983). Persson and von Bothmer (2000) showed higher diversity and larger within-population isozyme loci variation compared to the variation among populations.

6.5
Utilization of Molecular Markers in Rye Systematics

Molecular phylogenetics is expected to clarify many patterns of rye evolution that have been hard to resolve by classical approaches. There are several reasons why molecular data are much more powerful than morphological and physiological data for evolutionary studies. First, DNA and protein sequences generally evolve in a much more regular fashion than do morphological and physiological characters and therefore can provide a clearer picture of organism

relationships. Second, molecular data are often much more amenable to quantitative treatments than are complex morphological data. Third, molecular data are becoming much more abundant (Nei and Kumar 2000). Avise (1994) pointed out several special advantages of molecular data for use in phylogeny estimation. First, molecular data are genetically inherited. Since phylogeny is the stream of heredity, only genetically transmitted traits are informative to phylogeny estimation. Second, molecular methods open the entire biological world for genetic scrutiny. Various molecular assays provide direct structural evidence for genes or their products and can be applied to the genetics of any organism from microbes to whales. And third, molecular data can distinguish homology from analogy.

A variety of molecular techniques have been developed that provide genetic diversity and genetic relationship information that can be used as DNA fingerprinting strategies. Each method has its own benefits and constraints. The most common techniques are RFLPs and numerous PCR-based genetic marker assays, such as randomly amplified polymorphism DNA (RAPDs), SSRs, and AFLPs. Many studies have shown that both SSRs and AFLPs are suitable tools for assessing genetic diversity and the genetic relationship among accessions within species.

6.6
A Review of Linkage Mapping in Rye

The gateway for genome studies was the development of large-scale genome sequencing technology, which has already been used to sequence the entire genomes of a number of plants and animals. However, most of the complete sequencing efforts in plants have focused on a few model species with relatively small genome sizes. Thus, for most cereal crops with their relatively large genome sizes, genetic (linkage) and physical mapping are still the fundamental genomic studies. In addition, map-based cloning and marker-assisted selection have proven particularly important for crop improvement. As a result, much effort has been applied to the development of genetic maps in various cereal crops over the last 15 years.

As with other cereals, rye has experienced rapid progress in map development. Schlegel et al. (1997) have updated the rye mapping data, which are publicly obtainable at http://www.desicca.de/plant_breeding/

Rye_map/rye_map.html. The substantial amount of data available makes a summary of rye mapping progress useful. Table 1 includes data from maps containing at least six linkage groups.

6.6.1
Mapping Population and Linkage Maps

Twelve major maps are listed in Table 1. These maps were developed from seven mapping populations in six laboratories. The E-line × R-line population was used by Loarce et al. (1996) to construct a map consisting of 89 loci spanning 339.7 cM on all the rye chromosomes except for 2R. The map generated from population UC90 × E-line (Ma et al. 2001) contains 184 loci, including seven genes of known function and one cytological marker, covering 727.3 cM with a relatively equal distribution of loci in each of the seven rye chromosomes. In addition, at least two thirds of the markers in this map were derived from other cereal crops, allowing for good integration and estimation of syntenic relationships with maps of other crops (Ma et al. 2001).

The Ds2 × RxL10 population was first used for restriction fragment length polymorphism (RFLP) mapping by Devos et al. (1993) and consists of 156 loci spanning about 1,000 cM. The map of Devos et al. (1993) gives the most detailed description of rye chromosomes relative to their wheat homoeologs but contains no rye genomic or cDNA markers. This map was later saturated with random amplified polymorphic DNA (RAPD) and isozyme markers, resulting in the largest rye linkage map containing 282 markers covering 1,140 cM (Masojć et al. 2001). One notable observation regarding this population is that the loci on the maps were heavily clustered around the centromeres.

The P87 × P105 is a pooled mapping population generated by combining F_2 individuals derived from a pair of reciprocal crosses of the two inbred parents. The population has been used for a series of mapping efforts from RFLP mapping with genomic and cDNA clones (Korzun et al. 1998) to simple sequence repeat (SSR) or microsatellite mapping (Korzun et al. 2001), and from general linkage development to locating genes and quantitative trait loci (QTL) (Börner et al. 2000). As a result, the final map contains the greatest number of known function genes and morphological traits, including 19 isozyme and protein markers, 10 known function sequences, and two mor-

Table 1. A summary of major mapping data in rye

Population	No. of loci	cM	Program	Marker type	Segregation distortion (%)	Reference
E-line × R-line	89	339.7	MAPMAKER 3.0	RFLP, RAPD	20.2	Loarce et al. 1996
UC90 × E-line	184	727.3	JoinMap 2.0	RFLP, SSR, cytology	72.8 (P < 0.01)	Ma et al. 2001
Ds2 × RxL10	156	~1,000.0	MAPMAKER 2.0	RFLP, protein		Devos et al. 1993
Ds2 × RxL10	282	1,140.0	MAPMAKER 3.0b	RAPD, isozyme		Masojć et al. 2001
P87 × P105	91	660.0	MAPMAKER 2.0	RFLP, isozyme, morphology	11.0 (P < 0.05)	Korzun et al. 1998
P87 × P105	113	1,018.0	MAPMAKER 3.0	RFLP, isozyme, morphology, QTL		Börner et al. 2000
P87 × P105	183	1,063.4	MAPMAKER 2.0	RFLP, SSR, isozyme, protein, morphology	12.0 (P < 0.05)	Korzun et al. 2001
Danko × Halo	60	~350.0	LINKAGE 1.0	RFLP, RAPD, isozyme, morphology	34.3 (P < 0.05)	Philipp et al. 1994
F₂*	127	~760.0	MAPMAKER JoinMap 1.4	RFLP, RAPD, isozyme, morphology	6.3	Senft and Wricke 1996
F₂*	102	#757.4	MAPMAKER 2.0	RFLP, RAPD, SSR, isozyme	Observed†	Saal and Wricke 1999
F₂*	182	1,062.0	MAPMAKER 3.0b	RFLP, RAPD, SSR, AFLP	5.0	Saal and Wricke 2002
9953**	56	685	JoinMap 3.0	SSR	67.9 (7R only)	Hackauf and Wehling 2001

* An F₂ mapping population originated from a cross between two inbred lines, which were selected from a self-fertile synthetic population based on their allelic constitution of 11 isozyme loci

** A BC₁ population

\# Two gaps are not counted

~ Approximate values

† Observed, but the value is not given

phological genes. In addition, 23 gene loci and 25 QTL were anchored on particular regions of the linkage frames (Korzun et al. 2001).

The map based on the population Danko × Halo was relatively small, but it was one of the earliest linkage maps covering all seven rye chromosomes (Philipp et al. 1994). Those mapping data were later integrated into those of another population created by Senft and Wricke (1996). The new mapping population originated from a cross between two inbred lines selected from a self-fertile synthetic population based on their allelic constitution of 11 isozyme loci. This population was the first to incorporate and extend the previous mapping data provided by Philipp et al. (1994) with more RFLP and RAPD markers (Senft and Wricke 1996). The map was then further extended with SSR (Saal and Wricke 1999) and amplified fragment length polymorphism (AFLP) (Saal and Wricke 2002) markers. The final map involving this population consists of 182 markers distributed through a mapping length of 1,062 cM, and it is the only rye map that contains AFLP markers (Saal and Wricke 2002).

Hackauf and Wehling (2001) developed a second-generation rye linkage map composed solely of expressed sequence tag (EST)-derived SSRs. The map covers 685 cM and it is the only map developed from a non-F_2 population. Since the SSR markers were derived from expressed sequences, the resulting map contains a number of markers directly related to known function genes; thus it provided the basic framework for a "functional map" (Hackauf and Wehling 2001). The utilization of EST markers opened up the possibility of constructing maps based solely on gene-rich regions of the rye genome. At present, there are well over 500,000 wheat ESTs in Genbank that can be used as PCR-based markers for high-saturation mapping in rye.

Similarly to other cereal crops, all the rye linkage maps have shown a nonuniform distribution of mapped loci, which are often typically clustered near the centromeres, suggesting that more recombination occurs in the distal regions of chromosomes. Centromere clustering results in inflated interval distances between distal loci, thus reducing overall map resolution and utility (Devos et al. 1993).

6.6.2
Markers

The maps showed that various kinds of markers, involving most of the major marker technologies including RFLP and PCR-based methods (RAPD, SSR, and AFLP), could be used for genetic mapping in rye. The mapping efficiency and the potential application could be very different for each kind of marker. Many RFLP markers have been used for mapping in rye and have been utilized as core markers for mapping frame establishment (Loarce et al. 1996; Ma et al. 2001; Masojć et al. 2001; Korzun et al. 2001; Saal and Wricke 2002). Most of the RFLP markers are genomic or cDNA clones with relatively low to moderate levels of polymorphism; however, the degrees of polymorphism (generally 30 to 70%) depend on the marker sources, populations, and the species involved. Since rye is an outcrossing species and is comprised of ca. 85 to 90% repetitive sequences, most of the genomic clones showed multiple bands, which increased the variability of mapping efficiency in different populations. In addition, any genomic DNA-derived loci mapped in each individual rye population could be different.

The PCR-based methods (RAPD, SSR, and AFLP) are getting more important and popular because they are straightforward and can be carried out with a small amount of DNA. One of the earliest PCR-based markers involved the utilization of RAPDs, which have already been used to saturate rye maps in many of the rye populations (Table 1). Compared to RFLP markers, RAPD markers often show low reproducibility from population to population and laboratory to laboratory due to their randomness of amplification. Therefore, RAPD primers were not only screened for mapping polymorphism but also selected for reliability to ensure reproducible patterns of amplicons generated; thus the overall mapping efficiency using RAPDs was decreased (Senft and Wricke 1996; Masojć et al. 2001). SSR markers have been shown to be superior to other markers due to their levels of polymorphism (Saal and Wricke 1999) and to the fact that they are codominant; they have also been used to increase overall mapping coverage in several populations (Saal and Wricke 1999; Korzun et al. 2001; Ma et al. 2001). However, SSR marker primer design needs sequence information, and the amplicons usually need to be sequenced again for confirmation. In one population, AFLP markers have been used to extend rye linkage maps (Saal and Wricke 2002). This is one of the most

efficient methods because primers used do not need prior knowledge of DNA sequence and multiple loci always could be mapped from a single pair of primers. However, AFLP markers are dominant markers, and the AFLP amplicons are anonymous sequences.

Overall, the rye mapping coverage has been significantly increased with the development of new marker technologies. Meanwhile, the mapping tendency has changed, with the most time-consuming method, RFLP and the low-reproducible method, RAPD, being replaced by SSR and AFLP methods.

6.6.3
Mapping Programs

The major mapping programs used for rye linkage establishment are MAPMAKER (Lander et al. 1987) and JoinMap (Stam 1993). MAPMAKER is widely used and is a very reliable program for codominant marker incorporation. JoinMap is particularly useful for mapping data, which involves mixing codominant and dominant markers on the same map, and for the integration of mapping data involving two or more different mapping populations. This map-integration feature had been used by Senft and Wricke (1996) to combine their mapping results with data originated from another population (Philipp et al. 1994). Remarkably, using the JoinMap Gustafson and Snape (2001) have integrated the mapping data from five rye linkage maps (Devos et al. 1993; Philipp et al. 1994; Loarce et al. 1996; Korzun et al. 1998; Ma et al. 2001), which has resulted in a comprehensive map that contains more than 500 markers within a mapping distance of only about 760 cM. The data allowed for the establishment of a higher-resolution map with an average distance of only about 1.5 cM between adjacent markers. JoinMap also turned out to be powerful for incorporating dominant with codominant rye markers, as well as markers showing a high degree of segregation distortion (Ma et al. 2001).

6.6.4
Segregation Distortion

Segregation significantly different from the expected Mendelian ratios, 1:2:1 for codominant alleles and 3:1 for dominant alleles is defined as segregation distortion. In general, most of the existing rye mapping populations suffer significantly from segregation dis-

tortion, and the phenomenon has been observed in all seven rye chromosomes (Philipp et al. 1994; Loarce et al. 1996; Senft and Wricke 1996; Korzun et al. 1998, 2001; Saal and Wricke 1999, 2002; Ma et al. 2001). However, one population showed segregation distortion occurring mainly in chromosome 7R (Hackauf and Wehling 2001). Rye, an out-crossing species, suffers from variable amounts of inbreeding depression, and there is a strong reduction in viability following selfing. Distorted segregation ratios are known to result from competition among gametes for preferential fertilization. Selection operating at any stage of development from zygote to seedling may introduce a bias to the progeny (Loarce et al. 1996; Ma et al. 2001). One population, UC90 × E-line, demonstrated a considerable degree (72.8%) of segregation distortion in all seven rye chromosomes skewed in two directions (Ma et al. 2001). It is unclear why such a high number of alleles deviated from the normal segregation in the UC90 × E-line population compared to other rye populations. However, this demonstrates that each population of a highly out-crossing species such as rye can be significantly different.

The key to productive mapping in rye depends upon several factors. First, good quality mapping populations that have been derived from highly inbred rye parents (doubled haploids if possible) are required. The difficulty is that rye suffers greatly from inbreeding depression. Consequently, the selection of suitable parents will be the primary challenge. Second, the development of a large mapping population suitable for high-resolution mapping (a minimum of 1,000 lines) is required for marker-assisted selection programs or for map-based cloning in rye. Third, suitable markers, preferably PCR-based markers, are required. Fourth, EST sequences from all of the cereals (wheat, barley, rice, etc.) can be used as sources of markers.

References

Avise JC (1994) Molecular Markers, Natural History and Evolution. Chapman and Hall, New York

Börner A, Korzun V, Voylokov AV, Worland AJ, Weber WE (2000) Genetic mapping of quantitative trait loci in rye (*Secale cereale* L.). Euphytica 116:203–209

Chikmawati T (2003) Phylogenetic relationships in *Secale* and PCR-based EST mapping in wheat. PhD Thesis, University of Missouri, Columbia, MO

Cuadrado A, Jouve N (1997) Distribution of highly repeated DNA sequences in species of the genus *Secale*. Genome 40:309–317

Cuadrado A, Jouve N (2002) Evolutionary trends of different repetitive DNA sequences during speciation in the genus *Secale*. Am Genet Assoc 93:339–345

de Bustos A, Jouve N (2002) Phylogenetic relationships of the genus *Secale* based on the characterization of rDNA ITS sequences. Plant Syst Evol 235:147–154

de Candolle A (1886) Origin of Cultivated Plants. Appleton, New York

Dedio W, Kaltsikes PJ, Larter EN (1969) Numerical chemotaxonomy in the genus *Secale*. Can J Bot 47:1175–1179

Devos KM, Atkinson MD, Chinoy CN, Francis HA, Harcourt RL, Koebner RMD, Liu CJ, Masojc P, Xie DX, Gale MD (1993) Chromosomal rearrangements in the rye genome relative to that of wheat. Theor Appl Genet 85:673–680

Frederiksen S, Petersen G (1997) Morphometrical analyses of *Secale* (Triticeae, Poaceae). Nord J Bot 17:185–197

Frederiksen S, Petersen G (1998) A taxonomic revision of *Secale*. Nord J Bot 18:399–420

Gustafson JP, Snape JW (2001) Integration of five rye genetic maps. XVIth EUCARPIA Congr, 10–14 September 2001, Edinburgh, UK (Abstr)

Hackauf B, Wehling P (2001) Development of microsatellite markers in rye: map construction. In: Proc EUCARPIA Rye Meeting, Radzikow, Poland, pp 333–340

Jain SK (1960) Cytogenetics of rye (*Secale* spp.). Bibliogr Genet 19:1–86

Khush G (1962) Cytogenetic and evolutionary studies in *Secale*. II. Interrelationships of the wild Species. Evolution 16:484–496

Khush G (1963) Cytogenetic and evolutionary studies in *Secale*. III.

Cytogenetics of weedy ryes and origin of cultivated rye. Econ Bot 17:60–71

Khush G, Stebbins GL (1961) Cytogenetic and evolution in *Secale*. I. Some new data on the ancestry of *S. cereale*. Am J Bot 48:723–730

Korzun V, Malyshev, S, Kartel, N, Westermann, T, Weber WE, Börner A (1998) A genetic linkage map of rye (*Secale cereale* L.). Theor Appl Genet 96:203–208

Korzun V, Malyshev S, Voylokov AV, Börner A (2001) A genetic map of rye (*Secale cereale* L.) combining RFLP, isozyme, protein, microsatellite and gene loci. Theor Appl Genet 102:709–717

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg I (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Linde-Laursen I, von Bothmer R, Jacobson N (1980) Giemsa C-banding in Asiatic taxa of *Hordeum* sect. Stenostachys with notes on chromosome morphology. Hereditas 93:235–254

Loarce Y, Hueros G, Ferrer E (1996) A molecular linkage map of rye. Theor Appl Genet 93:1112–1118

Ma X-F, Wanous MK, Houchins K, Rodriguez Milla MA, Goicoechea PG, Wang Z, Xie M, Gustafson JP (2001) Molec-

ular linkage mapping in rye (*Secale cereale* L.). Theor Appl Genet 102:517–523

Masojć P, Myskow B, Milczarski P (2001) Extending a RFLP-based genetic map of rye using random amplified polymorphic DNA (RAPD) and isozyme markers. Theor Appl Genet 102:1273–1279

Nei M, Kumar S (2000) Molecular Evolution and Phylogenetics. Oxford University Press, New York

Peres de la Vega M, Allard RW (1983) Mating system and genetic polymorphism in populations of *Secale cereale* and *S. vavilovii*. Can J Genet Cytol 26:308–317

Persson K, von Bothmer R (2000) Assessing the allozyme variation in cultivars and Swedish landraces of rye (*Secale cereale* L.). Hereditas 132:7–17

Petersen G (1991) Intergeneric hybridization between *Hordeum* and *Secale* (Poaceae). 1. Crosses and development of hybrids. Nord J Bot 11:253–270

Petersen G, Doebley F (1993) Chloroplast DNA variation in the genus *Secale* (Poaceae). Plant Syst Evol 187:115–125

Philipp U, Wehling, P Wricke G (1994) A linkage map of rye. Theor Appl Genet 88:243–248

Pozo JC, Figueiras AM, Benito C, de la Pena A (1995) PCR derived molecular markers and phylogenetic relationships in the *Secale* genus. Biol Plant 37:481–489

Reddy P, Appel R, Baum BR (1990) Ribosomal DNA spacer-length variation in *Secale* spp. (Poaceae). Plant Syst Evol 171:205–220

Riley R (1955) The cytogenetics of the differences between some *Secale* species. J Agric Sci 46:377–383

Roshevitz RI (1947) Monographie of the genus *Secale* L. Acta Inst Bot Acad Sci USSR I 6:105–163

Saal B, Wricke G (1999) Development of simple sequence repeat markers in rye (*S. cereale* L.). Genome 42:964–972

Saal B, Wricke G (2002) Clustering of amplified fragment length polymorphism markers in a linkage map of rye. Plant Breed 121:117–123

Schiemann E (1948) Weizen, roggen, gerste, systematik, geschichte und verwendung. Fusher, Jena

Schlegel R, Melz G, Korzun V (1997) Genes, marker and linkage data of rye (*Secale cereale* L.). 5th updated inventory. Euphytica 101:23–67

Sencer HA (1975) Study of variation in the genus *Secale* L. and on the origin of the cultivated rye. PhD Thesis, Univ of Birmingham, UK

Sencer HA, Hawkes JG (1980) On the origin of cultivated rye. Biol J Linn Soc 13:299–313

Senft P, Wricke G (1996) An extended genetic map of rye (*Secale cereale* L.). Plant Breed 115:508–510

Singh RP, Röbbelen G (1977) Identification by giemsa technique of the translocations separating cultivated rye from three wild species of *Secale*. Chromosoma 59:217–225

Stam P (1993) Construction of integrated linkage maps by means of a new computer package: JOINMAP. Plant J 5:739–744

Stutz HC (1957) A cytogenetic analysis of the hybrid *Secale cereale* L.x *Secale montanum* Guss. and its progeny. Genetica 42:199–221

Stutz HC (1972) On the origin of cultivated rye. Am J Bot 59:59–70

Tumania MC (1929) A contribution to the study of the cereals in the region of Van. Bull Appl Bot Genet Plant Breed 22:297–327

Vavilov NI (1917) On the origin of cultivated rye. Bull Appl Bot Genet Plant Breed 10:561–590

Vavilov NI (1926) Studies on the origin of cultivated plants. Bull Appl Bot Genet Plant Breed 16:1–248

Vences FJ, Vaquero F, Peres de la Vega M (1987) Phylogenetic relationships in *Secale* (Poaceae): an isozymatic study. Plant Syst Evol 157:33–47

Wang RC, Bothmer R, Dvorak J, Fedak G, Linde-Laursen I, Muramatsu M (1996) Genome symbols in the Triticeae (Poaceae). In: RC Wang, Jensen KB, Jaussi C (eds) Proc 2nd Intl Triticeae Symp, Utah State University Press, Logan, Utah, pp 29–34

Zhukovsky PM (1928) A new wild growing form of rye in Anatolia. Bull Appl Bot 19:49–52

Zhukovsky PM (1933) Roggen. Vsesoiuzn akad selsk khoz nauk lenina, Inst Rastenievod, Leningrad, USSR

7 Sorghum

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7.1
Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is the fifth most important cereal crop, after wheat, rice, maize, and barley. A largely self-pollinated crop, it is grown on over 40 million hectares (USDA 2004) in both temperate and tropical regions. Sorghum is mainly grown as a rainfed crop by subsistence farmers in the semiarid tropical regions of Africa and Asia as well as by other farmers in the USA and Latin America. It is a suitable crop for drought and heat-stressed environments and can be grown from sea level to elevations in excess of 300 m, in high rainfall areas, in semiarid regions, and in different seasons.

7.1.1
Center of Origin

The origin of sorghum, an African grass, and its diversification into five major races and thousands of different genotypes began in the distant human past and is only partially known. However, the work of botanists, plant breeders, archaeologists, and geographers has uncovered the probable evolutionary pathway in the domestication of sorghum and the probable spatial dynamics of that evolution under cultural control. A great deal has been learned in the last few about the origins of the cereal and the people responsible for the domestication of sorghum races years. The Ethiopian region of Africa is the center of origin of sorghum (Mann et al. 1983) as it is rich in the number of snowdenian species and also contains several varieties of the durra type, which represents the highly evolved varieties among the cultivated races. From Ethiopia sorghum was taken to West Africa across the Sudan, from where it was first grown among the Mande people of the upper Niger. Also from Ethiopia sorghum was taken to East Africa, from where it was distributed among the Nilotic and Bantu people. From East Africa

the sorghum spread to India during the first millennium and was taken from there to China in the early Christian era (Doggett 1976). Sorghum races in India are closely related to those in northeast Africa. From West Africa sorghum was distributed to the USA and other parts of the world through slave trade around the mid-19th century. Before 1900 full-scale cultivation of sorghum had started in the southern great plains of the USA.

7.1.2
Domestication

Sorghum has been carried to many new habitats in different environments to become a staple grain for millions of people. Sorghum has also been diversified into a sugar source, a construction material, a raw material for household implements, and a raw material for industry. The change from a harvested wild plant with much internal variability to an important resource for use and improvement is the result of management. Cultivated races of sorghum originated by disruptive selection and domestication in east central Africa from the wild snowdenian species, *Sorghum arundinaceum*. Human selection for cultivated characters (mainly nonshattering heads, large seeds and ears, easy threshability, and suitable height and maturity) and natural selection for wild type character resulted in divergence into polymorphic populations in the presence of considerable gene flow between the wild and cultivated types. These processes seem to have contributed to the evolution of durra, kafir, bicolor, cernum, and caudatum and other intermediate types. According to Doggett (1976), most of these types might have migrated to India and China around 4000 BC and 2000 BC, respectively.

Sorghum is adapted to a wide range of environmental conditions but is particularly adapted to drought. It has a number of morphological and physiological characteristics that contribute to its adap-

tation to dry conditions, including an extensive root system, waxy bloom on the leaves that reduces water loss, and the ability to stop growth in periods of drought and resume it again when conditions become favorable. It is also tolerant to water logging and can be grown in high rainfall areas. It is, however, primarily a crop of hot, semiarid tropical environments with 400 to 600 mm rainfall that are too dry for maize. It is also widely grown in temperate regions and at altitudes of up to 2,300 m in the tropics.

7.1.3
Taxonomic Position

All commercial groups of sorghum such as grain sorghum, fodder sorghum, broomcorns, and sorgos are classified under a single botanical species *Sorghum bicolor* (L.) Moench. The genus *Sorghum* belongs to one of the 16 subtribes of the tribe Andropogoneae of the subfamily Panicoidae of the family Poaceae.

Classification of the Genus Sorghum

Among all the classification attempts, Snowden's (1936) is the most comprehensive and practicable to a certain extent.

```
Section   Eusorghum
            Subsection   Arundinaceae
            Series          Spontanea and Sativa
            Subsection   Halepensia
Section   Para-sorghum
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Members of the subsection Arundinacea are diploids with $2n = 20$ chromosomes. The series Spontanea comprises wild species or races, and the series Sativa, the cultivated races. Using this basic structure, Snowden (1936) described 31 cultivated and 17 related wild species. These species are more appropriately considered as races of a single species.

Garber (1950) and Celarier (1959) divided the genus into six subgenera based on cytotaxonomic data: Eusorghum, which is the same as Snowden's section = Eusorghum, Chaetosorghum, Heterosorghum, Sorghastrum, Parasorghum, and Stiposorghum. Variation within these subgenera can best be described from the key outlined by Celarier (1959):

AA Nodes glabrous or minutely pubescent, first bloom of sessile spikelet many nerved (>10)

A   Sorghum: pedicellate spikelets staminate or neuter, awns small or wanting.

B   Pedicellate spikelets with glumes only, awns prominent.

1. Heterosorghum: primary branch of panicle simple and not whorled, glumes of pedicellate spikelets subequal, lodicules ciliate

2. Chaetosorghum: primary branch of panicle simple and not whorled, glumes of pedicellate spikelets unequal, lodicules glabrous

BB Nodes with distinct ring of hairs, first glume of sessile spikelet few nerved (<10)

1. Parasorghum: callus obtuse, awns <65 mm in length

2. Stiposorghum: callus pointed, awns >65 mm in length

Sun et al. (1994) used internal transcribed spacers of nuclear ribosomal DNA to evaluate the phylogenetic relationships within the genus *Sorghum*. They found that Chaetosorghum and Heterosorghum appear to be closely related to each other, and these two are more closely related to sorghum than to Parasorghum.

A simplified classification scheme of cultivated sorghums was proposed by Harlan and de Wet (1972) based on morphological characteristics that most present-day breeders have come to recognize and utilize. The International Plant Genetic Resources Institute (formerly IBPGR) Advisory Committee on sorghum and millet germplasm has recommended this classification to be used in describing sorghum germplasm. Their system of classification of cultivated races into five basic races and ten intermediate races and those of wild races into six spontaneous races is presented below:

1. Basic races:
    – Race 1 bicolor (B)
    – Race 2 guinea (G)
    – Race 3 caudatum (C)
    – Race 4 kafir (K)
    – Race 5 durra (D)
2. Intermediate races: (all combinations of basic races)
    – Race 6 guinea-bicolor (GB)
    – Race 7 caudatum-bicolor (CB)
    – Race 8 kafir-bicolor (KB)
    – Race 9 durra-bicolor (DB)
    – Race 10 guinea-caudatum (GC)
    – Race 11 guinea-kafir (GK)

Table 1. Characteristics of commercial grain sorghum types

Grain sorghum type	Brief morphological description	Geographical location
Durra	Hairy, rachises, flattened kernels and dry stalks	Mediterranean, Near East, Middle East
Shallu	Partly pubescent involute glumes, cone-shaped lax panicles, corneous kernels, dry and non-sweet stalks	India, tropical Africa
Guineense	Involute and nearly glabrous glumes and compact panicles	Central and Western Africa
Kafir	Awnless, compact cylindrical panicles and juicy non-sweet stalks	South Africa
Kaoliang	Stiff stalks, thick hard rind, stiff spreading and few panicle branches, and dry and no-sweet stalks	Eastern Asia
Milo	Yellow midrib, transverse wrinkle of the glumes, compact, awned panicles, large round kernels	East Africa
Feterita	Large kernels, brown testa, and dry and non-sweet stalks	Sudan
Hegari	Rounded kernels, brown testa midcompact ellipsoid and branched panicles, and white kernels with a bluish-white appearance	Sudan

- – Race 12 guinea-durra (GD)
- – Race 13 kafir-caudatum (KC)
- – Race 14 durra-caudatum (DC)
- – Race 15 kafir-durra (KD)
3. Spontaneous races: *S. bicolor* ssp. *arundinaceum*
    - – Race 1 arundinaceum
    - – Race 2 aethiopicum
    - – Race 3 virgatum
    - – Race 4 verticilliflorum
    - – Race 5 propinquum
    - – Race 6 shattercane

Classification within the subgenera was further developed by de Wet (1978). The three species in the subgenera sorghum were recognized: Sorghum, two rhizomatous taxa, *S. halepense* and *S. propinquum*, and *S. bicolor*, representing all annual wild, weedy, and cultivated taxa. *S. bicolor* was broken down further into three subspecies: *S. bicolor* ssp. bicolor, *S. bicolor* ssp. *drummondii*, and *S. bicolor* ssp. *verticilliflorum* (formerly ssp. *arundinaceum*).

A commercial type of classification is used in the United States. Several commercial types occur and are given regional names. Extensive breeding has eroded the clear-cut differences among the various types. However, popular regional types such as durras, shallus, guineas, kafirs, kaoliangs, milos, feteritas, and hegaris are common in grain sorghum literature. These groups differ in their genetic characters as evidenced by the diversity resulting from intercrosses between the groups. Certain factors for disease reaction, insect resistance, heterosis, cytoplasmic male sterility, fertility restoration, and tillering tend to be associated with particular groups. Details of some of the more popular groups are given in Table 1.

7.1.4
Brief Morphology

Sorghum is a vigorous grass that varies between 0.5 and 5.0 m in height. It is usually an annual. It produces one or many tillers, which emerge initially from the base and later from stem nodes. The root system consists of fibrous adventitious roots that emerge from the lowest nodes of the stem, below and immediately above ground level. Roots are normally concentrated in the top 0.9 m of soil but may extend to twice that depth and can extend to 1.5 m in lateral spread. The stem is solid, usually erect. Its center can be dry or juicy, insipid or sweet to taste. The center of the stem can become pithy with spaces. Leaves vary in number from 7 to 24, depending on the cultivar. They are borne alternately in two ranks. Leaf sheaths vary in length from 15 to 35 cm and encircle the stem with their margins overlapping. The leaf sheath often has a waxy bloom. Leaves are from 30 to 135 cm long and 1.5 to 13 cm wide, with flat or wavy margins. Midribs are white or yellow in dry pithy cultivars or green in juicy cultivars. The flower is a panicle, usually erect,

but sometimes recurved to form a gooseneck. The panicle has a central rachis, with short or long primary, secondary, and sometimes tertiary branches, which bear groups of spikelets. The length and closeness of the panicle branches determine panicle shape, which varies from densely packed conical or oval to spreading and lax. Grain is usually partially covered by glumes. The seed is rounded and bluntly pointed, from 4 to 8 mm in diameter and varying in size, shape, and color with cultivar.

7.1.5
Cytogenetic Structure

Sorghum bicolor has a haploid chromosome number of 10, and it is classified as a diploid ($2n = 2x = 20$). Most species in the genus *Sorghum* are diploid with $2n = 20$, but several species, most notably *S. halepense*, are tetraploid ($2n = 4x = 40$). As the basic chromosome number in the Sorghastrae is five, it has often been hypothesized that sorghum may be of tetraploid origin. Meiotic chromosome pairing analysis did not provide any strong evidence of a tetraploid origin (Brown 1943; Endrizzi and Morgan 1955), but the large number of complementary gene loci seems to indicate a tetraploid origin. The application of fluorescent in situ hybridization (FISH) to sorghum chromosomes indicates that single-copy probes consistently identify two loci on separate chromosomes. This provides strong evidence that sorghum does in fact have tetraploid origins (Gomez et al. 1997).

Differences between chromosomes in subgenera of sorghum are detectable, but karyotypic analysis of sorghum chromosomes has been difficult due to similarities in chromosome size and structure (Huskins and Smith 1932; Doggett 1988). Karyotype analysis of several subgenera of the genus *Sorghum* indicates that chromosomes in the subgenus Eusorghum are distinctly different and smaller than chromosomes in the subgenera Parasorghum and Stiposorghum (Garber 1950; Celarier 1959; Gu et al. 1984). Gu et al. (1984) described the karyotype of *S. bicolor*, but only chromosome I (nucleolar organizing region) and chromosome IV (characteristic arm ratio) could be identified distinctly. Yu et al. (1991) were able to identify all ten chromosomes in *S. bicolor* using a combination of chromosome size, arm ratio, and C-banding patterns. C-banded karyotype for somatic metaphase chromosomes of sorghum (Combined Kafir 60) is presented

in Fig. 1. Later, Kim et al. (2002) used fluorescence in situ hybridization (FISH) and integrated structural genomic resources, including large insert genomic clones in bacterial artificial (BAC) libraries, to identify ten chromosomes simultaneously. Recently, they (Kim et al. 2004) have determined linkage group identities and homologies for metaphase chromosomes of *Sorghum bicolor* ($2n = 20$) by FISH of landed BACs. They used relative lengths of chromosomes in FISH-karyotyped metaphase spreads of the elite inbred BT × 623 to estimate the molecular size of each chromosome and to establish a size based nomenclature for sorghum chromosomes (SBI-01 to SBI-10) and linkage groups (LG1 to LG10) (Table 2 and Fig. 2).

The genome size for *S. bicolor* and *S. halepense* has been reported to be 735 and 1,617 Mb, respectively (Laurie and Bennett 1985). Later Arumunganathan and Earle (1991) estimated the genome size of *S. bicolor* to be ca. 750 Mb while Peterson et al. (2002) reported 692 Mb.

7.1.6
Economic Importance

Sorghum is the fifth most important cereal crop in the world after wheat, rice, maize, and barley. It is cultivated annually on ca. 45 million ha, producing ca. 60 million MT of grain (USDA 2004) (Table 3). Sorghum grain is a major food in much of Africa, South Asia, and Central America and an important animal feed in the USA, Australia, and South America. In addition to these uses of the grain, sorghum crop residues and green plants also provide sources of animal feed, building materials, and fuel, particularly in dryland areas of the semiarid tropics (SAT). Grain sorghum is well known for its capacity to tolerate conditions of limited moisture and to produce during periods of extended drought, in circumstances that would impede production in most other grains. Sorghum leaves roll along the midrib when moisture-stressed, making the plant more drought resistant than other grain plants. Like corn, sorghum can be grown under a wide range of soil and climatic conditions. Unlike corn, however, sorghum's yield under different conditions is not so varied. Consequently, it is grown primarily in arid areas where corn would not make it without substantial irrigation.

Sorghum is an important part of the diets of many people in the world and is nutritionally rich (Table 4). It is made into unleavened breads, boiled porridge
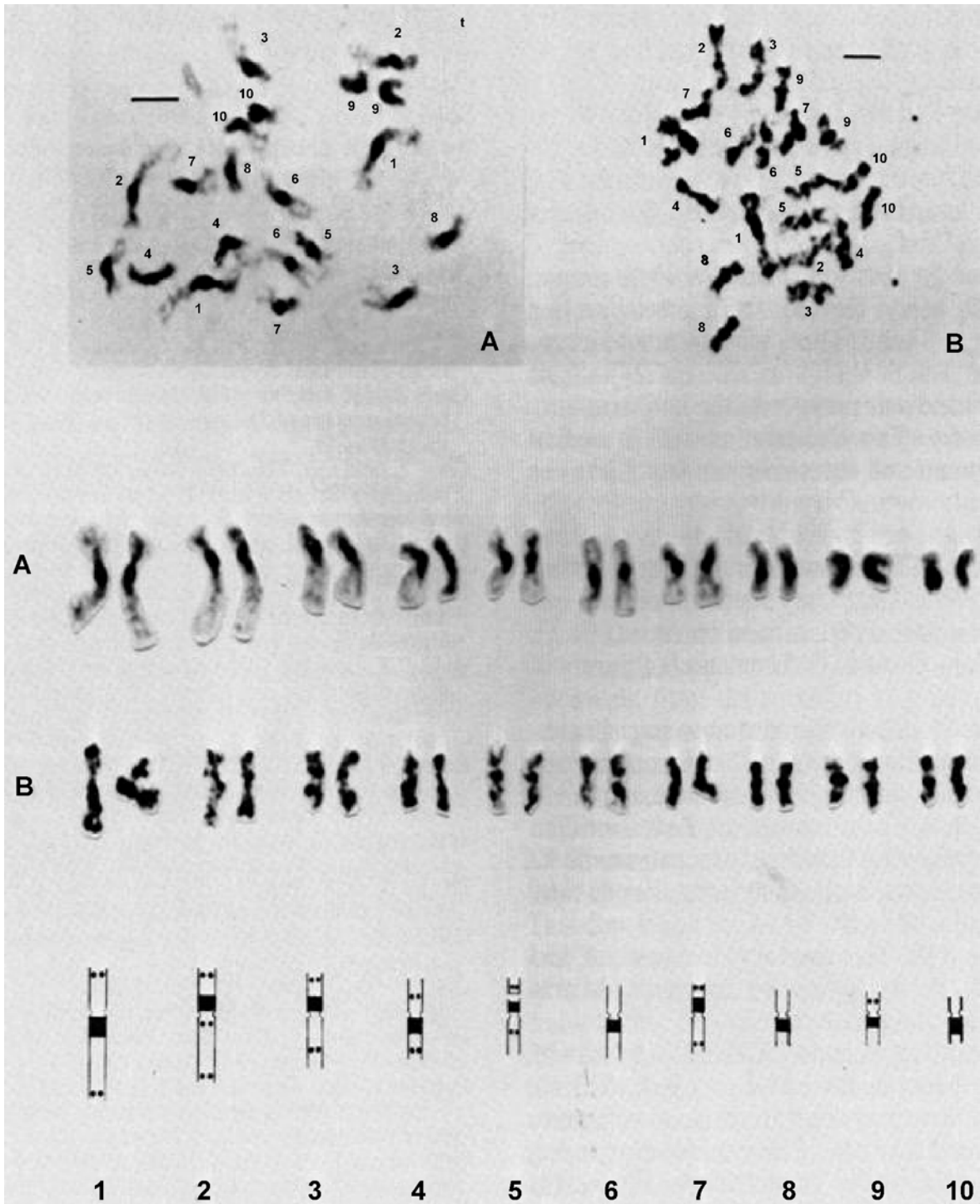
Fig. 1. C-band karyotype for somatic metaphase chromosomes of Combine Kafir 60, sorghum (Reprinted, with permission of Crop Science Society of America, from Yu et al. 1991)

Table 2. Relationship of FISH-based karyotype of sorghum and linkage groups comprising various linkage maps of sorghum genome. (Reprinted with permission of Genetics Society of America; from Kim et al. 2004)

Chromosome number[1] Linkage group (LG)	SBI-01 LG-01	SBI-02 LG-02	SBI-03 LG-03	SBI-04 LG-04	SBI-05 LG-05	SBI-06 LG-06	SBI-07 LG-07	SBI-08 LG-08	SBI-09 LG-09	SBI-10 LG-10
LG in Menz et al. 2002[2]	A	B	C	D	J	I	E	H	F	G
LG in Pereira et al. 1994	C	F	G	D	J	B	A	I	E	H
LG in Bowers et al. 2003[3]	C	B	A	F	H	D	J	E	G	I
LG in Crasta et al. 1999	G, K	D	A	C	J	F	E	H	I	B
LG in Boivin et al. 1999[4]	C, K	F	G	D, L	J	B	A	I	E	H
LG in Whitkus et al. 1992	B, C	D	F, M	H	G	E	A	K, L	I	J
Fish Karyotype	See	Fig. 2								
Total length (μm)	5.11	3.87	3.85	3.5	3.44	3.15	3.13	3.07	2.98	2.94
Standard error[6]	0.047	0.035	0.038	0.032	0.037	0.029	0.028	0.026	0.029	0.023
Relative length[7]	14.59	11.06	10.98	9.99	9.82	9.00	8.92	8.75	8.51	8.39
Estimated DNA content[8]	119.3	90.5	89.8	81.7	80.3	73.6	73.0	71.6	69.6	68.6
Arm ratio[9]	1.32	1.16	1.13	1.14	1.02	1.42	1.06	1.10	1.02	1.04

[1] Chromosomes were ordered and numbered according to their rank of the total length at metaphase (full contraction)

[2] Linkage group designations are identical to those described in Peng at al. (1999), Kong et al. (2000), Bhattramakki et al. (2000) and Haussmann et al (2002a)

[3] Linkage group designations are identical to those described in Chittenden et al. (1994) and Tao et al. (2000)

[4] Linkage group designations are identical to those described in Dufour et al. (1997)

[5] The chromosomes are displayed according to cytogenetic convention with the short arm at the top of the vertical chromosomes

[6] The sample size for measurement was 40

[7] Relative length = 100* (chromosome length/genome length)

[8] Estimated DNA content = Relative length × estimated genome size, i.e., 818 Mbp (Price et al. 2005)

[9] Arm ratio = length of arm/length of short arm

Table 3. Global area and production of Sorghum (USDA 2004)

Country	Area harvested (1,000 HA)	Production (1,000 MT)	Country	Area harvested (1,000 HA)	Production (1,000 MT)
Argentina	525	2,600	Lesotho	10	10
Australia	700	1,900	Mauritania	150	70
Benin	170	150	Mexico	1,800	6,300
Botswana	50	8	Morocco	25	15
Brazil	950	2,200	Mozambique	500	300
Burkina	1,450	1,300	Nicaragua	62	103
Burundi	55	65	Niger	1,500	650
Chile	0	0	Nigeria	6,800	8,050
China; Peoples Republic of	820	3,300	Norway	0	0
Colombia	60	170	Pakistan	400	230
Cote d'Ivoire	60	30	Paraguay	30	40
Dominican Republic	9	38	Peru	1	1
Ecuador	5	10	Philippines	0	0
Egypt	160	750	Romania	5	5
El Salvador	89	141	Rwanda	150	155
Eritrea	150	130	Saudi Arabia	180	200
Ethiopia	1,500	1,400	Senegal	210	160
EU-25	110	650	Somalia	225	150
Gambia; The	20	25	South Africa, Republic of	100	220
Ghana	300	320	Sudan	6,000	4,350
Guatemala	45	55	Swaziland	1	1
Guinea-Bissau	50	45	Taiwan	5	20
Haiti	115	90	Tanzania	750	580
Honduras	40	40	Thailand	160	280
India	9,900	8,500	Uganda	280	350
Iran	10	20	United States	2,799	11,050
Iraq	5	5	Uruguay	20	60
Israel	0	0	Venezuela	140	340
Japan	0	0	Yemen	320	260
Kenya	140	130	Zambia	40	25
Korea, Republic of	1	1	Zimbabwe	140	80
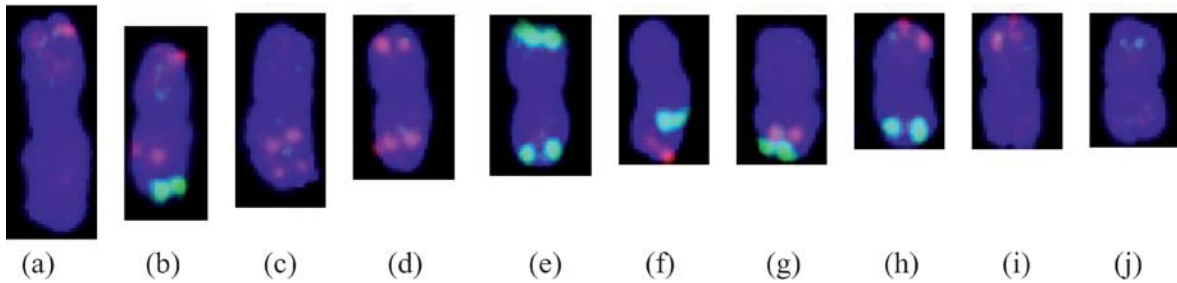
Fig. 2. FISH-based karyotype of sorghum. (**a**) LG-01. (**b**) LG-02. (**c**) LG-03. (**d**) LG-04. (**e**) LG-05. (**f**) LG-06. (**g**) LG-07. (**h**) LG-08. (**i**) LG-09. (**j**) LG-10. (Reprinted, with permission of Genetics Society of America, from Kim et al. 2004)

or gruel, malted beverages including beer, and specialty foods such as popped grain and syrup from sweet sorghum. In Africa, the straw of traditional tall sorghums is used to make palisades in villages or around a homestead. The plant bases are an important source of fuel for cooking, and the stems of wild varieties are used to make baskets or fish traps. Dye extracted from sorghum is used in West Africa to color leather red.

Some quantities of grain sorghums go into industrial uses. Sorghum starch is manufactured in the USA by a wet-milling process similar to that used for corn starch, then made into dextrose for use in foods. Starch from waxy sorghums is used in adhesives and for sizing paper and fabrics and is an ingredient in oil drilling "mud". The grain can be a source of butyl alcohol.

7.1.7
Breeding Objectives

Sorghum is grown in a wide range of physical conditions in locations ranging from the equator to over 50° N and 30° S. The crop is therefore subjected to a wide variety of temperature, day-length, and moisture regimes. Improved sorghum cultivars for a particular environment always involve breeding for adaptation to the specific climatic conditions found there. This is usually indicated by the appropriate crop duration for that environment and by acceptable and stable yield levels and appropriate grain qualities. The type of cultivar required for a target location also influences the objectives of the plant breeder. For example, the height of a pure-line variety for a specific environment and the heights of the parental lines of a hybrid for the same environment are likely to be different. In addition, improved cultivars for specific locations must

Table 4. Nutritional composition of sorghum*

Nutrient	Amount	Unit
Water	9.2	g
Energy	339.0	Kcal
Protein	11.3	g
Total lipid	3.3	g
Carbohydrate	74.6	g
Fiber, total dietary	n/a	g
Ash	1.57	g
Calcium	110.0	mg
Iron	3	mg
Magnesium	n/a	mg
Phosphorus	287.00	mg
Potassium	350	mg
Sodium	6	mg
Zinc	n/a	mg
Copper	n/a	mg
Manganese	n/a	mg
Selenium	n/a	mcg
Vitamin C	0	mg
Thiamin	0	mg
Riboflavin	0.26	mg
Niacin	3.53	mg
Pantothenic acid	n/a	mg
Vitamin B-6	n/a	mg
Folate	150.0	mcg
Vitamin B-12	0	mcg
Vitamin A	2,205	IU
Vitamin E	0.00	mg-ATE
Vitamin D	n/a	IU
Iodine	n/a	mcg

*Average values (per 100 g), taken from U.S. Department of Agriculture, Agricultural Research Service (USDA:ARS) 1998 USDA Nutrient Database, Release 12, Laboratory Home Page (http://www.nal.usda.gov/fnic/foodcomp)

possess resistance to the major constraints to production encountered and grain- and stover-quality factors appropriate for sorghum there. These constraints include biotic stresses such as diseases, insects, and parasitic weeds, and abiotic stresses, the requirements for which are usually quite different from one location to another. Resistance to these constraints is deliberately bred into cultivars by crossing resistant types with cultivars possessing other desirable traits and selecting plants with both resistance and desirable traits. Increased yields and improvement of quality are the main concerns of sorghum-breeding programs. On a global basis, sorghum breeding aims at specific objectives including high grain yields, higher fodder yields, disease resistance, insect resistance, drought tolerance, high temperature resistance, striga resistance, nutritional quality, cooking quality, and good stalk quality. In addition, development of suitable varieties to fit into various cropping patterns (intercropping and sequence cropping) in developing countries is another objective.

7.1.8
Classical Breeding Achievements

Kharif Sorghum

With the release of CSH I, the first commercial hybrid in 1964, sorghum became the second crop after maize in developing high-yielding hybrids using a cytoplasmic-genic male sterility system. Since CSH I, a total of 18 more hybrids have been released. The hybrids played a major role in raising productivity and production, particularly in the case of kharif sorghum. Yield potential shown by the hybrids CSH 5 to CSH 18 requires special mention. CSH 5 and CSH 6 had a yield potential of 34 q/ha, while CSH 9 produces 40 q/ha in. This further increase to 42 to 45 q/ha in CSH 16–CSH 18 recently.

Besides hybrids, 15 high-yielding varieties (CSV 1 to CSV 15) have also been released with medium maturity (Table 5). Higher preference was shown for dual-purpose varieties such as CSV 10, CSV 13, SPV 462, and CSV 15. A major advantage of varieties over hybrids is their relatively better grain quality and multiple resistance or tolerance against major pests and diseases. The recently released variety CSV 15 has established higher grain and fodder yield potential than hybrids CSH 5 and CSH 6 released two decades ago.

Rabi Sorghum

Improvement of rabi sorghum did not receive as much emphasis and effort as the kharif sorghum until the 1990s. However, some of the hybrids and varieties listed in Table 5 are specifically developed and recommended for rabi season where the fodder yield is more important than that in kharif sorghum. Therefore, rabi grain productivity must be accompanied by normal or better fodder productivity. From this point of view, gradual success was achieved from the first rabi hybrid CSH 7R to the latest hybrids CSH 15R and 18R.

7.1.9
Limitations of Classical Endeavors and Utility of Molecular Mapping

Plant-breeding efforts over the past six decades have contributed tremendously to the genetic improvement of cereals in terms of yield and quality. However, traditional approaches to crop improvement have several limitations, and increase in yield and productivity cannot be sustained indefinitely (Vasil 1994). Most sorghum-breeding programs have focused on agronomic performance to insure food security; however, grain quality is also an essential requirement for the development of improved cultivars. Sorghum proteins are not of superior quality. Limited lysine and the excess of leucine, which affects the leucine–isoleucine balance, are the primary limiting factors of sorghum protein quality. The hopes raised by those of the Ethiopian high-lysine sorghums that are late, photosensitive, and possess shriveled seeds, as well as those of P7212, an opaque mutant and N94 with shriveled seeds, have not been realized so far. Also, little is known about the genetic control of grain-quality parameters and their relationships with the main component of sorghum productivity.

Improving drought tolerance is an important objective in a sorghum-breeding program. Early breeding for host plant resistance to sorghum midge, shoot fly, and stem borers brought about worthwhile resistance in sorghum; however, fast evolving races require incorporation of multiple resistance genes, which has not been possible through classical breeding efforts.

The genetic improvement of sorghum through classical plant breeding has resulted in the successful development and deployment of highly adapted high-yielding cultivars that are stable across years.

Table 5. List of released sorghum hybrids

No.	Name	Parentage	Year of release	Duration (d)	Plant ht (cm)	Grain yield (q/ha)	Fodder yield (q/ha)
1.	CSH 1	CK 60A × IS 84	1964	105	150	28–31	80
2.	CSH 2	CL 60A × IS 3691	1965	110	150	30–32	95
3.	CSH 3	2219A × IS 3691	1970	110	145	33–35	105
4.	CSH 4	1036A × Swarna	1972	110	175	34–35	90
5.	CSH 5	2077A × CS 3541	1975	115	185	35–38	95
6.	CSH 6	2219A × CS 3541	1977	100	155	32–35	75
7.	CSH 7R	36A × 168	1977	110	130	27–29	24
8.	CSH 8R	36A × PD3-1-11	1977	110	120	33–35	97
9.	CSH 9	296A × CS 3541	1981	115	190	38–40	95
10.	CSH 10	296A × SB 1085	1984	110	235	36–38	130
11.	CSH 11	296A × MR 750	1986	110	190	38–40	95
12.	CSH 12R	296A × M 148-138	1986	115	205	25–28	50
13.	CSH 13R	296A × RS 29	1990	115	180	31–32	55
14.	CSH 14	AKMS14A × AKR-150	1989	103	178	30–32	75
15	CSH 15R	104A × RS 585	1996	110	195	32–33	56
16	CSH 16	27A × C 43	1997	110	210	42–45	90
17	CSH 17	AKMS 14A × RS 673	1999	105	205	42–45	105
18	CSH 18	IMS 9A × INDORE 12	1999	115	210	40–44	130
19	CSH 19R	104A × R 354	2000	125	165	25–28	45

However, to further enhance productivity, quality, and resistance to the constraints such as drought, *striga*, grain mold, and insect pests that are so common on farm fields in the tropics, much more needs to be done. The resistance level available in cultivated sorghum types is not adequate to build durable resistance to some of the constraints, especially those caused by insect pests.

Therefore, biotechnological tools like DNA markers, genome mapping, identification, characterization and expression of genes, and genetic engineering have been adopted from the crop improvement perspective to address limitations of classical breeding efforts. It will accelerate identification and incorporation of useful genes into cultivars, facilitate positional cloning of genes, provide new opportunities for assessing and expanding the gene pool in sorghum through comparative mapping of related and unrelated taxa, and contribute to the understanding of the biological basis of complex traits and phenomena important to crop improvement and in the development of transgenics.

7.2
Construction of Genetic Maps

7.2.1
First-Generation Genetic Maps

Construction of a linkage map is the most fundamental step required for a detailed genetic study and marker-assisted breeding approach in any crop (Tanksley et al. 1989). Sorghum genome mapping based on DNA markers began in the early 1990s, and since then several genetic maps of sorghum have been constructed. All the sorghum molecular maps generated to date are summarized in Table 6. Initially, the genetic maps of sorghum were based largely on DNA probes previously mapped in the maize genome (Hulbert et al. 1990; Binelli et al. 1992; Whitkus et al. 1992; Melake-Berhan et al. 1993; Pereira et al. 1994). Later, three more maps were constructed using mainly sorghum genomic DNA probes (Chittenden et al. 1994; Raghab et al. 1994; Xu et al. 1994). Another sorghum map published was based on both maize and sugarcane probes (Dufour et al. 1997). All of these were developed using RFLP markers, and most of the mapping populations were F_2, with the exception of the maps of Dufour et al. (1997) and Peng et al. (1999). Dufour et al. (1997) used two recombinant

inbred line (RIL) populations for the construction of a composite map, which was later extended by Boivin et al. (1999) with the addition of a large number of RFLP and AFLP markers to the map of Dufour et al. (1997). Tao et al. (1998a) constructed a sorghum map using an RIL population and variety of probes, including sorghum genomic DNA, maize genomic DNA and cDNA, sugarcane genomic DNA and cDNA, cereal anchor probes, and eight SSR loci. They attempted to review and compare their map with other published maps, which is supposed to enhance the effectiveness of mapping information and facilitate efforts to map agronomically important traits in sorghum. However, Subudhi and Nguyen (2000) completely aligned all ten linkage groups of all major sorghum RFLP maps using a common RIL population and sorghum probes from all three sources (Chittenden et al. 1994; Raghab et al. 1994; Xu et al. 1994) along with many cereal anchor and maize probes.

Kong et al. (2000) mapped 31 polymorphic SSR loci obtained from 51 clones isolated from a size-fractionated genomic DNA library of *S. bicolor* (L.) Moench that had been probed with four radiolabeled di- and trinucleotide oligomers using an RI population BT × 623 × IS3602C. Taramino et al. (1997) have characterized a total of 13 SSR loci in *S. bicolor* and mapped seven of these using an existing sorghum RFLP map.

Haussmann et al. (2004) have mapped molecular markers for resistance of sorghum to the hemiparasitic weed *Striga hermonthica* in two recombinant inbred populations (RIP-1, -2) of $F_{3.5}$ lines developed from the crosses IS9830 × E36-1 (1) and N13 × E36-1 (2). The resistant parental lines were IS9830 and N13; the former is characterized by a low stimulation of striga seed germination, the latter by "mechanical" resistance. The genetic maps of RIP-1 and RIP-2 spanned 1,498 cM and 1,599 cM, respectively, with 137 and 157 markers distributed over 11 linkage groups.

7.2.2
Integrated Genetic Maps

An integrated SSR and RFLP linkage map of the sorghum was reported by Bhattramaki et al. (2000) using 18 diverse sorghum lines. They designed SSR loci from clones isolated from two sorghum bacterial artificial chromosome (BAC) libraries, their enriched sorghum genomic DNA (gDNA), and sorghum DNA sequences present in public databases. The linkage

Table 6. Sorghum genetic maps developed to date

S. no.	Cross	Mapping population	Types of marker	Number of linkage group	Reference
1.	Shanqui Red × M91051	F2	Cloned maize DNA fragments from 14 characterized genes and 91 random fragments	8	Hulbert et al. (1990)
2.	Shanqui Red × M91051	F2	Maize DNA fragments	15	Melake-Berhan et al. (1993)
3.	S2482C × IS18809	F2	Isozymnes and maize nuclear sequebces	13	Whitkus et al. (1992)
4.	IS 18729 × IS 24756	F2	Maize DNA probes	5	Binelli et al. (1992)
5.	CK 60 × PI 229828	F2	Maize and sorghum DNA probes	10	Pereira et al. (1994)
6.	BSC35 × BTx623	F2	Sorghum and maize DNA probes	11	Ragab et al. (1994)
7.	IS 3620C × BT × 623	F2	Sorghum RNA probes	14	Xu et al. (1994)
8.	BT × 623 × *Sorghum propinquum*	F2	Sorghum DNA probes	10	Chittenden et al. (1994)
9.	IS2807 × 379	RILs	Maize, sugarcane, and cereal anchor probes	13	Dufour et al. (1997)
	IS2807 × 249	RILs	—do—	12	Dufour et al. (1997)
10.	IS2807 × 379	RILs	Sorghum cDNA probes, rice, oat, barley, pearlmillet, wheat and maize probes	12	Boivin et al (1999)
	IS2807 × 249	RILs	AFLPs	12	Boivin et al (1999)
11.	QL36 × QL41	RILs	Sorghum, maize, and sugarcane probes	21	Tao et al. (1998a)
12.	B35 × Tx430	RILs	Maize, sorghum, cereal anchor probes	14	Crasta et al. (1999)
13.	BT × 623 × 153620C	RILs	Sorghum, maize genomic DNA clones Rice, maize, barley, oat, and rice cDNA clones	10	Peng et al. (1999)
14.	CK60 × PI22898	F2	SSRs	–	Taramino et al. (1997)
15.	BT × 623 × IS3620C	RILs	SSRs	–	Kong et al. (2000)
16.	IS9830 × E36-1	RIPs	ALFP, SSR,RFLP, and RAPD	10	Haussmann et al. (2004)
	N13 × E36-1			12	
17.	BT × 623 × IS3620C	RILs	AFLP, RFLP, SSRs	10	Menz et al. (2002)
18.	BT × 623 × *S. propinquum*	F2	RFLP	10	Bowers et al. (2003)

map spanned 1,406 cM and consisted of 147 SSR loci and 323 RFLP loci. Klein et al. (2000) constructed an integrated genetic and physical map of the sorghum genome (750 Mbp). They have developed a new high-throughput PCR-based method for building BAC contigs and locating BAC clones on the sorghum genetic map. Subudhi and Nguyen (2000) attempted alignment and integration of all major molecular maps previously developed for sorghum. To achieve this objective, a genetic map of 214 loci with a total map of 1,200 cM was constructed using 98 F_7 sorghum recombinant inbred lines from a cross between B35 and T × 700. Five major restriction fragment length polymorphism (RFLP) maps independently developed were used for alignment purposes.

A high-density genetic map using AFLP technology was constructed by Menz et al. (2002). The 1,713-cM map encompassed 2,926 loci distributed on 10 linkage groups; 2,454 of those loci were AFLP products; 136 SSRs previously mapped in sorghum and 203 were cDNA and genomic clones from rice, barley, oat and maize. Besides, a comprehensive reference map of the sorghum genome (Fig. 3) was also constructed from two recombinant inbred populations using AFLP, SSR, RFLP, and RAPD markers (Haussmann et al. 2002a). Recently, Bowers et al. (2003) reported a genetic recombination map for sorghum of 2,512 loci spaced at average 0.4-cM (∼300-kb) intervals based on 2,050 RFLP probes, including 865 heterologous probes from sugarcane, maize, *Oryza, Pennisetum* (pearl millet, buffle grass), the Triticeae (wheat, barley, oat, rye), and *Arabidopsis.*

7.2.3
Comparative Mapping

Geneticists and evolutionary biologists have a long-held interest in the mechanisms involved in chromosomal evolution. Until recently, the primary means of addressing questions surrounding this issue has been via cytological analysis of interspecific hybrids and surveys of naturally occurring chromosomal diversity within populations (Stebbins 1971; Jackson 1984; Grant 1987). Comparative genome mapping adds a powerful new technique for investigating the mode and tempo of chromosomal evolution. This approach involves the use of molecular markers such as restriction fragment length polymorphisms (RFLPs) to map the genomes of two species for a common set of markers (loci). Although a labor-intensive and expensive

method, comparative genome mapping allows one to determine the extent and nature of chromosomal rearrangements between cross-incompatible species. This method thus opens up comparisons among distantly related genomes that are not amenable to analysis by traditional cytogenetic techniques. This approach was pioneered by Tanksley and coworkers using tomato RFLP probes to map the tomato (Tanksley et al. 1988). Recognition of the considerable conservation of features within sets of plants such as rice, wheat, and maize (Ahn et al. 1993); sorghum and maize (Pereira et al. 1994; Paterson et al. 1995b); wheat, barley, and rye (Devos et al. 1993); tomato, pepper, and potato (Tanksley et al. 1988, 1992); and *Arabidopsis* and *Brassica* (Teutonico and Osborn 1994) has inspired the suggestion of considering such groups as single genetic systems (Bennetzen and Freeling 1993; Helentjaris 1993). The recent discovery of small chromosomal regions retaining similar gene order in sorghum and two dicot species (*Arabidopsis* and cotton) suggests that comparative mapping may ultimately reach across a much greater "evolutionary distance" than has been spanned to date (Paterson et al. 1996). This concept should have considerable merit and mutual advantages for both breeders and geneticists.

The comparative mapping results between sorghum and closely related grass species are described below.

Sorghum, Maize, and Rice

Within the tribe Andropogoneae, comparative mapping facilitates an understanding of sorghum genetics. Several groups established the relationship between the sorghum and maize genomes (Hulbert et al. 1990; Whitkus et al. 1992; Melake-Berhan et al. 1993; Grivet et al. 1994; Pereira et al. 1994; Paterson et al. 1995b; Dufour et al. 1997). Gene orders appear to be largely conserved between sorghum and maize; only a limited number of rearrangements have been identified. With the exception of major evolutionary translocations, which characterize the Panicoideae, extreme colinearity also appears to have been maintained with rice. An RFLP linkage map of *S. bicolor* (L.) Moench was constructed (Peng et al. 1999) in a population of 137 F_{6-8} recombinant inbred lines using sorghum, maize, oat, barley, and rice DNA clones. The map consisted of 10 linkage group and 323 markers. Comparison of the map with RFLP maps of maize, rice, and oat produced evidence for sorghum-maize linkage group rearrangements and homoeologies not reported pre-

LINKAGE GROUP A   (130.1 cM,   333 Loci)

```
0       —— CSU482 (0 8) BCD0926b
2.3     —— CDSR018a UMC032a
3.8     —— DM065b HHU33 pPAP07D12
6.2     —— HHUK29 PRC1080b pSB0129
9.2     —— pSB0068 pSB1287 pSB1718
10.8    —— pSB0443a pSB1342% pSB1432 (11 5) pSB1889*
12.3    —— pPAP09D04
15.4    —— pSB0581%
20      —— CSU016 HHU03 pSB1652
21.6    —— pSB1306b pSB1661 pSHR0186.3a
23.1    —— CDO0328 pSB1323 pSB1409b pSB1462 pSHR0033 (23 9) pPAP07H07a
24.6    —— PRC0241 pSHR0158.1
29.3    —— pSB0311 pSB1368
30.8    —— pSB0739 pSHR0149 1b
32.3    —— AEST045 pPAP09C09b (33 1) pPAP01C03
34.7    —— pSB0017 pSB0688 pSB1157 UMC103 (35 4) CDSB62 CSU585 HHU61 PRC0012b PRC0144a PRC0167 see below
37      —— pSB0024b pSB0350 pSHR0070c
39.3    —— CSU532 (40) CSU386b G0107
40.8    —— PRC1058%
42.4    —— S13048
43.9    —— pPAP01E10a (44 7) pHER1A01% pHER5A09% PRC0039b
45.4    —— pHER5A04 PRC1080a (46 2) PRC0181b RZ543
47      —— pSB0777 (47 7) BNL09.11 pSB0854 pSB1792
48.5    —— R2727
50      —— pPAP10C08 PRC1044 (50 8) AEST047b CDSR125b pSB0485% pSB1361
51.6    —— CSU740 M713 pSB1706 SG293 (52 4) pPAP02A06
53.1    —— CDSR015 CSU029 CSU621b pHER1D07 pPAP07A06 PRC0297 PRC0354 pSB0988 pSB1433a* (53 9) see below
54.7    —— AEST163 HHU36 pPAP04B07 pPAP05F11 pPAP09G01 pPAP10B12a PRC0402a pSB1910 pSHR0076 see below
57      —⊕— AEST142b R2167b (57 7) DM064 pSB1421 pSB1693%
58.5    —— DM003 R2635 S13994 (59 3) CDO0455 CDO0920 CDSC55 CDSR154 PRC1120 PRC1151* pSB0526c see below
60      —— CDO0226b CSU036b pSB1048 pSB1120 (60 8) CSU058 CSU460a DM062 HHU09a% M696 pSB0032 see below
61.6    —— pSB0378 pSB0497 pSB0528% pSB0779 pSB0807b pSB0980a RZ776 UMC152 (62 4) HHU17b pSB0613* see below
63.1    —— 5C05H06 Sh2-1000E3
64.7    —— pSB1231 (65 4) CDSC30 CDSR128 CSU397 PRC0038a pSB1233 UMC063
66.2    —— CSU031 CSU456 HHU48* PRC1091b pSB0746b pSB0904 pSB1319 pSB1938 R0886 (67) pSB1920
67.7    —— C0122 C1264 CSU744 G0193a PRC0049 PRC0371 PRC0372 (68 5) pSB0289
70      —— pPAP12D11 (70 8) C0922a CDO0470 CDSB23 CSU673 pPAP08G07 PRC0407 pSB0046 pSB0073 pSB1812 see below
71.6    —— CDSR067a pSB0293 pSB1611a (72 4) UMC016a
74.7    —— PRC1157c (75 4) CDO1160b pSB0085b pSB0951c pSB1720b pSHR0139.2
77      —— pSB0794 (77 7) AEST109 PRC0287
80.1    —— CSU451 (80 8) AEST008b pPAP01D04 pPAP05D01b pPAP07C04 pSB0044 pSB0148 pSB1739
82.4    —— PRC0120 pSB1200 SHO46% UMC040 (83 1) CSU706 pPAP04B06 pPAP07CC5 pSB1827 RZ161 S02245
83.9    —— CSU690 (84 7) DM011
85.4    —— PRC1128b pSB0035 pSB0469 pSB1138 pSHR0149.3a
87.7    —— CSU096a (88 5) pSHR0157 5
90.8    —— JA2 RG598a
92.4    —— CSU591 pSB1591 (93 1) CSU351 pPAP08E07 PRC0336
93.9    —— AEST239a RZ444 (94 7) AEST171e CDSR087 CSU469a PRC0165 UMC060a
95.4    —— CSU110 CSU776 (96 2) PRC1109b pSB0079% pSB1545
97      —— CDSR126 PRC0106b pSB1698b S10846
98.5    —— pPAP03A08a pSB0175 pSB1075 (99 3) pSB0401
100.8   —— AEST137d C0086
103.1   —— pSB0443b
104.7   —— CDSR019 pSB1261 (105 4) CDO0345 HHU18 pSB0620 pSB0757 pSB1473 pSB1700
106.2   —— DM033a% pPAP07A07 pSB1140c (107) PRC0271
107.8   —— C3120 (108 5) PRC1102
109.3   —— DM110 R2414 (110 1) pSB0109
110.8   —— pSB0273 (111 6) pSB1289% pSB1728 S10526
112.4   —— CSU328 CSU344 PRC0096b pSB0464b pSB1337 RZ014 RZ237 (113 1) pSB1359
114.7   —— CDSC56 pPAP10D07 (115 4) CDSR153c
117     —— pHER1C11 PRC1050 pSHR0143.7 (117 8) HHU46% pHER1A07 pPAP08G04 PRC1067 PRC1125 PRC1145
120.1   —— CSU579a CSU621c pSB0527 pSB0940 pSB0956b pSB0960b pSB1500 pSB1802 UMC071 (120 8) pPAP07B12
121.6   —— pPAP08C09 (122 4) AEST069b CSU783
123.9   —— PRC1108 (124 7) pSB1277
125.4   —— C1456a
127.8   —— CSU387 PRC0166% PRC0389 pSB0243 pSB0333b (128 5) pSB0414
129.3   —— pSB0242a (130 1) C0112 PRC0068

(35.4)  PRC1142
(53.9)  pPAP01A09 pPAP01D10 pSHR0109 S01959b
(54.7)  pSHR0149.4 RZ995a S01959a (55 4) DM001c PRC1130a
(59.3)  pSB0614 pSB0789 pSHR0110.2b pSHR0110.4 RZ244 SG161 SG168
(60.8)  pSB0231 pSB0548 pSB0847a pSB1306a pSHR0164.3 UMC010
(62.4)  RZ323
(70.8)  SHO65
```

Fig. 3. Sorghum genetic map (Reprinted, with permission of Genetics Society of America, from Bowers et al. 2003)

LINKAGE GROUP B (120.8 cM, 331 Loci)

```
0       C2942b pSB1679
1.5     PRC0367 (2 3) AEST141 PRC1130d pSB1028 UMC045a
3.1     UMC035 (3 8) pSB0720% pSB1616
4.6     CDO0038 CSU163 CSU633 HHUK09 HHUK30b pPAP01F01a PRC0005b PRC0019b% PRC0163b pSB0700c see below
6.2     CDO0533 CSU005 CSU477b C6U621a pPAP08H03 PRC0199 pSB1458 pSB1490• pSHR0147 2 S10363  •  see below
7.7     C1127 CSU027 PRC0100% PRC0236a pSB0783 pSB1357 pSB1362 pSB1433b• pSB1468
9.2     AEST007a PRC1081b pSB1465 (10) pSB1766%
10.8    BCD0855 CDSB12 CDSR077 PRC0085 PRC0136a
12.3    CSU652a pPAP12B03 PRC0093 PRC0106a PRC0320 pSB0432 pSB0793b pSB1675 pSB1678
14.6    CSU702 (15 4) PRC0035% PRC0037% pSB1124 pSHR0157.1 R1245a

17.7    RZ395
19.2    MItalyE pSB1332 (20) C0848 pHER1C01
20.8    AEST054 CSU579b CSU581 pPAP07G04b pSB1267
22.3    R2672 (23 1) CSU534 CSU535 HHU16 PRC0148b pSB1070d

24.6    CSU460c HHU12b HHU17a
26.2    C1268

28.5    pSB1224 (29.2) pSB1144%
30      PRC0011 PRC0047 (30 8) PRC0039a

32.3    pSB0077
33.9    C0147 (34 6) AEST005 PRC0009 PRC0139 PRC0358 pSB0494
35.4    pSB1476a (36 2) C0285 MR0505 pSB0998 pSB1663 R3089
36.9    AEST157c CSU154 pPAP07D05 PRC1101 pSB0266 pSB0934a UMC004 (37 7) CSU784
38.5    S01723 (39.2) CDO0385 pPAP04F04 pSB0074b
40      C0006 pPAP06D04 (40 8) HHU17c pPAP09H09
41.5    CSU460b (42 3) pPAP12C03a PRC0409% PRC1065
43.1    AEST047a C0506 PRC0201 R2710 RG598b (43 9) pSB0103
44.6    PRC0117 pSB1374
46.2    C1454a CSU705 HHU26 HHU43 pSB0027b pSB0075 pSB1522b (46 9) pSB1382

48.5    pHER1A03 PRC0267 (49.2) pSB0361 pSB1561a pSHR0035
50      pPAP09A06 PRC0176 RZ456b (50 8) C1115a pHER1E04 pPAP10D08 PRC0127

53.1    pSB1089 pSB1497% (53 9) C0356a HHU50
54.6    C0846
56.2    BCD0926a pSB0500 pSB1450b pSB1859 (56 9) pSB0101 pSB0609 pSB1631b pSB1913
57.7    CDO0412 PRC0233 PRC0364 pSB1683 (58 5) PRC0403 pSB0407 UMC005
59.2    pSB1253
60.8    pPAP10F04 pSB0037 pSB0093a pSB0418 pSB1801 S10622 (61 6) pSB0605% pSB1355d pSB1400• see below
62.3    S13922b
63.9    pSB0805 (64.6) PRC0227
65.4    C0397 PRC0130c% PRC0151b PRC0152 PRC0211b PRC1150a pSB0413b pWal10001

69.3    CSU385 CSU401 pPAP09E04 PRC0190b PRC1045 pSB0080 pSB0986% pSB1051a pSB1803 pSB1832 see below
70.8    PRC1129 pSB0787e pSB1076 pSB1098b pSB1596b pSB1743b (71 6) AEST001b CDSC49 CDSR041 see below
73.1    pSB0521b AEST111 C0449 CDO0407a CSU422 HHU38% PRC0084a pSB1283 pSB1537 SG298a (73 9) see below
74.6    DM007 PRC1152b (75.4) CDSB07 CSU747 DM017 DM027 pPAP08C02 pPAP11D08 PRC0062 PRC0304 see below
76.2    pSB0925 pSHR0113.2 (76.9) pSB0147b

78.5    CSU109 pSB1037a pSB1529a (79 7) pSB0495 pSB0539

80.8    pSB0918 UMC032b UMC036
82.3    CSU013 PRC1098 (83 1) PRC0296a PRC1169a pSB1807
83.9    pSB1008 (84 6) pSB1898 pSB1944
85.4    CSU129 pSHR0056
86.9    S00851
88.5    PRC0383a pSB1311b (89 3) pPAP09D09 PRC1156 pSB1521 pSB1561b pSB1716a

91.6    pSB0054% pSB1019

95.4    HHU05 (96 2) pSB1765
97      AEST206b (97 7) RZ387a

99.3    PRC1056 PRC1149 pSHR0179.2a
100.8   DM088 (101 6) pSB1369

103.1   pSB0456 (103 9) pSB0799
104.7   PRC0380 pSB1037b pSB1529b (105 4) pSB0053 pSB0606•
106.2   HHU09b% pSB0853 pSB1524a
107.7   pSB1158a (108 5) pSB1926
109.3   pSB0990

111.6   UMC014b

114.7   pSB0018
116.2   AEST031b
117.7   pSB0174 R2677 (118.5) pSB1742a
120     RG418 (120 8) M190 PRC1127 pSHR0124.2 S10742


        (4.6) pSB1014 (5.4) pPAP10H08 PRC1088 pSHR0114.1
        (6.9) CDO0405
        (61.6) UMC139
        (69.3) pSHR0186.2 (70) PRC1081a pSB1476b pSB1894 S00831
        (71.6) CDSR074 CSU081 CSU603 PRC1162 pSB0091a pSB0457c pSB0774% pSB0951b pSB1448b pSB1716b
        (71.6) pSHR0122.2c pSHR0123.5b pSHR0130.1b pSHR0139.3b RZ413 SHO14b SHO38
        (73.9) pSB1452b pSB1456b% pSB1932 SHO70a UMC055
        (75.4) pSB0150% pSB0669 pSB0694 pSB0844b pSB1153
```

Fig. 3. (continued)

LINKAGE GROUP C   (118.5 cM,   499 Loci)

```
0        CSU527 PRC0094a pSB1846%  (0 6) CSU537 PRC1052%
1.5      CDSR018b pSB0978%  pSB1916 (1 1) pPAP05B03
3.1      pPAP08F02 (3 6) PRC0181a
4.6      AEST055 AEST137e CSU448 CSU682 pPAP02C03 pPAP05D01a PRC1063% pSB1365 RZ614 (5 4) AEST025 see below
6.2      AEST171b Pcp8c pPAP10H05a pSB0878 pSB0897 (6 9) pSB1070b

9.2      CSU134 CSU149 DM024 (10) pPAP09E02a PRC0148a pSB0041% pSB1659
10.8     CSU033 CSU536 CSU663 pHER1C12 pSB1387 pSB1451a pSB1478a pSB1719 (11 5) BNL08 29 FPC1215 see below
12.3     C1468 CSU604b pSB1298b
13.8     AEST039 CSU063a M466 M477 PRC0016 (14 6) CSU662 pSB1301 pSB1381 pSB1447
15.4     HHU34 PRC0378 pSB0406 (16 2) AEST031a

17.7     BCD0450 CDO0036b phyca PRC0156b PRC1064 pSB0158 pSB1059 pSB1463% pSB1467 pSB1656 see below
19.2     CSU399 pPAP09C09a PRC0143% PRC0370 pSB0065 pSB0105 pSB0875 pSB0929 pSB1140b RZ630 see below
20.8     PRC0144b (21 5) AEST018d pSB0948% pSB1172 pSB1698d UMC167a
22.3     phya (23 1) CDO0337
23.9     BCD1072b CDO0542 HHUK21a pPAP01C05 pPAP09A09 pPAP10G11a pSB0021 pSB0183 pSB1484a see below
25.4     pPAP09C10 PRC0021 R0654b (26 2) HHU21% PRC0305 pSB1237 pSHR0114.2
26.9     CO746 PRC1144 (27 2) CSU532 pSHR0119.2a
28.5     pSB1911 (29 2) UMC140a
30       PRC0187 RZ474 (30 8) CSU574 PRC1099 S10074a

32.3     pSB0097 pSB0399 pSB0611 UMC027
33.9     pSB1909e (34 4) CDO0795 pSB0167 pSB1126% pSB1431% pSB1544% pSB1615 pSB1729 RZ404 SG202 see below
35.4     pSB0195 (36 2) HHUK03a M848 M858 PRC0084c PRC0214 pSB1452a pSB1742c pSHR0177.3 pSHR0178.1
36.9     pPAP08A07a (37 7) pPAP10C12 pPAP11B11 PRC1073%
38.5     pSB1760 (39 2) pSB1411c

40.8     BCD0207 (41 5) pSB0874
42.3     PSB0770 S12564 (43 1) HHU28a HHUK20 PRC0273 PRC1116% pSB1563
43.9     BCD0386 (44 6) pSB0071
45.4     CSU507 pPAP03H01 pPAP07A01 PRC0186 PRC1141 pSB0951a (46 2) AEST006b AEST075 AEST137a see below
46.9     PRC0020a pSB0081 pSB0239 SHO59 SHO68 SHO87 (47 7) CSU111b pSB0352 pSB1345 pSB1406 see below
48.5     5C04E10 5C05H05 PRC1093 R1245b RZ892 (49 2) CSU145b CSU653 M096a pPAP07F07 pSB1391 see below

51.5     CDO0020b CDSR155 RZ421 (52 3) AHD225 CDO0066 PRC0031
53.1     PRC1072
54.6     PRC0324 pSB0800% RZ786 (55 4) pSB1086
56.2     pSB0033 (56 9) pPAP09B11

58.5     CSU694 PRC0393% pSB1814 RZ500a
60       pPAP07H09a PRC0321c PRC1199 pSB0062 pSB0761a pSB1469 pSB1743c RZ995b UMC014a (60 8) see below

63.1     ISU078
64.6     pSB0709
66.2     pSB1862

69.2     pSB1423 (70) S01764

71.6     CSU435 UMC116 (72 3) pPAP06H03 pSB1409a pSB1798
73.1     CSU389 CSU649 CSU737b DM010b% pHER1B05 PRC0137 pSB0395b pSB1776 RZ672 (73 9) C2942c
75.4     G0181 pSB0050 pSB1051b pSB1317 (76 2) pSB0569 SG370
76.9     C0901 C1454c CDSC57 CSU669a pPAP08F01 PRC0043 PRC0281 PRC1078 pSB0300a pSB0712 pSB1411b see below
78.5     AEST157a CSU392 phyb pPAP02A08 pPAP03D01 pPAP08C11 PRC0092 PRC0125 PRC1130c pSB0529 see below
80       PRC1119a pSB1298a SHO81% (80 8) AEST171c CDSR066 CSU710 PRC0154b% pSB1872 pSHR0189.3
81.6     BCD1381 CDO1081a CDSB06 CSU059 CSU145a CSU219 HHU55 pPAP06B07 PRC0248 PRC033? pSB0558% see below
83.1     CSU028 PRC0321d pSB0989a pSB1250 pSB1278% pSB1338 (83 9) AEST007b CDO1387 pPAP07G04c see below

85.4     pSHR0176.2

88.5     UMC081
90       C0245 PRC1203% pSB1223 UMC076 (90 8) BNL14.28 CDO0860 pPAP01F01b pPAP03F08 pPAP07E06 see below
91.6     AEST018b AEST022 CDSR035 CSU469b CSU513 pPAP10E11 PRC0209 PRC0270 pSB0771 pSB0928 see below

93.9     CDSB15a HHU35 pSB1196% pSB1777 (94 6) HHUK04 pSHR0123.3
95.4     PRC1055 PRC1105
96.9     pSB1024 (97 7) HHU60
98.5     pSB0600%
100      CSU111a pSB0088 pSB1187 pSB1797 RZ329
101.6    CDSR097 pPAP03A06 pSB1864 RG944 (102 3) PRC0109b RZ561a
103.1    pPAP10B01
104.6    pPAP07A05b pSB0186 (105 4) PRC1054a
106.2    pSHR0103.1 (106 9) C1454b CSU453 M096b pSB1669 pSB1790%
107.7    CDO0020a CDO0344b PRC0398 pSB0847b pSB1334
109.3    AEST122a CDO0507b pSHR0180.2b (110) CSU680a HHU13
110.8    RG348
112.3    pSB1159

114.6    CSU347 pPAP07A09 PRC0309 PRC0402b (115 4) pSB0508%

118.5    AEST069a PRC0046b
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(5.4)   AEST239c PRC0028 PRC0045 pSB1018 UMC084
(11.5)  pSB0102 pSB1106 SG305
(17.7)  S01912a (18 5) PRC1061 pSB1503a
(19.2)  UMC090 (20) PRC0057a pSB0446
(23.9)  pSB1621 pSB1733 (24 6) PRC0007% PRC0071a PRC0246 pSB0851
(34.6)  SG212
(46.2)  AEST256 AEST602a C0152a C0222 CDO0098 CDO0226a CDO0312 CDO0516b CDSR131 CSU009b CSU096b
(46.2)  CSU567 CSU654b CSU669b CSU716b HHU07a HHU41a KMG2 M869 Pcp8b pHER5F02c phycb pPAP05H06
(46.2)  pPAP07A08b pPAP07B03a pPAP07C06a pPAP07G04a pPAP08A05b pPAP08A07b pPAP08D04a pPAP09B03b
(46.2)  pPAP09C03 pPAP09E02b pPAP09F06 pPAP09G04b pPAP09H02a pPAP09H03 pPAP12G07b PRC0015a
(46.2)  PRC0033 PRC0038b PRC0071b PRC0077a PRC0094b PRC0312a PRC1054b PRC1077 PRC1189 pSB0641a
(46.2)  pSB079Ja pSB0880b pSB0915a pSB1330a pSB1489b pSB1704 pSB1743a R0404 R3202 R3330 RG433b
(46.2)  RG463b RG482a RZ053 RZ400 RZ561b RZ777a S01623 S14158 UMC016b UMC083 UMC085b UMC107
(46.2)  UMC133 UMC166 pYRL2C15
(47.7)  pSB1487 pSB1488 R2447 S10
(49.2)  pSHR0143.3
(60.8)  PRC0230
(76.9)  pSB1422 pSB1472 pSB1726 S11433 UMC095 (77 7) CO137a CSU455 DM002% DM056% HHUK30a PRC0012a
(77.7)  PRC0050a PRC1095
(78.5)  pSB1596d pSB1632 (79 2) CSU523 CSU742 PRC0140 pSB1016%
(81.6)  pSB0604 pSB1320 pSB1630 pSB1722a pSB1818 pSB1909a R0549 (82 3) pPAP07D07 pPAP09H11
(82.3)  pPAP10F10
(83.9)  pSB1034
(90.8)  PRC0108 PRC0159 pSB1281
(91.6)  pSB0965b pSB1021 pSB1098a pSHR0110.2a (92 1) BNL05.09
```

Fig. 3. (continued)

LINKAGE GROUP D (81.6 cM, 187 Loci)

```
0       ─── pSB0444 pSB1869 (0.8) PRC0329
1.5     ─── pSB0908 pSB1460%
3.1     ─── CSU781b (3.8) PRC1075

6.2     ─── C0107 (6.9) CDO0036a CSU048 HHU11
7.7     ─── PRC1146 (8.5) C1991b
9.2     ─── pPAP06B02 PRCC164b pSB0040b pSB0725 pSB1795
10.8    ─── C0513
12.3    ─── pPAP04G01 pSB0487 pSHR0066 (13.1) AEST051 HHU39 pPAP10B03
13.8    ─── CSU642 PRC1041 (14.6) AEST038 pSHR0180.4

16.9    ─── pSB0421 pSHR0111.4 (17.7) CDO0524 HHU28b pPAP08H05b pSB1175 R1534c RZ017
18.5    ─── CSU458 PRC0390 PRC1197 pSB1901 pSB1917
20      ─── pSB0700b pSB1411a pSB1895 R1534b RZ387b (20.8) PRC0318
21.5    ─── CSU405 CSU741 pPAP12C05 (22.3) pPAP10B07b PRC0368 pSB0838 pSB1220 pSB1363 pSHR0121.2

23.9    ─── pSB1764a%

27.7    ─── pPAP08E09 (28.5) UMC006
29.2    ─── pSB0523 pSB1619 (30) BNL10.13b M428 pSB0365a pSB0482 pSB1450c UMC044
30.8    ─── pSB1824% UMC045b (31.6) pSB1015
32.3    ─── PRC0064 (33.1) CDSR125a PRC1126

34.6    ─── pSB1850 (35.4) pSB1773
36.2    ─── pSB1568 (36.9) pSB0187a
37.7    ─── CSU745 pSB0647 (38.5) CSU410 PRC0253 pSB0140 pSB1047 pSB1589 UMC034

40.8    ─── HHU27 (41.6) pSB0095 pSB1151 RZ740a

43.1    ─── pSB0091b pSB0911 pSB1104 (43.9) pSB0242b pSB1442
44.6    ─── DM066b (45.4) CSU040
46.2    ─── pSB1436% pSHR0180.2a (46.9) CDSR109 CSU086 pHER5D10 pPAP0JC01a pSB1916 S13322
47.7    ─⊕─ pPAP11F10a (48.5) AEST136a CDO1328 CSU393 DM066c PRC0168b pSB0552 pSB1736 RZ467
49.2    ─── C0058 pHER1C10 PRC0151a (50) CSU518 pSB0745 pSB1456a%
50.8    ─── AEST018c pSB1817 (51.6) CSU063c PRC1163 pSB1854

53.9    ─── DM109 R0093

56.2    ─── PRC1096b pSB0314 pSHR0063 (56.9) DM057 pSB0747 pSB1343 RZ782a
57.7    ─── CDSC05 CDSR084 Pcp8a pPAP03A07 pPAP11G02 PRC0162a PRC0219 pSB1847 (58.9) pPAP07E10 see below
59.3    ─── pSB0866a pSB1450d (60) CDSR046 CDSR063 CSU034 PRC0090 PRC0185 PRC0247 PRC1132 pSB0520b see below
60.8    ─── pSB0428a pSB0521a pSB0580 pSB0806 (61.6) pSB0550 pSB0643 pSB0787d pSB1655b pSB1737 see below
62.3    ─── pPAP09F11
63.9    ─── pSB0900 pSB1379
65.4    ─── PRC0362 pSB1355b pSB1813d (66.2) pSB1113

69.3    ─── BCD0348 CDO0456

72.3    ─── CSU423 CSU430 PRC0136b pSB1611b (73.1) PRC0130b%

80.1    ─── pPAP08F03
81.6    ─── pSB1622


        (58.5) pSB0161
        (60)   pSB0692 pSB1456c pSB1657c RZ069
        (61.6) pSHR0091b pSHR0100.2 R2558 SHO74
```

Fig. 3. (continued)

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LINKAGE GROUP E    ( 84.7 cM,   146 Loci)
0        PRC0053
1.5      PRC1137 pSB1010 pSB1654
3.1      PRC0036
4.6      BCD0349 pSB1355c pSB1482 (5.4) pSB0607b
6.2      CSU108 (6.9) pSHR0032 S45
8.5      pSB0880a

11.5     CDSR095a (12.3) CDSR091
13.1     S10074b
14.6     pSB0861
16.2     pSB1699 S08
17.7     CDSR049a
19.2     pSB1311c

21.5     pSB1871%


29.3     CDSR095b pPAP08A05a pSB0506 pSB0544 pSB1007% pSB1502 pSB1799 (30.1) pSB0147a

32.4     PRC0303 pSB1004 pSHR0070a
34.7     RG463a RG482c
36.2     pSB0419a (37) pSB0866e
38.5     CDO0459

41.6     pPAP03C08 pSB1166 (42.4) pSB1203 RZ143a
43.9     AEST171d CSU781a HHU10 M036 M044 pSHR0172.4 rRGH08 S06 UMC102 (44.7) CSU736
45.5     M866 pSB1617b pWal1l RZ296
47       CSU462 HHU19 HHU62 M265 MZY14.1 pHER5C07 pPAP09H05 pPAP10D04 PRC0020b PRC0182 PRC0251 see below
48.5     pSB0818 (49.3) CSU439 CSU539 CSU681 pSB1066a pSB1397c pSB1647 RZ141a
50.1     CSU030 (50.9) pSB0638
51.6     AEST137b CSU103b PRC0190c pSB1549% pSHR0144.1
53.2     pPAP03H12 PRC1165


57.8     BCD0263
59.3     DM023a (60.1) DM023b
60.9     pSB1285b% (61.6) pHER1E07%
63.2     PRC0154a% pSB0318%
64.7     pSB0200 (65.5) CSU692a
67       CSU728 (67.8) DM023c pHER1B02 pHER5A03 PRC0103% PRC0236b pSB0845 pSB1610 pSB1617a see below

70.1     DM074 DM095 PRC0113b pSB1396a pSB1444a R2123 (70.9) AEST060

73.2     PRC0338b
74.7     HHUK27%

77.8     pSB0030
79.3     CDSR160b

82.4     G0243 pSB0047% pSB0063 UMC002b
84.7     PRC1178a


    (47) pSB1450e S00894 S02083 (47.8) BCD1072a pPAP12G07a pSB0182% pSB0504 pSB0787c pSB0804 pSB0839c
    (47.8) pSB1101 pSB1123 pSB1285c% pSB1330b pSB1344b pSB1397a pSB1478c pSB1503b pSB1514c pSB1550
    (47.8) pSB1720c pSB1758 pSB1809a pSB1823 pSHR0156.4a RZ261 UMC130
    (67.8) pSB1837
```

Fig. 3. (continued)

LINKAGE GROUP F   (127.8 cM,   275 Loci)

0	pSB0120
1.5	SHO60%
3.1	pSB0740
6.9	pPAP06C01 [7.7] HHU37c pSB0907
8.5	PRC1182 pSB0057 R2816
10.8	G1234 PRC0141 PRC1076 PRC1128a PRC1133 pSB1480 [11.5] CSU525 pPAP01D02
12.3	CSU651a pSB0871 RZ446 UMC019 [13.1] PRC0126
13.9	pPAP04F02 [14.6] pSB0979
16.2	pSB1094%
19.2	PRC1157e [20] PRC1096a
20.8	DM022a pPAP10A11 S02089 [21.6] DM054 pSB1839
26.9	pSB0883 pSB0956a pSB0960a
28.5	CDO0516a PRC1096c
30	pPAP10H07b PRC1062 pSB0179 pSB1794
31.6	CSU672 [32.3] PRC0268
33.1	pSB0367 [33.9] pSB1489a
37.7	AEST157t UMC126 UMC156
39.3	AEST001a MZY16.1
41.6	pSB1182 [42.3] UMC054
43.1	CDSB53
44.7	BNL10.13a
46.2	CSU661
47.7	CSU440
50	R3393 [50.8] pSB0107
53.1	C0479b PRC0096a R2266 [53.9] C0479a CSU460 CSU643 PRC0004 PRC115-a PRC1186 pSHR0120.2 see below
54.7	AEST122b BCD1631 CDO0507a CSU342 M443b pPAP08H11 PRC1191 pSB0170 pSB0236 pSB0455 see below
56.2	PRC1109a [57] pHER1D10 S13922a
57.7	pPAP08H10 PRC0079 PRC0162b PRC0163a R0654a [58.5] AEST048
59.3	PRC0312b PRC1198% pSB0425 pSB0896% pSB0920 [60] CDO0686 HHU44%
60.8	C0624 CDO0204 PRC0208 pSBC405 pSB0641b [61.6] pPAP09H02b pSB0201 pSB0635 pSB0796
62.3	CDO0400 CLSB0C8 [63.1] CSU358 pPAP08G09 pSB0163 pSB0458 pSB0915c pSB1804 pSHR0149.2
64.7	CDO0497 CDO1380 CSU593 ISU032b PRC0169 [65.4] CSU173 pPAP03E07 pPAP06F06 PRC0052 PRC0132% see below
66.2	CDSR017 [67] HHU14
68.5	DM066a pSB0866d pSB1698c [69.3] RZ782b
70.8	pSB1354 [71.6] CSU600 PRC0002 PRC0077b PRC0118 PRC0343 PRC0394% pSB0083 pSB0341 pSB1217 see below
73.9	pSB0464a S02577 S10847 [74.7] PRC0109a pSHR0149.3b RZ144b PZ74Cb
75.4	CSU039 pPAP07C06b pPAP08B04 PRC0375 pSB0038 pSB0176 pSB1524b pSB1548a pSB1677b pSB1703 see below
77	CDSB22 pPAP10H03 pSB0116 pSB1596c RZ500b [77.7] pPAP05H08 PRC0115 PRC0360 PRC1157d see below
78.5	HHU42 pHER5F09 pPAP01B05 pPAP05C03 pPAP09D01 [79.3] AEST123b HHU52:b pHR22075 pPAP0/C36c see below
81.6	pSB0512% [82.4] C0132
83.1	DM005a pHER1B19c pPAP01H04 PRC1100 pSB1331% pSB1642 pSB1727 pSB1826 [83.9] CDO0078b
85.4	pSB0093b [86.2] PRC1104b
87.7	HHU37b pPAP10A04 pSB1502 RZ087
90	pSB1446 pSB1720a pSHR0147.1 [90.8] C0915 CSU652b CSU670 CSU774 CSU782 pHER5A08
93.1	AEST127b pPAP09G08 pSB1060% [93.9] CSU413
94.7	pSB0187b [95.4] CSU377a
97.7	pSB0094% [98.5] HHUK22 PRC0075
99.3	pSB1056
101.6	CSU036a pSB1457b [102.4] pSB1457a
104.7	AEST036b pPAP09H10
107.8	pSHR0029 [108.5] CDSB15b
110.1	S01912b
111.6	pSB1135 [112.4] CDSC42 M738
113.9	pPAP05H10 pSB0927
115.4	PRC0381
117	AEST023 pPAP08G01 pSB0084 pSB1565%
118.5	PRC0383b pSB1445
120.1	pSB1723 [120.8] AEST240
121.6	CDSR029 [122.4] pHER1D12
123.1	PRC0346 [123.9] pSB0639a
124.7	PRC1104a
127.8	PRC0345a

(53.9) R2955 RZ058 RZ567
(54.7) pSB0615 pSB0823 [55.4] pSB0713a pSB0991 RZ260 UMC108
(65.4) PRC0192 pSB0193 pSB0435 RZ141b
(71.6) pSB1394 pSB1451c PSB1562 pSB1603 pSB1629a pSB1685 RZ166
(75.4) pSB1725 pSB1861 pSB1883 pSHR0070b pSHR0075 PSHR0085c pSHR0116.3 RZ783 [76.2] C0358
(77.7) pSB1424 pSB1763a
(79.3) PRC0170 pSB0934b pSB1221 pSB1601 pSB1780 pSHR0166.3b RZ476b

Fig. 3. (continued)

LINKAGE GROUP G   (107 cM,   196 Loci)

0	CDSB59 pSB0974 pSB1057 pSB1757* (0 8) UMC134
1.5	UMC140b
3.1	pSB1372
5.4	pSB1333 (6 2) UMC167b
7.7	CDO0202
9.2	C1991a (10) R0844
10.8	pSB1698a
12.3	PRC0164a (13 1) CDO1160a CDSB32
13.8	G0193b (14 6) pSB0769
17.7	CDSR153b pSB0989b
19.2	pSB0945 (20) pSHR0121.1
20.8	CDSC19 CSU066 M669 pSHR0117.1 UMC062
23.9	C2782 PRC0083b (24 6) pSB0416
25.4	C0922b CDSR067b pSB0045 pSB0772 SG155 (26 2) CSU660 pSHR0123.2b R2174 UMC132
26.9	C0597 pSB0122 (27 7) CSU402
28.5	pSB1781 (29 2) CSU549
30	pHER5C04 pPAP08G08 pSB0023 pSB1738 S02678
33.1	PRC0107 (33 9) pSB0395c pSB1189
34.6	pPAP09F03 (35 4) PRC0344
36.2	CDO0348 (36 9) pSB0169 pSHR0111.1
37.7	AEST110 BCD0738 pSB0082 pSB0445 pSB1147 pSB1811 (38 5) PRC1130b pSB1809b
39.2	AEST171a CDSB58 DM006 pHER5C08 pPAP06H09 pPAP08A02 PRC0261 pSB0024a pSHR0031a pSHR0156 4b see below
43.1	PRC0050b pSHR0074 (43 9) PRC1123 PRC1148b
44.6	pSB0714 pSB1140a SHO62 (45 4) HHU30 PRC1066
46.2	C1286 PRC0046a (46 9) PRC0130d* pSB1205
48.5	M319 pSB0535a* R0188 (49 3) pSB0404
50	BCD0454 CSU155 CSU760 HHU01 HHUK11 HHUK13 pPAP09B06 (50 8) R1436
51.6	pSB0061 (52 3) pSB0681 pSB1001
53.1	CDO0407b CSU060 DM065a HHU24c HHU40 pPAP01A11 pPAP03C01b pPAP07H07b pPAP10H05b PRC0083a see below
54.6	CDSR153a pPAP07G03 PRC0179 (55 4) pSB0069% pSHR0179.2b
56.9	CSU063b HHU58b pPAP06F11 pSB1866
58.5	CSU437 (59 3) pPAP04A06 PRC0147 pSB1408a
60.8	pSHR0123.2a
62.3	PRC1183 pSHR0146.2 (63 1) pSB1393
64.6	CDSR120 pPAP08D04b pSB1451b pSB1631a (65 4) DM001a DM104 M371
66.2	M386 pPAP07A08a pSB0903
67.7	AEST142a CDSB31 DM001b PRC0005a PRC0019a* R2167a UMC064 (68 5) pSB1905
69.3	CDSR094 pSB1865 SCA22
71.6	PRC0397 (72 3) pSB1002
73.1	CDO0580 (73 9) pPAP03B10
76.2	pSB0347
78.5	CDSR112 (79 3) pSB1505
82.3	pSB1945
89.3	UMC089
92.4	pSB0333a
94.7	ISU032a S02649
96.2	AEST090a pSB1158b
98.5	PRC1148a S00782 (99 3) C1456b pHER5E08
101.6	pSB0847c SHO35 UMC012 (102 4) AEST137c pSB0134
106.3	pSB1115 (107) PRC0242

(39.2) SG442 (40) AEST239b C0137a CDSR049b pSB1155 pSB1742b UMC060b
(53.1) PRC0111 PRC0156a pSB0980d pSB1229 pSB1307 pSB1635 pSB1842 pSHR0031c pSHR0113.3 RZ599
(53.1) SG322 UMC128 (53 9) M716

Fig. 3. (continued)

LINKAGE GROUP H (85.4 cM, 191 Loci)

```
0        — pSB1396b
2.3      — PRC0013% pSB0526b pSB1070a (3.1) pPAP09B03a PRC1178b pSB0517 UMC002a
3.8      — M476

6.9      — pSB0965a
8.5      — G0124  KSU3 pSB0860 (9.2) PRC0113a
10       — M180a

12.3     — PRC0338a

14.6     — HHUK05

18.5     — CDSR160a (19.2) CSU692b pSB1478b
20       — CDO0127 PRC1164 pSB0809a
21.6     — pSB0089 (22.3) pSB1925

24.6     — R2918
26.2     — UMC015
27.7     — AEST009 (28.5) pSB0616
29.3     — pSB0718 (30) CSU103a
31.6     — AEST137f DM097 pSB1407 (32.3) AEST602b
33.1     — pPAP07C08 R1534a (33.9) pSB1822c
34.6     — C0152b DM077 pPAP04E03 PRC1171% pSB1062 (35.4) CDSC24 pSB1263 pSB1464
36.9     — pSB0607a (37.7) pPAP10B12b PRC1135 pSB1522a RZ141c
38.5     — CSU050 pPAP11C07 (39.3) pSB0457a pSB0510% pSB0536 pSB0538% pSB0630 pSB1285a% pSB1541
40       — pTRL2C16 AEST006a AEST259 CDSR146 CSU6C4a HHU07b HHU12a HHU41b ISU036 pHER5F02b see below
41.6     — PRC1079 PRC1113 pSB0839b (42.3) RZ797
43.9     — CDO0365 CSU166 pSB0300b SHO89 (44.6) SHO88
45.4     — AEST136b CSU009a pSB0807c pSB0844a RZ537 (46.2) CSU704 pSB1434
46.9     — pPAP02C02 (47.7) CSU654a

50       — pSB1228

53.1     — pPAP07B03b pSB1249 pSB1316 (53.9) HHUK15 HHUK18
54.6     — pSB0713b (55.4) CDO0520
57       — pPAP07A05a pSB0240 pSB0396 pSB1673 pSB1750

60       — CDSR070 DM103 pSB1355a
62.3     — PRC1169b pSB1275 pSB1484b pSB1829
64.7     — DM099 (65.4) DM073

68.5     — HHU49 (69.3) DM113 PRC0350 pSHR0129

73.1     — PRC1057% (73.9) PRC1124 S35
75.4     — pSB0395a pSB0543 (76.2) pHER5D11 pPAP12A03
77       — pSB0343 pSB0915b (77.7) DM010a% pSB0572
78.5     — C4A DM014 DM021% DM034 pSB0914 S12886 (79.3) DM028 S02344 UGT1976
80.8     — pSB0944

83.1     — HHU45 pHER5B09
84.7     — HHU56 PRC1205 pSB0809b pSB1218 PSHR0085b RZ143b S10003 (85.4) F12 PRC1211%
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(40) pPAP07A03 pPAP09G04a pPAP12C02 PRC0008 PRC0015b PRC0057b PRC0160 PRC0250 PRC0345b PRC1071
(40) PRC1176 pSB0051 pSB0064 pSB0413a pSB0415 pSB0419b pSB0535b pSB0640 pSB0767a pSB0787a
(40) pSB0816% pSB0844c pSB0866b pSB0880c pSB0980c pSB1070c pSB1248 pSB1397b pSB1408b pSB1448a
(40) pSB1450a pSB1494 pSB1529c pSB1596a pSB1600 pSB1677d pSB1732 pSB1779b pSB1813c RG482b
(40) RZ777b SHO14a SHO70b (40.8) PRC0313 PRC1119b

Fig. 3. (continued)

LINKAGE GROUP I   (107 cM,   216 Loci)

```
0        ── CSU698 FLS2 pSB0115% pSB1438 RZ508

3.1      ── pPAP01A02 (3.9) RG433a
4.6      ── M345 (5.4) PRC1213

6.9      ── pPAP08B06

9.2      ── AEST090c pSB0632 pSB1511% (10) C1338 CSU679b CSU680b
10.8     ── pSB1689 pSHR0186.4
12.3     ── CDO0344c (11.1) pSHR0149.1a


17.7     ── AEST036a CSU400 (18.5) pSB1657

20       ── RG653

23.1     ── CSU395
24.6     ── AEST127a CSU391 CSU419 HHU04% (25.4) PRC0128 pSB1185
26.2     ── pSB1771% (26.9) pSB1459

28.5     ── CSU758
30       ── pSB1470 (30.8) CDO0226c

33.1     ── pSB1791% (33.9) CSU377b pSB1418


38.5     ── pSB0355 pSB0373
40       ── CSU056 M815 (40.8) pSB0761b
42.3     ── pSB0301% (43.2) pSB0142
44.7     ── PRC1090 (45.4) pSHR0122.2b

47       ── pSB0742
48.5     ── Hm1a PRC0296b RZ682
50       ── CDO0078a CDO0099b PH100.43misco PRC0168a pSB1161 pSB1403 pSHR0164.2 UMC114 (50.8) see below
51.6     ── BCD0147b pSB0127 pSB0518% (52.4) C0235
53.1     ── pSB1073
54.7     ── AEST126 CDO0017 CSU559 CSU646 HHU02 PRC0155 pSB0596 PSB0703 PSB0707 pSB1633a R3235
56.2     ── AEST123a CDO1081b CSU095 HHU37b M443a pHER5H01 pPAP07H07c pPAP20G11r PRC0095 PRC0211a see below
57.7     ── DM005b M180b PRC1131 PRC1204 pSB0428b
59.3     ── pSB1050 (60) AEST203 pPAP10H07a pSB0767b pSB1684 RZ588
60.8     ── pPAP06G10 PRC0231 pSB0746a pSB1311a pSB1384% pSB1822b pSHR0085a S01520a
62.4     ── pSB0520a (63.1) pSB0526a
63.9     ── 5C04A07 pSB0005 (64.7) PRC1207
65.4     ── PRC1091a (66.2) CSU651b PRC0190a PRC0302
67       ── HHU37d PRC1150b (67.7) PRC0404

69.3   ──⊕── PRC1157b
70.8     ── pSB1475 pSHR0080 pSHR0082a pSHR0123.5a (71.6) RZ002
72.4     ── PRC1152a pSHR0082b
73.9     ── PRC0202 pSB1810%

77       ── pSHR0116.1
78.5     ── AEST008a CSU415 pPAP02D06 PRC1167 RZ516

80.8     ── pSB0027a (81.6) R2749

83.1     ── CDO0475 pSB0457b pSB1070e pSB1348 (83.9) PRC0213

85.4     ── PRC0071c pSB0653 pSB1822a
87       ── pPAP09H08b (87.7) PRC0193
88.5     ── pSB1274 (89.3) CSU158 MZY14.2 pSB1722b pSB1909b

90.8     ── HHU58a pSB1546

93.1     ── PRC0274 PRC0295 (93.9) pSB0764

95.4     ── pSB0085a
97       ── pSHR0130.1a
98.5     ── pPAP01F12
100.1    ── pPAP11F11 (100.8) pSB0365b pSB1499%
101.6    ── pSB0074a pSB1336

103.9    ── HHU15 PRC1138

107      ── AEST132 CSU407 pSB0305%
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    (50.8) BCD0178 CDO0087 CSU147 CSU360 HHU23 ISU128 MZY19.1 pPAP03D12 pPAP09C11 PRC0237 PRC1181
    (50.8) pSB0138 pSB0666 pSB1236 pSB1633b pSB1775% R0518 RG716
    (56.2) PRC0405 PRC1190 pSB0951d pSB0980b pSB1066b pSB1344a pSB1410 pSB1435 pSB1444r pSB1481
    (56.2) pSB1655a pSB1677a pSB1763b pSB1779a pSB1813a pSB1822d pSHR0071 pSHR0139.3a pSHR0163.2
    (56.2) RG123 RZ144a RZ476a RZ612 SG298b UMC065 (57) pHER5F02a pSB0106 pSB0156 pSB0646 pSB0917

Fig. 3. (continued)

LINKAGE GROUP J   (96.3 cM,   138 Loci)

cM	Loci
0	AEST146 (0.8) CSU061 R1985
1.5	CSU092
3.8	C0356b CSU675
6.2	PRC1155 (6.9) R3188
8.5	pSB0204%
10.8	5C05E06
13.1	PRC0122 pSB0491% pSB1401a
14.6	pSB0541
20	RZ028
21.6	CSU590
24.6	CSU737a (25.4) CSU608 HHUK03b
26.2	pSB0700a pSB0811
27.7	pSB0108 (28.5) pSHR0110.1
29.3	C1115b S14003 (30) DM026
31.6	DM033b% pPAP11F10b (32.3) PRC0315
33.1	pSB1163 RZ456a
35.4	PRC0121 (36.2) pPAP09H08a
37	BCD0098 CDO0099a pPAP03A08b pPAP10B05 pSB1629b pSHR0115.6 pSHR0119.2c pSHR0120.1 see below
38.5	B73 pSB1755%
40	Hm1b PRC0084b pSB1764b%
43.1	CSU386a pSB0716%
44.7	pSB0815% (45.4) AEST018a
46.2	AEST056 (47) pPAP12C03b pSB0164
47.7	pSB1226 pSB1556 (48.5) pSB1397d
50	PRC0376 S14085 (50.8) CDSR085 pPAP10B07a PRC0046c pSB0019 pSB0464c pSB0501 pSB0533 see below
51.6	pSB0402 (52.4) Bt2 CSU542 CSU716a pPAP09A08 PRC1177 pSB0067 pSB0540% pSB0787b pSB08C7a see below
53.1	pPAP09E08 pPAP09G11 (53.9) CDSB29
54.7	BCD0147a HHU07c pPAP01E10b pPAP06A09 pPAP09E10 pSB1813b (55.4) pHER1D05% pPAP02F07 see below
56.2	pPAP08H05a (57) CSU613 pSB0639
57.7	pSB0784
59.3	pPAP07H09b pSB0562 pSB1548b pSB1858 (60) pSHR0122.2a
61.6	PRC1046 (62.4) PRC1048+ pSB0760
63.9	CDSR133
66.2	SHO29
67.7	HHU24a
73.9	AEST206a pSB1662 UMC085a
75.5	CDO0344a CSU679a DM075 pPAP04A10 pSHR0119.2b (76.2) CSU474a
77	pPAP09C04 (77.8) pPAP07C09 pSB1517a%
79.3	pPAP06E08
83.9	AEST090b DM106 pHER5D09 PRC0130a+ pSB0040a
85.5	HHU32 (86.2) C2942a pSB1108
93.2	HHU20%
94.8	CSU354 (95.5) AEST144
96.3	pSB0381

(37) R1683
(50.8) pSB0866c pSB1401b pSB1517b% pSB1796
(52.4) pSB0895 pSB0919 pSB1430 UMC047
(55.4) pSB0124 pSB0419c pSB1254

Fig. 3. (continued)

viously. Comparative maps of rice and maize (Ahn and Tanksley 1993) may help to link rice and sorghum using maize as a bridge. This may be extended similarly to wheat (Ahn et al. 1993). Comparative maps should make it possible to begin uniting the genetics of these species and allow for transfer of mapping information (including centromere positions) and molecular-marker resources (e.g., RFLP probes) between species. In addition, such maps should shed light on the nature of chromosome evolution that accompanied the radiation of grasses in the early stages of plant diversification.

The extent of colinearity and other aspects of genome structure in cereals were investigated by cloning *Sh2* homologs from sorghum and rice using the maize *Sh2* gene as a probe in screening rice and sorghum bacterial artificial chromosome libraries (Woo et al. 1994; Zhang et al. 1996). In maize, the *Sh2* and *Al* loci are separated by about 140 kbp (Civardi et al. 1994). In both sorghum and rice, an *Al* homolog is near the *Sh2* homologs, but the *Al* and *Sh2* genes are about seven times closer together than in maize (Chen et al. 1997). In addition, the sorghum *Al* homolog was tandemly duplicated. Sequencing these regions indicated that the same genes were present in all three species, but the gene density was about one per 45 kb in maize and about one per 10 kb in sorghum and rice (Chen et al. 1998). A third gene encoding a putative transcription factor was located between these two loci, but no other sequences in the region were conserved except the genes. Comparative analysis of the orthologous *adh1* regions of sorghum and maize revealed the presence of nine known or candidate genes, including *adhl,* in a 225-kbp maize sequence, whereas the homolog of the same nine genes was identified in colinear order along with five additional genes in a 78-kbp space in sorghum (Tikhonov et al. 1999).

Significantly, it was discovered that only the genes cross-hybridized between these two colinear segments of the sorghum and maize genomes. Intergenic regions are likely to have accumulated species-specific sequences, which prohibit prediction of physical distances between homologous genes in related species. This made the genomic cross-referencing technique (i.e., cross-hybridization between homologous segments) (Avramova et al. 1996) a better method for gene identification than either transcript identification (Avramova et al. 1995) or enrichment for single-copy DNA (San Miguel et al. 1996). The combined *Al-Sh2* and *adh1* regions show that grasses often exhibit extensive colinearity and similar gene content at the 50- to 300-kbp level. Therefore, map-based cloning, genomic sequencing, and gene identification using the smaller rice and sorghum genomes will usually be simpler in these species than in maize, barley, or wheat. Thus, a successful and efficient way to find genes in a large region of a complex genome is to use a homologous colinear clone from another species.

To gain insight into the relationship between spatial organization of the genome and genome function, Avramova et al. (1998) identified the locations of the matrix attachment regions (MARs) in the colinear *sh2/a1* homologous chromosome segments of rice and sorghum (30 and 50 kbp, respectively), which could serve as anchors for individual structural units or loops. All identified genes were placed in individual loops of comparable size for homologous genes. Hence, gene composition, gene orientation, gene order, and the placement of genes into structural units have been conserved evolutionarily in this region. Their analysis demonstrated that the occurrence of various "MAR motifs" is not indicative of MAR location. However, most of the MARs discovered in the two genomic regions were found to colocalize with miniature inverted repeat transposable elements (MITEs), suggesting that MITEs preferentially insert near MARs and/or that they can serve as MARs.

The nature, timing, and lineages of most of the genic rearrangements that have differentiated the chromosome segment that is orthologous to the maize *adh1* region of sorghum, rice, and *adh1* homologous region of maize, a remnant of the tetraploid history of the *Zea* lineage over the last 60 million years, was described by Ilic et al. (2003). The rice genome has been the most stable, sharing 11 orthologous genes with sorghum and exhibiting only one tandem duplication of a gene in this region. The lineage that gave rise to sorghum and maize acquired a two-gene insertion (containing the *adh* locus), whereas sorghum received two additional gene insertions after its divergence from a common ancestor with maize. The two homoeologous regions of maize have been particularly unstable, with complete or partial deletion of three genes from one segment and four genes from the other segment. As a result, the region now contains only one duplicated locus compared with the eight original loci that were present in each diploid progenitor. Deletion of these maize genes did not remove both copies of any locus. This study suggests that grass genomes are generally unstable in local genome

organization and gene content but that some lineages are much more unstable than others.

Maize, probably because of its polyploidy origin, has exhibited extensive gene loss so that it is now approaching a diploid state. *Al* toxicity is a major constraint to crop production on acidic soils. To assess the possible ancestral relationship between *Al* tolerance genes in the grasses, Magalhaes et al. (2004) conducted a molecular genetic analysis of *Al* tolerance in sorghum and integrated their findings with those from previous studies performed in crop species belonging to different grass tribes. A single locus, AltSB, was found to control *Al* tolerance in two highly *Al*-tolerant sorghum cultivars. Significant macrosynteny between sorghum and the Triticeae was observed for molecular markers closely linked to putatively orthologous *Al* tolerance loci present in the group 4 chromosomes of wheat, barley, and rye. However, AltSB was not located within the homoeologous region of sorghum but rather mapped near the end of sorghum chromosome 3. Thus, AltSB not only is the first major *Al* tolerance gene mapped in a grass species that does not belong to the Triticeae, but it also appears to be different from the major *Al* tolerance locus in the Triticeae. Intertribe map comparisons suggest that a major *Al* tolerance QTL on rice chromosome 1 is likely to be orthologous to AltSB, whereas a rice QTL on chromosome 3 is likely to correspond to the Triticeae group 4 *Al* tolerance loci. Therefore, this study demonstrates a clear evolutionary link between genes and QTLs encoding the same trait in distantly related species within a single plant family.

To provide a phylogenetic context to two maize genes *r*1 and *b*1, which have been a rich source for studying transposition, Swigonova et al. (2004) sequenced orthologous regions from maize and sorghum (>600 kb) surrounding these genes and compared them with the rice genome. This comparison showed that the homoeologous regions underwent complete or partial gene deletions, selective retention of orthologous genes, and migration of nonorthologous genes.

Rp1 is a complex resistance (R) locus in maize conferring race-specific resistance to a fungal pathogen, common leaf rust (*Puccinia sorghii*). A 268-kb chromosomal segment containing sorghum (*S. bicolor*) genes that are orthologous to the maize (*Zea mays*) *Rp1* disease resistance (R) gene complex was sequenced (Ramakrishna et al. 2002a) to determine structural variation for an R gene cluster that has diverged at least since the ancestral divergence of maize

and sorghum. A region of approx. 27 kb in sorghum was found to contain five *Rp1* homologs, but most have structures indicating that they are not functional. In contrast, maize inbred B73 has 15 *Rp1* homologs in two nearby clusters of 250 and 300 kb. As at maize *Rp1*, the cluster of R gene homologous in sorghum is interrupted by the presence of several genes that appear to have no resistance role, but these genes were different from those found within the maize *Rp1* complex.

Conservation of gene order between sorghum and rice is well documented, which helped to enhance our understanding of cereal genome structure and evolution (Moore et al. 1995; Shimano et al. 1995; Paterson et al. 1995a). Multani et al. (1998) demonstrated that in sorghum and rice, the homologs of a pair of unlinked duplicate genes *Hml* and *Hm2* conferring resistance to *C. carbonum race* 1 in maize map to two chromosomal regions that are syntenic with the regions in maize harboring these loci, indicating that they are related to maize genes by vertical descent. These results suggest that the Hm-encoded resistance is of ancient origin and probably is conserved in all grasses. A direct comparison of the genetic linkage maps of sorghum and rice was done by Ventelon et al. (2001). It was based on the mapping of a common set of 123 RFLP probes scattered on the genomes of both species. For each species a composite map was established by merging two individual maps comprising many common loci. This enabled them to confirm the global correspondence scheme that had previously been established between the chromosomes of sorghum and rice. Morishige et al. (2002) have developed a "gene-island" sequencing strategy that expedites the targeted acquisition of orthologous gene sequences from related species for comparative genome analysis. A 152-kb bacterial artificial chromosome (BAC) clone from sorghum (*S. bicolor*) encoding phytochrome A (*PHYA*) was fully sequenced, revealing 16 open reading frames with a gene density similar to many regions of the rice (*Oryza sativa*) genome. The sequences of genes in the orthologous region of the maize (*Zea mays*) and rice genomes were obtained using the gene-island sequencing method. BAC clones containing the orthologous maize and rice *PHYA* genes were identified, sheared, subcloned, and probed with the sorghum *PHYA*-containing BAC DNA. Comparative mapping of rhizomatousness between rice and *Sorghum propinquum*, a wild relative of cultivated *Sorghum*, indicated that each gene closely corresponds to two major quantitative trait loci (QTL) (Hu et al. 2003). Correspondence of these genes in rice

and sorghum, which diverged from a common ancestor ca. 50 million years ago, suggests that the two genes may be key regulators of rhizome development in many poaceae.

Sequence-based alignment of sorghum and rice chromosomes was attempted by Klein et al. (2003) for refining the sorghum genetic/physical map based on the rice genome sequence. A framework of 135 BAC contigs spanning ca. 33 Mbp was anchored to sorghum chromosome 3. A limited number of sequences was collected from 118 of the BACs and subjected to BLASTX analysis to identify putative genes and BLASTN analysis to identify sequence matches to the rice genome. Extensive conservation of gene content and order between sorghum chromosome 3 and the homologous rice chromosome 1 was observed (Fig. 4). One large-scale rearrangement was detected involving the inversion of an approx. 59-cM block of the short arm of sorghum chromosome 3. Several small-scale changes in gene colinearity were detected, indicating that single genes and/or small clusters of genes have moved since the divergence of sorghum and rice. Additionally, the alignment of the sorghum physical map to the rice genome sequence allowed sequence-assisted assembly of an approx. 1.6-Mbp sorghum BAC contig.

Using bacterial artificial chromosome sequence analysis Ramakrishna et al. (2002b) have studied four orthologous regions in barley, rice, sorghum, and wheat and observed general microcolineariry to shared genes in this region. However, three genic rearrangements were observed. First, the rice region contains a cluster of 48 predicted small nucleolar RNA genes, but the comparable region from sorghum contains no homologous loci. Second, gene 2 was inverted in the barley lineage by an apparent unequal recombination after the ancestors of barley and wheat diverged 11 to 15 million years ago (mya). Third, gene 4 underwent direct tandem duplication in a common ancestor of barley and wheat 11 to 29 mya.

A duplication or diploidization event that predates divergence of taxa from a common ancestor may account for some incongruence in "comparative maps". Specifically, if gene loss were still continuing at an appreciable rate after taxon divergence occurred, then differential gene loss in independent lineages would cause incongruities in their comparative maps. To test this possibility, Paterson et al. (2004) examined a sorghum–rice comparative map developed by BLASTing sequences from 2509 genetically mapped sorghum loci (Bowers et al. 2003) against the genome

assembly. The positions of 1626 corresponding loci could be plotted based on the rice physical location and sorghum genetic location. This revealed much colinearity, with eight sorghum linkage groups (A, D, E, F, G, H, I, and J) corresponding to single rice chromosomes (1, 4, 12, 2, 5, 11, 6, and 8) and two sorghum linkage groups (B and C) differing from rice by translocations between chromosomes 7/9 and 3/10, respectively.

Sorghum and Sugarcane

The first comparison between the sorghum and sugarcane genomes was mostly indirect, in which maize was used as an intermediate, but it hinted at a large degree of synteny between the genomes of two species (D'Hont et al. 1994; Grivet et al. 1994; McIntyre et al. 2004). Grivet et al. (1994) determined the syntenic genomic regions in maize, sorghum, and sugarcane according to the existing bridge loci. The distribution of these synteny clusters closely matched the duplication pattern in maize. There appear to be common chromosome rearrangements between maize and sugarcane and between maize and sorghum. In this respect, sugarcane and sorghum appear to be more closely related than either is with maize. Distances between genes were similar in maize and sorghum, whereas sugarcane tended to display less recombination.

Existence of large colinear regions among the three species (sugarcane, maize, and sorghum) was also revealed in a study involving comparative genetic mapping between duplicated segments on maize chromosomes 3 and 8 and homologous regions in sorghum and sugarcane (Dufour et al. 1996). Their results emphasize that those duplications will considerably complicate precise comparative mapping at the whole genome scale between maize and other Poaceae. A more elaborate analysis by Dufour et al. (1997) revealed a straight synteny between two pairs of sorghum and sugarcane linkage groups and a large array of colinear probes with sugarcane along the other sorghum linkage groups (Fig. 5). Similarly, colocation of RFLP markers associated with stalk number and suckering in sugarcane with QTLs associated with tillering and rhizomatousness in sorghum was reported by Jordan et al. (2004). Guimaraes et al. (1997) also observed striking colinearity between *Sorghum* and *Saccharum* genomes.

Alignment of complex polyploid genomes of three *Saccharum* species with the compact diploid genome of sorghum ($2n = 2x = 20$) was also reported by

Fig. 4. Sequence-based alignment of sorghum chromosome 3 and rice chromosome 1 (Reprinted, with permission of Blackwell Publishing, from Klein et al. 2003)
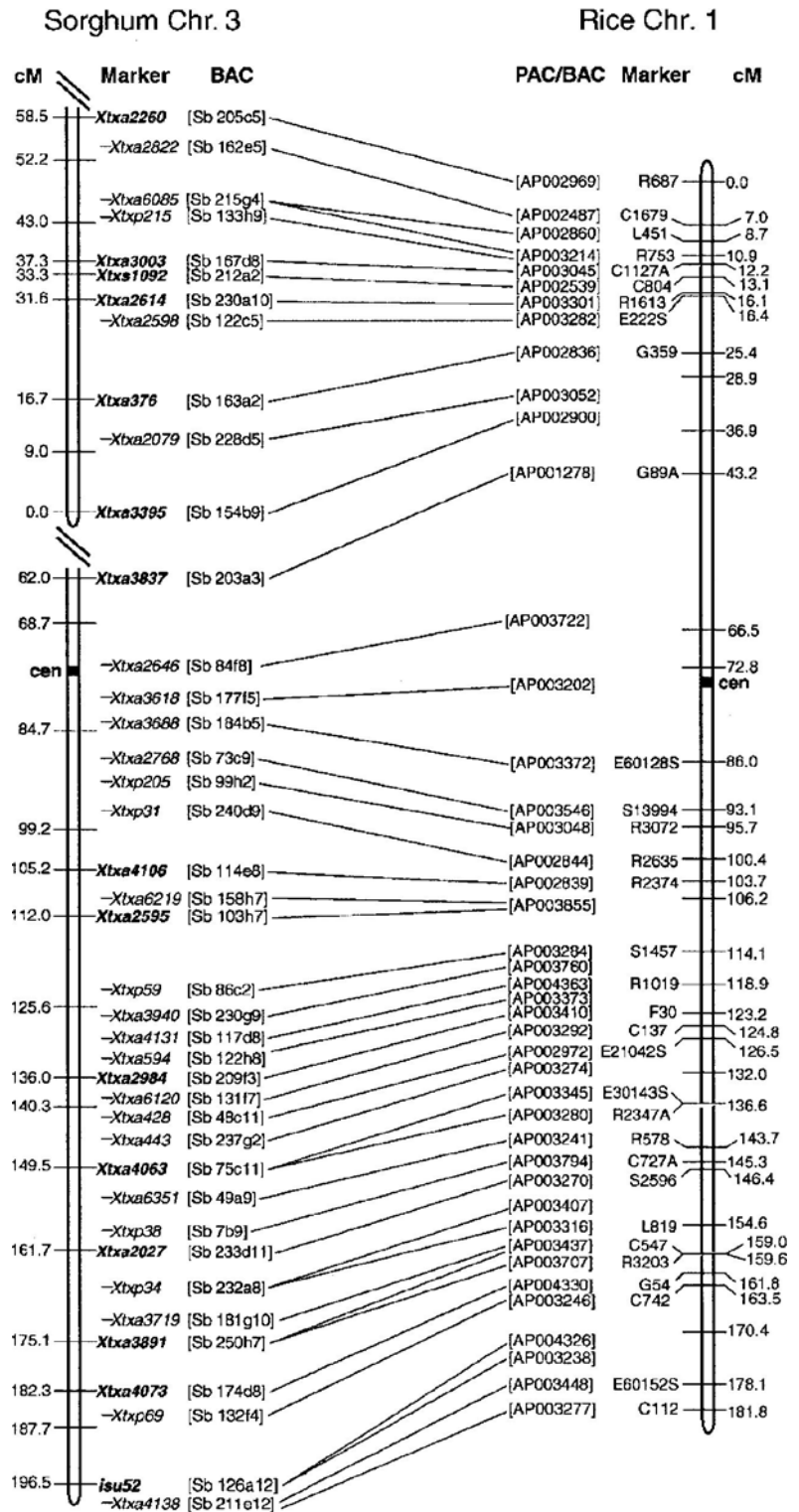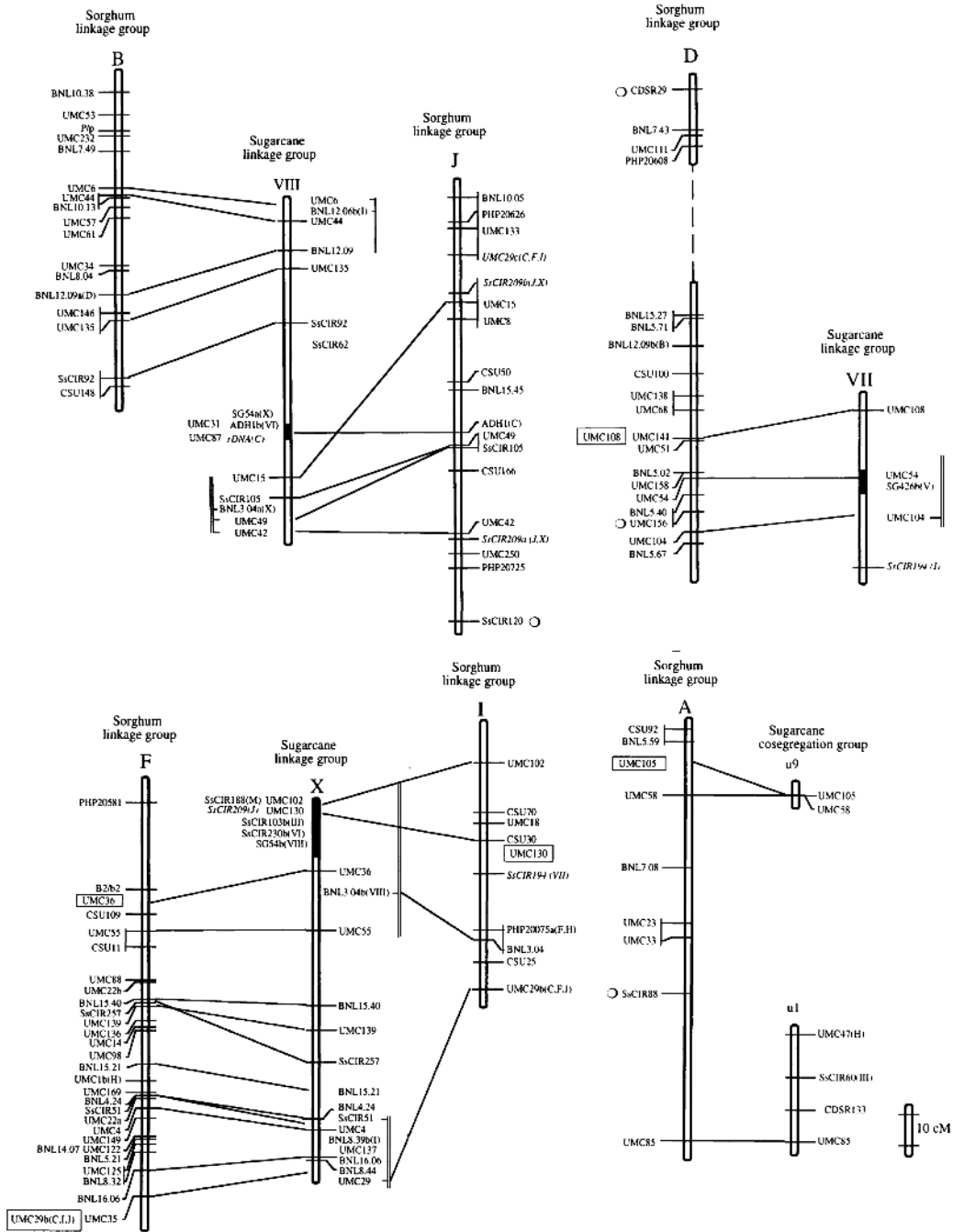
Fig. 5. Comparative mapping between sorghum and sugarcane (Reprinted, with permission of Springer, from Dufour et al. 1997)
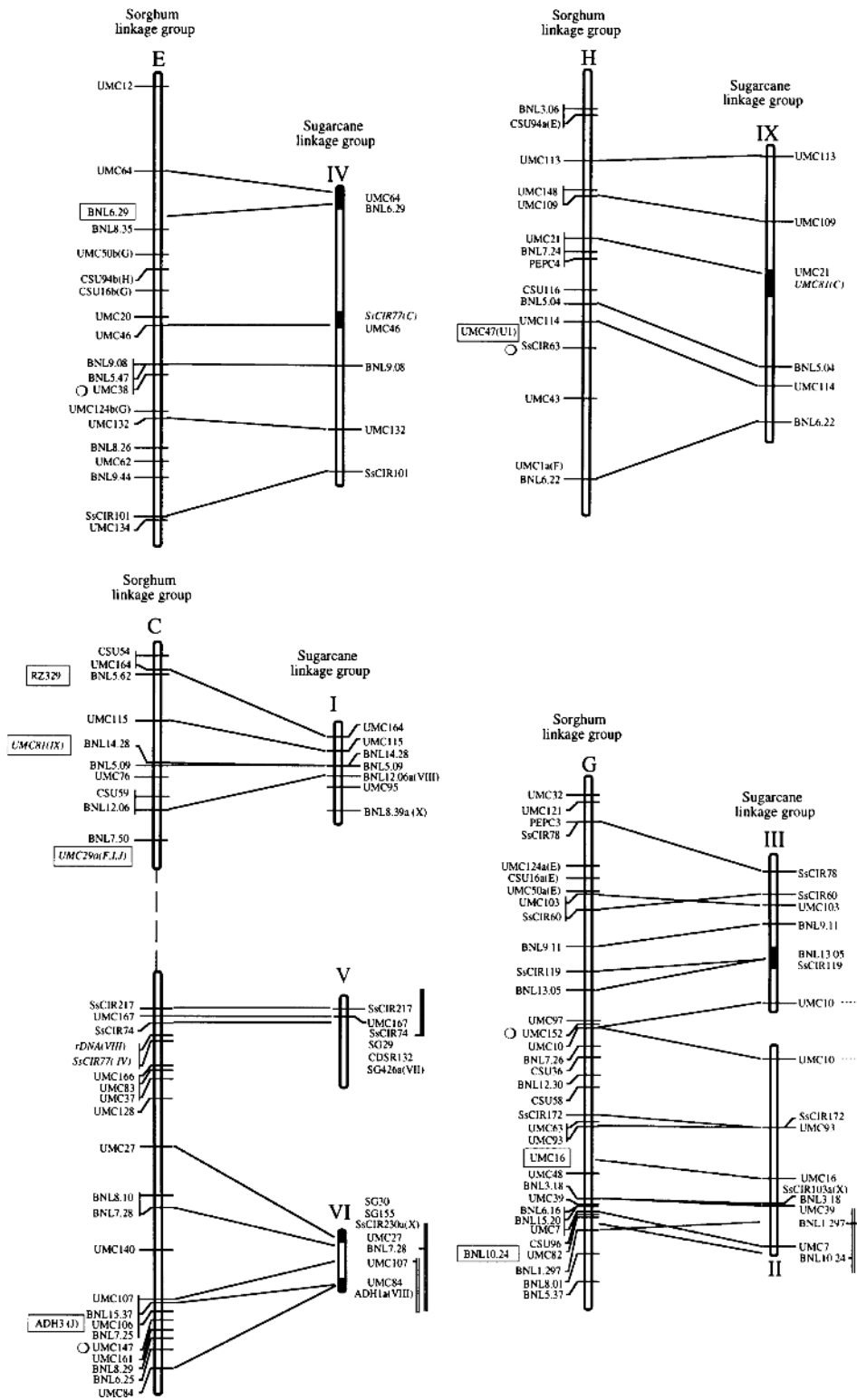
Fig. 5. (continued)

Table 7. Major genes tagged by molecular markers in Sorghum

Trait	Closely linked markers	Reference
Heat smut resistance	RFLP (TXS 560)	Oh et al. (1994)
Shattering	RFLP (PSB 766 and PSB 195)	Paterson et al. (1995b)
Organophosphate insecticide reaction	RFLP (TXS 713)	Toure et al. (1997)
Awn	RFLP (SSCIR 203)	Tao et al. (1998a)
Mesocarp thickness	RFLP (TXS 636)	Tao et al. (1998a)
Juicy midrib	RFLP (CSU6 and UMC34)	Xu et al. (2000)
Red coleoptile	RFLP (UMC 44)	Xu et al. (2000)
Red pericarp	RFLP (TXS 584)	Xu et al. (2000)
Leaf blight resistance	RAPD (OPD12)	Boora et al. (1999)
Male sterility	AFLP	Wen et al. (2002)
Pollen fertility	AFLP; SSR	Klein et al. (2001)
Yield	RFLP	Jordan et al. (2003)
Downy mildew resistance	RFLP	Gowda et al. (1995)
Acremonium wilt, downy mildew, and smut resistance	RFLP, RAPD	Oh et al. (1996)

Ming et al. (1998). Genetic maps of the six *Saccharum* genotypes, constituting up to 72 linkage groups, were assembled into homologous groups based on parallel arrangements of duplicated loci. About 84% of the loci mapped by 242 common probes were homologous between *Saccharum* and sorghum. One interchromosomal and two intrachromosomal rearrangements differentiated *S. officinarum* and *S. spontaneum* from sorghum, but 11 additional cases of chromosome structural polymorphism were found within *Saccharum*. Cross utilization of microsatellites or single sequence repeats developed from sugarcane ESTs between sugarcane and sorghum revealed lower level of polymorphism in sugarcane and a significantly higher level of polymorphism in a related genus *Sorghum sp.* (Cordeiro et al. 2001).

McIntyre et al. (2004) mapped a sugarcane cDNA clone with homoeology to the maize *Rp1–D* rust resistance gene in sorghum. The cDNA probe hybridized to multiple loci, including one on sorghum linkage group E in a region where a major rust resistance QTL had been previously mapped. Partial sorghum *Rp1–D* homologs were isolated from genomic DNA of rust resistance and susceptible progeny selected from a sorghum mapping population. Sequencing of the *Rp1–D* homologs revealed five discrete sequence classes: three from resistant progeny and two from susceptible progeny. Cluster analysis of these sorghum sequences and available sugarcane, maize, and sorghum *Rp1–D* homolog sequences showed that the maize *Rp1–D* sequence and the partial sugar-

cane *Rp1–D* homolog were clustered with one of the sorghum resistant progeny sequence classes.

Sorghum and Foxtail Millet
Comparative mapping revealed a very close relationship between foxtail millet (Setaria italica) with haploid chromosome $n = 9$ and sorghum with $n = 10$ (Devos and Gale 1997). The difference in chromosome number is accounted for by the synteny of foxtail millet chromosome III with sorghum chromosomes E and I (Devos et al. 1998; Wang et al. 1998). Elsewhere, only one inversion was detected in sorghum chromosome D and one translocation involving foxtail millet chromosomes III and VII, which differentiate the two species.

7.3
Gene Mapping

Determination of the relative positions of genes on a DNA molecule (chromosome or plasmid) and of the distance, in linkage units or physical units, between them is critical for marker-assisted selection, gene cloning, and elucidating the functions of these genes, thereby contributing to accelerated crop improvement. Sorghum is an important target of plant genomics because of its unusual tolerance to adverse environments, a small genome (750 Mbp) relative to most other grasses, a diverse germplasm, and util-

ity for comparative genomics with rice, maize, and other grasses. Efforts are under way for discovery and mapping of genes in sorghum (Table 7). Boora et al. (1999) analyzed the genetic basis for resistance to leaf blight, which revealed resistance was transmitted as a dominant single-gene trait. By combining the random amplified polymorphic DNA (RAPD) technique with bulked-segregant analysis, it was possible to identify PCR amplification products that segregated with disease response. Primer OPD12 amplified a 323-bp band (D12R) that segregated with resistance.

Molecular mapping of a gene for pollen fertility in *Al* (milo) type cytoplasm of sorghum using AFLP and SSR marker analysis was reported by Klein et al. (2001) that will facilitate the selection of pollen fertility restoration in sorghum inbred-line development and provide the foundation for map-based gene isolation. Fifteen AFLP markers were linked to fertility restoration from the initial screening with 49 unique AFLP primer combinations (+3/+3 selective basis). As many of these AFLP markers had been previously mapped to a high-density genetic map of sorghum, the target gene (*rf1*) could be mapped to linkage group H. Confirmation of the map location of *rf1* was obtained by demonstrating that additional linkage group-H markers (SSR, STS, AFLP) were linked to fertility restoration. The closest marker, AFLP *Xtxa2582,* mapped within 2.4 cM of the target loci, while two SSRs, *Xtxp and Xtxp250,* flanked the *rf1* locus at 12 cM and 10.8 cM, respectively. Wen et al. (2002) also reported three RFLP markers suitable for mapping rf4 linked to restoration of male fertility in the sorghum IS 1112 (A3) male sterile cytoplasm.

7.4
Detection of Quantitative Trait Loci (QTL)

Quantitative phenotypes have been a major area of genetic study for over a century because they are a common feature of natural variation in populations of all eukaryotes. They include commercially important traits in crop plants and domestic animals as well as in vital traits in humans from hypertension to intelligence (Kearsey and Farquhar 1998). The first attempt to study individual determinants of quantitatively inherited characters in plants date back to Sax (1923). The studies on quantitative variation suffered from a lack of precision in the absence of complete ge-

netic maps (Thoday 1961). This limitation was overcome with the advent of DNA markers detected as restriction fragment length polymorphism (Paterson et al. 1988). The advent of RFLPs and subsequent PCR-based markers has revolutionized the field of genetic mapping and gene identification in both animals and plants. The basis of all QTL detection is the identification of association between genetically determined phenotypes and specific genetic markers. In sorghum several QTLs have been associated with plant height (Lin et al. 1995) and pre- and postflowering drought tolerance (Tuinstra et al. 1996, 1997). Later Tao et al. (1998b) mapped four regions, each in a separate linkage group, associated with rust resistance (Table 8).

Subudhi et al. (2000) determined the consistency of quantitative trait loci (QTLs) controlling stay-green in sorghum, which is characterized by the plant's ability to tolerate postflowering drought stress by reevaluating the recombinant inbred line (RIL) mapping population from the cross B35 × Tx7000 in two locations over 2 years and compared it with earlier reports. Analysis using the combined stay-green-rating means of seven environments and the expanded molecular map reconfirmed all four stay-green QTLs *(Stgl, Stg2, Stg3,* and *Stg4)* that had been identified earlier by Xu et al. (2000). Similarly, comparison of the stay-green QTL locations with earlier reported results indicated that all four stay-green QTLs showed consistency across different genetic backgrounds. Sanchez et al. (2002) also identified four genomic regions associated with the stay-green trait using an RIL population developed from B35 × Tx7000, whereas Kebede et al. (2001) reported nine QTLs located over seven linkage groups for stay-green using the method of composite interval mapping. In addition, three and four major QTLs responsible for lodging tolerance and preflowering drought tolerance, respectively, were detected. Haussmann et al. (2002b) reported five to eight QTLs for the stay-green trait in two recombinant inbred populations (IS 9830 × E 36-1 and N 13 × E 36-1), and three QTLs present on linkage groups A, E, and G were common to both crosses.

Preharvest sprouting (PHS), one of the important agronomic problems in the production of sorghum [*Sorghum bicolor* (L.) Moench] in humid climates, was studied by Lijavetzky et al. (2000). A molecular linkage map was developed using 112 molecular markers in an F_2 mapping population derived from a cross between IS 9530 (high resistance to PHS) and Redland B2 (susceptible to PHS). Two years' phenotypic data were obtained. By means of interval mapping analy-

Table 8. List of QTLs identified in sorghum

S. no.	Trait	Population	Marker type	No. of QTLs	Reference
1	Stay green	RILs (B35 × TX7000)	RFLP, SSR, RAPD	4	Subudhi et al. 2000
2	Plant height	*S. bicolor* × *S. propinquum*	RFLP	6	Lin et al. 1995
3	Flowering			3	
4	Pre-harvest sprouting	F2 (IS9530x Redland B2)	RFLP	2	Lijavetzky et al. 2000
5	Tiller number	BC1 and F2 (BTx623 × S. propinquum)	RFLP	4	Paterson et al. 1995a
6	Rhizomatousness			3	Paterson et al. 1995a
7	Ratooning ability			6	
8	Stay green	RILs (SC56 × TX7000)	RFLP	9	Kebede at al. 2001
9	Lodging tolerance			3	
10	Pre-flowering drought tolerance			4	
11	Flowering time	RILs (IS2807 × TS 7680)	RFLP	1	Chantereau et al. 2001
12	Photoperiod sensitivity			2	
13	Height of main culm	RILs (BTX623 × IS3620C)	RFLP & SSR	3	Hart et al. 2001
14	Tallest basal tiller height			2	
15	Number of basal tillers			2	
16	Panicle length			3	
17	Panicle width			7	
18	Leaf angle			3	
19	Maturity			2	
20	Awn length			1	
21	Greenbug resistance and tolerance	RILs (GBIK × Redlan)	SSR and RAPDs	9	Agrama et al. 2002
22	Staygreen	RILs (IS9830 × e36-1 and N13 × H36-1)	AFLP, RFLP, SSR, RAPD	5–8	Haussmann et al. 2002b
23	Staygreen	RILs (B35 × TX70000)	–	4	Sanchez et al. 2002
24	Midge resistance (Antixamosis)	RILs (ICSV 745 × 90562)	RFLP SSR	2	Tao et al. 2003
25	*Striga hermonthica*	RIPs (IS9830 × E36-1 and N13 × E36-1)	RFLP AFLP SSRs	11 (RIP1) 9 (RIP2)	Haussmann et al 2004
26	Grain mold	RTx430x Sureno	–	5	Rooney and Klein 2000
27	Rust Resistance	QL 39 × QL 41	RFLP	4	Tao et al. 1998b

sis, two significant QTLs were detected in two different linkage groups with LOD scores of 8.77 and 4.39. Each of these two QTLs individually explained ca. 53% of the phenotypic variance, but together, in a two-QTL model, they explained 83% of the phenotypic variance with a LOD score of 12.37.

The plant *vp1* gene, which encodes a transcription factor originally identified in maize, participates in the control of the transition from embryogenesis to seed germination. Different lines of evidence suggest that *vp1* participates in preharvest sprouting resistance in cereals. Carrari et al. (2003) studied the con-nection between *vp1* and formerly documented QTLs (Lijavetzky et al. 2000) for PHS in sorghum. Linkage analysis revealed that the sorghum *vp1* (*sbvp1*) locus is linked to markers on chromosomes 3 and 8 in maize, and this gene is not correlated with PHS.

Chantereau et al. (2001) investigated the genetic control of flowering time in sorghum using a recombinant inbred line population derived from a cross between IS 2807, a slightly photoperiod-sensitive tropical caudatum landrace, and TS 7680, a highly photoperiod-sensitive tropical guinea landrace. Emphasis was placed on identifying the most relevant

traits to account for basic vegetative phase (BVP) and photoperiod sensitivity *sensus stricto*. One QTL was detected on linkage group (LG) F for the traits related to BVP. Two QTLs were detected on LGs C and H for the traits related to the photoperiod sensitivity *sensus stricto*. For nine morphological traits, including the presence vs. the absence and the height of basal tillers, number of tillers, plant height, and time of anthesis, Hart et al. (2001) mapped a minimum of 27 unique QTLs.

For resistance and tolerance to green bug (*Schizaphids grami-num* Rondani) biotypes I and K, Agrama et al. (2002) mapped 113 markers (38 SSRs and 75 RAPDs) in 12 linkage groups covering 1,530 cM. In general, nine QTLs were detected affecting both resistance and tolerance to green bug (GB) biotypes I and K. The phenotypic variance explained by each QTL ranged from 5.6 to 38.4%. For green bug biotypes C, E, I, and K, Katsar et al. (2002) also reported at least nine loci, dispersed on eight linkage groups. Tao et al. (2003) identified two and one quantitative trait loci associated with two of the mechanisms of midge resistance, antixenosis, and antibiosis, respectively, in an RI population from the cross of sorghum lines ICSV745 × 90562. Haussmann et al. (2004) detected 11 and nine QTLs in two recombinant inbred populations IS9830 × E 36-1 and N13 × E36-1, respectively, for resistance to *Striga hermonthica*

Comparative Mapping of QTLs

Conversion of gene order along the chromosomes is well known to transgress species boundaries, but the extent of correspondence in the QTLs that account for variation in complex phenotypes has been a point of conjuncture. Paterson et al. (1995b) hypothesized that if QTLs in separate taxa mapped to corresponding locations more often than would be expected by chance, such a finding would strongly suggest that corresponding genes were involved in the evolution of the relevant phenotypes. They tested the hypothesis by assessing correspondence between QTLs that affect seed mass, temperate (day-neutral) flowering, and disarticulation of the mature inflorescence (shattering) in crosses between divergent sorghum, O*ryza* and Z*ea* taxa. Three QTLs that affect seed mass (size) correspond closely in sorghum, rice, and maize, and at least five additional QTLs correspond between two of these genera. Among seven QTLs that account for 52% of phenotypic variance explained (PVE) in sorghum

seed mass, five (on linkage groups A, C, E, F, and I) correspond to five of the eight QTLs that account for 78% of PVE in rice. Four of the sorghum QTLs (on linkage groups A, B, C, and F) correspond to four of the eight QTLs that account for 69% of PVE in maize. Five maize QTLs correspond to rice QTLs. Only four QTLs (two on maize chromosome 2, one on rice chromosome 5, and one on sorghum LG J) showed no correspondence. The probability that seed mass QTLs in sorghum, rice, and maize would correspond so frequently by chance is conservatively estimated as 0.1 to 0.8%. QTLs that affect seed dispersal show similar correspondence across taxa. Shattering mapped to a single locus (ca. 100% PVE) in sorghum, three loci (24% PVE) in rice, and ten loci (60% PVE) in maize. The discrete sorghum locus corresponds to rice QTLs on chromosome 9 and to maize QTLs on duplicated regions of chromosomes 1 and 5. Rice QTLs on chromosomes 2 and 3 correspond to maize QTLs on chromosome 4 and 1. Six additional QTLs influence shattering in maize but not in rice or sorghum.

The ability of many cultivated cereals to flower in the long days of summer temperatures may be largely the result of mutations at a single ancestral locus. Sorghum LG D QTL (probably *Ma1*) explains about 86% of PVE in flowering time and accounts for the dichotomy of F_2 phenotypes in our day-neutral (*S. bicolor*) × short-day (*S. propinquum)* cross. It also accounts for short-day flowering in each of the five races of *S. bicolor* (Lin et al. 1995). Short-day flowering of sugarcane is closely associated with the DNA probe PSB188 (Paterson et al. 1995b), which lies near *Ma1*. The corresponding region of maize chromosome 10 accounts for up to 26% of PVE in the flowering of a temperate/tropical cross (Koester et al. 1993). The corresponding region in wheat and barley, the short arm of the group 2 homologs, all harbor photoperiodic flowering mutants (Laurie et al. 1994). In rice, the orthologous (directly descended from a common ancestral locus) region on chromosome 4 harbors no known flowering mutants; however, short-day flowering mutations *Se1* and *Se3* both map to a region of chromosome 6 (Mackill et al. 1993; Causse et al. 1994), that is, are orthologous to sorghum LG I and paralogous (derived by duplication and subsequent divergence from a common ancestral locus) to the sorghum LG D region of *Ma1*. The *Sel/Se3* region of rice corresponds to a region of maize chromosome 9 that harbors QTLs that affect flowering in at least four populations (Lin et al. 1995). This model implies an-

cient duplication of regions of maize chromosomes 9 and 10 and regions of rice chromosomes 4 and 6 equivocally supported by the correspondence of *Pi2* and *Pi5t* genes that influence rice blast reaction (Causse et al. 1994). These day-length-insensitive flowering mutations are not in any of at least three genes for phytochrome, a key regulator of photomorphogenesis (Paterson et al. 1995b).

Comparative mapping has provided the basis for parallel investigations of other genetic factors. The first report of detection of orthologous QTLs with the greatest effects on seed weight in mungbean and cowpea was provided by Fatokun et al. (1992). In a similar manner, comparative mapping in maize and sorghum has revealed four putatively orthologous regions for plant height (Pereira and Lee 1995; Lee 1996) and other possible instances of orthologous QTL included regions for maturity and tillering. The putative orthologous regions for plant height are on linkage group A and the long arm of chromosome 1, D and chromosome 5, E and the long arm of chromosome 6, H and chromosome 9 of the sorghum linkage map and maize chromosome, respectively. The regions of the maize plant height QTL also contain genetic loci defined by mutants with qualitative effects on stature, such as *br1* and *an1* on chromosome 1, *na1* and *td1* on chromosome 5, *py1* on chromosome 6, and *d3* on chromosome 9. The effects of some of these maize mutants strongly resemble those of the sorghum plant height QTL and *dw* loci. At least three of the maize loci, *an1*, *br1*, and *d3*, have been tagged with transposons or cloned by various laboratories. These sequences could be used to isolate the related gene from sorghum and further assess the degree and nature of conservation between these two genomes. In sorghum, each region has a major effect on that trait and on a unique suite of other traits (e.g., tillering, panicle dimensions, leaf length, and width), much like some of the *dw* loci in sorghum. Interestingly, plant height mutants at maize genetic loci in related regions have pleiotropic effects on some of the same combinations of traits as the sorghum QTL and the candidate *dw* loci. Possible duplication of QTLs that affect the height of sorghum and maize has also been reported (Lin et al. 1995).

Evidence for several other orthologous regions has also been provided through comparative QTL analysis (Lee 1996). For example, a region of linkage group A (*isu033* to *isul23)* was strongly associated with tillering and production of lateral branches. This region of the sorghum genome is most closely related to the long arm of chromosome 1 of maize. This region

of the maize genome is the site of a genetic locus, *tb1*. The mutant phenotype at that locus is characterized by the production of many tillers and lateral branches in a manner strongly resembling the tillering QTL in sorghum. Other possible instances of orthologous QTL included regions for maturity. These observations suggest that the conservation of the maize and sorghum genomes encompass sequence homology, colinearity, and function despite their divergence millions of years ago and subsequent evolution in different hemispheres with contrasting ecogeographical conditions. Thus, comparative QTL mapping provides a means to unify, and thereby simplify, molecular analysis of complex phenotypes.

7.5
Marker-Assisted Breeding

7.5.1
Marker Conversions

Molecular markers help unravel patterns of diversity in crops and their wild relatives. DNA markers are used to evaluate the genetic variation in gene banks as well as to identify phylogenetic and molecular structure of crops and their associated wild species. Molecular-assisted genetic analysis provides a means to locate and select genes controlling important agronomic, pest-resistance, stress-tolerance, and food quality traits.

For leaf blight resistance, Boora et al. (1999) developed RAPD primer OPD12, and a 332-bp PCR band has been converted into SCAR, which resulted in the amplification of a single major band of the predicted size from all the resistant F_2 progeny and the resistant parent SC326-6, but not from BT × 623 or 24 of 29 susceptible F_2 progeny. The SCAR primers also amplified a single band with DNA from TS3620C, the female parent in a cross with BT × 623 that has been used to produce a recombinant inbred population for RFLP mapping. An equivalent band was amplified from all 137 recombinant inbred progeny, indicating that organelle DNA is the amplification target in this cross.

The gene *rf4* restores fertility in IS1112 (A3) male sterile cytoplasm, for which three AFLP markers were identified and subsequently converted to STS/CAPS markers, two of which are codominant (Wen et al. 2002). Markers LW8 and LW9 were used to screen sorghum BAC libraries to identify the genomic region

encoding *rf4*. A contig of BAC clones flanking the LW9 marker represents seed clones on linkage group E, from which fine mapping of the *rf4* locus and chromosome mapping can be initiated.

7.5.2
Marker-Assisted Selection

Conventional plant breeding is primarily based on phenotypic selection of superior individuals among segregating progenies resulting from hybridization. Although significant strides have been made in crop improvement through phenotypic selections for agronomically important traits, considerable difficulties are often encountered during this process primarily due to genotype-environment interactions. Molecular-marker-assisted selection (MAS) involves selection of plants carrying genomic regions that are involved in the expression of traits of interest through molecular markers. With the development and availability of an array of molecular markers and dense molecular genetic maps in crop plants, MAS has become possible for traits governed by both major genes and by quantitative trait loci (QTLs).

Grain mold caused by *Curvularia lunata* (Wakker) Boedijn is a serious disease on sorghum especially when grain development coincides with wet and warm weather conditions. Rooney and Klein (2000) identified five QTLs on linkage groups D, E, F, G, and I using a mapping population consisting of 125 F_5 RILs from a cross between RT × 430 × Sureno. Five populations were developed using Sureno as grain mold resistant parent. From each cross, F_2 progeny were selected based on maturity and short plant height. A total of 1,000 $F_{2:3}$ lines were evaluated for agronomic desirability and grain mold resistance. From this evaluation, a total of 100 $F_{3:4}$ lines were selected and advanced. In the F_4 generation, an array of molecular markers linked to the sorghum grain mold QTL was screened. To test the effectiveness of MAS, lines from each population were classified for QTL marker alleles at each of the five loci. This comparison indicated that only one of the five QTLs enhanced selection for grain mold resistance. The presence of the Sureno allele in LG-F enhanced mold resistance. MAS was clearly effective in the population derived from crosses with RT × 430 since these QTLs were developed in this population (Rooney and Klein 2000).

Drought is another major limiting factor in sorghum productivity. Moisture stress during both pre- and postflowering stages reduces sorghum yield drastically. Therefore, improvement in both pre- and postflowering drought tolerance is necessary to improve and stabilize productivity of sorghum in stress environments. Subudhi et al. (2000) have identified QTLs for stay-green, postflowering drought tolerance trait using three random inbred lines (RILs). Near-isogenic lines (NILs) for stay-green QTLs have been developed using MAS to dissect the QTL regions and to determine the effect of QTLs in stress environments.

Jordan et al. (2003) investigated the value of molecular-marker-based distance information to identify high-yielding grain sorghum hybrids in Australia. Data from 48 trials were used to produce hybrid performance estimates for four traits (yield, height, maturity, and stay-green) for 162 hybrid combinations derived from 70 inbred parent lines. Each line was screened with 113 mapped RFLP markers. The researchers utilized the concept of using diversity on linkage groups to predict hybrid performance. Using data from just two linkage groups, 38% of the variation in hybrid performance for grain yield could be explained. A model combining phenotypic trait data and parental diversity on particular linkage groups explained 71% of the variation in grain yield and has potential for use in the selection of heterotic hybrids.

7.6
Physical Mapping in Sorghum

Molecular physical mapping will provide an invaluable, readily accessible system for many detailed genetic studies. The development of large DNA fragment (>100 kb) manipulation and cloning technologies, such as pulsed-field gel electrophoresis (PFGE), and yeast artificial chromosome (YAC) (Burke et al. 1987) and bacterial artificial chromosome (BAC) (Shizuya et al. 1992) cloning have provided the powerful tools needed to generate molecular physical maps for genomes of higher organisms. Once generated, the physical map will provide a virtually unlimited number of DNA markers from any chromosomal region for gene tagging, gene manipulation, and genetic studies. It will also provide an online framework for studies in genome molecular structure, genome organization, evolution, and gene regulation. The identification, isolation, characterization, and manipula-

tion of genes will become far more user feasible than ever before. The physical map, therefore, will become central to all types of genetic and molecular enquiry and manipulation, including genome analysis, gene cloning, and crop improvement.

The first construction and characterization of a 2.7 × BAC library from *S. bicolor* cultivar BT × 623 with 13,440 ordered clones and average insert size of 157 kbp was reported by Woo et al. (1994). Sorghum inserts of up to 315 kbp were isolated and shown to be stable when grown for over 100 generations in liquid media. No chimeric clones were detected as determined by fluorescence in situ hybridization of 10 BAC clones to metaphase and interphase *S. bicolor* nuclei. Lin et al. (1999) constructed and characterized a 6.6× BAC library of *Sorghum propinquum,* with 38,016 clones and average insert size of 126 kbp. This wild relative of sorghum has been utilized in RFLP linkage mapping and QTL analysis of many important traits related to domestication and productivity (Chittenden et al. 1994; Lin et al. 1995; Paterson et al. 1995a,b). Further, *S. propinquum* appears to have been the ancestor that conferred many "weediness" traits to johnsongrass (*S. helepense)* and so offers opportunities to pursue new dimensions in agricultural research (Paterson et al. 1995a). This *S. propinquum* library is a valuable complement to an established *S. bicolor* BAC library (Woo et al. 1994) for the cloning of genes associated with domestication and many other traits. Six traits related to domestication were analyzed in the F₂ of a cross between *S. bicolor* cultivar BT × 623 and *S. propinquum*. *S. propinquum* possessed most of the dominant alleles at five traits (grain shattering, plant height, flowering time, tiller number, and rhizomatousness). Dominant and additive alleles have an advantage over recessive alleles in physical mapping, and the testing of candidate DNA sequences for mutant complementation requires that the candidate sequence be genetically dominant or additive. Thus, BAC libraries of wild species offer unique advantages for map-based cloning that harbor dominant and additive alleles for many traits of agronomic importance. Bowers et al. (2001) reported their efforts toward the construction of two physical maps of sorghum based on a 6 × coverage BAC library of *S. propinquum* and 14× coverage BAC library of *S. bicolor*. Markers from a 2,600-loci RFLP-based genetic map of sorghum are being used to probe the BAC libraries either as individual plasmid probes or by using synthetically designed overgo probes. Attempts at constructing robust physical maps of sorghum using a high-density RFLP map

as a framework were also reported by Draye et al. (2001); such a map is being assembled by integrating hybridization and fingerprint data with comparative data from related taxa such as rice and using new methods to resolve genomic duplications into locus-specific groups. By taking advantage of allelic variation revealed by heterologous probes, the positions of corresponding loci on the wheat (*Triticum aestivum*), rice, maize, sugarcane, and Arabidopsis genomes are being interpolated on the sorghum physical map. Bacterial artificial chromosomes for the small genome of rice are shown to close several gaps in the sorghum contigs. Characterwise positional cloning efforts are discussed below.

Seed dispersal via disarticulation of inflorescence, or shattering, is an important agronomic trait contributing to significant yield loss in many common cereal crops. Isolation of shattering genes can enhance our understanding of the seed dispersal process and perhaps help us to reduce grain losses. Lin (1998) focused on positional cloning of the sorghum shattering gene, *Sh1*, and used substitution mapping to narrow down the chromosome segment associated with *Shl* to 0.8 cM. Based on these data, *Shl* cosegregates with RZ474 and is flanked by pSB097 and BCD1072b. These three RFLP markers were used to screen the *S. propinquum* BAC library. Twelve BAC clones with an average size of 113 kbp were identified, and nine of them formed a contig spanning the region of pSB097 and RZ474 (*Shl*). Wise et al. (2002) also screened the *S. propinquum* BAC library with DNA markers closely linked to *sh1* for the fine mapping of a chromosomal segment associated with *sh1*. Interval mapping showed that *sh1* cosegregated with one marker, SOG0128, that is located between markers SOG0251 and SOG1273 in a genetic interval of 0.42 cM. Thirteen BACs that hybridized markers in the region formed one contig. One BAC, 39E21, spanned a large part of the contig with SOG0251 at one end, and the *sh1* cosegregation marker SOG0128 near the middle. Sequencing revealed this BAC to be 220 kb in size. But the researchers were unable to extend the BAC contig at satisfactory stringency to include the BAC hybridizing marker SOG1273.

Lin (1998) studied characteristics of photoperiodic-sensing genes in sorghum, a short-day plant, focusing on positional cloning of the sorghum photoperiodic flowering gene, *Ma*1. Previous work on comparative mapping of flowering-time QTLs in the Poaceae has revealed that *Ma1* may be homologous to sugarcane, maize, barley, and wheat

photoperiodic flowering genes and paralogous to rice photoperiodic flowering genes. Substitution mapping was used to narrow down the chromosomal segment containing *Ma1* to 0.5 cM. The two most closely linked RFLP markers, pSB1113 and CDSR084, were used to screen a *S. propinquum* BAC library. These two markers hybridized to ten BAC clones with an average size of 190 kbp, which set the stage for chromosome walking to clone *Ma1*. Positional cloning and subsequent analysis of the sorghum photoperiodic flowering gene will pave the way to understanding how photoperiodic genes regulate flowering in response to day length.

Stay-green is an important postflowering drought resistance trait in sorghum. With the objective of isolating the drought resistance genes in sorghum, markers linked to stay-green QTLs (Xu et al. 2000) were used for screening the BAC libraries in Henry Nguyen's laboratory. Several positive BAC clones corresponding to the stay-green QTL 1 and 2 regions were identified, and these positive BACs fall entirely into five contigs. Simultaneously, large mapping populations have been developed using near-isogenic lines for the stay-green QTL regions for fine mapping. Identification of BACs in conjunction with the NIL mapping populations will be a useful starting point for chromosome walking toward the stay-green genes.

The liguleless (*lg-1*) linkage group is a highly conserved region of the rice and maize genome (Ahn and Tanksley 1993). Zwick et al. (1998) used fluorescent in situ hybridization (FISH) for physical mapping of BACs to analyze the *liguleless (lg-1)* linkage group in sorghum and compared it to the conserved region in rice and maize. Six *liguleless*-associated rice RFLP markers were used to select 16 homoeologous sorghum BACs, which were in turn used to physically map the *liguleless* linkage group in sorghum. Results show a basic conservation of the *liguleless* region in sorghum relative to the linkage map of rice. Selected BACs, representing RFLP loci, were end-cloned for RFLP mapping, and the relative linkage order of these clones was in full agreement with the physical data. Similarities in locus order and the association of RFLP-selected BAC markers with two different chromosomes were found to exist between the linkage map of the *liguleless* region in maize and the physical map of the *liguleless* region in sorghum.

Fertility restorer gene *Rf1* in sorghum is very important because of its critical role in hybrid seed production. Klein et al. (2004) utilized four BAC libraries from two unique sorghum genotypes to create an integrated genetic, physical, and cytological map of the sorghum genome targeting *Rf1* gene for positional cloning. Initial cytological examination of this genomic region suggested that the physical size of the trait locus was amenable to positional cloning. A minimum tiling path of BAC clones spanning the *Rf1* locus was subsequently assembled. A key feature in physical map closure in the *Rf1* region was the exploitation of the synteny between rice and sorghum to identify sorghum BACs that span gaps in the sorghum physical map. A 0.5-Mbp genomic region surrounding *Rf1* was sequenced. The development of a high-resolution map for the *Rf1* locus was accomplished in part by identifying sequence polymorphisms in overlapping BACs derived from two unique sorghum genotypes. The culmination of these efforts was the identification of a member of the pentatricopeptide repeat gene family that cosegregates with *Rf1*.

Development of modified cDNA selection protocol to aid the discovery and mapping of genes across an integrated genetic and physical map of the sorghum genome has been reported by Childs et al. (2001). BAC DNA from the sorghum genome map was isolated and covalently bound in arrayed tubes for efficient liquid handling. Amplifiable cDNA sequence tags were isolated by hybridization to individual sorghum BACs, cloned, and sequenced. Analysis of a fully sequenced sorghum BAC indicated that about 80% of known or predicted genes were detected in the sequence tags, including multiple tags from different regions of individual genes. Data from cDNA selection using the fully sequenced BAC indicate that the occurrence of mislocated cDNA tags is very low. Analysis of 35 BACs (5.25 Mb) from sorghum linkage group B revealed (and therefore mapped) two sorghum genes and 58 sorghum ESTs. Additionally, 31 cDNA tags that had significant homologies to genes from other species were also isolated. The modified cDNA selection procedure described will be useful for genomewide gene discovery and EST mapping in sorghum and for comparative genomics of sorghum, rice, maize, and other grasses.

7.7
Structural Genomics

Structural genomic resources for *S. bicolor* (L.) Moench were applied by Islam-Faridi et al. (2002) to target and develop multiple molecular cytogenetic

probes that would provide extensive coverage for a specific chromosome of sorghum. Bacterial artificial chromosome (BAC) clones containing molecular markers mapped across sorghum linkage group A were labeled as probes for fluorescence in situ hybridization (FISH). Signals from single-, dual-, and multiprobe BAC-FISH to spreads of mitotic chromosomes and pachytene bivalents were associated with the largest sorghum chromosome, which bears the nucleolus organizing region (NOR). The order of individual BAC-FISH loci along the chromosome was fully concordant with that of marker loci along the linkage map. In addition, the order of several tightly linked molecular markers was clarified by FISH analysis. The FISH results indicated that markers from the linkage map positions 0.0 to 81.8 cM reside in the short arm of chromosome 1 whereas markers from 81.8 to 242.9 cM are located in the long arm of chromosome 1. The centromere and NOR were located in a large heterochromatic region that spans ~60% of chromosome 1. In contrast, this region represents only 0.7% of the total genetic map distance of this chromosome. Variation in recombination frequency among euchromatic chromosomal regions also was apparent. The integrated data underscore the value of cytological data because minor errors and uncertainties in linkage maps can involve huge physical regions. The successful development of multiprobe FISH cocktails suggests that it is feasible to develop chromosome-specific "paints" from genomic resources rather than flow sorting or microdissection and that, when applied to pachytene chromatin, such cocktails provide an especially powerful framework for mapping. Such a molecular cytogenetic infrastructure would be inherently cross-linked with other genomic tools and thereby establish a cytogenomics system with extensive utility in development and application of genomic resources, cloning, transgene localization, development of plant "chromonomics", germplasm introgression, and marker-assisted breeding. In combination with previously reported work, the results indicate that a sorghum cytogenomics system would be partially applicable to other gramineous genera but recent publication by Kim et al. (2004) has changed this notion completely. They have used FISH-based karyotyping in metaphase chromosomes of elite inbred BT × 623 to estimate the molecular size and to establish a size-based nomenclature for sorghum chromosomes. This size-based nomenclature for BT × 623 represents a reasonable choice as the standard

for a unified chromosome nomenclature. Adoption of such a common reference for nomenclature of sorghum chromosomes and a related nomenclature for linkage groups would definitely facilitate development of gramineous genomics, e.g., by enhancing communication between research groups and data usage across genome maps. The unified nomenclature system for chromosomes and linkage groups of line BT × 623 provides a reasonable basis for a genomic nomenclature for *S. bicolor* in that this line is readily available, highly inbred, and extensively used for genetic, breeding, and genomics research. However, caution must be exercised in applying the nomenclature to other mapping endeavors because the incidence of structural rearrangements in sorghum is inadequately studied, so it remains reasonably likely that genomes of mapping parents differ structurally (Kim et al. 2004)

7.8
Functional Genomics

The complete sequence of the Arabidopsis [*Arabidopsis thaliana* (L.) Hyenh.] and rice (*Oryza sativa* L.) genomes ushered plant biology into the postgenomic era. From being largely a genetic black box, the genome sequence is revealing all the possible genes that make up a flowering plant. Now the goal for plant biologists in the postgenome era is to understand the function of every gene and how individual gene products interact and contribute to major plant processes. This new challenge for plant functional genomics is destined to become the most difficult hurdle in plant biology and requires the systematic application of global molecular approaches integrated through bioinformatics. Several tools are now required to decipher gene function including the traditional methods of random mutagenesis, gene knockout and silencing, and the new high-throughput "omic" disciplines of transcriptomics, proteomics, and metabolomics. In the last few years, new techniques for the global analysis of gene expression (including microarrays and DNA chips) using thousands of sequences at a time have been rapidly changing the way to do research to determine gene expression and function for both basic and applied objectives. This shift from the analysis of one gene at a time to thousands at a time has created opportunities to dramatically increase the rate of gene discovery in higher plants and animals. For

an important agronomic crop such as sorghum, the traits of interest include preharvest sprouting, shattering, flowering and fertility, nutritional quality, disease and insect resistance, photosynthesis, drought tolerance, and many others.

7.8.1
Development of ESTs

Expressed sequence tags (ESTs) are currently the most widely sequenced nucleotide commodity from plant genomes in terms of the number of sequences and the total nucleotide count. ESTs provide a robust sequence resource that can be exploited for gene discovery, genome annotation, and comparative genomics (Rudd 2003). To date, 190,949 ESTs in *S. bicolor*, 21,387 in *S. propinquum*, and 1,641 in *S. halepense* (Johnsongrass) have been submitted to GenBank (http://www.ncbi.nlm.nih.gov/dbEST/dbEST_summary.html; as of 26 November 2004) from various global EST sequencing projects.

7.8.2
Gene Function Analysis

With the advancement of bioinformatics, sequence analysis of molecular probes to assign function has been realized. Schloss et al. (2002) collected and analyzed DNA sequence data for 789 previously mapped RFLP probes from *S. bicolor* (L.) Moench. DNA sequences, comprising 894 nonredundant contigs and end sequences, were searched against three GenBank databases, nucleotide (nt), protein (nr), and EST (dbEST), using BLAST algorithms. Matching ESTs were also searched against nt and nr. Translated DNA sequences were then searched against the conserved domain database (CDD) to determine if functional domains/motifs were congruent with the proteins identified in previous searches. More than half (500/894 or 56%) of the query sequences had significant matches in at least one of the GenBank searches. Overall, proteins identified for 148 sequences (17%) were consistent among all searches, of which 66 sequences (7%) contained congruent coding domains.

The 3-deoxyanthocyanidins, a unique class of flavonoid phytoalexins, have been reported to be synthesized in sorghum in response to fungal infection. Lo et al. (2002) studied the biosynthetic pathways for 3-deoxyflavonoids, which are known to involve tran-

scriptional activation of chalcone synthase (CHS). CHS, or naringenin CHS, catalyzes the formation of naringenin, the precursor for different flavonoids. They have isolated seven sorghum CHS genes, CHS1 7, from a genomic library on high-density filters. CHS1 7 genes are highly conserved and closely related to the maize C2 and Whp genes. Several of them are also linked in the genome. These findings suggest that they are the result of recent gene-duplication events. Expression of the individual CHS genes was studied *in silico* by examination of EST data available in the public domain. Analyses suggested that CHSl 7 genes were not differentially expressed in the various growth and developmental conditions represented by the cDNA libraries used to generate the EST data. However, a CHS-like gene, CHS8, was identified with significantly higher EST abundance in the pathogen-induced library. CHS8 shows only 81 to 82% identity to CHSl 7 and forms a distinct subgroup in the phylogenetic analysis. In addition, the active site region contains substitutions that distinguish CHS8 from naringenin CHS. The researchers proposed that CHS8 has evolved new enzymatic functions that are involved in the synthesis of defense-related flavonoids, such as the 3-deoxyanthocyanidins, during fungal infection.

Complete sequences of mitochondrial (mt) genomes or chondrions are now available from *Arabidopsis thaliana*. As a consequence of recombination, the order and localization of mitochondrial genes differ largely among plant chondrions. But cotranscripts for two mt genes, *nad*3 and *rps*12, are conserved within angiosperms and also in gymnosperms. The *nad*3 gene codes for a subunit of the mitochondrial NADH-ubichinonoxidoreductase complex, while the *rps*12 gene product is a protein of the mitochondrial small ribosomal subunit. Howad and Kempken (1997) have cloned and sequenced the *nad3-rps12* genes from *S. bicolor*. The DNA sequence was very similar to known sequences from wheat or maize. Both genes were cotranscribed. A total of 17 RNA editing sites in *nad3* and six editing sites in *rps12* were detected. Cotranscripts exhibited a low degree of RNA editing, which was the same in four different fertile and cytoplasmic male sterile lines. In contrast to *atp6* RNA editing, no cell-type specific loss of RNA editing was observed.

Photosynthesis depends upon the strict compartmentalization of the CO_2-assimilatory enzymes of the C_4 and Calvin cycle in two different cell types, mesophyll and bundle-sheath cells. A differential accumulation is also observed for enzymes of other metabolic

pathways, and mesophyll and bundle-sheath chloroplasts of NADP-malic enzyme type C4 plants differ even in their photosynthetic electron transport chains. A large number of studies indicate that this division of labor between mesophyll and bundle-sheath cells is the result of differential gene expression. To investigate the extent of this differential gene expression and thus gain insight into the genetic basis of C_4 photosynthesis, Wyrich et al. (1998) cataloged genes that are differentially expressed in the mesophyll and bundle-sheath cells in the NADP-malic enzyme type C_4 grass *S. bicolor.* A total of 58 cDNAs were isolated by differential screening. Using a tenfold difference in transcript abundance between mesophyll and bundle-sheath cells as a criterion, 25 cDNAs were confirmed to encode mesophyll-specific gene sequences, and eight were found to encode bundle-sheath-specific sequences. Eight mesophyll-specific cDNAs showed no significant similarities within GenBank and may therefore represent candidates for the elucidation of hitherto unknown functions in the differentiation of mesophyll and bundle-sheath cells. The chromosomal location of 50 isolated cDNAs was determined by RFLP mapping using an interspecific sorghum cross.

Bak et al. (1998) have isolated a cDNA encoding the multifunctional cytochrome P450, CYP71EI, involved in the biosynthesis of the cyanogenic glucoside dhurrin from *S. bicolor* (L.) Moench. A PCR approach based on three consensus sequences of A-type cytochromes P450 – (V/T) KEX (L/F) R, FXPERF, and PFGXGRRXCXG – was applied. Three novel P450 cytochromes (CYP71E1, CYP98, and CYP99), in addition to a PCR fragment encoding sorghum cinnamic acid 4-hydroxylase, were obtained. Reconstitution experiments with recombinant CYP71E1 heterologously expressed in *Escherichia coli* and sorghum NADPH-cytochrome P450-reductase in L-a-dilaurylphosphatidyl choline micelles identified CYP71E1 as the P450 cytochrome that catalyzes the conversion of p-hydroxyphenylacetaldoxime top-hydroxymandelonitrile in dhurrin biosynthesis. In accordance with the proposed pathway for dhurrin biosynthesis, CYP71E1 catalyzes the dehydration of the oxime to the corresponding nitrile, followed by a C-hydroxylation of the nitrile to produce p-hydroxymandelonitrile. In vivo administration of oxime to *E. coli* cells results in the accumulation of the nitrile, which indicates that the flavodoxin/flavodoxin reductase system in *E. coli* is only able to support CYP71E1 in the dehydration

reaction and not in the subsequent C-hydroxylation reaction. CYP79 catalyzes the conversion of tyrosine to p-hydroxyphenylacetaidoxime, the first committed step in the biosynthesis of the cyanogenic glucoside dhurrin. Reconstitution of both CYP79 and CYP7 IE1 in combination with sorghum NADPH-cytochrome P450-reductase resulted in the conversion of tyrosine to p-hydroxymandelonitrile, i.e., the membranous part of the biosynthetic pathway of the cyanogenic glucoside dhurrin. Isolation of the cDNA for CYP71E1 together with the previously isolated cDNA for CYP79 provided important tools necessary for the tissue-specific regulation of cyanogenic glucoside levels in plants to optimize food safety and pest resistance.

Preharvest sprouting (PHS) in sorghum is related to the lack of a normal dormancy level during seed development and maturation. Carrari et al. (2001) used a PCR-based approach to isolate two *S. bicolor* genomic and cDNA clones from two genotypes exhibiting different PHS behavior and sensitivity to abscisic acid (ABA). The two 699 amino-acid-predicted protein sequences differ in two residues at positions 341 (Gly or Cys within the repression domain) and 448 (Pro or Ser) and show over 80, 70, and 60% homology to maize, rice, and oat *vp1* proteins, respectively. Expression analysis of the sorghum *vp1* gene in the two lines shows a slightly higher level of *vp1* mRNA in the embryos susceptible to PHS than in those resistant to PHS during embryogenesis. However, timing of expression was different between these genotypes during this developmental process. Whereas for the former the main peak of expression was observed at 20 d after pollination (DAP), the peak in the latter was found at later developmental stages when seed maturation was almost complete. Under favorable germination conditions and in the presence of fluridone (an inhibitor of ABA biosynthesis), sorghum *vp1* mRNA proved to be consistently correlated with sensitivity to ABA but not with ABA content and dormancy.

Sorghum is attacked by *Colletrotrichum sublineolum,* which causes leaf blight. Goodwin et al. (2004) analyzed the types of genes being expressed and their level of expression by conducting single-pass, partial sequencing of cDNA clones to generate expressed sequence tags (ESTs). They compared expressed sequence tag redundancy between EST collections from resistant and susceptible *S. bicolor* inoculated with *C. sublineolum.* Differences in expressed sequence redundancy between interactions included a greater

abundance of heat shock protein ESTs in the suscep-tible interaction and a greater abundance of cystine proteinase ESTs in the resistant interaction.

7.9
Future Prospects

Population trends predict increasing food needs, while progress in developmental and genomic plant sciences offer new opportunities for crop improve-ment. Sorghum is an important target for molecular genetic studies because of its adaptation to harsh environments, diverse germplasm collection, smaller genome size, and value for comparing the genomes of grass species such as corn, rice, and sugarcane. Concerted efforts over the past one and a half decades have greatly helped in the construction of integrated and highly saturated molecular maps in sorghum, and the majority of the agronomically important genes have been tagged. Successful utilization of this information in sorghum genetic improvement has not yet been realized. This is largely due to lack of application of marker information in marker-assisted breeding. Molecular breeders must reassess their strategies and design efficient MAS programs to augment efforts in breeding for better plant types to meet the growing needs of modern agriculture.

The most noted accomplishment is in the filed of comparative genomics as sorghum stands central in the Andropogoneae tribe. Sorghum has also served as a model to bridge the comparative analysis between the grass relatives. Conservation of gene order across cereal genomes is evident from several studies. How-ever, very little information is available on chromo-some walking and positional cloning of agriculturally important genes in sorghum to facilitate isolation of orthologous genes in the related crop species and vice versa. Physical mapping efforts were initiated (Woo et al. 1994; Lin 1998; Klein et al. 2000; Bowers et al. 2001) and are near completion, which will eventu-ally provide innumerable number of DNA markers from any chromosomal region for map-based gene isolation and a better understanding of genome orga-nization, evolution, and gene regulation.

Recent programs to understand the function of every gene and how individual gene products interact and contribute to major plant processes resulted in the development and deposition of 190,949 sorghum ESTs in GenBank. Utilization of corresponding cDNA clone libraries in large-scale expression profiling will prove to be a valuable resource for gene discovery im-plicated in plant development processes, disease and insect resistance, drought tolerance, and nutritional qualities.

With the availability of these efficient molecular biology tools in hand, there is a great potential for the exploitation of large genetic diversity as yet un-tapped so far in sorghum. Furthermore, application of novel gene-combining techniques has the potential to meet the challenges of increasing the productivity of sorghum.

References

Agrama A, Widle E, Reese C, Campbell R, Tuinstra R (2002) Genetic mapping of QTLs associated with greenbug resis-tance and tolerance in *Sorghum bicolor*. Theor Appl Genet 104:1373–1378

Ahn SN, Tanksley SD (1993) Comparative linkage maps of the rice and maize genomes. Proc Natl Acad Sci USA 90:7980–7984

Ahn SN, Anderson JA, Sorrels ME, Tanksley SD (1993) Homoe-ologous relationships of rice, wheat and maize chromo-somes. Mol Gen Genet 241:483–490

Arumunganathan K, Earle ED (1991) Nuclear DNA content of some important species. Plant Mol Biol Rep 9:208–218

Avramova Z, SanMiguel P, Georgieva E, Bennetzen JL (1995) Matrix attachment regions and transcribed sequences within a long chromosomal continuum containing maize Adh1. Plant Cell 7:1667–1680

Avramova Z, Tikhonov A, SanMiguel P, Jin YK, Liu C, Woo SS, Wing RA, Bennetzen JL (1996) Gene identification in a complex chromosomal continuum by local genomic cross-referencing. Plant J 10:1163–1168

Avramova Z, Tikhonov A, Chen M, Bennetzen JL (1998) Ma-trix attachment regions and structural collinearity in the genomes of two grass species. Nucleic Acids Res 26:761–767

Bak S, Kahn RA, Nielsen HL, Moller BL, Halkier BA (1998) Cloning of three A-type cytochromes P450, CYP71E1, CYP98, and CYP99 from *Sorghum bicolor* (L.) Moench by a PCR approach and identification by expression in *Es-cherichia coli* of CYP71E1 as a multifunctional cytochrome

P450 in the biosynthesis of the cyanogenic glucoside dhurrin. Plant Mol Biol 36:393–405

Bennetzen JL, Freeling M (1993) Grasses as a single genetic system: genome composition, collinearity, and compatibility. Trends Genet 9:259–261

Bhattramakki D, Donj J, Chhabra AK, Hart GE (2000) An integrated SSR and RFLP linkage map of *Sorghum bicolor* (L.) Moench. Genome 43:988–1002

Binelli GL, Gianfranceschi L, Pe ME, Taramino G, Busso C, Stenhouse J, Ottaviano E (1992) Similarity of maize and sorghum genomes as revealed by maize RFLP probes. Theor Appl Genet 84:10–16

Boivin K, Deu M, Rami J-F, Trouche G, Hamon P (1999) Towards a saturated sorghum map using RFLP and AFLP markers. Theor Appl Genet 98:320–328

Boora KS (1999) A molecular marker that segregates with sorghum leaf blight resistance in one cross is maternally inherited in another. Mol Gen Genet 261:317–322

Bowers JE, Burow GB, Kaivin C, Draye X, Hooks CA, Lemke C, Marler B, Presting GG, Begum D, Blackmon B, Wing RA, Paterson AH (2001) Development of a BAC based physical map of sorghum. In: Plant and Animal Genome IX Conf, San Diego

Bowers JE, Abbey C, Anderson S, Chang C, Draye X, Hoppe AH, Jessup R, Lemke C, Lennington J, Li Z, Lin YR, Liu SC, Luo L, Marler BS, Ming R, Mitchell SE, Qiang D, Reischmann K, Schulze SR, Skinner DN, Wang YW, Kresovich S, Schertz KF, Paterson AH (2003) A high-density genetic recombination map of sequence-tagged sites for sorghum, as a framework for comparative structural and evolutionary genomics of tropical grains and grasses. Genetics 165:367–86

Brown MS (1943) Haploid plants in sorghum. J Hered 34:163–166

Burke DT, Carle GFa, Olson MV (1987) Cloning of large segments of exogenous DNA into yeast by means of artificial chromosome vectors. Science 236:805–811

Carrari F, Perez-Flore L, Lijavetzky D, Enciso S, Sanchez R, Benech-Arnold R, Iusem N (2001) Cloning and expression of a sorghum gene with homology to maize *vp1*. Its potential involvement in pre-harvest sprouting resistance. Plant Mol Biol 45:631–640

Carrari F, Benech-Arnold R, Osuna-Fernandez R, Hopp E, Sanchez R, Iusem N, Lijavetzky D (2003) Genetic mapping of the *Sorghum bicolor vp1* gene and its relationship with preharvest sprouting resistance. Genome 46:253–8

Causse MA, Fulton TM, Cho YG, Ahn SN, Chunwongse J, Wu K, Xiao J, Yu Z, Ronald PC, Harrington SE et al (1994) Saturated molecular map of the rice genome based on an interspecific backcross population. Genetics 138:1251–74

Celarier RP (1959) Cytotaxonomy of the andropogoneae. III. Sub-tribe Sorgheae, genus, Sorghum. Cytologia (Tokyo) 21:272

Chantereau J, Trouche G, Rami JF, Deu M, Barro C, Grivet L (2001) RFLP mapping of QTLs for photoperiod response in tropical sorghum. Euphytica 120:183–194

Chen M, San Miguel P, de Oliveira AC, Woo SS, Zhang H, Wing RA, Bennetzen JL (1997) Microcolinearity in *sh2*-homologous regions of the maize, rice, and sorghum genomes. Proc Natl Acad Sci USA 94:3431–3435

Chen M, SanMiguel P, Bennetzen JL (1998) Sequence organization and conservation in sh2/a1-homologous regions of sorghum and rice. Genetics 148:435–43

Childs KL, Klein RR, Klein PE, Morishige DT, Mullet JE (2001) Mapping genes on an integrated sorghum genetic and physical map using cDNA selection technology. Plant J 27:243–55

Chittenden LM, Schertz KF, Lin YR, Wing RA, Paterson AH (1994) A detailed RFLP map of *Sorghum bicolor × Sorghum propinquum*, suitable for high density mapping, suggests ancestral duplication of sorghum chromosomes or chromosomal segments. Theor Appl Genet 87:925–933

Civardi L, Xia Y, Edwards KJ, Schnable PS, Nikolau BJ (1994) The relationship between genetic and physical distances in the cloned *a1-sh2* interval of the *Zea mays* L. genome. Proc Natl Acad Sci USA 91:8268–72

Cordeiro GM, Casu R, McIntyre CL, Manners JM, Henry RJ (2001) Microsatellite markers from sugarcane (*Saccharum* spp.) ESTs cross transferable to erianthus and sorghum. Plant Sci 160:1115–1123

Crasta OR, Xu WW, Rosenow DT, Mullet J, Nguyen HT (1999) Mapping of post-flowering drought resistance traits in grain sorghum: association between QTLs influencing premature senescence and maturity. Mol Gen Genet 262:579–588

de Wet JMJ (1978) Systematics and evolution of sorghum sect. Sorghum (Gramineae). Am J Bot 65:477–484

Devos KM, Gale MD (1997) Comparative genetics in the grasses. Plant Mol Biol 35:3–15

Devos KM, Milan T, Gale MD (1993) Comparative RFLP maps of the homoeologous group-2 chromosomes of wheat, rye, and barley. Theor Appl Genet 85:784–792

Devos KM, Wang ZM, Beales J, Sasaki T, Gale MD (1998) Comparative genetic maps of foxtail millet (*Setaria italica*) and rice (*Oryza sativa*). Theor Appl Genet 96:63–68

D'Hont A, Lu YH, Gonzalez-dey-Leon D, Grivet L, Geldmen P, Lanaud C, Glaszmann JC (1994) A molecular approach to unravelling the genetics of sugarcane, a complex polyploid of the Andropogoneae tribe. Genome 37:222–230

Doggett H (1976) Sorghum. In: Simmonds NW (ed) Evolution in Crop Plants. Longman, Essex, UK, pp 112–117

Doggett H (1988) Sorghum, 2nd edn. Wiley, New York

Draye X, Lin YR, Qian XY, Bowers JE, Burow GB, Morrell PL, Peterson DG, Presting GG, Ren SX, Wing RA, Paterson AH (2001) Toward integration of comparative genetic, physical, diversity, and cytomolecular maps for grasses and grains, using the sorghum genome as a foundation. Plant Physiol 125:1325–41

Dufour P, Grivet L, D'Hont A, Deu M, Trouche G, Glaszmann JC, Hamon P (1996) Comparative genetic mapping between duplicated segments on maize chromosomes 3 and 8 and

homoeologous regions in sorghum and sugarcane. Theor Appl Genet 92:1024–1030

Dufour P, Deu M, Grivet L, D'Hont A, Paulet F, Bouet A, Lanaud C, Glaszmann JC, Hamon P (1997) Construction of a composite sorghum genome map and comparison with sugarcane, a related complex polyploid. Theor Appl Genet 94:409–418

Endrizzi JE, Morgan DTJ (1955) Chromosomal interchanges and evidence for duplication in haploid *Sorghum vulgare*. J Hered 46:201–208

Fatokun CA, Menancio-Hautea DI, Danesh D, Young ND (1992) Evidence for orthologous seed weight genes in cowpea and mungbean based on RFLP mapping. Genetics 132:841–846

Garber ED (1950) Cytotaxonomic studies in the genus sorghum. Univ California Publ Bot 23:283–362

Gomez MI, Islam-Faridi MN, Woo S, Schertz KF, Czeschin DG, Zwicj MS Jr, Wing RA, Stelly DM, Price HJ (1997) FISH of a maize sh2-selected sorghum BAC to chromosomes of *Sorghum bicolor*. Genome 40:475–478

Goodwin PH, Oliver RP, Hsiang T (2004) Comparative analysis of expressed sequence tags from *Malva pusilla, Sorghum bicolor,* and *Medicago truncatula* infected with *Colletotrichum* species. Plant Sci 167:481–489

Gowda PSB, Magill CW, Frederiksen RA, Xu GW (1995) DNA markers for downey mildew resistance genes in sorghum. Genome 38:823–826

Grant WF (1987) Genome Differentiation in Higher Plants. Academic, London

Grivet L, D'Hont A, Dufour P, Hamon P, Roques D, Glaszmann JC (1994) Comparative genome mapping of sugarcane with other species within the andropogoneae tribe. Heredity 73:500–508

Gu MH, Ma HT, Liang GH (1984) Karyotype analysis of seven species in genus sorghum. J Hered 75:196–202

Guimaraes CT, Sills GR, Sobral BWS (1997) Comparative mapping of Andropogoneae: *Saccharum* L. (sugarcane) and its relation to sorghum and maize. Proc Natl Acad Sci USA 94:14262–14266

Harlan JR, de Wet JMJ (1972) A simplified classification of cultivated sorghum. Crop Sci 12:172

Hart GE, Schertz KF, Peng Y, Syed NH (2001) Genetic mapping of *Sorghum bicolor* (L.) Moench QTLs that control variation in tillering and other morphological characters. Theor Appl Genet 103:1232–1242

Haussmann G, Hess E, Seetharama N, Welz G, Geiger H (2002a) Construction of a combined sorghum linkage map from two recombinant inbred populations using AFLP, SSR, RFLP, and RAPD markers, and comparison with other sorghum maps. Theor Appl Genet 105:629–637

Haussmann BI, Mahalakshmi V, Reddy BV, Seetharama N, Hash CT, Geiger HH, Haussmann G, Hess E, Welz G, Geiger H (2002b) QTL mapping of stay-green in two sorghum recombinant inbred populations. Theor Appl Genet 106:133–142

Haussmann BI, Hess DE, Omanya GO, Folkertsma RT, Reddy BV, Kayentao M, Welz HG, Geiger HH (2004) Genomic regions influencing resistance to the parasitic weed *Striga hermonthica* in two recombinant inbred populations of sorghum. Theor Appl Genet 109:1005–16

Helentjaris T (1993) Implications for conserved genomic structure among plant species. Proc Natl Acad Sci USA 90:8308–8309

Howad W, Kempken F (1997) Cell type-specific loss of atp6 RNA editing in cytoplasmic male sterile *Sorghum bicolor*. Proc Natl Acad Sci USA 94:11090–11095

Hu FY, Tao DY, Sacks E, Fu BY, Xu P, Li J, Yang Y, McNally K, Khush GS, Paterson AH, Li ZK (2003) Convergent evolution of perenniality in rice and sorghum. Proc Natl Acad Sci USA 100:4050–4054

Hulbert S, Richter T, Axtell J, Bennetzen J (1990) Genetic mapping and characterization of sorghum and related crops by means of maize DNA probes. Proc Natl Acad Sci USA 87:4251–4255

Huskins CL, Smith SG (1932) A cytological study of the genus sorghum Pers. I. The somatic chromosomes. J Genet 25:241–249

Ilic K, SanMiguel PJ, Bennetzen JL (2003) A complex history of rearrangement in an orthologous region of the maize, sorghum, and rice genomes. Proc Natl Acad Sci USA 100:12265–12270

Islam-Faridi MN, Childs KL, Klein PE, Hodnett G, Menz MA, Klein RR, Rooney WL, Mullet JE, Stelly DM, Price HJ (2002) A molecular cytogenetic map of sorghum chromosome 1. Fluorescence *in situ* hybridization analysis with mapped bacterial artificial chromosomes. Genetics 161:345–353

Jackson RC (1984) Chromosome pairing in species and hybrids. In: Grant WF (ed) Plant Biosystematics. Academic, Toronto, pp 67–86

Jordan DR, Tao Y, Godwin ID, Henzell RG, Cooper M, McIntyre CL (2003) Prediction of hybrid performance in grain sorghum using RFLP markers. Theor Appl Genet 106:559–67

Jordan DR, Casu RE, Besse P, Carroll BC, Berding N, McIntyre CL (2004) Marker associated with stalk number and suckering in sugarcane colocate with tillering and rhizomatousness QTLs in sorghum. Genome 47:988–993

Katsar CS, Paterson RH, Teetes GL, Peterson GC (2002) Molecular analysis of sorghum resistance to the greenbug (Homoptera: Aphididae). J Econ Entomol 95:448–457

Kearsey MJ, Farquhar AG (1998) QTL analysis in plants; where are we now? Heredity 80 (Pt 2):137–142

Kebede H, Subudhi PK, Rosenow DT, Nguyen HT (2001) Quantitative trait loci influencing drought tolerance in grain sorghum (*Sorghum bicolor* L. Moench). Theor Appl Genet 103:266–276

Kim JS, Childs KL, Islam-Faridi MN, Menz MA, Klein RR, Klein PE, Price HJ, Mullet JE, Stelly DM (2002) Integrated karyotyping of sorghum by *in situ* hybridization of landed BACs. Genome 45:402–412

Kim JS, Klein PE, Klein RR, Price HJ, Mullet JE, Stelly DM (2004) Chromosome identification and nomenclature of *Sorghum bicolor*. Genetics 104.035980

Klein PE, Klein RR, Cartinhour SW, Ulanch PE, Dong J, Obert JA, Morishige DT, Schlueter SD, Childs KL, Ale M, Mullet JE (2000) A high-throughput AFLP-based method for constructing integrated genetic and physical maps: progress toward a sorghum genome map. Genome Res 10:789–807

Klein RR, Klein PE, Chhabra AK, Dong J, Pammi S, Childs KL, Mullet JE, Rooney WL, Schertz KF (2001) Molecular mapping of the *rf1* gene for pollen fertility restoration in sorghum (*Sorghum bicolor* L.). Theor Appl Genet 102:1206–1212

Klein PE, Klein RR, Vrebalov J, Mullet JE (2003) Sequence-based alignment of sorghum chromosome 3 and rice chromosome 1 reveals extensive conservation of gene order and one major chromosomal rearrangement. Plant J 34:605–21

Klein RR, Klein PE, Stelly DM, JE M (2004) Positional cloning of the sorghum fertility restoration gene *rf1* utilizing large insert DNA libraries and associated genomics technology. In: Plant and Animal Genome XII Conf, San Diego, CA, USA

Koester RP, Sisco PH, Stuber CW (1993) Identification of quantitative trait loci controlling days to flowering and plant height in to near isogenic lines of maize. Crop Sci 33:1209–1216

Kong L, Dong J, Hart GE (2000) Characteristics, linkage-map positions, and allelic differentiation of *Sorghum bicolor* (L.) Moench DNA simple-sequence repeats (SSRs). Theor Appl Genet 101:438–448

Laurie DA, Bennett MD (1985) Nuclear DNA content in the genera *Zea* and *Sorghum*: intergeneric, interspecific, and intraspecific variation. Heredity 55:307–313

Laurie DA, Pratchett N, Bezant JH, Snape JW (1994) Genetic analysis of a photoperiod response gene on the short arm of chromosome 2(2H) of *Hordeum vulgar*e (Barley). Heredity 72:619–627

Lee M (1996) Comparative genetic and QTL mapping in sorghum and maize. In: Heslop-Harrison JS (ed) Unifying Plant Genomes, Symp Soc Exp Biol, No 50, The Company of Biologists, Cambridge, UK, pp 31–38

Lijavetzky D, Martinez MC, Carrari F, Hopp E (2000) QTL analysis and mapping of pre-harvest sprouting resistance in sorghum. Euphytica 112:125–135

Lin Y (1998) Construction of *Sorghum propinquum* BAC library, towards positional cloning of sorghum shattering gene (*Sh1*) and the sorghum photoperiodic gene (Ma1). PhD Thesis, Texas A&M University, College Station, TX

Lin YR, Schertz KF, Paterson AH (1995) Comparative analysis of QTLs affecting plant height and maturity across the Poaceae, in reference to an interspecific sorghum population. Genetics 141:391–411

Lin YR, Zhu L, Ren S, Yang J, Schertz KF, Paterson AH (1999) A *Sorghum propinquum* BAC library, suitable for cloning

genes associated with loss-of-function mutations during crop domestication. Mol Breed 5:511–520

Lo C, Coolbaugh RC, Nicholson RL (2002) Molecular characterization and in silico expression analysis of a chalcone synthase gene family in *Sorghum bicolo*r. Physiol Mol Plant Pathol 61:179–188

Mackill DJ, Salam MA, Wang ZY, Tanksley SD (1993) A major photoperiod-sensitivity gene tagged with RFLP and isozyme markers in rice. Theor Appl Genet 85:536–540

Magalhaes JV, Garvin DF, Wang Y, Sorrells ME, Klein PE, Schaffert RE, Li L, Kochian LV (2004) Comparative mapping of a major aluminum tolerance gene in sorghum and other species in the poaceae. Genetics 167:1905–1914

Mann JA, Kimber CT, Miller FR (1983) The origin and early cultivation of sorghums in Africa, Bulletin No. 1454. Texas A & M University, College Station, TX

McIntyre CL, Hermann SM, Casu RE, Knight D, Drenth J, Tao Y, Brumbley SM, Godwin ID, Williams S, Smith GR, Manners JM (2004) Homologues of the maize rust resistance gene *Rp1-D* are genetically associated with a major rust resistance QTL in sorghum. Theor Appl Genet 109:875–883

Melake-Berhan A, Hulbert SH, Butler LG, Bennetzen JL (1993) Structure and evolution of the genomes of *Sorghum bicolor* and *Zea mays*. Theor Appl Genet 86:598–604

Menz MA, Klein RR, Mullet JE, Obert JA, Unruh NC, Klein PE (2002) A high-density genetic map of *Sorghum bicolor* (L.) Moench based on 2926 AFLP, RFLP and SSR markers. Plant Mol Biol 48:483–99

Ming R, Liu SC, Lin YR, da Silva J, Wilson W, Braga D, van Deynze A, Wenslaff TF, Wu KK, Moore PH, Burnquist W, Sorrells ME, Irvine JE, Paterson AH (1998) Detailed alignment of Saccharum and Sorghum chromosomes: comparative organization of closely related diploid and polyploid genomes. Genetics 150:1663–1682

Moore G, Devos KM, Wang Z, Gale MD (1995) Grasses, line up and form a circle. Curr Biol 5:737–739

Morishige DT, Childs KL, Moore LD, Mullet JE (2002) Targeted analysis of orthologous phytochrome A regions of the sorghum, maize, and rice genomes using comparative gene-island sequencing. Plant Physiol 130:1614–1625

Multani DS, Meeley RB, Paterson AH, Gray J, Briggs SP, Johal GS (1998) Plant-pathogen microevolution: molecular basis for the origin of a fungal disease in maize. Proc Natl Acad Sci USA 95:1686–1691

Oh BJ, Frederiksen RA, Magill CW (1994) Identification of molecular markers linked to head smut resistance gene (*Shs*) in sorghum by RFLP and RAPD analyses. Phytopathology 84:830–833

Oh BJ, Frederiksen RA, Magill CW (1996) Identification of RFLP markers linked to a gene for downy mildew resistance (*Sdm*) in sorghum. Can J Bot 74:315

Paterson AH, Lander ES, Hewitt JD, Peterson S, Lincoln SE, Tanksley SD (1988) Resolution of quantitative traits into Mendelian factors by using a complete linkage map

of restriction fragment length polymorphisms. Nature 335:721–726

Paterson AH, Schertz K, Lin Y, Liu S, Chang Y (1995a) The Weediness of wild plants: molecular analysis of genes influencing dispersal and persistence of Johnsongrass, *Sorghum halepense* (L.). Proc Natl Acad of Sci USA 92:6127–6131

Paterson AH, Lin YR, Li Z, Schertz KF, Doebly JF, Pinson SRM, Liu SC, Stansel JW, Irvine JE (1995b) Convergent domestication of cereal crops by independent mutations at corresponding genetic loci. Science 269:1714–1718

Paterson AH, Lan TH, Reischmann KP, Chang C, Lin YR, Liu SC, Burow MD, Kowalski SP, Katsar CS, DelMonte TA, Feldman KA, Schertz KF, Wendel JF (1996) Toward a unified genetic map of higher plants, transcending the monocot-dicot divergence. Nat Genet 14:380–382

Paterson AH, Bowers JE, Chapman BA (2004) Ancient polyploidization predating divergence of the cereals, and its consequences for comparative genomics. Proc Natl Acad Sci USA 101:9903–9908

Peng Y, Schertz KF, Cartinhour S, Hart GE (1999) Comparative genome mapping of *Sorghum bicolor* (L.) Moench using an RFLP map constructed in a population of recombinant inbred lines. Plant Breed 118:225–235

Pereira MG, Lee M (1995) Identification of genomic regions affecting plant height in sorghum and maize. Theor Appl Genet 90:380–388

Pereira MG, Lee M, Bramel-Cox P, Woodman W, Doebley J, Whitkus R (1994) Construction of an RFLP map in sorghum and comparative mapping in maize. Genome 37:236–243

Peterson DG, Schulze SR, Sciara EB, Lee SA, Bowers JE, Nagel A, Jiang N, Tibbitts DC, Wessler SR, Paterson AH (2002) Integration of Cot analysis, DNA cloning, and high-throughput sequencing facilitates genome characterization and gene discovery. Genome Res 12:795–807

Price HJ, Dillon SL, Hodnett G, Rooney W, Ross L (2005) Genome evolution in the genus *Sorghum* (Poaceae). Ann Bot *95:219–227*

Raghab RA, Dronvalli S, Saghai Maroof MA, Yu YG (1994) Construction of sorghum RFLP linkage map using sorghum and maize DNA probes. Genome 37:590–594

Ramakrishna W, Emberton J, San Miguel P, Ogden M, Llaca V, Messing J, Bennetzen JL, Dubcovsky J, Park YJ, Busso C (2002a) Comparative sequence analysis of the sorghum Rph region and the maize *Rp1* resistance gene complex. Plant Physiol 130:1728–1738

Ramakrishna W, Dubcovsky J, Park YJ, Busso C, Emberton J, San Miguel P, Bennetzen JL (2002b) Different types and rates of genome evolution detected by comparative sequence analysis of orthologous segments from four cereal genomes. Genetics 162:1389–1400

Rooney WL, Klein RR (2000) Potential of marker-assisted selection for improving grain mold resistance in sorghum. ICRISAT, Patancheru, India, pp 183–194

Rudd S (2003) Expressed sequence tags: alternative or complement to whole genome sequences? Trends Plant Sci 8:321–329

San Miguel P, Tikhonov A, Jin YK, Motchoulskaya N, Zakharav D, Melake-Berhan A, Sprienger P, Edwards K, Lee M, Avramova Z, Bennetzen JL (1996) Nested retrotransposons in the inter-genic regions of the maize genome. Science 274:765–768

Sanchez AC, Subudhi PK, Rosenow DT, Nguyen HT (2002) Mapping QTLs associated with drought resistance in sorghum (*Sorghum bicolor* L. Moench). Plant Mol Biol 48:713–726

Sax K (1923) The association of size differences with seed-coat pattern and pigmentation in *Phaseolus vulgaris*. Genetics 8:552–560

Schloss J, Mitchell E, White M, Kukatla R, Bowers E, Paterson H, Kresovich S (2002) Characterization of RFLP probe sequences for gene discovery and SSR development in *Sorghum bicolor* (L.) Moench. Theor Appl Genet 105:912–920

Shimano T, Inove T, Antonio B, Kajiya H, Shomura A, Yang Lin S, Kuboki Y, Nagamura N, Yano M, Sasaki S (1995) Extensive conservation in linkage alignement of RFLP markers between rice chromosomes 11 and 12. In: Plant Genome III Conf, San Diego

Shizuya H, Birren B, Kim U, Mancino V, Slepak T, Tachiiri Y, Simon M (1992) Cloning and stable maintenance of 300-kilobase-pair fragments of human DNA in *Escherichia coli* using an F-factor-based vector. Proc Natl Acad Sci USA 89:8794–8797

Snowden JD (1936) Cultivated Races of Sorghum. Adlard & Sons, London

Stebbins GL (1971) Chromosomal Evolution of Higher Plants. Edward Arnold, London

Subudhi PK, Nguyen HT (2000) Linkage group alignment of sorghum RFLP maps using a RIL mapping population. Genome 43:240–249

Subudhi PK, Rosenow DT, Nguyen HT (2000) Quantitative trait loci for the stay green trait in sorghum (*Sorghum bicolor* L. Moench): consistency across genetic backgrounds and environments. Theor Appl Genet 101:733–741

Sun Y, Skinner DZ, Liang GH, Hulbert SH (1994) Phylogenetic analysis of sorghum and related taxa using internal transcribed spacers of nuclear ribosomal DNA. Theor Appl Genet 89:26–32

Swigonova Z, Bennetzen JL, Messing J (2004) Structure and evolution of the r/b chromosomal regions in rice, maize, and sorghum. Genetics 2004: doi: 10.1534/genetics.104.034629

Tanksley SD, Bernatzky R, Lapitan NL, Prince JP (1988) Conservation of gene repertoire but not gene order in pepper and tomato. Proc Natl Acad Sci USA 85:6419–6423

Tanksley SD, Young ND, Paterson AH, Bonierbale MW (1989) RFLP mapping in plant breeding: new tool for an old science. Bio/Technology 7:257–264

Tanksley SD, Ganal MW, Prince JP, deVincente MC, Bonierbale MW, Broun P, Fulton TM, Giovannoni JJ, Grandilo S,

Martin GB, Messenger R, Miller L, Paterson AH, Pineda O, Roder MS, Wing RA, Wu W, Young ND (1992) High density molecular linkage maps of the tomato and potato genomes. Genetics 132:1141–1160

Tao YZ, Henzell RG, McIntyre CL (1998a) Construction of a genetic map in a sorghum RIL population using probes from different sources and its comparison with other sorghum maps. Aust J Agric Sci 49:729–736

Tao YZ, Jordan DR, Henzell RG, McIntyre CL (1998b) Identification of genomic regions for rust resistance in sorghum. Euphytica 103:287–292

Tao YZ, Henzell RG, Jordan DR, Butler DG, Kelly AM (2000) Identification of genomic regions associated with slay green in sorghum by testing RILs in multiple anvironments. Theor Appl Genet 100:1225–1232

Tao YZ, Hardy A, Drenth J, Henzell RG, Franzmann BA, Jordan DR, Butler DG, McIntyre CL (2003) Identifications of two different mechanisms for sorghum midge resistance through QTL mapping. Theor Appl Genet 107:116–22

Taramino G, Tarchini R, Ferrario S, Lee M, Pe ME (1997) Characterization and mapping of simple sequence repeats (SSRs) in *Sorghum bicolor*. Theor Appl Genet 95:66–72

Teutonico RA, Osborn TC (1994) Mapping of RFLP and quantitative trait loci in Brassica rapa and comparison to the linkage maps of *B. napus*, *B. oleracea*, and *Arabidopsis thaliana*. Theor Appl Genet 89:885–893

Thoday JM (1961) Location of polygenes. Nature 191:368–369

Tikhonov AP, San Miguel PJ, Nakajima Y, Gorenstein NM, Bennetzen JL, Avramova Z (1999) Colinearity and its exceptions in orthologous adh regions of maize and sorghum. Proc Natl Acad Sci USA 96:7409–7414

Toure A, Xu W, Rosenow DT, Peterson GC, Nguyen HT (1997) Inheritance of insecticide phytotoxicity in sorghum. In: Plant and Animal Genome V Conf, San Diego

Tuinstra MR, Grote EM, Goldsbrough PB, Ejeta G (1996) Identification of quantitative trait loci associated with pre-flowering drought tolerance in sorghum. Crop Sci 36:1337–1344

Tuinstra MR, Grote EM, Goldsbrough PB, Ejeta G (1997) Genetic analysis of post-flowering drought tolerance and components of grain development in *Sorghum bicolor* (L.) Moench. Mol Breed 3:439–448

USDA (2004) Sorghum – Production, Consumption, Exports, and Imports Statistics – 2004. http://www.usda.gov/wps/portal/usdahome

Vasil V, Castillo AM, Fromm ME, Vasil IK (1994) Herbicide resistant fertile transgenic wheat plants obtained by microprojectile bombardment of regenerable embryogenic callus. Bio/Technology 10:667–674

Ventelon M, Deu M, Garsmeur O, Doligez A, Ghesquière A, Lorieux M, Rami JF, Glaszmann JC, Grivet L (2001) A direct comparison between the genetic maps of sorghum and rice. Theor Appl Genet 102:379–386

Wang ZM, Devos KM, Liu CJ, Wang RQ, Gale MD (1998) Construction of RFLP-based maps of foxtail millet, *Setaria italica* (L.) P. Beauv. Theor Appl Genet 96:31–36

Wen L, Tang HV, Chen W, Chang R, Pring DR, Klein PE, Childs KL, Klein RR (2002) Development and mapping of AFLP markers linked to the sorghum fertility restorer gene *rf4*. Theor Appl Genet 104:577–585

Whitkus R, Doebley J, Lee M (1992) Comparative genome mapping of sorghum and maize. Genetics 132:119–130

Wise MG, Schulze SR, Lin YR, Bowers JE, Okuizumi H, Schertz KF, Paterson AH (2002) Progress toward the positional cloning of the sorghum grain shattering gene (*sh1*). In: Plant, Animal & Microbe Genomes X Conf, San Diego

Woo SS, Jiang J, Gill BS, Paterson AH, Wing RA (1994) Construction and charaterization of a bacterial artificial chromosome library for *sorghum bicolor*. Nucleic Acids Res 22:4922–4931

Wyrich R, Dressen U, Brockmann S, Streubel M, Chang C, Qiang D, Paterson AH, Westhoff P (1998) The molecular basis of C4 photosynthesis in sorghum: isolation, characterization and RFLP mapping of mesophyll- and bundle-sheath-specific cDNAs obtained by differential screening. Plant Mol Biol 37:319–35

Xu GW, Magill CW, Schertz KF, Hart GE (1994) A RFLP linkage map of *Sorghum bicolor* (L.) Moench. Theor Appl Genet 89:139–145

Xu W, Subudhi PK, Crasta OR, Rosenow DT, Mullet JE, Nguyen HT (2000) Molecular mapping of QTLs conferring stay-green in grain sorghum (*Sorghum bicolor* L. Moench). Genome 43:461–469

Yu H, Liang GH, Kofoid KD (1991) Analysis of C-banding chromosome patterns of sorghum. Crop Sci 31:1524–1527

Zhang HB, Choi SD, Woo SS, Li Z, Wing RA (1996) Construction and characterization of two rice bacterial artificial chromosome libraries from the parents of a permanent recombinant inbred mapping population. Mol Breed 2:11–14

Zwick MS, Islam-Faridi MN, Czeschin DG Jr, Wing RA, Hart GE, Stelly DM, Price HJ (1998) Physical mapping of the liguleless linkage group in *Sorghum bicolor* using rice RFLP-selected sorghum BACs. Genetics 148:1983–1992

8 Pearl Millet

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8.1
Introduction

8.1.1
Brief History

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is an important cultivated species in the tribe Paniceae, the largest tribe in the Gramineae family consisting of 71 genera with over 1,400 species (Roshevits 1980). Pearl millet has a complex taxonomic history with cited references to as early as 1576 (Brunken 1977; Jauhar 1981). In the second half of the 18th century, taxonomists placed the species in five different genera. In the 20th century, taxonomists agreed that it belonged in the genus *Pennisetum*; however, the species underwent several name changes, e.g., *americanum*, *typhoides*, and *glaucum* (Jauhar and Hanna 1998). Today, most researchers recognize its correct name as *Pennisetum glaucum* (L.) R. Br. Pearl millet most likely originated in northern Africa in the region from western Sudan to Senegal (Harlan 1971). Archaeological evidence for the use of domesticated pearl millet as long ago as 3,500 years B.P. has been summarized (Zach and Klee 2003). Preservation was primarily observed as carbonized remains and imprints in potsherds from multiple locations on the southern edge of the Sahara and in sub-Saharan West Africa.

The genus *Pennisetum* is divided into five sections: Gymnothrix, Eupennisetum, Penicillaria, Heterostachya, and Brevivalvula (Stapf and Hubbard 1934). Cultivated pearl millet belongs to the section Penicillaria.

Pearl millet grows best on well-drained light sandy soils. Although it grows better than most grain crops under poor fertility and low moisture, it readily responds to more favorable growing conditions. It can tolerate acid subsoils with a pH as low as 4 and high in exchangeable Al (National Research Council 1996).

8.1.2
Botanical Description

Pearl millet is morphologically diverse. It is a robust annual grass that usually ranges from 1.5 to 3 m tall but can grow to 5 m. Inflorescences are cylindric, stiff, and very dense and usually range from 15 to 45 cm in length but can be 150 cm long. Spikelets are short-pediceled, come two in a fascicle, and are 3.5 to 4.5 mm long, ovate, and turgid. Leaf blades are flat, cordate, and up to 1 m long and 5 cm wide (Hitchcock 1950).

Pennisetum is reported to consist of more than 140 species (Brunken 1977). However, this is misleading because some taxonomists have assigned species names to races. Base chromosome numbers of $x = 5$, 7, 8, and 9 with ploidy levels ranging from diploid to octoploid can be found in this genus. Sexual, apomictic, and facultative apomictic species occur in the genus. *Pennisetum* species with a base chromosome number of $x = 9$ and that reproduce by apomixis are most common in *Pennisetum*. Pearl millet is an annual, sexual diploid ($2n = 2x = 14$), and its chromosomes are designated as the A genome (Jauhar and Hanna 1998). Napiergrass (*Pennisetum purpureum* Schum.), a member of the secondary gene pool (Harlan and de Wet 1971), is a perennial, sexual tetraploid ($2n = 4x = 28$) with A′ and B genomes. It is probably the closest relative of pearl millet, and they readily hybridize (Harlan 1975).

The genome size of pearl millet first was estimated to be 4.9 pg/2C by Bennett and Smith 1976, who used the technique of microdensitometry with Feulgen-stained nuclei and *Senecio vulgaris* as a reference species. Subsequently, pearl millet and 14 other *Pennisetum* species were surveyed for DNA content using flow cytometry of ethidium-bromide-stained nuclei from leaves and alfalfa as an internal standard (Martel et al. 1997). Their measurement of 2C DNA content for pearl millet was 4.71 pg/2C, which was similar to that of Bennett and Smith (1976). Other es-

timates ranging from 2.2 to 5.4 pg/2C previously have been reviewed by Roche et al. 2002, who independently estimated the genome size of pearl millet to be 4.0 pg/2C using DAPI-stained leaf nuclei and rice as a reference species. Rice and pearl millet have similar GC contents (~44%) (Martel et al. 1997; Tyagi et al. 2004). The pearl millet genome, therefore, is about five times the size of rice and only slightly less than that of maize (Bennett and Smith 1976; Laurie and Bennett 1985).

8.1.3
Economic Importance

Pearl millet is an important crop that reliably produces food and fodder for millions of people where the growing conditions are too dry and too infertile to grow most other grain crops. Pearl millet is grown on over 28 million hectares (National Research Council 1996), mainly in Africa and India. However, it is also an important crop in Pakistan, USA, Australia, and South America. Although the grain is used mainly as a human food crop, it is also used to feed livestock. Additionally, the plant is used for grazing, hay, silage, as building material, and as a source of fuel. Pearl millet forage is highly digestible in the vegetative stage and does not produce hydrocyanic acid.

Pearl millet produces nutritious grain and is a major human food for people living in the semiarid, low input, dryland agriculture regions of Africa and southeastern Asia. It is high in oil, protein, and energy, has balanced amino acids (except low in S-containing amino acids), is high in Ca and Fe (Malhotra and Dhindsa 1984; Serna-Saldivar and Rooney 1995), and contains no tannins. People in northern Namibia are almost entirely dependent on pearl millet for food. Four countries in the Sahel region of Africa, with a total population of 38 million, depend on pearl millet to provide over 1,000 calories per person per day (Dendy 1995). Pearl millet is consumed in many different ways: porridges, breads, fermented and nonfermented beverages, snacks, popped grain, etc. (Murty and Kumar 1995).

8.1.4
Breeding Objectives and Achievements

Pearl millet is grown in areas of Africa and India where it is too dry and the soil fertility is too low to grow most other grain crops. This crop performs better than most other grain crops under these conditions

because it has evolved over thousands of years in the Sahel of West Africa. Therefore, it is a key grain crop for human nutrition for a large portion of the world's population.

Breeding objectives may vary depending on whether the plant is used for grain or forage production. Traits for grain production include seed size, weight, and color; inflorescence length and diameter; floret compactness; stalk strength; pest resistance; tillering ability; days to seed maturity; and plant height. Traits for forage production include dry matter yield, dry matter digestibility, response to daylength, regrowth potential, tillering ability, and pest resistance. The known plant, ear, disease, and seed characteristics considered important in pearl millet have been summarized (Anand Kumar and Andrews 1993). Greater productivity and reliability of pearl millet cultivars have been achieved by using population improvement approaches (Witcombe 1999). These approaches will continue to be important in the improvement of pearl millet. However, utilizing hybrid vigor to improve pearl millet forage and grain cultivars is an effective approach to maximizing the potential of this crop. Hybrid vigor for grain yield in pearl millet was recognized in the mid-1940s with the development and release of two chance hybrids, X-1 and X-2, that yielded 45% more grain than the local types (Rao et al. 1951). Unfortunately, these hybrids did not become popular because only 40% of the seed produced were hybrid seed. Gahi 1, a first-generation synthetic with four male fertile inbreds that yielded 52% more dry matter than Common and 35% more than Starr, was released in 1962 (Burton 1962). This chance hybrid was more successful because four inbreds were used instead of two. However, these hybrids demonstrated the need for cytoplasmic-nuclear male sterility to produce commercial pearl millet hybrids. Since then, hybrids have been used almost entirely to produce forage cultivars and emerging grain cultivars in the USA. Tifleaf 3 is the latest release in a series of improved semidwarf leafy forage cultivars in the USA (Hanna et al. 1997). It is a three-way hybrid where commercial F_1 seed is produced on a cytoplasmic-nuclear male sterile F1 female parent to make commercial seed production of Tifleaf 3 more economical. TifGrain 102 is a newly released drought-resistant, dwarf F_1 grain hybrid for the USA (Hanna et al. 2005a,b).

Use of hybrids in India has increased since the mid-1960s, even though the total area planted with pearl millet has remained static. Dave (1987) esti-

mated that 40% of the pearl millets in India are F_1 hybrids. Grain production in India has increased by 50% because of hybrids. The popularity and use of hybrids have evolved because of development of superior inbreds. Most pearl millet inbreds are vigorous and have both good general and specific combining ability to produce hybrids in a number of different combinations as well as in specific combinations. Characteristics such as good seed set and yields, plant vigor, disease resistance, standability, plant height, and earliness have enhanced the use of hybrids (Hanna and Rai 1999). Hybrids are beginning to be used in Africa, especially Nigeria, because significant grain yield increases are being realized. The main constraint on the use of pearl millet hybrids in Africa, however, is the lack of infrastructure for seed distribution.

8.1.5
Classical Mapping Efforts

An excellent comprehensive review of qualitative traits identified in pearl millet has been published (Anand Kumar and Andrews 1993). As the authors state, their review "reports 167 studies, since 1934, on 145 characters in 12 categories: chlorophyll deficiencies, foliage striping, leaf characters, pubescence, plant form, pigmentation, earhead characters, reproductive structures and gamete formation, sterility, seed characters, earliness and maturity, and disease resistance." We will include a few additional studies in the present review, which is not intended to be comprehensive for qualitative traits except where genetic characterization has been carried out.

One of the more recent genetic studies for morphological traits analyzed red and purple plant color and linkage to trichomeless, yellow, female sterile, light green, and dwarf (Hanna and Burton 1992). When crossed independently with green plants, red (Rp^1) and purple (Rp^2) segregated as single, dominant genes. Red was shown to be allelic to and dominant over purple because F_1 progeny of red × purple crosses displayed only the red character, but one quarter of the F_2 progeny were purple. It already had been shown that yellow, light green, and female sterile loci were linked with each other (Hanna et al. 1978). Linkage of Rp^1 and Rp^2 to the other traits listed above was detected only for the dwarf (d_2) locus. The linkage between purple foliage color (P) and d_2 was independently verified by Azhaguvel et al. (2003), who also showed that the two loci mapped to LG 4, a link-

age group known to contain disease resistance genes (see below). Furthermore, the same study showed that d_1 was unlinked with d_2. Although linkage of trichomeless with other morphological markers has not been detected, trichomeless is useful not only as a genetic marker, but it also has a positive pleiotropic effect on smut resistance (Wilson 1995; Wilson and Hanna 1998). Near-isogenic trichomed and trichomeless lines showed up to 50% difference in their susceptibility to smut but no difference in yield (Wilson and Hanna 1998). A floral, phylloid homeotic mutant that was simply inherited as a recessive trait has been described (Wilson 1996). In this mutant, the staminate floret formed normally and produced pollen, but the hermaphroditic floret became vegetative, producing leaves and occasionally plantlets. No linkage studies have yet incorporated this homeotic mutant. Other floral mutants characterized because of their potential for apomixis include female sterile and stubby head (Morgan et al. 1997). Although both mutants display aposporous embryo sac development, the mutant loci are not linked.

In addition to the limited number of classical mapping studies with morphological traits, several studies have involved isozymes. Most of these were conducted prior to 1990 and were reviewed in Anand Kumar and Andrews (1993) under the category of biochemical genetic markers. According to their summary, several isozymes were simply inherited (thus would be useful as genetic markers) including esterase (*Est*), alcohol dehydrogenase (*Adh*), phosphoglucomutase (*Pgm*), phosphoglucose isomerase (*Pgi*), catalase (*Cat*), shikimate dehydrogenase (*Skdh*), glutamate oxaloacetate transaminase (*Got*), and endopeptidase (*Ep*). There may be multiple forms of an isozyme, each derived from its own locus such as Adh_1 and Adh_2, although in this example, the two loci are closely linked (Banuett-Bourrillon 1982). Furthermore, *Adh* was reported to be linked with *Skdh* and the dwarfing gene d_2 (Tostain 1985). By extrapolation, these two isozymes should also be linked with one gene for red/purple pigmentation since red was shown to be linked with d_2 (Hanna and Burton 1992). In pearl millet, isozymes have been more extensively used for diversity rather than mapping studies since allelic polymorphism is sufficient for diversity (Tostain et al. 1987; Tostain 1992) and breeding system/gene flow (Sandmeier 1993; Renno et al. 1997) analyses. However, nucleotide polymorphism at the *Adh1* locus of pearl millet was shown to be less than in maize and not significantly different between cultivated and wild populations (Gaut

and Clegg 1993). Most of the nucleotide changes either were synonymous substitutions or found in introns.

Although there have been only a few well-designed mapping studies to detect genetic linkage with isozymes, isozymes in pearl millet have been analyzed for their correlation with disease resistance (Shetty et al. 2001; Chhabra et al. 2001). These include esterases, peroxidases, and ß-1,3-glucanases, some forms of which may be involved in disease resistance reactions. They have not provided good genetic markers, however, since their level of expression shows considerable quantitative variation.

8.1.6
Classical vs. Molecular Maps in Pearl Millet

As in other plant species, the number of morphological or isozyme traits that can be used for classical genetic mapping in pearl millet is limited. Genetic maps often combine phenotypic traits and molecular markers, and both are dependent on polymorphism in mapping populations (Tanksley et al. 1989). Phenotypes often are influenced by the environment, however, and are not the markers of choice. Parents of mapping populations typically are chosen to optimize the level of polymorphism while allowing for mapping of a trait (phenotype) of interest such as disease resistance. Molecular polymorphisms are unlimited in practice and can be screened using a variety of DNA-based detection methods (Young 2001). An early example of polymorphism detection in pearl millet is drawn from the analysis of *Adh1* and ribosomal RNA restriction fragment length polymorphisms (Gepts and Clegg 1989). This study included 24 wild and 54 cultivated accessions. The cultivated accessions had much less polymorphism for ribosomal RNA genes than the wild accessions, but no difference was observed between the two for *Adh1*. In conclusion, sufficient DNA polymorphism does exist to allow the development and implementation of molecular genetic maps in pearl millet for the purpose of breeding and gene isolation.

8.2
Construction of Genetic Maps

8.2.1
Brief History of Mapping Efforts

The first application of restriction fragment length polymorphism (RFLP) markers for the construction of genetic maps was carried out by Botstein and colleagues in humans in 1980 (Botstein et al. 1980). By the mid to late 1980s, RFLP maps had been generated for many of the major crops (Helentjaris et al. 1986; Bernatzky and Tanksley 1986; Landry et al. 1987; McCouch et al. 1988; Gebhardt et al. 1989; Chao et al. 1989). However, it was not until 1994 that the first genetic map of pearl millet was published (Liu et al. 1994). The map contained some 200 loci mapped with *Pst*I genomic clones. Seven linkage groups (LGs) were obtained, which most likely corresponded to the seven pearl millet chromosomes. The big surprise, however, was the short genetic map length, which was only 300 cM over the seven LGs. This contrasted with the genetic length of wheat, rice, and maize chromosomes, which averaged 100 to 180 cM per chromosome (Causse et al. 1994; Gale et al. 1995; Harushima et al. 1998; Davis et al. 1999). The apparent lack of recombination seen in the first linkage map was also a feature of the maps subsequently constructed using a variety of different crosses. The addition of new markers finally demonstrated that recombination in pearl millet is not reduced relative to that of other species but is extremely localized toward the chromosome ends (Qi et al. 2004).

The genetic maps formed the basis for a number of trait studies, including analysis of the domestication syndrome (Poncet et al. 1998, 2000, 2002), drought tolerance (Yadav et al. 2002, 2004), rust resistance (Morgan et al. 1998), and downy mildew resistance (Jones et al. 1995, 2002). The QTL results were quickly taken up by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) to improve the resistance of elite pearl millet lines to drought and downy mildew infection using marker-assisted breeding. Marker-assisted breeding using RFLP markers is, however, cumbersome and time consuming. This prompted the development of simple sequence repeat (SSR) markers (Qi et al. 2001) and, more recently, single nucleotide polymorphism (SNP) markers (Bertin et al. 2005). The most recent genetic map, published in 2004, is a consensus map of four different crosses

and contains 353 RFLP and 65 SSR markers (Qi et al. 2004).

8.2.2
First-Generation Genetic Maps

The first genetic map of pearl millet was constructed in an F_2 population generated from a cross between the lines LGD 1-B-10 and ICMP 85410. One hundred and 66 polymorphic *Pst*I genomic RFLP probes, detecting a total of 181 loci, were mapped over six main LGs. In addition, one group of two markers (*Xpsm160* and *Xpsm190*) and an independently segregating locus (*Xpsm870*) were obtained. The largest LG, which carried 46% of the markers, was subsequently broken down in two LGs. The two-point linkage data had indicated that linkage between the two groups was most likely caused by the presence of a translocation that differentiated LGD 1-B-10 and ICMP 85410 (Liu et al. 1994). Transfer of the markers to a new F_2 mapping population, generated from the cross Tift 23DB$_1$ × WSIL (IP 18292), indeed revealed that the map now consisted of seven LGs, presumably corresponding to the seven pearl millet chromosomes (Liu et al. 1994).

A subset of the markers was subsequently transferred to a range of different crosses to address a number of biological questions related to recombination. In animals as well as plants, recombination rates have been shown to vary in male and female gametogenesis. In most animals, recombination is lower in male gametogenesis (Johnson et al. 1987; Donis-Keller et al. 1987; Graf 1989), while in plants the results vary. In maize, for example, less recombination occurs in female gametogenesis, while the opposite is true in tomato (Robertson 1984; de Vicente and Tanksley 1991). Using reciprocal intraspecific three-way crosses, Busso et al. (1995) showed that in pearl millet overall recombination rates were comparable in male and female gametogenesis. Similar results were obtained in reciprocal interspecific pearl millet crosses (Liu et al. 1996). In contrast, a cytogenetic analysis of the chiasma frequency and distribution in pollen and embryo sac mother cells showed a higher number of chiasmata in female (average 2.3 per chromosome) compared to male cells (average 1.7 per chromosome). Moreover, chiasma formation in the male cells took place mainly in the distal chromosome regions, while in the female cells mostly interstitial chiasmata were observed (Koul et al. 2000). Nevertheless, the average chiasma frequency of two per chromosome would suggest a genetic map length of some 100 cM per pearl millet chromosome. Such a length would be in line with the results of mapping exercises in other species, which have shown that, irrespective of their DNA amount, most chromosomes have a genetic length of 100 to 180 cM (Causse et al. 1994; Gale et al. 1995; Harushima et al. 1998; Davis et al. 1999). The length of the pearl millet maps constructed in the LGD 1-B-10 and ICMP 85410 cross, however, varied between 13.3 cM for LG 7 and 77.1 cM for LG 1. Maps constructed subsequently in other intervarietal pearl millet crosses were equally short. However, it should be noted that these maps were constructed by transfer of the markers originally used in the LGD 1-B-10 × ICMP 85410 cross and did not incorporate new markers. The discrepancy between the chiasma frequency and map length suggested that the pearl millet maps were, as yet, incomplete. This was further supported by the fact that two linked markers, *Xpsm160* and *Xpsm190*, remained unlinked to any of the seven LGs, and that in all crosses tested.

In 1994, a new core mapping population was generated. The population consisted of 157 F_2 progeny generated from a cross between the inbred lines 81B and ICMP 451. Both parents were completely colinear, and transfer of a core set of markers from the original LGD 1-B-10 and ICMP 85410 population generated seven LGs. The map was populated with additional pearl millet RFLP markers, heterologous RFLP markers, and simple sequence repeat (SSR) markers. SSRs were generated using two different methods. A first set of 44 SSR markers was isolated from a $(CA)_n$-enriched small insert library (Qi et al. 2004). A second set of 42 $(GT)_n$ and eight $(CT)_n$ markers was extracted from pearl millet BAC clones using a PCR approach (Qi et al. 2001). All SSRs were tested for variation in a sample of 20 pearl millet inbred lines that were being used in mapping and/or breeding. Polymorphism information content (PIC) values varied from 0 (monomorphic) to 0.92 (Allouis et al. 2001; Qi et al. 2001, 2004). The SSRs isolated from the BAC clones displayed overall lower levels of variation compared to the SSRs developed from the enriched library. This difference was almost certainly due to the fact that the latter comprised a larger number of repeat units (Qi et al. 2004). A total of 63 SSR markers, 35 from the enriched library and 28 from the BAC clones, were mapped in the 81B × ICMP 451 cross or one of the other available mapping populations. The 81B × ICMP 451 and most recent LGD 1-B-10 × ICMP 85410 maps are shown in Fig. 1. The relative location in the 81B X ICMP

Fig. 1. Genetic maps constructed in crosses 81B × ICMP 451 and LGD 1-B-10 × ICMP 85410. *Vertical lines*: range of markers that could not be unambiguously placed on map. *Stars* (*): markers that deviate from Mendelian segregation ratios (*, 0.01 < P ≤ 0.5; **, P ≤ 0.01). *Dotted lines* on 81B X ICMP 451 map: relative position of SSR markers mapped in other pearl millet crosses. Also shown on this map are regions of pearl millet genome with known orthology to rice. *Vertical bars* on right-hand side of LGD 1-B-10 X ICMP 85410 cross: major QTLs identified for domestication-related traits (*Dom*), grain yield (*Gy*), stover yield (*Sy*), harvest index (*Hi*), and downy mildew resistance (*Dm*)
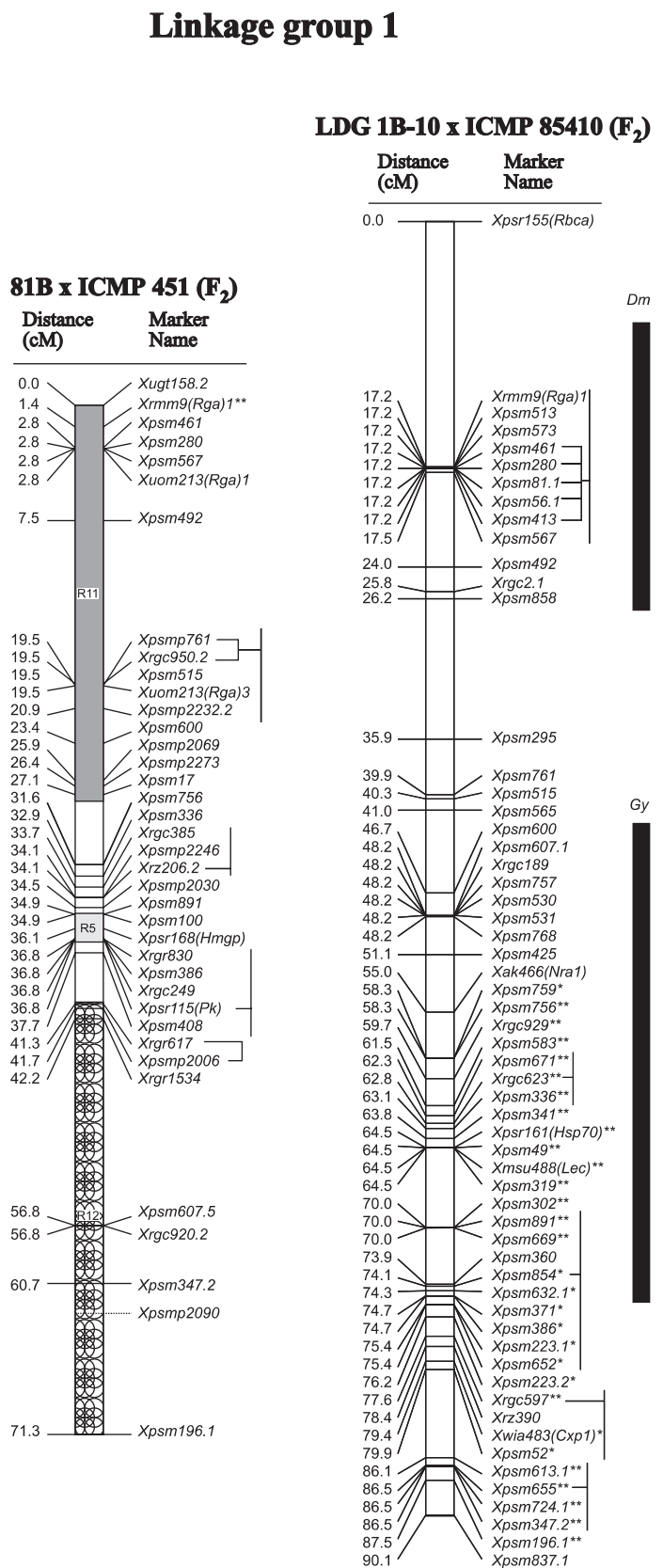
Linkage group 1

Fig. 1. (continued)

Linkage group 2

81B x ICMP 451 (F$_2$)

Distance (cM)	Marker Name
0.0	Xpsm708.1
32.0	Xpsm651.1**
33.3	Xpsm322*
34.0	Xpsmp2211
35.3	Xpsm706*
35.3	Xpsmp2237
37.3	Xpsm848*
37.3	Xpsmp2088
37.3	Xpsmp2072
37.3	Xumc107*
37.3	Xcdo122
37.3	Xrz474*
38.4	Xpsmp2066
40.2	Xpsr467*
41.0	Xrgc1286
41.0	Xpsm25
41.4	Xpsm380
42.2	Xrgr2447
43.6	Xpsmp2077
44.4	Xpsm321
46.8	Xpsmp2255
46.8	Xnpi253*
46.8	Xpsm592*
46.8	Xphp10005*
47.7	Xrgc903.1**
47.7	Xpsmp2232.1*
47.7	Xpsmp2206
47.7	Xpsm443*
47.7	Xugt158.1
47.7	Xpsmp2231
47.7	Xpsmp2050
47.7	Xpsmp2201
47.7	Xpsmp2225
47.7	Xrgc630.2
48.2	Xpsmp2059
48.2	Xpsr477(Pgk1-2)
51.5	Xrz87*
51.5	Xrgc1361*
93.2	Xpsm708.2*
97.5	Xrgr2510
109.4	Xpsmp2089

R3, R10, R6, R2

LDG 1B-10 x ICMP 85410 (F$_2$)

Distance (cM)	Marker Name
0.0	Xpsm708.1
26.7	Xpsm672.2**
26.7	Xpsm651.1**
29.0	Xpsm322**
29.7	Xpsm227.1**
30.4	Xpsm227.2**
31.5	Xpsm706**
31.5	Xpsm394**
31.5	Xpsm466**
31.5	Xpsm856**
31.5	Xpsm477**
31.7	Xpsm416.2***
31.9	Xpsm215.3**
32.3	Xpsm458**
37.9	Xpsm875.1*
37.9	Xpsm848*
37.9	Xpsm176*
38.3	Xpsm214*
40.2	Xpsm359*
40.2	Xpsm556
41.0	Xpsm215.2
41.8	Xumc83
42.6	Xpsm634.2
43.4	Xpsm380
43.4	Xrz614*-
44.6	Xpsm758
50.8	Xpsm527.2**
50.8	Xpsr487(C1)1*
50.8	Xpsr490(Ss1)*
52.3	Xpsm592*
52.8	Xpsm403*
52.8	Xpsm421.2*
54.6	Xpsm607.4*
54.6	Xpsb38*
54.6	Xpsm239*
54.6	Xpsm443*
54.6	Xugt159*
56.0	Xpsm662.1*
56.0	Xpsm1246*
56.0	Xpsm356
56.0	Xpsm632.2
62.9	Xpsm738
94.0	Xpsm708.2

Dm   Gy   Sy   Hi

Fig. 1. (continued)

Linkage group 3

81B x ICMP 451 (F₂)

Distance (cM)	Marker Name
0.0	Xpsm37
5.0	Xpsmp2056*
5.0	Xpsmp2251*
6.7	Xpsm174
9.3	Xrgr418
10.0	Xpsm868
10.3	Xpsm850
10.3	Xpsm724.3
10.3	Xpsm510.2*
	Xpsmp2070
11.5	Xpsmp2214
11.5	Xpsb193
11.5	Xpsm248
11.5	Xrgc621
11.5	Xpsm473
12.1	Xpsmp2267
12.1	Xhhu27
12.1	Xrgc975
12.1	Xpsm119
12.1	Xpsr390
12.7	Xpsm599
12.7	Xpsm428
13.3	Xpsm391
13.3	Xpsm525
13.6	Xpsmp2071
13.6	Xpsm662.2
	Xpsmp2227
26.6	Xrgr416

LDG 1B-10 x ICMP 85410 (F₂)

Distance (cM)	Marker Name
0.0	Xpsm37
4.1	Xpsm174
7.0	Xpsm108
7.5	Xugt185
8.4	Xpsm634.4
8.4	Xpsm133
8.4	Xpsm18
8.4	Xpsm325
8.4	Xpsm51
8.4	Xpsm180
8.4	Xpsm96
8.4	Xpsr2(α-Amy-1)
8.4	Xhhu44
9.0	Xpsm678
10.1	Xrgc1419
10.5	Xpsm409.2
10.5	Xpsm850
11.8	Xpsm317
13.1	Xpsm473
14.6	Xpsm376
15.7	Xpsm476
15.7	Xpsm248
16.7	Xrgc949.1
17.2	Xpsm869
17.2	Xpsm686
17.2	Xrgc777
17.2	Xsho89
17.2	Xrgc131.2
19.0	Xrgc178
19.4	Xpsm634.3
19.4	Xpsm410
19.4	Xpsm428
19.4	Xrgc131.4
19.4	Xpsr142(Prk)
19.6	Xpsm617
19.8	Xpsm525
19.8	Xrgc600
19.8	Xpsr487(C1)2
23.0	Xpsm451.1
35.2	Xpsm416.1

Hi

Fig. 1. (continued)

Linkage group 4

LDG 1B–10 x ICMP 85410 (F$_2$)

Distance (cM)	Marker Name
0.0	Xrgc901
6.6	Xpsm409.1
15.8	Xpsm464**
39.1	Xpsm716**
43.4	Xpsm265**
43.4	Xpsm866.1**
44.3	Xrgc2.2**
45.1	Xpsm397**
45.4	Xpsm131.3**
45.7	Xpsm306**
45.7	Xpsr105**
46.0	Xrgr2285**
46.3	Xpsr476(Gapd2)**
46.7	Xpsr690**
46.7	Xpsm743.1**
47.8	Xpsm568**
47.8	Xpsm532**
47.8	Xpsm421.3**
48.8	Xpsm648**-
49.7	Xpsm364**-
53.5	Xpsm640.1*
54.9	Xpsm512*
55.8	Xpsb67*
55.8	Xpsm305*
57.0	Xpsm344*
57.7	Xpsm612*
58.1	Xpsm56.2**
58.1	Xpsm84**
58.1	Xpsm634.5**
61.4	Xrgc131.1
65.7	Xpsm875.3
68.6	Xpsm330.1**

Dm

81B x ICMP 451 (F$_2$)

Distance (cM)	Marker Name
0.0	Xpsm1004
0.8	Xpsm1005
2.1	Xpsm464
16.7	Xpsm1003
31.1	Xrgc1069*
32.8	Xpsmp2076*
32.8	Xpsmp2081**
33.7	Xpsm1001**
33.7	Xpsm1007*
34.8	Xpsm866.1**
35.7	Xpsm1009**
36.6	Xrgc630.1**
36.6	Xpsr803(Fed)**
36.6	Xrz900**
36.6	Xhhu20**
37.5	Xrgc390**
37.5	Xugt225**
37.5	Xpsr165(Sdh)**
37.7	Xrgc950.3**
37.9	Xpsmp2085**
38.3	Xpsm568**
40.0	Xpsm364**
41.8	Xpsm344**
41.8	Xpsm305**
42.1	Xrgr1963**
42.4	Xwaxc2(Acl1.3)**
42.7	Xpsm730**
43.7	Xpsr144(Lhcb5)**
43.7	Xhhu12**
	Xpsmp2084
44.4	Xpsmp2008**
51.5	Xrgc920.1*
53.5	Xrgr642**
56.8	Xrgr3202*
61.9	Xrgc903.2*
67.8	Xpsm196.2
89.9	Xpsmp2086

R12

R8

R12

Fig. 1. (continued)

Linkage group 5

LDG 1B-10 x ICMP 85410 (F₂)

81B x ICMP 451 (F₂)

Fig. 1. (continued)

Linkage group 6

81B x ICMP 451 (F₂)

Distance (cM)	Marker Name
0.0	Xhhu33
17.0	Xpsmp2048
28.7	Xpsm202
35.9	Xpsm696
	R1
49.9	Xpsmp2213
50.2	Xpsm107
50.2	Xbnl13.05
51.1	Xpsr56(Caa)
51.1	Xhhu18
51.4	Xpsm713
51.4	Xpsmp2018
51.4	Xpsm1002
51.4	Xrgc601
51.4	Xpsm765
51.4	Xpsmp2248
51.4	Xrgc950.1
51.7	Xpsm97
51.7	Xpsr116(Pp)
52.1	Xumc63
52.8	Xpsm288
57.5	Xpsmp2270

LDG 1B-10 x ICMP 85410 (F₂)

Distance (cM)	Marker Name
0.0	Xpsm202
3.3	Xpsm459.1
5.3	Xpsm588
12.6	Xugt1*
13.0	Xpsm696*
22.2	Xpsm87.1
26.6	Xpsm516.1
26.6	Xpsm713
28.2	Xpsm514
28.2	Xpsr17.1
28.2	Xpsm95.1
28.6	Xpsm548
28.6	Xpsm575
29.7	Xpsr804(Sbp)
30.0	Xpsm81.2
30.3	Xpsm524
30.3	Xrgc949.2
30.3	Xpsm737
30.3	Xpsm579
64.9	Xpsm870**

Sy

Hi

Dom

Fig. 1. (continued)

Linkage group 7

LDG 1B-10 x ICMP 85410 (F₂)

81B x ICMP 451 (F₂)

451 map of SSR loci that have been mapped in other crosses is also presented. This shows that the distribution pattern of both types of SSR markers is similar, and comparable to that of RFLP markers.

The most interesting markers, from a genetic point of view, are the dozen or so that extended the original maps produced by Liu et al. (1994). At last, linkage was also obtained for the two-marker LG *Xpsm160 – Xpsm190* to LG 7. Interestingly, in most of the LGs, the distal markers mapped at a genetic distance of 30 cM or more from the core "centromeric cluster" (Fig. 1). In an attempt to fill these gaps, a pilot AFLP analysis was conducted on bulked lines that were either homozygous for the 81B allele in the *Xpsm866.1 – Xpsm464* interval (81B bulks) on LG 4 and heterozygous for the remainder of the genome or homozygous for the ICMP 451 allele in the target interval (ICMP 451 bulks) and again heterozygous for the remainder of the genome. AFLP bands that were differentially amplified in the 81B and ICMP 451 bulks were cloned and mapped as RFLP probes. From the six AFLP fragments cloned, three were tightly linked to *Xpsm866.1*, two mapped closely to *Xpsm464*, and only one marker, *Xpsm1003*, mapped in the actual gap (Fig. 1). This suggests that the gaps may be regions of high recombination that are largely devoid of markers. Confirmation of this hypothesis will need to await physical mapping of the markers to BAC clones.

Segregation distortion has been observed in both the interspecific and intervarietal maps. In the wild X cultivated crosses, most of the regions were skewed toward maintenance of the wild-wild and cultivated-cultivated nuclear-cytoplasmic allelic associations (Liu et al. 1996), a tendency that had previously been observed by Robert et al. (1991). In the intervarietal crosses, however, there appeared to be little correspondence between the regions that carried markers that deviated from Mendelian segregation ratios and/or the direction of the distortion (Table 1). The only exception may be LG 4, where distortion is present in the same region in four of the six crosses. The fact that distortion is observed in the (81B × ICMP 451) X BKM 1163 population, but not in the reciprocal BKM 1163 X (81B × ICMP 451) cross, suggests that gametophytic rather than sporophytic viability may have played a role in the preferential transmission of one type of parental alleles on LG 4.

8.2.3
Comparative Genetic Mapping in Pearl Millet

The incorporation of heterologous RFLP markers that had previously been mapped in rice and other grasses allowed the establishment of a pearl millet-rice comparative map (Devos et al. 2000) (Fig. 1). The pearl millet genome appears to have undergone many rearrangements since its divergence from rice some 60 million years ago. That most rearrangements have occurred in pearl millet rather than rice can be derived from the fact that the rice genome has remained largely colinear with the foxtail millet genome, a Panicoideae species closely related to pearl millet (Devos et al. 2000). Nevertheless, regions in which gene orders have remained conserved between pearl millet and rice can be identified. This allows the exploitation of the sequenced rice genome as a source of new markers or even candidate genes for traits that are of agronomic importance in millet. Drought tolerance, domestication, and stover quality are a few examples of traits that are under study in pearl millet and for which extensive data are available in rice and other cereal crops. Extrapolation of this information to pearl millet will greatly assist in the genetic analysis and agronomic improvement of this regionally very important cereal.

8.3
Quantitative Trait Loci (QTL) Analyses

8.3.1
Domestication Syndrome

Pearl millet is thought to have been domesticated some 4,000 to 5,000 years ago in sub-Saharan Africa (Harlan 1971). The main characters selected for during domestication include reduced tillering, nonshattering, increased panicle size, and larger seed size. QTL mapping of the differences observed in plant architecture, spike, and spikelet structure between wild and cultivated pearl millet has shown that the majority of the characters are controlled by a small number of loci with relatively large effect (Poncet et al. 2000). The domestication-related loci are mostly located on LGs 2, 5, 6, and 7 (Fig. 1), with the latter two LGs carrying most of the genes involved in spikelet architecture and shattering habit. The regions on LGs 6 and 7 that appear to have played a major role in the pearl mil-

Table 1. Chromosomal regions showing segregation distortion in six pearl millet crosses

Cross	LG	Region	Overrepresented allele
LGD 1-B-10 and ICMP 85410	1	*Xpsm756-Xpsm196.1*	H*
	2	*Xpsm672.2-Xpsm458*	H*
	4	*Xpsm716-Xpsm512*	ICMP 85410
	5	*Xpsm616-Xpsm735*	ICMP 85410
	7	*Xpsm618-Xpsm526*	ICMP 85410
(81B X ICMP 451) × BKM 1163	1	*Xpsm280-Xpsm858*	ICMP 451
	4	*Xpsm409-Xpsm56*	ICMP 451
	7	*Xpsm618-Xpsm526*	ICMP 451
BKM 1163 × (81B × ICMP 451)	None		
81B × ICMP 451	4	*Xpsmp2081-Xrgr642*	ICMP 451
ICMB 841 × 863B	3	*Xpsm325-Xpsmp2227*	ICMB 841
	6	*Xpsm459.1-Xwg110*	ICMB 841
PT 732B × P1449	2	*Xpsm706-Xpsm592*	P1449
	4	*Xspm716-Xrgc903.2*	PT 732B
	5	*Xpsm73.1-Xpsm749*	H

*Distortion most likely due to the presence of a translocation between LGs 1 and 2 that differentiates LGD 1-B-10 and
 ICMP 85410

let domestication process correspond to regions of the rice and maize genome that were also selected for during domestication (Poncet et al. 2002). Domestication clearly took place long after the divergence of rice, maize, and millet and thus occurred independently in the different cereal crops. The fact that potentially orthologous genes were selected for suggests that the number of genes controlling these traits is small, as has been suggested by the QTL studies, and/or that only a limited number of genes can be modified to give agronomically acceptable phenotypes.

8.3.2
Drought Tolerance

Enhancing drought tolerance is a main focus for many pearl-millet-breeding programs. Identification of the genomic regions involved in grain and stover yield production under drought conditions has been the target of several QTL studies (Yadav et al. 2002, 2003, 2004). In a first study, testcrosses of mapped progeny from the cross H 77/833-2 × PRLT 2/89-33 were grown in three separate field trials and exposed to terminal drought stress (Yadav et al. 2002). Control trials were grown under irrigation. All drought trials gave lower grain and biomass yields compared to the irrigated controls, and QTLs for these traits were identified on LGs 1, 2, 3, 4, 6, and 7. Two of the regions, on LGs

1 and 2, were associated with a higher grain yield or increased yield stability under terminal drought conditions. The LG 1 QTL appeared to act mainly when mild late-onset drought stress is applied, while the LG 2 QTL was effective under conditions of interrupted early-onset drought stress. The QTL on LG 2 could also improve yield stability under uninterrupted severe drought stress, although at the cost of stover yield when drought conditions were less severe (Yadav et al. 2002). An evaluation of hybrids derived from five different seed parents crossed to near-isogenic lines of H 77/833-2 that carried the drought resistance QTL from PRLT 2/89-33 on LG 2 showed a consistent yield advantage associated with the presence of the PRLT 2/89-33 LG 2 segment under a range of moisture regimes (Serraj et al. 2004).

A second drought study was conducted on testcrosses of mapped progeny from the cross ICMB 841 × 863B (Yadav et al. 2004). The plants were grown in replicated field trials during two consecutive years, and each trial was conducted in three environments – a nonstress (control) environment and early and late postflowering drought stress environments. The main genomic regions associated with grain yield were located on LGs 2, 5, and 7. These regions were also associated with higher stover yield, although the parental alleles contributing to increased grain and stover yield were different. This suggests that, under drought stress, higher grain yields were achieved

by repartitioning assimilates from stover to filling grain. Nevertheless, it may be possible to improve both grain and stover yield under stress conditions. In addition to the genomic regions on LGs 2, 5, and 7, two regions were identified on LGs 3 and 6 that affected harvest index and thus the partitioning of dry matter from stover to grains. Neither of these QTLs comapped with QTL for grain yield. Therefore, selection for parental alleles associated with reduced harvest index would lead to increased stover yields (Yadav et al. 2004).

8.3.3
Downy Mildew Resistance

The introduction of high-yielding but downy mildew susceptible pearl millet hybrids in India in the late 1960s led to devastating epidemics. Although *Sclerospora graminicola*, the fungus causing downy mildew, can be chemically controlled, host-plant resistance provides a more attractive and cost-efficient method of disease management. The prospective of enhancing classical breeding programs with marker-assisted selection for the introduction of downy mildew resistance into elite cultivars led to QTL studies to identify chromosomal regions associated with resistance (Jones et al. 1995, 2002). F_4 progeny from the cross LGD 1-B-10 × ICMP 85410 were screened either in the field or in a glasshouse for resistance to pathogen populations collected from pearl millet in Patancheru in India, Maiduguri in Nigeria, Bengou in Niger, and Doffane and Dimbetaba in Senegal (Jones et al. 1995). Major QTLs, explaining between 19.4 and 48.9% of the variation, were detected on LG 1 for the Indian pathogen population, on LG 2 for the Senegalese population, and on LG 4 for the populations from Niger and Nigeria. A number of putative QTLs of smaller effect were also identified. None of the QTLs was effective against all pathogen populations tested. A similar analysis of the effects of infection with a Patancheru (India) downy mildew population was carried out in two field and glasshouse screens on F_4 families derived from the cross 7042(S)-1 × P 7-3 (Jones et al. 2002). Again, a major QTL was detected on LG 1. It is expected that the same gene underlies the resistance in ICMP 85410 and P 7-3. A minor recessively inherited QTL on LG 2 was also identified in all four screens. Recessive inheritance is unusual for downy mildew, as most resistance QTLs were inherited in

a dominant fashion. In recent years, a number of other mapping populations, based on the parental lines ICMP 451, H77/833-2, PRLT 2/89-33, P310-17, W 504-1-1, P 1449-2, and PT 732, have been screened against multiple pathogen populations from Asia and Africa (Hash and Bramel-Cox 2000). Several of the identified resistance QTLs are being transferred to hybrid parental, maintainer, and pollinator lines using marker-assisted breeding.

8.3.4
Mendelization of QTLs

A BC_4F_1 line that was heterozygous for a small segment of LG 1, carrying the dominant downy mildew resistance QTL derived from the inbred line P7-3, in an 843B background was identified using the sequence-tagged-site markers PSMP567, PSMP461, and PSMP858. Selfing of this line generated a BC_4F_2 population of which 135 BC_4F_3 families (40 plants/family) were screened in a glasshouse for resistance to the downy mildew pathogen population from Patancheru, India (Allouis 2000). Using an inoculum concentration of 3×10^5 sporangia/ml, infection rates of 89.9% were obtained in the susceptible parent, 843B, while P7-3 remained disease free. The progeny could be classified unambiguously as homozygous resistant (<8% of infected seedlings over 3 replicates), homozygous susceptible (>60% infection), or heterozygous (15 to 30% infection). Downy mildew resistance mapped as a single Mendelian gene at the top of LG 1, flanked by the AFLP markers P71M20-1 (0.7 cM) and P64M21-3 (0.6 cM) (Allouis 2000).

8.4
Marker-Assisted Breeding

Marker-assisted selection (MAS) can be particularly useful for screening backcross progeny for the presence of apomixis-linked markers. Apomixis is a genetically controlled reproductive mechanism whereby a chromosomally unreduced egg cell can develop into an embryo without fertilization by a sperm cell (Koltunow 1993). Progeny of an apomictic plant are genetically identical to the maternal parent. The apomictic phenotype can only be determined at anthesis by clearing of ovules and microscopic examination of embryo sac development. Numerous

PCR-based markers [SCARs or sequence characterized amplified regions (Paran and Michelmore 1993)] have been identified that completely cosegregate with apomixis in the gametic contribution of *P. squamulatum* to an interspecific hybrid with pearl millet (Ozias-Akins et al. 1998, 2003). The trait for apomixis is transmitted at a low frequency (2 to 5%) in advanced backcross generations when pearl millet is used as the recurrent parent (Ozias-Akins et al. 1993); therefore, linked markers are useful for identifying the infrequently occurring apomictic plants prior to flowering (Hanna et al. 1993). The number of markers available for MAS for apomixis is large because the genomic region associated with this reproductive trait is physically large and highly hemizygous (many genomic sequences are found only on the chromosome transmitting apomixis and not on its homo(eo)logs) (Goel et al. 2003; Akiyama et al. 2004). Most traits for which breeders might use MAS have been introgressed not from a wild species but rather from another cultivar or subspecies of pearl millet. The level of polymorphism associated with traits of intraspecific origin will most likely be less.

Marker-assisted breeding has not yet been applied extensively in pearl millet. The technique has been widely used in maize with both isozyme- and DNA-based markers (Stuber 2001). MAS is most cost efficient for quantitative traits that have low heritabilities. An example of this in pearl millet is the application of MAS with RFLPs to the improvement of forage quality. Forage/stover quality characteristics often are assessed in vitro but need to be validated by testing for a correlation with ruminant nutritional quality in feeding trials. The characteristics that can be measured include in vitro dry matter digestibility, mineral content, protein content, and protein composition; however, all of these are subject to genotype × environment effects (Zerbini and Thomas 2003), which requires mapping studies to be carefully designed and replicated in different environments. An indirect assay for digestibility is the measurement of gas production during fiber digestion (Zerbini and Thomas 2003). A QTL accounting for ~20% of the variation in gas production was identified in a pearl millet mapping population and located at the top of LG 7 (Hash et al. 2003). The putative QTL for leaf-blade gas production has been transferred from the donor to the recipient by MAS, and near-isogenic lines containing the alternate forms of the gas production QTL are being produced. Such lines will facilitate testing in multiple environments for in vitro digestibility char-

acteristics as well as ruminant nutritional quality and performance. Similar studies for straw yield, drought tolerance, and disease resistance have been initiated (Hash et al. 2003).

Marker-assisted breeding in pearl millet could be used for several disease resistance traits, but probably would be cost effective only for QTL such as downy mildew (Jones et al. 1995, 2002) and slow-rusting or partial rust resistance (Wilson 1997). Witcombe and Hash (2000) described breeding strategies that could be facilitated by MAS and used to pyramid disease resistance genes and reduce the probability of a breakdown in resistance. MAS can now be more effectively applied to large populations of pearl millet with the development of PCR-based markers such as SSRs and SNPs.

8.5
Map-Based Cloning

Map-based cloning has not yet been achieved in pearl millet, although one group is approaching success (Faure et al. 2002). Two bacterial artificial chromosome (BAC) libraries have been constructed recently with several-fold coverage of the pearl millet genome. One library is from Tift 23DB and contains 159,100 clones with an average insert size of 90 kb and totaling 14,200 Mb of genomic DNA (Allouis et al. 2001). The estimated 5.8-haploid genome equivalents was verified by the average number of clones (7.2) identified with 12 probes. This library will be useful for map-based cloning of agriculturally important genes. A second library has been constructed from an interspecific hybrid between pearl millet and *P. squamulatum* for the purpose of analyzing the genomic region linked with apomixis (Roche et al. 2002). The hybrid plant was polyhaploid with 7 chromosomes from pearl millet and 14 from *P. squamulatum* (Dujardin and Hanna 1983, 1986). From the published data we can estimate that the library contains ~1.9 haploid genome equivalents of pearl millet, genotype Tift 239DB.

Whether candidate genes are cloned using positional information or expressed sequences, it will be necessary to verify their gene action by complementation of a null phenotype. This can only be accomplished by transformation of pearl millet. Pearl millet, being a minor cereal, has not received the highest priority for transformation research even though it was one of the first cereals for which tissue culture meth-

ods were extensively developed (Vasil 1987). Repellin et al. (2001) reviewed the state of cereal transformation and listed numerous examples for rice, maize, wheat, barley, and oat, but pearl millet was notably absent. Only recently have reports emerged that describe methods for the recovery of transgenic pearl millet plants using hygromycin resistance (Lambe et al. 2000), phosphinothricin resistance (Girgi et al. 2002; Goldman et al. 2003), or phosphomannose isomerase (O'Kennedy et al. 2004) as selectable marker genes. The highest transformation frequency achieved thus far (~3 transgenic lines per bombardment on average) has been with the use of "shaved" inflorescences as the explant for microprojectile bombardment followed by phosphinothricin selection (Goldman et al. 2003). Insufficient selection pressure with this herbicide can lead to the recovery of a high frequency of escapes as shown by Girgi et al. (2002), who recovered mostly (99%) escapes. The escape frequency was less (20%) with phosphomannose isomerase as the selectable marker gene when immature zygotic embryos were bombarded (O'Kennedy et al. 2004), but this is still an undesirable level. The highest transformation frequency reported using currently published protocols is sufficient for testing of candidate genes, but remains too low for the use of pearl millet transformation in high-throughput functional genomics projects.

8.6
Future Scope of Work

Apomictic millet could revolutionize the opportunities for capturing hybrid vigor and producing unique genotypes through true-breeding hybrids (Hanna 1995). True-breeding hybrids would make hybrid grain production more feasible in African countries where infrastructure for hybrid seed distribution is lacking. Opportunities exist for improving processing of grain and developing new products from the grain. The multiple uses of pearl millet provide opportunities for improvement of not only grain but also vegetative characteristics. Forage and stover quality could be greatly improved with the low-lignin, brown-midrib mutants (Cherney et al. 1988), although effects on yield must also be considered. Molecular biology, including gene transfer, offers the prospect to discover and more directly introduce value-added traits into locally adapted cultivars. Although diverse ge-

netic resources and breeding techniques are making major contributions to pearl millet improvement, international pricing and markets need to be developed for opportunities in pearl millet to be fully realized (National Research Council 1996).

References

Akiyama Y, Conner JA, Goel S, Morishige DT, Mullet JE, Hanna WW, Ozias-Akins P (2004) High-resolution physical mapping in *Pennisetum squamulatum* reveals extensive chromosomal heteromorphism of the genomic region associated with apomixis. Plant Physiol 134:1733–1741

Allouis S (2000) Construction of a bacterial artificial chromosome library and gene targeting in pearl millet. PhD dissertation, University of East Anglia, UK

Allouis S, Qi X, Lindup S, Gale MD, Devos KM (2001) Construction of a BAC library of pearl millet, *Pennisetum glaucum*. Theor Appl Genet 102:1200–1205

Anand Kumar K, Andrews DJ (1993) Genetics of quantitative traits in pearl millet: a review. Crop Sci 33:1–20

Azhaguvel P, Hash CT, Rangasamy P, Sharma A (2003) Mapping the d_1 and d_2 dwarfing genes and the purple foliage color locus P in pearl millet. J Hered 94:155–159

Banuett-Bourrillon F (1982) Linkage of the alcohol dehydrogenase structural genes in pearl millet (*Pennisetum typhoides*). Biochem Gen 20:359–367

Bennett MD, Smith JB (1976) Nuclear DNA amounts in angiosperms. Phil Trans Roy Soc Lond Ser B 274:227–274

Bernatzky R, Tanksley SD (1986) Toward a saturated linkage map in tomato based on isozymes and random cDNA sequences. Genetics 112:887–898

Bertin I, Zhu JH, Gale MD, (2005) SSCP-SNP in pearl millet – a new marker system for comparative genetics. Theor Appl Genet 110:1467–1472

Botstein D, White RL, Skolnick M, Davis RW (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet 32:314–331

Brunken JN (1977) A systematic study of *Pennisetum* sect. *Pennisetum* (Gramineae). Am J Botany 64(2):161–176

Burton GW (1962) Registration of varieties of other grasses: Gahi I pearl millet (Reg. No. 6). Crop Sci 2:355–356

Busso CS, Liu CJ, Hash CT, Witcombe JR, Devos KM, de Wet JMJ, Gale MD (1995) Analysis of recombination rate in female and male gametogenesis in pearl millet (*Pennisetum glaucum*) using RFLP markers. Theor Appl Genet 90:253–257

Causse MA, Fulton TM, Cho YG, Ahn SN, Chunwongse J, Wu K, Xiao J, Yu Z, Ronald PC, Harrington SE, Second G, McCouch SR, Tanksley SD (1994) Saturated molecular map of the rice genome based on an interspecific backcross population. Genetics 138:1251–1274

Chao S, Sharp PJ, Worland AJ, Warham EJ, Koebner RMD, Gale MD (1989) RFLP-based genetic maps of wheat homoeologous group 7 chromosomes. Theor Appl Genet 78:495–504

Cherney JH, Axtell JD, Hassen MM, Anliker KS (1988) Forage quality characterization of chemically induced brown midrib mutant pearl millet. Crop Sci 28:783–787

Chhabra AK, Behl RK, Bassam NE (2001) Role of isozymes in pearl millet improvement (*Pennisetum glaucum*). Landbauforschung Volkenrode 4:165–173

Dave HR (1987) Pearl millet hybrids. In: Witcombe JR, Beckerman SR (eds) Proc Int Pearl Millet Workshop, ICRISAT, Patancheru, India, pp 121–126

Davis GL, McMullen MD, Baysdorfer C, Musket T, Grant D, Staebell M, Xu G, Polacco M, Koster L, MeliaHancock S, Houchins K, Chao S, Coe EH (1999) A maize map standard with sequenced core markers, grass genome reference points and 932 expressed sequence tagged sites (ESTs) in a 1,736-locus map. Genetics 152:1137–1172

de Vicente MC, Tanksley SD (1991) Genome-wide reduction in recombination of backcross progeny derived from male versus female gametes in an interspecific cross of tomato. Theor Appl Genet 83:173–178

Dendy DA (1995) Sorghum and millets: production and importance. In: Denby DAV (ed) Sorthum and Millets: Chemistry and Technology. American Association of Cereal Chemists, St. Paul, MN, pp 11–26

Devos KM, Pittaway TS, Reynolds A, Gale MD (2000) Comparative mapping reveals a complex relationship between the pearl millet genome and those of foxtail millet and rice. Theor Appl Genet 100:190–198

Donis-Keller H, Green P, Helms C, Cartinhour S, Weiffenbach B, Stephens K, Keith TP, Bowden DW, Smith DR, Lander ES, Botstein D, Akots G, Rediker KS, Gravius T, Brown VA, Rising MB, Parker C, Powers JA, Watt DE, Kauffman ER, Bricker A, Phipps P, Mullerkahle H, Fulton TR, Ng S, Schumm JW, Braman JC, Knowlton RG, Barker DF, Crooks SM, Lincoln SE, Daly MJ, Abrahamson J (1987) A genetic-linkage map of the human genome. Cell 51:319–337

Dujardin M, Hanna WW (1983) Apomictic and sexual pearl millet × *Pennisetum squamulatum* hybrids. J Hered 74:277–279

Dujardin M, Hanna W (1986) An apomictic polyhaploid obtained from a pearl millet × *Pennisetum squamulatum* apomictic interspecific hybrid. Theor Appl Genet 72:33–36

Faure S, Allouis S, Breese W, Devos KM (2002) Fine mapping a downy mildew resistance gene in pearl millet. http://www.intl-pag.org/pag/10/abstracts/PAGX_P447.html

Gale MD, Atkinson MD, Chinoy CN, Harcourt RL, Jia J, Li QY, Devos KM (1995) Genetic maps of hexaploid wheat. In: Li ZS, Xin ZY (eds) Proc 8th Int Wheat Genet Symp. China Agricultural Scientech Press, Beijing, pp 29–40

Gaut BS, Clegg MT (1993) Nucleotide polymorphism in the *Adh*1 locus of pearl millet (*Pennisetum glaucum*) (Poaceae). Genetics 135:1091–1097

Gebhardt C, Ritter E, Debener T, Schachtschabel U, Walkemeier B, Uhrig H, Salamini F (1989) RFLP analysis and linkage mapping in *Solanum tuberosum*. Theor Appl Genet 78:65–75

Gepts P, Clegg MT (1989) Genetic diversity in Pearl Millet (*Pennisetum glaucum* [L.] R. Br.) at the DNA sequence level. J Hered 80:203–208

Girgi M, O'Kennedy MM, Morgenstern A, Mayer G, Lorz H, Oldach KH (2002) Transgenic and herbicide resistant pearl millet (*Pennisetum glaucum* L.) R.Br. via microprojectile bombardment of scutellar tissue. Mol Breed 10:243–252

Goel S, Chen Z, Conner JA, Akiyama Y, Hanna WW, Ozias-Akins P (2003) Physical evidence that a single hemizygous chromosomal region is sufficient to confer aposporous embryo sac formation in *Pennisetum squamulatum* and *Cenchrus ciliaris*. Genetics 163:1069–1082

Goldman JJ, Hanna WW, Fleming GH, Ozias-Akins P (2003) Fertile transgenic pearl millet [*Pennisetum glaucum* (L.) R. Br.] plants recovered through microprojectile bombardment and phosphinothricin selection of apical meristem-, inflorescence-, and immature embryo-derived embryogenic tissues. Plant Cell Rep 21:999–1009

Graf JD (1989) Genetic-mapping in Xenopus-Laevis – 8 linkage groups established. Genetics 123:389–398

Hanna WW (1995) Use of apomixis in cultivar development. Adv Agron 54:333–350

Hanna WW, Burton GW (1992) Genetics of red and purple plant color in pearl millet. J Hered 83(5):386–388

Hanna WW, Burton GW, Powell JB (1978) Genetics of mutagen induced non-lethal chlorophyll mutants in pearl millet. J Hered 69:273–274

Hanna WW, Dujardin M, Ozias-Akins P, Lubbers EL, Arthur L (1993) Reproduction, cytology and fertility of pearl millet × *Pennisetum squamulatum* BC$_4$ plants. J Hered 84:213–216

Hanna WW, Hill GM, Gates RN, Wilson JP, Burton GW (1997) Registration of Tifleaf 3 pearl millet. Crop Sci 37:1388

Hanna WW, Rai KN (1999) Inbred Line Development. In: Khairwal IS, Rai KN, Andrews DJ, Harinarayana G (eds) Pearl Millet Breeding. Science Publishers, Enfield, pp 257–267

Hanna W, Wilson J, Timper P (2005a) Registration of pearl millet parental lines tift 99D$_2$A$_1$/B$_1$ Crop Sci 45:2671

Hanna W, Wilson J, Timper P (2005b) Registration of pearl millet parental line tift 454 Crop Sci 45:2670–2671

Hanna W, Wilson J, Timper P, Hill G, Buntin D (2005) Registration of TifGrain 102 pearl millet. Crop Sci 45 (in press)

Harlan JR (1971) Agricultural origins: centers and non-centers. Science 174:468–474

Harlan JR (1975) Crops and Man. American Society of Agronomy, Madison, WI

Harlan JR, de Wet JMJ (1971) Toward a rational classification of cultivated plants. Taxon 20:509–517

Harushima Y, Yano M, Shomura A, Sato M, Shimano T, Kuboki Y, Yamamota T, Lin SY, Antonio BA, Parco A, Kajiya H, Huang N, Yamamoto K, Nagamura Y, Kurata N, Khush GS,

Sasaki T (1998) A high density-rice genetic linkage map with 2,275 markers using a single F$_2$ population. Genetics 148:479–494

Hash C, Bramel-Cox P (2000) Marker applications in pearl millet. In: Haussman BIG, Geiger HH, Hess DE, Hash CT, Bramel-Cox PJ (eds) Application of Molecular Markers in Plant Breeding. ICRISAT, Patancheru, India, pp 113–127

Hash CT, Bhasker Raj AG, Lindup S, Sharma A, Beniwal CR, Folkertsma RT, Mahalakshmi V, Zerbini E, Blu M (2003) Opportunities for marker-assisted selection (MAS) to improve the feed quality of crop residues in pearl millet and sorghum. Field Crops Res 84:79–88

Helentjaris T, Slocum M, Wright S, Schaefer A, Nienhuis J (1986) Construction of genetic linkage maps in maize and tomato using restriction fragment length polymorphisms. Theor Appl Genet 72:761–769

Hitchcock AS (1950) Manual of grasses of the United States. US Dept of Agriculture, Washington, DC

Jauhar PP (1981) Cytogenetics and breeding of pearl millet and related species. Liss, New York

Jauhar PP, Hanna WW (1998) Cytogenetics and breeding of pearl millet and related species. Adv Agron 64:1–26

Johnson KR, Wright JE, May B (1987) Linkage relationships reflecting ancestral tetraploidy in salmonid fish. Genetics 116:579–591

Jones ES, Liu CJ, Gale MD, Hash CT, Witcombe JR (1995) Mapping quantitative trait loci for downy mildew resistance in pearl millet. Theor Appl Genet 91:448–456

Jones ES, Breese WA, Liu CJ, Singh SD, Shaw DS, Witcombe JR (2002) Mapping quantitative trait loci for resistance to downy mildew in pearl millet: Field and glasshouse screens detect the same QTL. Crop Sci 42:1316–1323

Koltunow AM (1993) Apomixis: embro sacs and embryos formed without meiosis or fertilization in ovules. Plant Cell 5:1425–1437

Koul KK, Nagpal R, Sharma A (2000) Chromosome behaviour in the male and female sex mother cells of wheat (*Triticum aestivum* L.), oat (*Avena sativa* L.) and pearl millet (*Pennisetum americanum* (L.) Leeke). Caryologia 53:175–183

Lambe P, Dinant M, Deltour R (2000) Transgenic pearl millet (*Pennisetum glaucum*). Biotechnology in Agriculture and Forestry Vol 46 (Bajaj, YPS). Springer, Berlin Heidelberg New York, pp 84–108

Landry BS, Kesseli RV, Farrara B, Michelmore RW (1987) A genetic map of lettuce (*Lactuca sativa* L.) with restriction fragment length polymorphism, isozyme, disease resistance and morphological markers. Genetics 116:331–337

Laurie DA, Bennett MD (1985) Nuclear DNA content in the genera Zea and Sorghum. Intergeneric, interspecific and intraspecific variation. Heredity 55:307–313

Liu CJ, Witcombe JR, Pittaway TS, Nash M, Busso CS, Hash CT, Gale MD (1994) An RFLP-based genetic map of pearl millet (*Pennisetum glaucum*). Theor Appl Genet 89:481–487

Liu CJ, Devos KM, Witcombe JR, Pittaway TS, Gale MD (1996) The effect of genome and sex on recombination rates in *Pennisetum* species. Theor Appl Genet 93:902–908

Malhotra S, Dhindsa KS (1984) Chemical composition an dother nutritive characteristics of bajra (*Pennisetum typhoides* L.) – a review. Int J Trop Agric 2:271–286

Martel E, DeNay E, Siljak-Yakovlev S, Brown S, Sarr A (1997) Genome size variation and basic chromosome number in pearl millet and fourteen related *Pennisetum* species. J Hered 88:139–143

McCouch SR, Kochert G, Yu ZH, Wang ZY, Khush GS, Coffman WR, Tanksley SD (1988) Molecular mapping of rice chromosomes. Theor Appl Genet 76:815–829

Morgan RN, Alvernaz J, Arthur L, Hanna WW, Ozias-Akins P (1997) Genetic characterization and floral development of female sterile and stubby head, two aposporous mutants of pearl millet. Sex Plant Reprod 10:127–135

Morgan RN, Wilson JP, Hanna WW, Ozias-Akins P (1998) Molecular markers for rust and pyricularia leaf spot disease resistance in pearl millet. Theor Appl Genet 96:413–420

Murty DS, Kumar KA (1995) Traditional uses of sorghum and millets. In: Denby DAV (ed) Sorghum and Millet: Chemistry and Technology. American Association of Cereal Chemists, St. Paul, MN, pp 185–221

National Research Council (1996) Lost Crops of Africa. Vol 1: Grains. National Academy Press, Washington, DC

O'Kennedy MM, Burger JT, Botha FC (2004) Pearl millet transformation system using the positive selectable marker gene phosphomannose isomerase. Plant Cell Reprod 22:684–690

Ozias-Akins P, Lubbers EL, Hanna WW, McNay JW (1993) Transmission of the apomictic mode of reproduction in *Pennisetum*: Co-inheritance of the trait and molecular markers. Theor Appl Genet 85:632–638

Ozias-Akins P, Roche D, Hanna WW (1998) Tight clustering and hemizygosity of apomixis-linked molecular markers in *Pennisetum squamulatum* implies genetic control of apospory by a divergent locus which may have no allelic form in sexual genotypes. Proc Natl Acad Sci USA 95:5127–5132

Ozias-Akins P, Akiyama Y, Hanna WW (2003) Molecular characterization of the genomic region linked with apomixis in *Pennisetum/Cenchrus*. Funct Integr Genom 3:94–104

Paran I, Michelmore RW (1993) Development of reliable PCR-based markers linked to downy mildew resistance genes in lettuce. Theor Appl Genet 85:985–993

Poncet V, Lamy F, Enjalbert J, Joly H, Sarr A, Robert T (1998) Genetic analysis of the domestication syndrome in pearl millet (*Pennisetum glaucum* L., Poaceae): inheritance of the major characters. Heredity 81:648–658

Poncet V, Lamy F, Devos KM, Gale MD, Sarr A, Robert T (2000) Genetic control of domestication traits in pearl millet (*Pennisetum glaucum* L., Poaceae). Theor Appl Genet 100:147–159

Poncet V, Martel E, Allouis S, Devos KM, Lamy F, Sarr A, Robert T (2002) Comparative analysis of QTLs affecting domestication traits between two domesticated × wild pearl millet (*Pennisetum glaucum* L., *Poaceae*) crosses. Theor Appl Genet 104:965–975

Qi X, Lindup S, Pittaway TS, Allouis S, Gale MD, Devos KM (2001) Development of simple sequence repeat markers from bacterial artificial chromosomes without subcloning. Biotechniques 31:355–362

Qi X, Pittaway T, Lindup S, Liu H, Waterman E, Padi F, Hash C, Zhu J, Gale M, Devos K (2004) An integrated genetic map and a new set of simple sequence repeat markers for pearl millet, *Pennisetum glaucum*. Theor Appl Genet 109:1485–1493

Rao PK, Nambiar AK, Manon PM (1951) Maximization of production by cultivation hybrid stains with special reference to Cumbu (pearl millet). Madras Agric J 38:95–100

Renno JF, Bezancon G, Bonnefous F, Winkel T (1997) Experimental study of gene flow between wild and cultivated *Pennisetum glaucum*. Can J Bot 75:925–931

Repellin A, Baga M, Jauhar PP, Chibbar RN (2001) Genetic enrichment of cereal crops via alien gene transfer: new challenges. Plant Cell Tiss Org Cul 64:159–183

Robert T, Lespinasse R, Pernes J, Sarr A (1991) Gametophytic competition as influencing gene flow between wild and cultivated forms of pearl millet. Genome 34:82–88

Robertson DS (1984) Different frequency in the recovery of cross-over products from male and female gametes of plants hypoploid for B-A translocations in maize. Genetics 107:117–130

Roche DR, Conner JA, Budiman MA, Frisch D, Wing R, Hanna WW, Ozias-Akins P (2002) Construction of BAC libraries from two apomictic grasses to study the microcolinearity of their apospory-specific genomic regions. Theor Appl Genet 104:804–812

Roshevits R (1980) Grasses: an introduction to the study of fodder and cereal grasses. Indian National Scientific Documentation Center, New Delhi

Sandmeier M (1993) Selfing rates of pearl millet (*Pennisetum typhoides* Stapf and Hubb.) under natural conditions. Theor Appl Genet 86:513–517

Serna-Saldivar S, Rooney LW (1995) Structure and chemistry of sorghum and millets. In: Dendy, DAV (ed) Sorghum and millers. American Association of Cereal Chemists, St. Paul, MN, pp 69–124

Serraj R, Hash C, Yadav R, Bidinger F (2004) Recent advances in the marker-assisted selection from drought tolerance in pearl millet. http://www.cropscience.org.au/icsc2004/poster/1/1/561_serrajr.htm

Shetty H, Vasanthi N, Sarosh B, Kini K (2001) Inheritance of downy mildew resistance, b-1,3-glucanases and peroxidases in pearl millet [*Pennisetum glaucum* (L.) R. Br.] crosses. Theor Appl Genet 102:1221–1226

Stapf O, Hubbard CE (1934) Pennisetum. In: Prain D (ed) Flora of Tropical Africa. Reeve, Ashford, pp 954–1070

Stuber CW (2001) Breeding multigenic traits. In: Phillips RL, Vasil IK (eds) DNA-Based Markers in Plants. Kluwer, Dordrecht, pp 115–137

Tanksley S, Young N, Paterson A, Bonierbale M (1989) RFLP mapping in plant-breeding – new tools for an old science (review). Biotechnology 7:257–264

Tostain S (1985) Mise en evidence d'une liaison genetique entre un gene de nainisme et des margueurs enzymatiques chez le mil penicillaire (*Pennisetum glaucum* L.). Can J Genet Cytol 27:751–758

Tostain S (1992) Enzyme diversity in pearl millet (*Pennisetum glaucum* L) 3. Wild millet. Theor Appl Genet 83:733–742

Tostain S, Riandey M-F, Marchais L (1987) Enzyme diversity in pearl millet (*Pennisetum glaucum* L.): I. West Africa. Theor Appl Genet 74:188–193

Tyagi AK, Khurana JP, Khurana P, Raghuvanshi S, Gaur A, Kapur A, Gupta V, Kumar D, Ravi V, Vij S, Khurana P, Sharma S (2004) Structural and functional analysis of rice genome. J Genet 83:79–99

Vasil IK (1987) Developing cell and tissue culture systems for the improvement of cereal and grass crops. J Plant Physiol 128:193–218

Wilson JP (1995) Mechanisms associated with the TR allele contributing to reduced smut susceptibility of pearl millet. Phytopathology 85:966–969

Wilson JP (1996) A recessive homeotic mutant in pearl millet. J Hered 87:66–67

Wilson JP (1997) Inheritance of partial rust resistance in pearl millet. Plant Breed 116:239–243

Wilson JP, Hanna WW (1998) Smut resistance and grain yield of pearl millet hybrids near isogenic at the Tr locus. Crop Sci 38:649–651

Witcombe JR (1999) Population improvement. In: Khairwal IS, et al. (eds) Pearl Millet Breeding. Oxford & IBH Publishing, New Delhi, pp 213–256

Witcombe JR, Hash CT (2000) Resistance gene deployment strategies in cereal hybrids using marker-assisted selection: Gene pyramiding, three-way hybrids, and synthetic parent populations. Euphytica 112:175–186

Yadav RS, Hash CT, Bidinger FR, Cavan GP, Howarth CJ (2002) Quantitative trait loci associated with traits determining grain and stover yield in pearl millet under terminal drought stress conditions. Theor Appl Genet 104:67–83

Yadav RS, Bidinger FR, Hash CT, Yadav YP, Yadav OP, Bhatnagar SK, Howarth CJ (2003) Mapping and characterization of QTL X E interactions for traits determining grain and stover yield in pearl millet. Theor Appl Genet 106:512–520

Yadav RS, Hash CT, Bidinger FR, Devos KM, Howarth CJ (2004) Genomic regions associated with grain yield and aspects of post-flowering drought tolerance in pearl millet across stress environments and tester background. Euphytica 136:265–277

Young ND (2001) Constructing a plant genetic linkage map with DNA markers. In: Phillips RL, Vasil IK (eds) DNA-Based Markers in Plants. Kluwer, Dordrecht, pp 31–47

Zach B, Klee M (2003) Four thousand years of plant exploitation in the Chad Basin of NE Nigeria II: discussion on the morphology of caryopses of domesticated *Pennisetum* and complete catalogue of the fruits and seeds of Kursakata. Veget Hist Archaeobot 12:187–204

Zerbini E, Thomas D (2003) Opportunities for improvement of nutritive value in sorghum and pearl millet residues in South Asia through genetic enhancement. Field Crops Res 84:3–15

9 Foxtail Millet

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9.1
Introduction

Millet is a generic term that refers to several small-seeded cereal crop species, most of them belonging to the panicoideae subfamily and the paniceae tribe of the Poaceae family. The main millet crops are pearl millet (*Pennisetum glaucum*), foxtail millet (*Setaria italica*), proso millet (*Panicum miliaceum*), and finger millet (*Eleusine coracana*). Taken together, these four species produced nearly 30 million tons (Mt) of grain in 2003 (FAOstat data 2004, http://faostat.fao.org/), which ranks them sixth among cereals, after maize (640 Mt), rice (590 Mt), wheat (550 Mt), barley (140 Mt), and sorghum (60 Mt). Millets are cultivated primarily on marginal lands in dry areas in temperate, subtropical, and tropical regions. Although their contribution to global food production is small (1.5% of cereal production), millets are essential for the sustainability of food production in many developing countries (particularly in drought-prone areas). Millets are indeed better adapted to dry, adverse soils than most other crops and are therefore often cultivated in difficult conditions as an alternative to maize or even sorghum. Most millets have strong, deep root systems and short life cycles and can grow rapidly when water is available. As a result, they can survive and reliably produce small quantities of grain in areas where mean annual precipitation is as low as 300 mm. This compares with a minimum water requirement of 400 mm for sorghum and 500 to 600 mm for maize.

Foxtail millet, *S. italica* (L.) Beauv., is grown primarily in China mostly for human consumption and also for forage. In Europe, its cultivation has been documented as far back as 4,000 years ago (Marinval 1992). It was traditionally grown as a summer crop until the 17th century, when it was gradually replaced by maize. It has now become a marginal crop on this continent, where it is used mainly for bird feed, game forage, and, to a smaller extent, for human consumption. Central Europe is today the main area of production (15,000 ha in 2003; FAOstat data 2004, http://faostat.fao.org/).

Setaria genus is known not only for the domesticated form *S. italica*, but also for several of its wild relatives: the green (*S. viridis*), yellow (*S. glauca*), giant (*S. faberi*), knotroot (*S. geniculata*), and bristly (*S. verticillata*) foxtails. Altogether, these five species constitute the worst weed group, causing a significant loss of agricultural production worldwide (Dekker 2003). Wild foxtails are characterized by seed dormancy, strong phenotypic plasticity, and tolerance to both mechanical and chemical (herbicide) damages. Interestingly, green foxtail (*S. viridis*) is the ancestor of the domesticated form *S. italica* (see below), and both species are still interfertile. Therefore, the genetic mapping of these two species (which form only one species from a genetic point of view) has two prospects: to improve the domesticated form and to help control the weedy form.

In this chapter, we will first provide a description of the *Setaria* species complex, including the genetic relationships among *Setaria* species. We will then give an overview of the genetic and molecular mapping of foxtail millet and finally conclude with some research perspectives, particularly in the context of the recent development of comparative genomics in cereals.

9.2
Setaria Complex

The genus *Setaria* comprises 125 species widely distributed throughout the world in both tropical and temperate climates. *Setaria* species have diverse breeding systems (self- to cross-pollinated) and life cycles (annual or perennial). The primary gene pool of foxtail millet is composed of both *S. italica*, the cultivated form, and *S. viridis* (the green foxtail)

Table 1. Ploidy level and DNA content (when available) of 18 *Setaria* species. For life cycle, A and P are ascribed to annual and perennial, respectively (data from Le Thierry d'Ennequin et al. 1998)

Species	Gene pool	Chromosome number and ploidy level	Genome size (pg/2C)	Life cycle
S. italica	I	$2x = 18$	1.03/1.04/1.02/1.03	A
S. viridis	I	$2x = 18$	1.04/1.03/1.00	A
S. verticillata	II	$4x = 36$	n/a	A
S. adhaerans	II	$2x = 18$	n/a	A
S. faberii	II	$4x = 36$	n/a	A
S. pumila	III	$6x = 54$	5.14/5.26	A
S. geniculata	III	$4x = 36$	n/a	A
S. holstii	III	$4x = 36$	1.7	P
S. woodii	III	$2x = 18$	1.66	n/a
S. chevalieri	III	$4x = 36$	4.46	P
S. incrassata	III	$4x = 36$	4.23	n/a
S. leiantha	III	$4x = 36$	2.40	n/a
S. neglecta	III	$4x = 36$	3.50	n/a
S. palmifolia	III	$4x = 36$	3.88	P
S. parviflora	III	$4x = 36$	4.82	P
S. queenslandica	III	$4x = 36$	2.76	n/a
S. sphacelata	III	$4x = 36$	2.06/3.31	P
S. macrostachya	III	$6x = 54$	3.60	P

its closest wild relative (Harlan and De Wet 1971; Jusuf and Pernes 1985). The secondary gene pool is composed of the three more remotely related wild species: *S. adhaerans* and the two weeds *S. verticillata* and *S. faberi*. Finally, the tertiary gene pool contains the remaining wild *Setaria* species (Table 1).

The genetic relationships within *Setaria* genus have only been studied in the primary and secondary gene pools. Cytogenetic studies have shown that two genome types (A and B) are present (Li et al. 1942, 1945): *S. italica* and *S. viridis* are diploid AA species, *S. adhaerans* is diploid BB, while *S. verticillata* and *S. faberii* are tetraploid AABB species. This was recently confirmed by fluorescent in situ hybridization (FISH) analysis using the 5S and the 18S-5.8S-25S rDNA genes as probes (Benabdelmouna et al. 2001a) and also by genomic in situ hybridization (GISH) analysis (Benabdelmouna et al. 2001b). Unfortunately, no data are available regarding the genome types found in the species of the tertiary gene pool. Le Thierry d'Ennequin et al. (1998) have determined the nuclear DNA content of several *Setaria* species using flow cytometry (Table 1). This study showed that the diploid A genome species have a size of 0.5 pg/1C, which is comparable to that of rice, making *S. italica* one of the smallest genomes among cereal species and the

smallest in the panicoid subfamily to which maize, sorghum, and pearl millet belong.

The genetic relationships within the primary gene pool have been quite well documented using a variety of molecular techniques. The most detailed study of the structure of *S. italica* genetic diversity and of the genetic relationships between the cultivated form and its wild relative *S. viridis* has been reported by Jusuf and Pernes (1985). The authors analyzed 222 varieties of *S. italica* and 45 accessions of *S. viridis* using 10 polymorphic isozymic loci. Their results suggest that the cultivated gene pool can be classified into three main cultivar groups: a Chinese (accessions from China, Japan, and Korea), a tropical (varieties from Okinawa, Taiwan, India, and Kenya), and a European group. China represents the largest center of diversity of the crop, with many wild populations of *S. viridis* that are closely related to cultivated varieties. China therefore can be considered the main center of domestication of foxtail millet. In addition, the authors show that the European cultivated foxtail millet is closer genetically to the European accessions of *S. viridis* than to the Chinese ones. Therefore, they concluded that a secondary center of domestication of the crop is located in Europe, although not ruling out the hypothesis of a single domestication center,

since the close relatedness of *S. italica* and *S. viridis* in Europe could be the result of gene flows between the two forms posterior to the domestication. As mentioned earlier, *S. viridis* is a very common weed that has now spread to almost all the temperate agrosystems. In addition, Wang et al. (1997) have shown that despite a low outcrossing rate (less than 2%), cross-pollination can occur in vivo as far as 24 m away (40 m in the case of a male sterile receiver).

More recent diversity studies using different marker systems do not support the hypothesis of two domestication centers. Fukunaga et al. (2002a) used 16 RFLP single-copy probes to test 62 landraces and proposed a classification into five groups (which are in fact in accordance with the classification of Jusuf and Pernes described above). Le Thierry d'Ennequin et al. (2000) tested 39 accessions of *S. italica* and 22 accessions of *S. viridis* with 160 polymorphic AFLP loci and failed to identify a clear structure of the diversity of either the cultivated or the wild accessions. Their data therefore support the hypothesis of a single center of origin of the crop in China. Previous studies involving the use of RAPD markers (Schontz and Rether 1999), RFLP markers using an rDNA probe (Schontz and Rether 1998), or the analysis of either the waxy gene (Fukunaga et al. 2002b) or the prolamine genes (Nakayama et al. 1999) provided more data on the molecular characterization of foxtail millet germplasm but failed to strongly support either of the two hypotheses (one vs. two centers of origin) regarding the domestication of the crop.

9.3
Molecular Maps of Foxtail Millet

The first comprehensive molecular maps of the species were reported by Wang et al. (1998). Two maps were constructed, one based on an intraspecific cross (Longgu25 × Pagoda flower green, two cultivars of *S. italica*) and the other based on a *S. italica* (cv. B100) × *S. viridis* (acc. A10) interspecific cross. In both cases, F$_2$ populations were used for linkage analyses. The two crosses generated similar map topologies, both in terms of marker order and genetic distances. Figure 1 shows a map of the interspecific cross. Nine linkage groups were obtained, corresponding to the nine chromosomes of the species (*S. italica* and *S. viridis* are 2n = 2x = 18). Linkage groups were assigned to chromosomes using

trisomic millet lines. The overall genetic distance of the map was found equal to 777 cM and 727 cM for the intra- and interspecific crosses, respectively. This is significantly shorter than the linkage map of rice (i.e., 1,520 cM, Harushima et al. 1998). However, the difference may simply be due to the contrasting levels in marker saturation between the maps. The rice map is comprised of 2,275 markers, while the millet map contains 160 markers.

The discovery, more than 10 years ago, of the conservation of the order of genetic markers on the genetic maps of maize and rice (Ahn and Tanksley 1993) opened new perspectives in the exploitation of genomic resources across the Poaceae family and particularly among cereal species (Gale and Devos 1998). In 1998, Devos et al. gathered and compiled enough cross-mapping data to align the molecular maps of seven cereal species, including foxtail millet. An anchored millet RFLP map was then published concomitantly with the two maps described above (Devos et al. 1998). The authors have added five markers from wheat (*Triticum aestivum*) and 111 markers from rice (*Oryza sativa*) onto the existing foxtail millet map (based on the interspecific cross), resulting in a map comprising 257 loci spanning 1,050 cM.

9.4
Mapping Genetic Factors Underlying Plant Architecture

In a recent report, Doust et al. (2004) presented some results on the genetic control of branching in *S. italica*. Vegetative branching can be considered one of the key traits in the domestication syndrome in cereals because reduced tiller numbers are often associated with reduced inflorescence numbers and increased panicle size (Poncet et al. 2000). The authors exploited the mapping population derived from the interspecific cross, i.e., between the domesticated and the wild forms of the same species. *S. italica* exhibits a variable number of basal tillers (usually a few), but no axillary branching in normal cultivation condition. Contrastingly, the wild form often presents many axillary branches as well as many basal tillers. The F$_2$ population derived from *S. italica* and *S. viridis* is therefore well suited for the study of the genetic factors underlying this particular component of the domestication syndrome. The authors found four significant and reproducible QTLs (quantitative trait loci) for the

Fig. 1. Current molecular map of foxtail millet (extracted from Doust et al. 2004). Correspondence between marker color and species from which they were originally cloned is as follows: *green*: foxtail millet, *blue*: rice, *blue-green*: pearl millet, *red*: maize, *purple*: wheat. See Wang et al. (1998), Devos et al. (1998), and Doust et al. (2004) for marker codes. *Boxes*: chromosomal regions where reproducible significant QTLs were found by authors for basal tillering and axillary branching
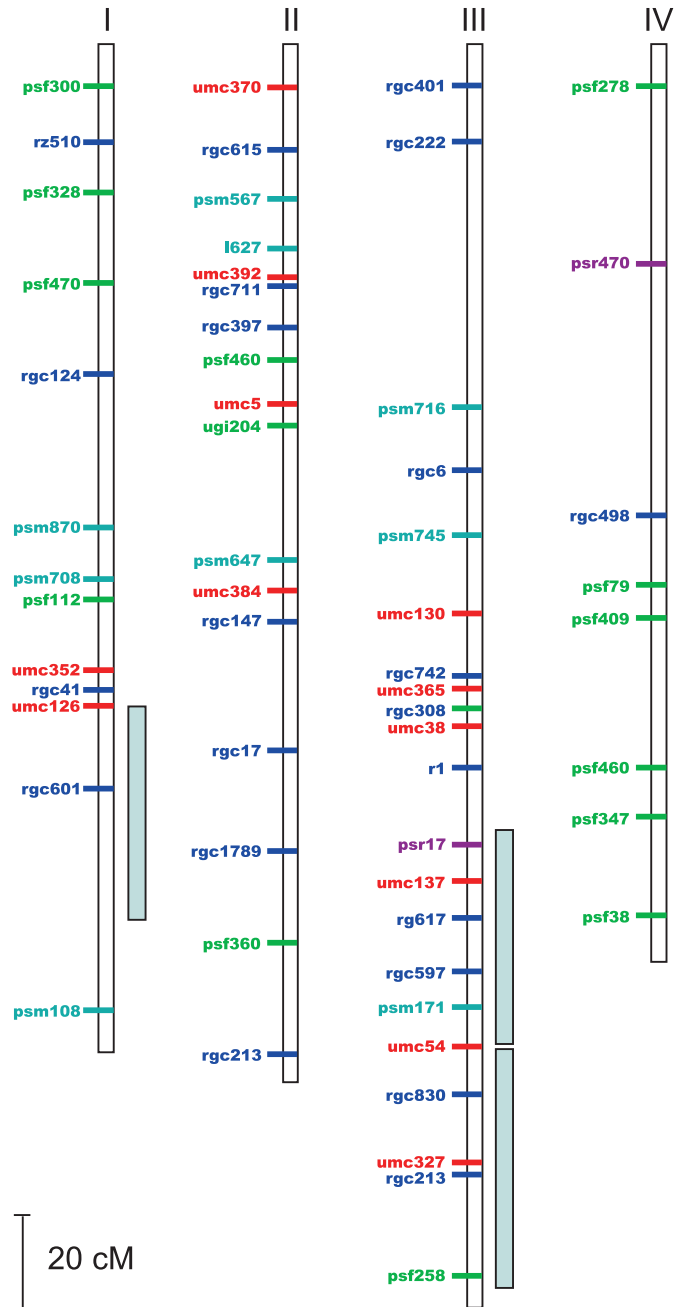
tillering (on chromosomes I, III, and V) and four QTLs for the axillary branching (on chromosomes V, VI, and IX), cf. Fig. 1.

This study was completed by a comparative mapping approach, made possible by the alignment of the millet map on the existing rice and maize maps (see above). The genetic control of axillary branching in maize has been studied in the context of the domestication of the crop (Doebley and Stec 1993). This work led to the cloning of *teosinte branching 1* (*Tb1*,

Wang et al. 1999). *Tb1* is a transcription factor carrying a basic helix-loop-helix type of DNA-binding motif. The allele of the domesticated maize constitutively represses the growth of axillary meristems (thus explaining the absence of secondary tillers in the crop). Following this work, the rice homolog of *Tb1*, *OsTB1*, was isolated and characterized (Takeda et al. 2003). The authors showed that *OsTB1* negatively regulates lateral branching in rice, as does *TB1* in maize. Moreover, a QTL involved in the control

Fig. 1. (continued)
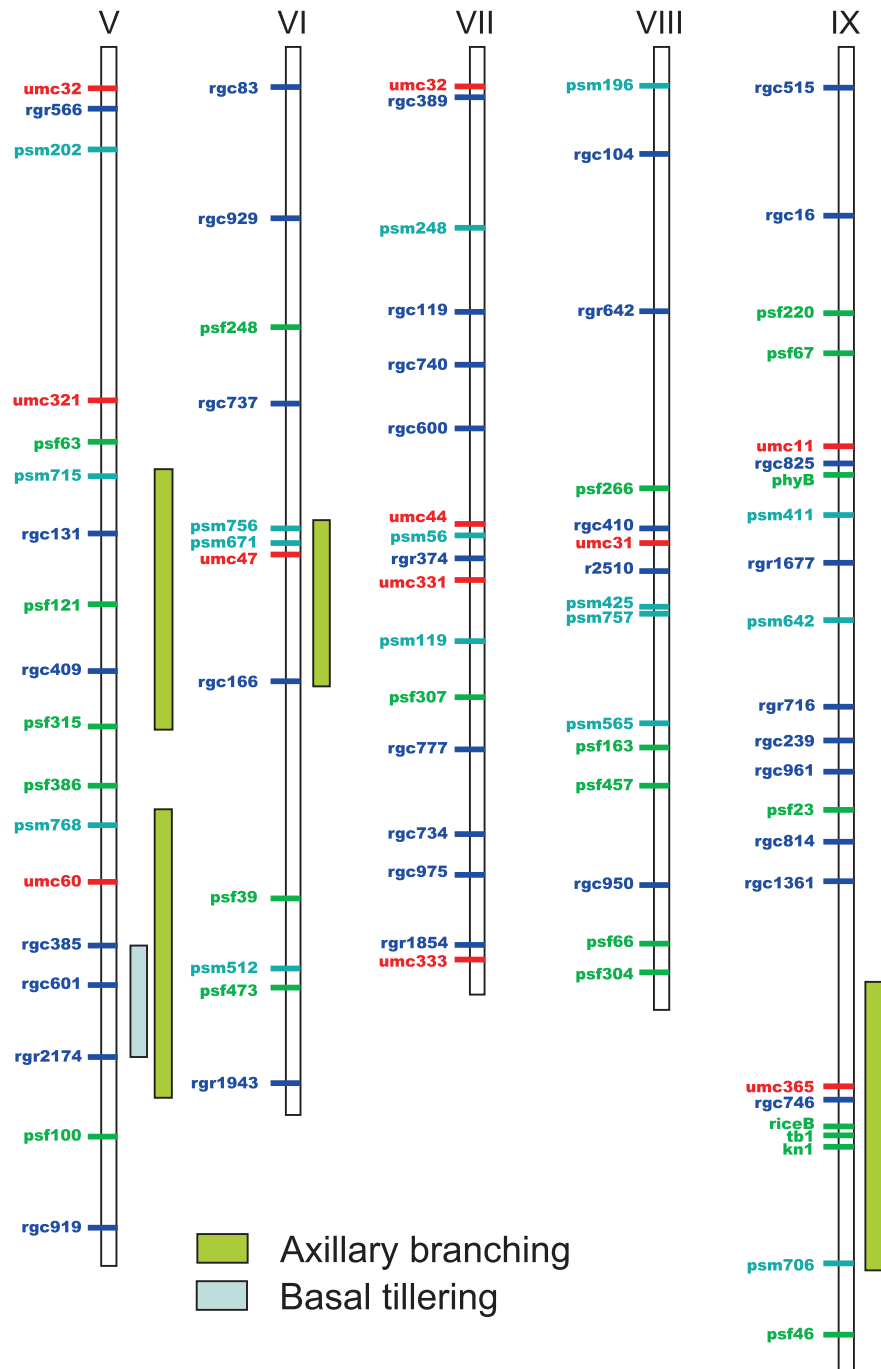
of basal tillering in rice, *monoculm1* (*moc1*), has recently been cloned (Li et al. 2003). *MOC1* is distinct from *OsTB1*. It encodes a protein that belongs to the plant-specific GRAS family. The *moc1* mutant exhibits no axillary buds because axillary meristems are repressed at an early stage.

The homolog of the maize *TB1* gene was positioned by Doust et al. (2004) on the foxtail millet map. A corresponding maize cDNA clone was used as a probe on millet digested genomic DNAs for the segregation analysis. The *MOC1* homolog was indirectly positioned on the map *in silico*, based on the assumption of total conservation of the synteny in the region of the foxtail millet genetic map harboring tillering QTLs. The authors clearly showed that both *TB1* and *MOC1* homologs are present in regions

where branching QTLs were identified (chromosomes IX and IV for *TB1* and *MOC1*, respectively). However, the percentage of variability explained by the QTL on chromosome IX is low, in contrast with maize, where *Tb1* is the predominant factor that represses secondary tillers. A similar conclusion can be drawn in the case of *MOC1* (the QTL on chromosome IV, where one *MOC1* homolog has been localized, has a minor effect on both primary and secondary tillerings). The authors also analyzed in detail all the predicted genes found in the rice genomic sequence regions that are collinear to the foxtail millet map areas harboring tillering QTLs. They found many putative genes, such as hormone biosynthesis pathway genes and transcription factors. However, these candidate genes still have to be validated.

This work illustrates both the potential and pitfalls of the comparative genomic approach in the case of complex traits such as plant architecture. Although the characterizations of *MOC1* and *TB1* are relevant to our knowledge of plant development, the comparative study described above shows that their direct use for gene discovery in other species (although from the same family, i.e., the Poaceae) is not straightforward. The results do not rule out a possible role of *TB1* and *MOC1* in foxtail millet tillering, but they suggest that neither of the two might have been the target of selection during the domestication of the crop. As a consequence, one may argue that the phenotypic diversity observed today in foxtail millet germplasm regarding tillering should find its origin in the molecular diversity at loci that are distinct from both *TB1* and *MOC1* homologs (although this last statement would certainly require more experimental proof).

9.5
Conclusion and Perspectives

As mentioned in the introduction, one interest in mapping the genome of foxtail millet stems from the close genetic relationships of the crop with several severe weeds. In fact, one of the main threats that these species represent is the spontaneous acquisition of herbicide tolerance in their populations. Table 2 shows the various cases of such events recorded over the last 22 years within the *Setaria* species of the primary and secondary gene pools. Twenty-seven independent events concerning 14 herbicides in total were recorded in North America and Europe. At present, only one

source of herbicide tolerance has been characterized in *Setaria* (Delye et al. 2002): this nuclear gene encodes a chloroplastic Acetyl-CoA Carboxylase that is the target of sethoxydim (a cyclohexanedione herbicide that blocks fatty acid metabolism). This gene was identified by homology to a maize ACCase gene (Egli et al. 1995), one allele of which also confers tolerance to cyclohexanedione and aryloxyphenoxypropionate herbicides (Parker et al. 1990). The construction of the genetic map of foxtail millet provides the opportunity to localize and then clone the genetic factors conferring tolerance to other herbicides. This should increase our knowledge of the dynamics of the genomic regions involved in the process, which may then enable the engineering of new herbicide molecules.

The work of Doust et al. (2004) described above shows that the use of comparative genomics is not straightforward as long as the molecular mechanisms and the genetic factors underlying complex traits are not fully characterized. Nevertheless, the vast amount of genomic resources presently available for the two model species *Arabidopsis thaliana* and the Asian rice *Oryza sativa* should provide, at least to a certain extent, some relevant information regarding the biology of minor crops, such as foxtail millet. In this regard, the alignment of the genetic map of the species with one of the major cereals by Devos et al. (1998) represents a highly valuable resource.

Rice is considered the reference species for comparative genomics (Gale and Devos 1998). Its small genome size has long been thought to help resolve the more complex genomic structure of larger genomes such as that of maize or wheat. The availability of the complete genomic sequence of Asian rice as well as the large genomic sequences of other cereals facilitated examination of the evolution of the structure of the genome in several orthologous loci. The results show that in many cases several rearrangements occurred since the radiation of the Bambusoideaes, the Panicoideaes, and the Pooideae subfamilies (Ilic et al. 2003). The three subfamilies radiated almost concomitantly with the origin of the family, i.e., nearly 60 million years ago. Foxtail millet belongs to the Panicoideae subfamily and thus is much closer phylogenetically to maize and sorghum than is rice. Given the small size of its genome (i.e., 450 Mbp, similar to that of rice), foxtail millet could serve as a reference genome in comparative genomics within this subfamily in cases where rice would be unsuitable due to numerous rearrangements.

Table 2. Records of natural occurrence of herbicide tolerance in weedy *Setaria* species populations from primary and secondary gene pools. *Asterisks*: occurrence of a multiple resistance in a single event. Data extracted from database of herbicide-resistant weeds at http://www.weedscience.org/

Species	Year	Origin of event	Class of herbicide	Name of herbicide
S. faberi	1984	USA (Maryland)	Photosystem II inhibitors	atrazine
S. faberi	1987	Spain	Photosystem II inhibitors	atrazine
S. faberi	1991	USA (Wisconsin)	ACCase inhibitors	fluazifop-p-butyl, and sethoxydim
S. faberi	1992	USA (Iowa)	Photosystem II inhibitors	atrazine
S. faberi	1994	USA (Iowa)	ACCase inhibitors	clethodim, fenoxaprop-p-ethyl, fluazifop-p-butyl, quizalofop-p-ethyl, and sethoxydim
S. faberi	1996	USA (Minnesota)	ALS inhibitors	imazethapyr, nicosulfuron, and primisulfuron-methyl
S. faberi	1999	USA (Wisconsin)	ALS inhibitors	imazethapyr, and nicosulfuron
S. faberi	2003	Canada (Ontario)	ALS inhibitors	imazethapyr
S. verticillata	1992	Spain	Photosystem II inhibitors	atrazine
S. viridis	1982	France	Photosystem II inhibitors	atrazine
S. viridis	1987	Spain	Photosystem II inhibitors	atrazine
S. viridis	1988	Canada (Manitoba)	Dinitroanilines	ethalfluralin, and trifluralin
S. viridis	1989	Canada (Alberta)	Dinitroanilines	trifluralin
S. viridis	1989	USA (North Dakota)	Dinitroanilines	trifluralin
S. viridis	1991	Canada (Manitoba)	ACCase inhibitors	diclofop-methyl, fenoxaprop-p-ethyl, sethoxydim, and tralkoxydim
S. viridis	1991	Canada (Saskatchewan)	Dinitroanilines	ethalfluralin, and trifluralin
S. viridis	1992	Canada (Manitoba)	ACCase inhibitors and Dinitroanilines*	diclofop-methyl, ethalfluralin, fenoxaprop-p-ethyl, sethoxydim, tralkoxydim and trifluralin
S. viridis	1996	Canada (Saskatchewan)	ACCase inhibitors	fenoxaprop-p-ethyl, and sethoxydim
S. viridis	1996	Canada (Alberta)	ACCase inhibitors	diclofop-methyl, fenoxaprop-p-ethyl, and sethoxydim
S. viridis	1996	Canada (Saskatchewan)	ACCase inhibitors and Dinitroanilines*	fenoxaprop-p-ethyl and trifluralin
S. viridis	1996	USA (Minnesota)	ALS inhibitors	imazethapyr, nicosulfuron, and primisulfuron-methyl
S. viridis	1999	USA (Wisconsin)	ALS inhibitors	imazamox
S. viridis	1999	Yugoslavia	Photosystem II inhibitors	atrazine
S. viridis	1999	USA (Minnesota)	ACCase inhibitors	fenoxaprop-p-ethyl, and fluazifop-p-butyl
S. viridis	1999	USA (Minnesota)	ACCase inhibitors	fenoxaprop-p-ethyl, fluazifop-p-butyl, and sethoxydim
S. viridis	2001	Canada (Ontario)	ALS inhibitors	imazethapyr
S. viridis	2002	Canada (Manitoba)	ALS inhibitors	imazethapyr, and sulfosulfuron

References

Ahn S, Tanksley SD (1993) Comparative linkage maps of the rice and maize genomes. Proc Natl Acad Sci USA 90(17):7980–7984

Benabdelmouna A, Abirached-Darmency M, Darmency H (2001a) Phylogenetic and genomic relationships in *Setaria italica* and its close relatives based on the molecular diversity and chromosomal organization of 5S and 18S-5.8S-25S rDNA genes. Theor Appl Genet 103:668–677

Benabdelmouna A, Shi Y, Abirached-Darmency M, Darmency H (2001b) Genomic *in situ* hybridization (GISH) discriminates between the A and the B genomes in diploid and tetraploid *Setaria* species. Genome 44:685–690

Dekker J (2003) The foxtail (*Setaria*) species-group. Weed Sci 51(5):641–656

Delye C, Wang T, Darmency H (2002) An isoleucine-leucine substitution in chloroplastic acetyl-CoA carboxylase from green foxtail (*Setaria viridis* L. Beauv.) is responsible for resistance to the cyclohexanedione herbicide sethoxydim. Planta 214(3):421–427

Devos KM, Wang ZM, Beales J, Sasaki T, Gale MD (1998) Comparative genetic maps of foxtail millet (*Setaria italica*) and rice (*Oryza sativa*). Theor Appl Genet 96:63–68

Doebley J, Stec A (1993) Inheritance of the morphological differences between maize and teosinte: comparison of results for two F$_2$ populations. Genetics 134(2):559–570

Doust AN, Devos KM, Gadberry MD, Gale MD, Kellogg EA (2004) Genetic control of branching in foxtail millet. Proc Natl Acad Sci USA 101(24):9045–9050

Egli MA, Lutz SM, Somers DA, Gengenbach BG (1995) A maize acetyl-coenzyme A carboxylase cDNA sequence. Plant Physiol 108(3):1299–1300

Fukunaga K, Wang Z, Kato K, Kawase M (2002a) Geographical variation of nuclear genome RFLPs and genetic differentiation in foxtail millet, *Setaria italica* (L.) P. Beauv. Genet Resource Crop Evol 49:95–101

Fukunaga K, Kawase M, Kato K (2002b) Structural variation in the *Waxy* gene and differentiation in foxtail millet (*Setaria italica* (L.) P. Beauv.): implications for multiple origins of the waxy phenotype. Mol Genet Genom 268:214–222

Gale MD, Devos KM (1998) Comparative genetics in the grasses. Proc Natl Acad Sci USA 95(5):1971–1974

Harlan JR, DeWet JMJ (1971) Towards a rational classification of cultivated plants. Taxon 20:509–517

Harushima Y, Yano M, Shomura A, Saito M, Shimano T, Kuboki Y, Yamamoto T, Lin SY, Antonio BA, Parco A, Kajiya H, Huang N, Yamamoto K, Nagamura Y, Kurata N, Khush GS, Sasaki T (1998) A high-density rice genetic linkage map with 2275 markers using a single F$_2$ population. Genetics 148(1):479–494

Ilic K, SanMiguel PJ, Bennetzen JL (2003) A complex history of rearrangement in an orthologous region of the maize, sorghum, and rice genomes. Proc Natl Acad Sci USA 100(21):12265–12270

Jusuf M, Pernes J (1985) Genetic variability of foxtail millet (*Setaria italica* P. Beauv.). Theor Appl Genet 63:117–119

Le Thierry d'Ennequin M, Panaud O, Brown S, Siljak-Yakovlev S, Sarr A (1998) First evaluation of nuclear DNA content in *Setaria* genus by flow cytometry. J Hered 89(6):556–559

Le Thierry d'Ennequin M, Panaud O, Toupance B, Sarr A (2000) Assessment of genetic relationships between *Setaria italica* and its wild relative *S. viridis* using AFLP markers. Theor Appl Genet 100:1061–1066

Li CH, Pao WK, Li HW (1942) Interspecific crosses in *Setaria*. J Hered 33:351–355

Li HW, Li CH, Pao WK (1945) Cytogenetical and genetical studies of the interspecific cross between the cultivated foxtail millet, *Setaria italica* (L.) Beauv. and the green foxtail millet *S. viridis* L. J Am Soc Agron 37:32–54

Li X, Qian Q, Fu Z, Wang Y, Xiong G, Zeng D, Wang X, Liu X, Teng S, Hiroshi F, Yuan M, Luo D, Han B, Li J (2003) Control of tillering in rice. Nature 422:618–621

Marinval P (1992) Archaeobotanical data on millets (*Panicum miliaceum* and *Setaria italica*) in France. Rev Palaeobot Palyn 73(1-4):259–270

Nakayama H, Namai H, Okuno K (1999) Genes controlling prolamin biosynthesis, *Pro1* and *Pro2*, in foxtail millet, *Setaria italica* (L.) Beauv. Genes Genet Syst 74:93–97

Parker WB, Marshall LC, Burton JD, Somers DA, Wyse DL, Gronwald JW, Gengenbach BG (1990) Dominant mutations causing alterations in acetyl-coenzyme A carboxylase confer tolerance to cyclohexanedione and aryloxyphenoxypropionate herbicides in maize. Proc Natl Acad Sci USA 87(18):7175–7179

Poncet V, Lamy F, Devos KM, Gale MD, Sarr A, Robert T (2000) Genetic control of domestication traits in pearl millet (*Pennisetum glaucum* L., Poaceae). Theor Appl Genet 100(1):147–159

Schontz D, Rether B (1998) Genetic variability in foxtail millet, *Setaria italica* (L.) P. Beauv. – RFLP using a heterologous probe. Plant Breed 117:231–234

Schontz D, Rether B (1999) Genetic variability in foxtail millet, *Setaria italica* (L.) P. Beauv.: identification and classification of lines with RAPD markers. Plant Breed 118:190–192

Takeda T, Suwa Y, Suzuki M, Kitano H, Ueguchi-Tanaka M, Ashikari M, Matsuoka M, Ueguchi C (2003) The *OsTB1* gene negatively regulates lateral branching in rice. Plant J 33(3):513–520

Wang TY, Chen HB, Reboud X, Darmency H (1997) Pollen-mediated gene flow in an autonomous crop: foxtail millet (*Setaria italica*). Plant Breed 116:579–583

Wang ZM, Devos KM, Liu CJ, Wang RQ, Gale MD (1998) Construction of RFLP-based maps of foxtail millet, *Setaria italica* (L.) P. Beauv. Theor Appl Genet 96:31–36

Wang RL, Stec A, Hey J, Lukens L, Doebley J (1999) The limits of selection during maize domestication. Nature 398(6724):236–239

10 Finger Millet

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10.1
Introduction

Finger millet, *Eleusine coracana* Gaertn L., is a cereal grown for food in Africa and Southern Asia, mainly India (the states of Uttar Pradesh, Bilhar, Tamil Nadu, Karnataka, and Andhra Pradesh) and Nepal. In Africa, the crop is principally grown in the eastern regions, mainly in Uganda, Kenya, and Tanzania and, to a lesser extent, in Ethiopia, Rwanda, Malawi, Sudan, Zambia, and Zimbabwe.

10.1.1
Brief History of the Crop

Finger millet originated and was domesticated in Africa. Archeological and linguistic evidences show that around 5,000 years ago, farming communities in eastern Africa were already cultivating this millet (Klichowska 1984). The exact area of domestication is unknown, and it has been suggested that it may have occurred anywhere between western Uganda and the Ethiopian highlands of Eastern Africa (de Wet 1995). From Africa the crop was transported to India about 3,000 years ago, whereupon the subcontinent became its secondary center of diversity.

Cultivated finger millet (*Eleusine coracana* subsp. *coracana*) is likely to have been derived from selection and domestication of a large-grained mutant of the wild *E. coracana* subsp. *africana*. Evidence for the ancestry of cultivated millet has been provided by cytological (Hiremath and Salimath 1992), morphological (Hilu and de Wet 1976), and molecular data (Dida 1998; Hilu 1988).

10.1.2
Botanical Descriptions

Finger millet (*E. coracana*) and related species belong to the subfamily Chloridoideae within the Poaceae family. The crop belongs to the genus *Eleusine*, which contains eight species, both annuals and perennials. Finger millet is a tufted annual growing from about 40 to 150 cm tall and takes from 3 to 6 months to mature. The stems are erect, compressed, and glabrous. The leaf blades are linear and taper to an acute point, folded, and striated and often have ciliated margins (Rachie and Peters 1977). The inflorescence consists of a variable number of spikes ranging from 3 to 20 arranged in a bird's foot style. It resembles fingers on a hand, hence its common name "finger millet". Each spike contains about 70 spikelets arranged alternately on the rachis, and each spikelet carries 4 to 7 seeds. The seeds vary in diameter from 1 to 2 mm. The caryopsis (seed) is globose and smooth, and the color can be brown, reddish brown, black, orange red, purple, and white (J. Duke, 1983, Handbook of Energy Crops. Unpublished, Purdue University).

The morphology of the finger millet inflorescence is highly variable and may be a consequence of farmers' selection preferences (de Wet 1995). Based on the inflorescence morphology, finger millet can be grouped into five races. The race *coracana* resembles the subspecies *africana* and has well-developed central spikes numbering from 5 to 20. The spikes are straight, slender, and up to 11 cm in length. The race *vulgaris* has inflorescences with incurved or straight spikes (Fig. 1). The *compacta* race (Cockscomb finger millet) has incurved spikes with lower finger branches divided in compacta. The lower inflorescence branches usually present in Indian cultivars may not be present in some African cultivars (Fig. 1). The race *plana* has large spikelets arranged in two even rows along the rachis, giving the head a ribbonlike appearance, and the *elongata* race has long slender spikes that are incurved at maturity, with lengths of up to 24 cm.

Finger millet (*Eleusine coracana* subsp. *coracana*) and the weedy wild relative *E. coracana* subsp. *africana* are allotetraploids with $2n = 4x = 36$
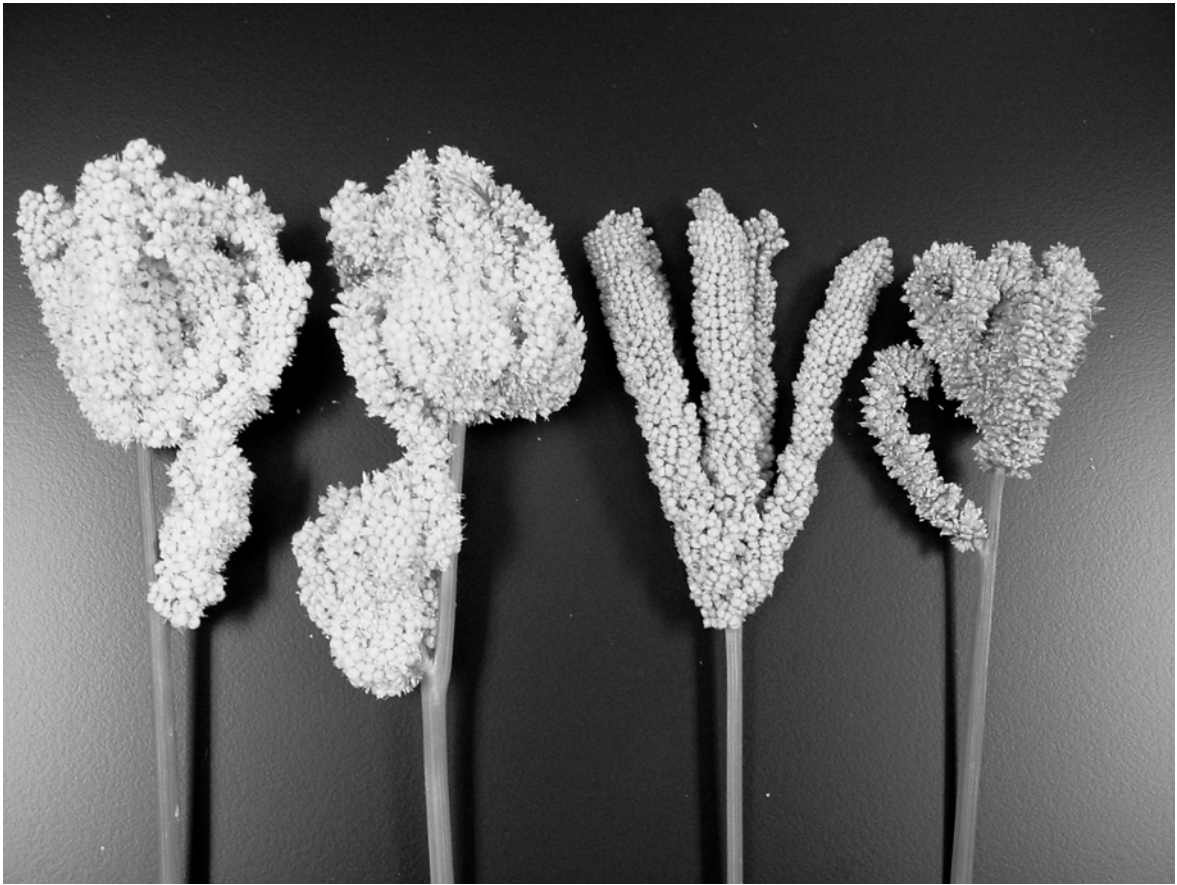
Fig. 1. Variation of finger millet head shapes. The first and second from *left* belong to the race Compacta, the third and fourth belong to races Coracana and Vulgaris, respectively

chromosomes. These two subspecies have been assigned the genomic notation AABB (Chennaveeriah and Hiremath 1974; Hiremath and Salimath 1992). It has been established that the diploid *E. indica* (wild goosegrass) is the source of the A genome in finger millet (Hilu 1988; Hiremath and Salimath 1992; Dida 1998; Bisht and Mukai 2001a). The source of the B genome, however, has not been unequivocally established. The results of recent genomic in situ hybridization studies suggest that the perennial *E. floccifolia* may be the B genome donor to both cultivated finger millet and the subspecies *africana* (Bisht and Mukai 2001a,b). Cultivated finger millet is crosscompatible with the wild subspecies *africana* and with another allotetraploid, *E. kigeziensis* ($2n = 4x = 38$) (Hiremath and Salimath 1991). These two wild allotetraploids are confined to the African continent with *E. kigeziensis* being endemic to southwestern Uganda (Kabale district) and Rwanda (Phillips 1974).

There are limited reports on the DNA content of finger millet and related species. A review by Bennett and Leitch (1995) reported that cultivated finger millet had a 2C (diploid) nuclear DNA content of 5.5 picograms (pg), whereas the wild subspecies *africana* had a value of 5.1 pg. These values were determined on root nuclei using a microdensitometry method with onion (*Allium cepa*) as standard (Hiremath and Salimath 1991). Later, another report of DNA contents of *Eleusine* species using laser flow cytometry and chicken red blood cell nuclei as standard gave comparatively lower values (Mysore and Baird 1997). Mysore and Baird reported 2C nuclear DNA value of 3.6 pg and 3.3 pg for *E. coracana* and *E. africana*, respectively. These authors further postulated that the earlier reported DNA values may have been overestimated owing to the use of onion with higher DNA content as standard and a frequent occurrence of root endopolyploidy. Based on these reports,

the finger millet 2C nuclear DNA content translates into ca. 3.6×10^9 base pairs.

10.1.3
Economic Importance

Finger millet is adapted to a wide range of environments. It is often grown from sea level up to 2,400 m on the slopes of the Himalayas in Nepal and in the Kabale district in Uganda. Finger millet can be grown as a dryland crop in areas with as little as 500 mm of annual rainfall. The crop is also adapted to a wide range of tropical soils, ranging from red lateritic to sandy loams and black heavy vertisols.

Table 1. Major nutrient composition of finger millet (per 100 g) (Sources: Rao 1994; FAO 1995; National Research Council 1996; Vandivoo et al. 1998)

Major component	Content
Proteins (g)	7 to 14
Fats (g)	1.5
Ash (g)	2.6
Crude fiber (g)	3.6
Carbohydrates (g)	73
Calcium (mg)	160 to 490
Iron (mg)	4 to 12
Phosphorus (mg)	200 to 320
Magnesium (mg)	140
Zinc (mg)	1.5 to 2.4
Copper (mg)	0.5
Manganese (mg)	1.9 to 5.5
Molybdenum (μg)	2
Potassium (mg)	314
Sodium (mg)	49
Iodine (μg)	10
Thiamine (mg)	0.24
Riboflavin (mg)	0.11
Energy (Kcal)	335

Finger millet is a staple food for millions of people in Africa, India, and Nepal. The estimated global annual production of finger millet is about 4.5 million tons of grain, of which approx. two million tons is produced in Africa while the Asian continent (mainly India and Nepal) produces the remainder (FAO 1996). African finger millet is grown mainly in eastern Africa, where the finger millet cultivation area encompasses at least one million hectares (ha), with ca. 405,000 ha

in Uganda, 320,000 ha in Tanzania, and 90,000 ha in Kenya (FAO 1996). In Nepal, finger millet, locally known as kodo, is the fourth staple food crop after rice, maize, and wheat. Here the crop is grown on about 26,000 ha of land with an average productivity of 1,100 kg/ha (Joshi and Joshi 2002). In this country finger millet is popular, mainly due to its adaptation to growing on marginal lands where subsistence farmers live. Its popularity is also due to its good response to low levels of fertilizer applications and the crop's tolerance to cold temperatures.

Finger millet is an outstanding subsistence food crop. Its small seeds can be stored for many years with minimal insect damage and with little loss of viability. Finger millet grain can be used in many ways. The ground flour is made into porridge or bread that represents nutritious and wholesome foods for diabetics and the elderly (Duke 1983). The grains can also be fermented into malt, which is highly nutritious and recommended for infants and the elderly (National Research Council 1996). The malted grains are also used to brew beers. Among the tropical cereals, finger millet provides the best malt for beer making and is better than either maize or sorghum. Finger millet straw makes good fodder for animals and contains up to 61% total digestible nutrients (Duke 1983).

The nutrient content of finger millet grain is given in Table 1. It has a protein content ranging from about 7 to 14%. Brown and red seeded cultivars generally have protein levels in the lower range, whereas levels in the white seeded cultivars and the wild subspecies *africana* are at the higher end of the spectrum (10 to 14%) (Rachie and Peters 1977; Rao 1994; FAO 1995; Vadivoo et al. 1998). Finger millet protein has a very favorable amino acid composition and is particularly rich in the essential amino acids tryptophan and methionine (National Research Council 1996). Compared to other cereals, finger millet grains also have a relatively higher content of minerals such as calcium, phosphorus, iron, and manganese. The calcium content, for example, is 16 times that of maize.

10.1.4
Breeding Objectives

Finger millet hybridization and breeding are often hampered by the crop's high rate of self-fertilization, coupled with extremely small flowers that are difficult to emasculate. However, this challenge can be

overcome by employing hot water emasculation and a contact method of crossing (Rachie and Peters 1977; Dida 1998). Some finger millet lines with male sterility have been discovered (Gupta et al. 1997; National Research Council 1996), and the availability of male sterile lines in finger millet should greatly facilitate cross breeding of different lines or cultivars. Most finger millet cultivars tend to lodge under high fertility and moisture conditions. Tall lines with heights of more than 120 cm tend to lodge more compared to shorter genotypes, and the ideal plant height for grain and fodder production is around 80 cm. Therefore, selection for reduced plant height and incorporation of dwarf and semidwarf genes into adapted lines are important breeding goals. Other major breeding objectives include the development of genotypes with profuse basal tillering and reduced number of nodal tillers. Developing genotypes with more and longer digits is required since there is a positive correlation between length, number of digits, and grain yield. For drier semiarid areas, early maturing genotypes that flower within 80 to 90 d after planting are the best adapted and, hence, the farmers' choice.

Breeding for improved grain quality is also a major objective. Most pearly white seeded grains of finger millet have been found to have high protein content and to be low in tannins compared to most brown and red seeded types. The tannins may have protective functions against fungi, birds, and other predators. However, they impart an astringent taste to the grains and reduce their palatability and nutritional quality. Therefore, the development of high protein and low tannin varieties should be emphasized.

A major biotic constraint on finger millet production in moist mid-altitude and higher elevations of East Africa is infection by the blast pathogen *Pyricularia oryzae*. Blast epidemics can result in significant losses in crop yields in susceptible varieties. In eastern Africa, only a few finger millet varieties with blast resistance have been developed. Identification and development of varieties with blast resistance should be a priority in eastern Africa.

Grain yield improvement is a major breeding objective in finger millet. Most current finger millet lines, particularly those grown in eastern Africa, are landraces or landrace selections with average grain yields of one to two tons per hectare. Breeding and improvement in agronomic practices can substantially raise the grain yields, potentially to 5 tons per hectare (Duke 1983).

Other breeding efforts in eastern Africa should be directed toward the development of drought and striga weed tolerance. For marginal and semiarid regions, shade-tolerant genotypes for relay and intercropping should be useful. These should fit in well in farming systems where farmers are already practicing relay and intercropping.

10.1.5
Classical Breeding Achievements

In India, finger millet breeding has been carried out mainly in the southern states of Tamil Nadu, Karnataka, and Andhra Pradesh. Breeding progress has been remarkable. In Tamil Nadu alone, more than 15 improved cultivars have been developed and released (Rachie and Peters 1977). In Nepal, examples of high-yielding varieties developed are Okhale I and Dalle I (Joshi and Joshi 2002).

In eastern and southern Africa, major breeding efforts have been reported in Uganda, Malawi, Zimbabwe, Kenya, Tanzania, and Ethiopia. In Uganda, several improved varieties have been developed including Gulu E, Serere 1, P283, Engeny, and P224 (PESE1). In Kenya, evaluation and screening of local collections and introductions have resulted in the identification of high-yielding cultivars such as Gulu E, P224, KA2, and KATFM1 (KARI 1990). In Ethiopia, screening of introductions has identified varieties with high yield potential such as KNE 409, KNE 1098, Acc 100057, and KNE 479 (Mulatu et al. 1985). Multilocation yield trials in eastern Africa have indicated that these improved lines have a yield potential of two tons per hectare (Mukuru and Guiragossian 1990). In Zimbabwe, a variety SDEV 87001 that yields up to 3.5 tons per hectare has been developed (Gupta and Mushonga 1994). However, there is still scope to further improve finger millet yields to attain the five-metric-tons-per-hectare target.

In India and Nepal, the area under finger millet production has been expanding. In Nepal, a growth rate of 8% per year has been reported (National Research Council 1996). In Africa, on the other hand, a general decline of the area under finger millet production has taken place within the last 50 years. This has been attributed to changing farming systems and competition with maize and other cereals (Oryokot 1990). It is believed that the decline may have stabilized (National Research Council 1996). Moreover, in eastern Africa, the crop is regaining its importance

and popularity. In Uganda, finger millet occupies 50% of the land area under cereal crops. In Kenya, finger millet grain fetches a premium price that is more than twice that of maize and sorghum.

Currently, in East Africa, there are a number of finger-millet-flour-based formulations for adult and infant porridge on the market. To satisfy the demands of diverse growers and users of this crop as a specialty food, current breeding efforts should be holistic. The integration of classical, molecular, and participatory breeding approaches will lead to the development of revolutionary finger millet lines that are adapted to local environmental niches and stresses, with nutritionally superior characteristics that are culturally acceptable.

10.2
Genetic Mapping in Finger Millet

10.2.1
Brief History of Mapping Efforts

Genetic mapping in finger millet is in its infancy. Although finger millet is an important food crop in regions of Africa and India, based on the limited interest from the research community and funding agencies, finger millet most definitely can be classified as a "neglected crop". The first partial finger millet genetic maps were produced in 1998 (Dida 1998). In early 2000, mapping efforts were renewed with funding from the McKnight Foundation, and maps covering 18 linkage groups, each larger than 20 cM, in addition to several smaller linkage groups have recently been constructed (MM. Dida, Srinivasachary, K.M. Devos, unpubl. obs.).

10.2.2
First-Generation Genetic Maps

Before embarking on a genetic mapping study, it is useful to have information on the extent of variation present within the target crop. Because such information was not available for finger millet, a small survey was conducted on the level of restriction fragment length polymorphism (RFLP) present within *E. coracana* germplasm. Eight *E. coracana* subsp. *coracana* lines from Kenya, Uganda, Ethiopia, and Nepal together with five Kenyan *E. coracana* subsp. *africana*

accessions were evaluated using 30 RFLP probes. Polymorphism levels within cultivated finger millet were limited to 15% despite the diverse origins of the lines. This may be a reflection of the relatively recent (some 2,000 to 3,000 years ago) introduction of finger millet into Asia. Variation between the wild accessions amounted to 28% (Dida 1998). Polymorphism levels between the two subspecies, however, were 72%. Therefore, a mapping population consisting of 151 F_2 progeny was generated from a cross between the cultivar Okhale I and the wild accession MD-20. In the first mapping phase, a total of 126 RFLP probes were placed onto the genetic map. In addition to the 182 RFLP loci, 15 amplified fragment length polymorphism (AFLP) markers, generated using the restriction enzyme combination *Pst*I/*Mse*I, were incorporated into the map (Dida 1998). The second mapping phase was conducted using finger millet expressed sequenced tags (ESTs) (Srinivasachary and K.M. Devos, unpubl. obs.). Primers were generated to the ESTs and amplification products were checked for single strand conformation polymorphisms (SSCP). This technique detects mainly single nucleotide polymorphisms (SNPs) and small insertion/deletions (indels). Polymorphism levels in the intersubspecific *coracana* × *africana* population were around 55%.

A preliminary analysis of the current mapping data set (RFLP, AFLP, and SSCP markers) has grouped the 379 loci into 18 large linkage groups (each exceeding 20 cM and containing a minimum of seven markers). In addition, several smaller linkage groups were formed, and 85 loci, including 27 AFLP markers, remained unlinked. The large number of unlinked markers is due to the fact that the maps were constructed at LOD (log of the odds ratio) 10 to avoid spurious linkages, and this precluded the inclusion of markers that link at distances of >25 cM. A more thorough analysis of the mapping data is required to incorporate at least a subset of the currently unlinked markers into the genetic maps.

Of the 18 major linkage groups, 9 belong to the B genome, 7 to the A genome and 2 are, as yet, unassigned. In wheat, linkage groups can be located to genomes and, indeed, chromosomes using sets of aneuploid lines. No such lines exist in finger millet. However, we can take advantage of the relatively low level of variation that exists between *E. indica*, the A genome donor of finger millet, and the present-day A genome of *E. coracana*. Following hybridization of RFLP probes to digested DNA of an *E. indica* accession, and of Okhale I and MD-20, the parents

of the mapping population, it is possible, at least for a number of probes, to identify common A genome fragments (Fig. 2). A fragment that is monomorphic between *E. indica*, Okhale I, and MD-20 will, most likely, belong to the A genome. A second, polymorphic fragment present only in the tetraploid *E. coracana* therefore represents a B genome locus (Fig. 2; PSE143). Alternatively, a fragment that is polymorphic between Okhale I and MD-20, but monomorphic either between Okhale I and *E. indica* or between MD-20 and *E. indica*, can be allocated to the A genome (Fig. 2; PSE84).
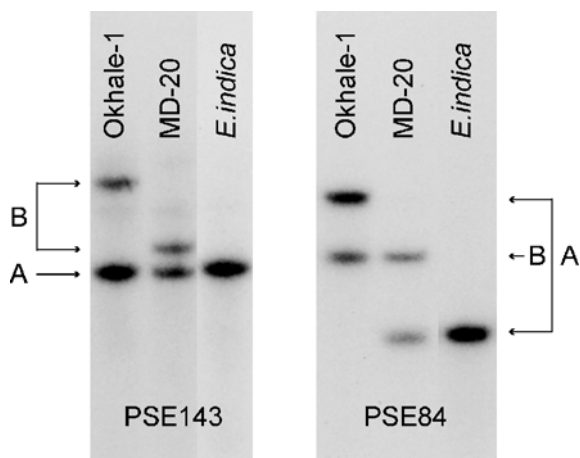
Fig. 2. Hybridization of RFLP probes PSE143 and PSE84 to digested DNA of tetraploid (AABB) *E. coracana* accessions Okhale I and MD-20, and to *E. indica*, the A genome donor

Homoeology between the A and B genome chromosomes has been established for seven pairs of linkage groups. Two examples of homoeologous linkage groups are presented in Fig. 3. In addition, one B genome linkage group shows homoeology with two A genome linkage groups. Although this may be an indication that the two genomes are rearranged with respect to one another, it is possible that the two A genome linkage groups will link when new markers are added to the genetic map.

One such further source of markers is microsatellites or simple sequence repeats (SSRs). Screening of some 18,000 *Hind*III, *Sal*I, and *Pst*I finger millet genomic clones containing insert sizes of 1 to 1.5 kb with selected di- and trinucleotide repeats has yielded 111 SSR sequences to which primers were designed (K.M. Devos, unpubl.). Some 70 primer pairs gave consistent amplification against a panel of eight fin-

ger millet varieties, including Okhale I and MD-20. Fifty SSRs that are polymorphic between Okhale I and MD-20 are being mapped.

10.2.3
Comparative Genetic Maps

The incorporation of heterologous RFLP markers that had previously been mapped in rice into the finger millet genetic maps allowed the construction of a rice–finger millet comparative framework. A further source of comparative markers was provided by mapped finger millet ESTs. Priority was given to mapping those ESTs for which a putative homolog could be identified in the rice genomic sequence produced by the International Rice Genomic Sequencing Project (IRGSP) (http://rgp.dna.affrc.go.jp/IRGSP/). Since the IRGSP sequenced the rice genome using a BAC by BAC approach of physically mapped BACs, many of which were anchored to the genetic map, the location of the putative homologs of the finger millet ESTs in the rice genome could be established.

Many of the preliminary established finger millet linkage groups correspond to a single rice chromosome, indicating that few rearrangements have taken place at the map level in the finger millet genome since its divergence from a common ancestor with rice (Fig. 3). Nevertheless, considering that rice has $2n = 2x = 24$ chromosomes and finger millet has $2n = 4x = 36$ chromosomes, one would expect at least some finger millet linkage groups to have orthology to two or more rice chromosomes. Two such linkage groups were identified among the current data set. One finger millet linkage group contained loci orthologous to both rice chromosome 5 and rice chromosome 12 (Fig. 3). A second linkage group showed orthology to both rice chromosomes 2 and 10.

A comparison with other grass genetic maps suggests that the observed rearrangements occurred either in the Chloridoideae lineage or in finger millet itself. The only other comparative map constructed in a species belonging to the Chloridoideae subfamily is *Eragrostis tef* (Zhang et al. 2001). However, due to the incompleteness of the tef maps, it is not possible to infer whether any of the putative rearrangements detected in finger millet are common to tef. No tef linkage groups with orthology to rice chromosomes 5, 10, and 12 were identified by Zhang and colleagues (Zhang et al. 2001).

The availability of rice–finger millet comparative maps should enhance the efficiency with which agronomic traits can be mapped and tagged in finger millet. This is particularly true for traits such as maturity and plant height for which QTLs are often conserved across species (e.g., Lin et al. 1995; Peng et al. 1999). However, blast resistance may be another trait that can benefit greatly from the available data in rice. Blast is caused by the fungus *Pyricularia oryzae*, which has a wide host range including rice and finger millet (Kato et al. 2000). In rice, some 30 genes for blast resistance have been identified, several of which have been isolated (e.g., Wang et al. 1999; Bryan et al. 2000; Jiang and Wang 2002; Zheng et al. 2004). It will be interesting to investigate whether homologous genes underlie QTL for blast resistance that map to orthologous positions in finger millet and rice. Particularly encouraging in this respect is a recent report by Chen and colleagues who identified four QTLs contributing resistance to *P. grisea* in orthologous positions in rice and barley. The orthologous QTLs had complete or partial conserved isolate specificity Chen et al. 2003).

10.3 Future Scope of Work

Finger millet genetic studies lag considerably behind those of major cereal crops. Nevertheless, a first step, the construction of genetic maps and establishment of their relationship to other cereal genomes, in particular the rice genome, has been accomplished. These maps will form the foundation for targeted improvement of finger millet. Being a recent polyploid, finger millet has a very narrow genetic base. It is envisioned that in the future, the genetic base of this crop can be widened using the wild relative gene pool. This strategy has been used successfully in other cereals, where wild species have been donors of novel genes, in particular to confer resistance to biotic stresses but also to enhance quality traits (Friebe et al. 1996; Tanksley and McCouch 1997). A systematic analysis of the biodiversity existing within cultivated and wild finger millet germplasm has not yet been conducted. However, a small-scale biodiversity study is under way, and some *E. coracana* subsp. *africana* accessions have been identified with relatively good levels of resistance to the blast fungus *P. oryzae* (M.M. Dida, N. Wanyera, K.M. Devos, unpubl.). Furthermore, in an analysis

of protein and calcium content of the finger millet grain, it was found that some of the highest values were present in the wild *E. africana* (Barbeau and Hilu 1993). Screening large collections of wild and cultivated germplasm therefore will most likely lead to the identification of lines containing high levels of protein, lysine, and minerals, in particular, calcium and iron. These efforts should also concomitantly aim at reducing the levels of grain tannins that are antinutritional factors. *E. africana* alleles could thus contribute to the improvement of finger millet for resistance to blast disease and the nutritional quality of the grain. Through hybridization, backcrossing, and selection, useful traits could be transferred from subspecies *africana* and, potentially, *E. kigeziensis* to the cultivated finger millet. However, gene introgression is carried out most efficiently in conjunction with marker-assisted selection. This requires knowledge of the location of quantitative trait loci (QTLs) conferring the phenotype and the availability of linked markers.

Remarkable yield improvements have occurred in other cereals such as wheat over the last 50 years. This was achieved in large part through the introduction of semidwarf genes. A similar approach could be applied to finger millet. Application of a combination of conventional and molecular breeding techniques will allow for a rapid development of high-yielding crop ideotypes adapted to specific environments and ecological niches and has the potential to more than double the current finger millet yields.

Finger millet improvement will require collaborative efforts between breeders, biotechnologists, and, importantly, funding agencies. Unfortunately, finger millet has been stigmatized as a food for the poor, and this negative label has contributed to the decline of finger millet production in recent decades. However, the elimination of finger millet has had serious health implications. In households where rice has replaced finger millet as the staple diet, anemia caused by nutritional unbalance has become widespread. It is clear that finger millet has a very important contribution to make to satisfy current and future nutritional needs in human food. The most important prerequisites to improve finger millet are a change in attitude, in particular by governments and funding agencies, toward finger millet as a famine food and a growth in interest of researchers and breeders to work on this underresourced crop.

Fig. 3. Two sets of homoeologous finger millet linkage groups. Markers are on *right-hand side*, genetic distances on *left-hand side*. A and B indicate whether the linkage groups belong to the A or B genome, respectively. *Dotted lines* between homoeologous groups connect homoeologous loci. The relationship with rice is shown by a *hatched bar*
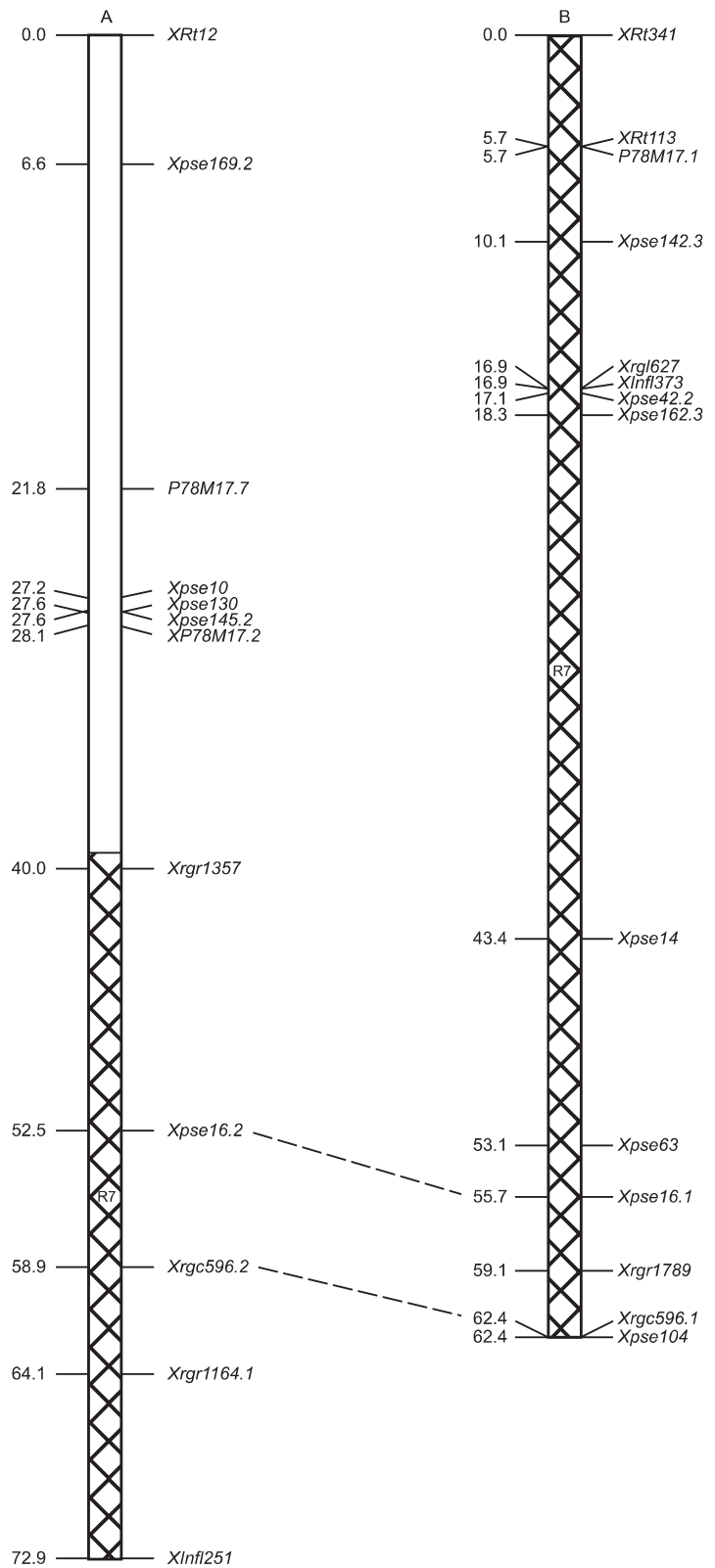
Fig. 3. (continued)
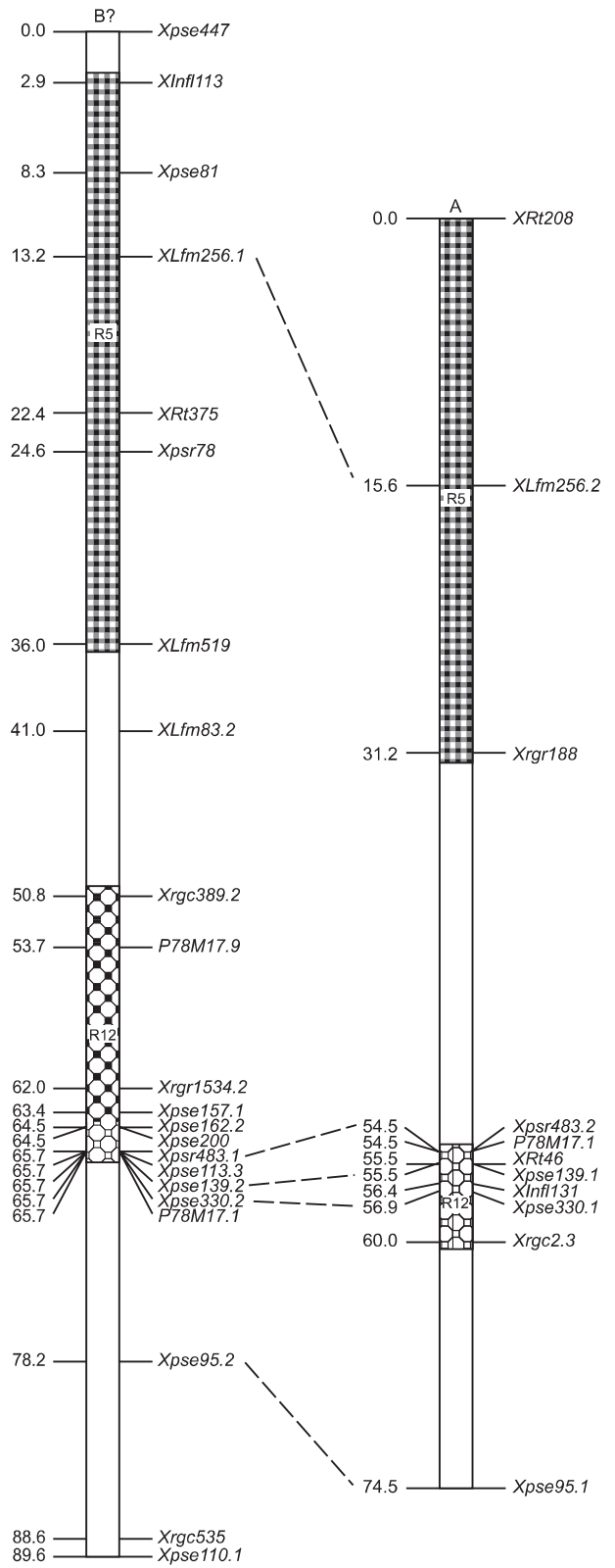
References

Barbeau WE, Hilu KW (1993) Protein, calcium, iron, and amino acid content of selected wild and domesticated cultivars of finger millet. Plant Foods Hum Nutr 43:97–104

Bennett MD, Leich IJ (1995) Nuclear DNA amounts in angiosperms. Ann Bot 76:113–176

Bist MS, Mukai Y (2001a) Genomic in situ hybridization identifies genome donor of finger millet (*Eleusine coracana*). Theor Appl Genet 102:825–832

Bisht MS, Mukai Y (2001b) Identification of genome donors to the wild species of finger millet, *Eleusine africana* by genomic in situ hybridization. Breed Sci 51:263–269

Bryan GT, Wu KS, Farrall L, Jia YL, Hershey HP, McAdams SA, Faulk KN, Donaldson GK, Tarchini R, Valent B (2000) A single amino acid difference distinguishes resistant and susceptible alleles of the rice blast resistance gene *Pi-ta*. Plant Cell 12:2033–2045

Chen H, Wang S, Xing Y, Xu C, Hayes PM, Zhang Q (2003) Comparative analyses of genomic locations and race specificities of loci for quantitative resistance to *Pyricularia grisea* in rice and barley. Proc Natl Acad Sci USA 100:2544–2549

Chennaveeriah MS, Hiremath SC (1974) Genome analysis of *Eleusine coracana* Gaertn. Euphytica 23:489–495

Dida MM (1998) Genome studies in finger millet (*Eleusine coracana* L.). PhD Dissertation, John Innes Centre, Norwich, UK

de Wet JMJ (1995) Finger millet, *Eleusine coracana*. In: Smartt J, Simmonds NW (eds) Evolution of Crop Plants. Longman, Singapore, pp 137–140

FAO (1995) Sorghum and millets in human nutrition. Food and Nutrition Series No. 27. FAO, Rome, Italy

FAO (1996) The world sorghum and millet economies. Facts, trends and outlook. ICRISAT, India and FAO, Rome, Italy

Friebe B, Jiang J, Raupp WJ, McIntosh RA, Gill BS (1996) Characterization of wheat-alien translocations conferring resistance to diseases and pests: current status. Euphytica 91:59–87

Gupta SC, Mushoga JN (1994) Registration of 'SDEY 87001' finger millet. Crop Sci 34:536

Gupta SC, Muza FR, Andrews DJ (1997) Registration of INFM 95001 finger millet genetic male-sterile line. Crop Sci 37:1409

Hilu KW (1988) Identification of the 'A' genome of finger millet using chloroplast DNA. Genetics 118:163–167

Hilu KW, de Wet JMJ (1976) Domestication of *Eleusine coracana*. Econ Bot 306:199–208

Hiremath SC, Salimath SS (1991) Quantitative nuclear DNA changes in *Eleusine* (Gramineae). Plant Syst Evol 178:225–233

Hiremath SC, Salimath SS (1992) The 'A' genome donor of *Eleusine coracana* (L) Gaertn (Gramineae). Theor Appl Genet 84:747–754

Jiang J, Wang S (2002) Identification of a 118-kb DNA fragment containing the locus of blast resistance gene *Pi-2(t)* in rice. Mol Genet Genom 268:249–252

Joshi BK, Joshi MR (2002) A field assesment of finger millet (Kodo) diversity in Sankhuwasabha district, Nepal. Rural Reconstruct Conservat Newslett 2(2):3–5. Lazimpat, Kathmandu, Nepal: http://www.rrn.org.np

KARI (1990) Kenya Agricultural Research Institute Annual Report, 1990, Nairobi, Kenya

Kato H, Yamamoto M, Yamaguchi-Ozaki T, Kadouchi H, Iwamoto Y, Nakayashiki H, Tosa Y, Mayama S, Mori N (2000) Pathogenecity, mating ability and DNA restriction fragment length polymorphism of *Pyricularia* populations isolated from Gramineae, Bambusideae and Zingiberaceae plants. J Gen Plant Pathol 66:30–47

Klichowska M (1984) Plants of the Neolithic Kadera (Central Sudan): a paleobotanical study of the plant impressions on pottery. In: Krzyniak l, Kobusiewics (eds) Origins and Early Development of Food Producing Cultures in North-Eastern Africa. Polish Academy of Sciences, Warsaw

Lin Y-R, Schertz KF, Paterson AH (1995) Comparative analysis of QTLs affecting plant height and maturity across the Gramineae, in reference to an interspecific Sorghum population. Genetics 141:391–411

Mukuru SZ, Guiragossian VY (1990) The performance of improved elite sorghum and cultivars in the 1989 EARSAM cooperative regional yield trials. In: Proc 7th EARSAM Regional Workshop on Sorghum and Millet Improvement in Eastern Africa, 25–28 June 1990, Nairobi, Kenya, pp 133–152

Mulatu T, Debelo A, Genetchu Z, Degu E (1985) Finger millet (*Eleusine coracana*): a potential crop in Ethiopia. In: Mukuru NZ, Ejeta G (eds) Proceedings of a Workshop Organized to Reestablish Sorghum and Millets Research in Eastern and Central Africa, 6–9 November 1985, Kampala, Uganda

Mysore KS, Baird V (1997) Nuclear DNA content in species of *Eleusine* (Gramineae): a critical re-evaluation using laser flow cytometry. Plant Syst Evol 207:1–11

National Research Council (1996) Lost Crops of Africa. Vol 1: Grains. National Academy Press, Washington, DC

Oryokot JOE (1990) Trends in finger millet production in Uganda. In: Proc 7th EARSAM Regional Workshop on Sorghum and Millet Improvement in Eastern Africa, 25–28 June 1990, Nairobi, Kenya, pp 93–104

Peng JR, Richards DE, Hartley NM, Murphy GP, Devos KM, Flintham JE, Beales J, Fish LJ, Worland AJ, Pelica F, Sudhakar D, Christou P, Snape JW, Gale MD, Harberd NP (1999) 'Green Revolution' genes encode mutant gibberellin response modulators. Nature 400:256–261

Phillips SM (1974) Eleusine in East Africa. In: Polhill RM, Clayton WD, Phillips SM, Renvoice SA (eds) Flora of Tropical East Africa. Gramineae (Part 2). White Friars Press, London, pp 260–266

Rachie KO, Peters LV (1977) The Eleusines. A review of World Literature. ICRISAT, Pattancheru, India

Rao PU (1994) Evaluation of protein quality of brown and white ragi (*Eleusine coracana*) before and after malting. Food Chem 51:433–436

Tanksley SD, McCouch SR (1997) Seed banks and molecular maps: unlocking genetic potential from the wild. Science 277:1063–1066

Vadivoo AS, Joseph R, Ganesan NM (1998) Genetic variability and diversity for protein and calcium contents in finger millet (*Eleusine coracana* (L.) Gaertn) in relation to grain colour. Plant Foods Hum Nutr 52:353–364

Wang ZX, Yano M, Yamanouchi U, Iwamoto M, Monna L, Hayasaka H, Katayose Y, Sasaki T (1999) The *Pib* gene for rice blast resistance belongs to the nucleotide binding and leucine-rich repeat class of plant disease resistance genes. Plant J 19:55–64

Zhang D, Ayele M, Tefera H, Nguyen HT (2001) RFLP linkage map of the Ethiopian cereal tef [*Eragrostis tef* (Zucc) Trotter]. Theor Appl Genet 102:957–964

Zheng X, Chen X, Zhang X, Lin Z, Shang J, Xu J, Zhai W-X, Zhu L (2004) Isolation and identification of a gene in response to rice blast disease in rice. Plant Mol Biol 54:99–109

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