Chittaranjan Kole *Editor*

Wild Crop Relatives: Genomic and Breeding Resources

Cereals

Springer

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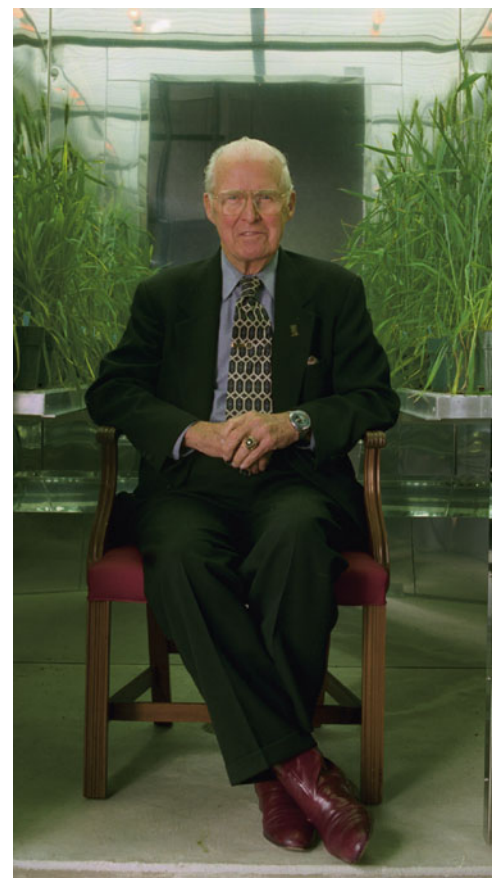
Dedication



Dr. Norman Ernest Borlaug,[1] the Father of Green Revolution, is well respected for his contributions to science and society. There was or is not and never will be a single person on this Earth whose single-handed service to science could save millions of people from death due to starvation over a period of over four decades like Dr. Borlaug's. Even the Nobel Peace Prize he received in 1970 does not do such a great and noble person as Dr. Borlaug justice. His life and contributions are well known and will remain in the pages of history of science. I wish here only to share some facets of this elegant and ideal personality I had been blessed to observe during my personal interactions with him.

It was early 2007 while I was at the Clemson University as a visiting scientist one of my lab colleagues told me that "somebody wants to talk to you; he appears to be an old man". I took the telephone receiver casually and said hello. The response from the other side was – "I am Norman Borlaug; am I talking to Chitta?" Even a million words would be insufficient to define and depict the exact feelings and thrills I experienced at that moment!

[1]The photo of Dr. Borlaug was kindly provided by Julie Borlaug (Norman Borlaug Institute for International Agriculture, Texas A&M Agriculture) the granddaughter of Dr. Borlaug.

I had seen Dr. Borlaug only once, way back in 1983, when he came to New Delhi, India to deliver the Coromandal Lecture organized by Prof. M.S. Swaminathan on the occasion of the 15th International Genetic Congress. However, my real interaction with him began in 2004 when I had been formulating a 7-volume book series entitled *Genome Mapping and Molecular Breeding in Plants*. Initially, I was neither confident of my ability as a series/book editor nor of the quality of the contents of the book volumes. I sent an email to Dr. Borlaug attaching the table of contents and the tentative outline of the chapters along with manuscripts of only a few sample chapters, including one authored by me and others, to learn about his views as a source of inspiration (or caution!) I was almost sure that a person of his stature would have no time and purpose to get back to a small science worker like me. To my utter (and pleasant) surprise I received an email from him that read: "May all Ph.D.'s, future scientists, and students that are devoted to agriculture get an inspiration as it refers to your work or future work from the pages of this important book. My wholehearted wishes for a success on your important job". I got a shot in my arm (and in mind for sure)! Rest is a pleasant experience – the seven volumes were published by Springer in 2006 and 2007, and were welcome and liked by students, scientists and their societies, libraries, and industries. As a token of my humble regards and gratitude, I sent Dr. Borlaug the Volume I on *Cereals and Millets* that was published in 2006. And here started my discovery of the simplest person on Earth who solved the most complex and critical problem of people on it – hunger and death.

Just one month after receiving the volume, Dr. Borlaug called me one day and said, "Chitta, you know I cannot read a lot now-a-days, but I have gone through only on the chapters on wheat, maize and rice. Please excuse me. Other chapters of this and other volumes of the series will be equally excellent, I believe". He was highly excited to know that many other Nobel Laureates including Profs. Arthur Kornberg, Werner Arber, Phillip Sharp, Günter Blobel, and Lee Hartwell also expressed generous comments regarding the utility and impact of the book series on science and the academic society. While we were discussing many other textbooks and review book series that I was editing at that time, again in my night hours for the benefit of students, scientists, and industries, he became emotional and said to me, "Chitta, forget about your original contributions to basic and applied sciences, you deserved Nobel Prize for Peace like me for providing academic foods to millions of starving students and scientists over the world particularly in the developing countries. I will recommend your name for the World Food Prize, but it will not do enough justice to the sacrifice you are doing for science and society in your sleepless nights over so many years. Take some rest Chitta and give time to Phullara, Sourav and Devleena" (he was so particular to ask about my wife and our kids during most of our conversations). I felt honored but really very ashamed as I am aware of my almost insignificant contribution in comparison to his monumental contribution and thousands of scientists over the world are doing at least hundred-times better jobs than me as scientist or author/editor of books! So, I was unable to utter any words for a couple of minutes but realized later that he must been too affectionate to me and his huge affection is the best award for a small science worker as me!

In another occasion he wanted some documents from me. I told him that I will send them as attachments in emails. Immediately he shouted and told me: "You know, Julie (his granddaughter) is not at home now and I cannot check email myself. Julie does this for me. I can type myself in type writer but I am not good in computer. You know what, I have a xerox machine and it receives fax also. Send me

the documents by fax". Here was the ever-present child in him. Julie emailed me later to send the documents as attachment to her as the 'xerox machine' of Dr. Borlaug ran out of ink!

Another occasion is when I was talking with him in a low voice, and he immediately chided me: "You know that I cannot hear well now-a-days; I don't know where Julie has kept the hearing apparatus, can't you speak louder?" Here was the fatherly figure who was eager to hear each of my words!

I still shed tears when I remember during one of our telephone conversations he asked: "You know I have never seen you, can you come to Dallas in the near future by chance?" I remember we were going through a financial paucity at that time and I could not make a visit to Dallas (Texas) to see him, though it would have been a great honor.

In late 2007, whenever I tried to talk to Dr. Borlaug, he used to beckon Julie to bring the telephone to him, and in course of time Julie used to keep alive all the communications between us when he slowly succumbed to his health problems.

The remaining volumes of the *Genome Mapping and Molecular Breeding in Plants* series were published in 2007, and I sent him all the seven volumes. I wished to learn about his views. During this period he could not speak and write well. Julie prepared a letter based on his words to her that read: "Dear Chitta, I have reviewed the seven volumes of the series on *Genome Mapping and Molecular Breeding in Plants*, which you have authored. You have brought together genetic linkage maps based on molecular markers for the most important crop species that will be a valuable guide and tool to further molecular crop improvements. Congratulations for a job well done".

During one of our conversations in mid-2007, he asked me what other book projects I was planning for Ph.D. students and scientists (who had always been his all-time beloved folks). I told him that the wealth of wild species already utilized and to be utilized for genetic analysis and improvement of domesticated crop species have not been deliberated in any book project. He was very excited and told me to take up the book project as soon as possible. But during that period I had a huge commitment to editing a number of book volumes and could not start the series he was so interested about.

His sudden demise in September 2009 kept me so morose for a number of months that I could not even communicate my personal loss to Julie. But in the meantime, I formulated a 10-volume series on *Wild Crop Relatives: Genomic and Breeding Resources* for Springer. And whom else to dedicate this series to other than Dr. Borlaug!

I wrote to Julie for her formal permission and she immediately wrote me: "Chitta, Thank you for contacting me and yes I think my grandfather would be honored with the dedication of the series. I remember him talking of you and this undertaking quite often. Congratulations on all that you have accomplished!" This helped me a lot as I could at least feel consoled that I could do a job he wanted me to do and I will always remain grateful to Julie for this help and also for taking care of Dr. Borlaug, not only as his granddaughter but also as the representative of millions of poor people from around the world and hundreds of plant and agricultural scientists who try to follow his philosophy and worship him as a father figure.

It is another sad experience of growing older in life that we walk alone and miss the affectionate shadows, inspirations, encouragements, and blessings from the fatherly figures in our professional and personal lives. How I wish I could treat my next generations in the same way as personalities like Mother Teresa and Dr. Norman Borlaug and many other great people from around the world treated me!

During most of our conversations he used to emphasize on the immediate impact of research on the society and its people. A couple of times he even told me that my works on molecular genetics and biotechnology, particularly of 1980s and 1990s, have high fundamental importance, but I should also do some works that will benefit people immediately. This advice elicited a change in my thoughts and workplans and since then I have been devotedly endeavoring to develop crop varieties enriched with phytomedicines and nutraceuticals. Borlaug influenced both my personal and professional life, particularly my approach to science, and I dedicate this series to him in remembrance of his great contribution to science and society and for all his personal affection, love and blessings for me.

I emailed the above draft of the dedication page to Julie for her views and I wish to complete my humble dedication with great satisfaction with the words of Julie who served as the living ladder for me to reach and stay closer to such as great human being as Dr. Borlaug and express my deep regards and gratitude to her. Julie's email read: "Chitta, Thank you for sending me the draft dedication page. I really enjoyed reading it and I think you captured my grandfather's spirit wonderfully. . .. So thank you very much for your beautiful words. I know he would be and is honored."

Clemson, USA Chittaranjan Kole

Preface

Wild crop relatives have been playing enormously important roles both in the depiction of plant genomes and the genetic improvement of their cultivated counterparts. They have contributed immensely to resolving several fundamental questions, particularly those related to the origin, evolution, phylogenetic relationship, cytological status and inheritance of genes of an array of crop plants; provided several desirable donor genes for the genetic improvement of their domesticated counterparts; and facilitated the innovation of many novel concepts and technologies while working on them directly or while using their resources. More recently, they have even been used for the verification of their potential threats of gene flow from genetically modified plants and invasive habits. Above all, some of them are contributing enormously as model plant species to the elucidation and amelioration of the genomes of crop plant species.

As a matter of fact, as a student, a teacher, and a humble science worker I was, still am and surely will remain fascinated by the wild allies of crop plants for their invaluable wealth for genetics, genomics and breeding in crop plants and as such share a deep concern for their conservation and comprehensive characterization for future utilization. It is by now a well established fact that wild crop relatives deserve serious attention for domestication, especially for the utilization of their phytomedicines and nutraceuticals, bioenergy production, soil reclamation, and the phytoremediation of ecology and environment. While these vastly positive impacts of wild crop relatives on the development and deployment of new varieties for various purposes in the major crop plants of the world agriculture, along with a few negative potential concerns, are envisaged the need for reference books with comprehensive deliberations on the wild relatives of all the major field and plantation crops and fruit and forest trees is indeed imperative. This was the driving force behind the inception and publication of this series.

Unlike the previous six book projects I have edited alone or with co-editors, this time it was very difficult to formulate uniform outlines for the chapters of this book series for several obvious reasons. Firstly, the status of the crop relatives is highly diverse. Some of them are completely wild, some are sporadically cultivated and some are at the initial stage of domestication for specific breeding objectives recently deemed essential. Secondly, the status of their conservation varies widely: some have been conserved, characterized and utilized; some have been eroded completely except for their presence in their center(s) of origin; some are at-risk or endangered due to genetic erosion, and some of them have yet to be explored. The third constraint is the variation in their relative worth, e.g. as academic model, breeding resource, and/or potential as “new crops.”

The most perplexing problem for me was to assign the chapters each on a particular genus to different volumes dedicated to crop relatives of diverse crops grouped based on their utility. This can be exemplified with *Arabidopsis*, which has primarily benefited the Brassicaceae crops but also facilitated genetic analyses and improvement in crop plants in other distant families; or with many wild relatives of forage crops that paved the way for the genetic analyses and breeding of some major cereal and millet crops. The same is true for wild crop relatives such as *Medicago truncatula*, which has paved the way for in-depth research on two crop groups of diverse use: oilseed and pulse crops belonging to the Fabaceae family. The list is too long to enumerate. I had no other choice but to compromise and assign the genera of crop relatives in a volume on the crop group to which they are taxonomically the closest and to which they have relatively greater contributions. For example, I placed the chapter on genus *Arabidopsis* in the volume on oilseeds, which deals with the wild relatives of Brassicaceae crops amongst others.

However, we have tried to include deliberations pertinent to the individual genera of the wild crop relatives to which the chapters are devoted. Descriptions of the geographical locations of origin and genetic diversity, geographical distribution, karyotype and genome size, morphology, etc. have been included for most of them. Their current utility status – whether recognized as model species, weeds, invasive species or potentially cultivable taxa – is also delineated. The academic, agricultural, medicinal, ecological, environmental and industrial potential of both the cultivated and/or wild allied taxa are discussed.

The conservation of wild crop relatives is a much discussed yet equally neglected issue albeit the in situ and ex situ conservations of some luckier species were initiated earlier or are being initiated now. We have included discussions on what has happened and what is happening with regard to the conservation of the crop relatives, thanks to the national and international endeavors, in most of the chapters and also included what should happen for the wild relatives of the so-called new, minor, orphan or future crops.

The botanical origin, evolutionary pathway and phylogenetic relationship of crop plants have always attracted the attention of plant scientists. For these studies morphological attributes, cytological features and biochemical parameters were used individually or in combinations at different periods based on the availability of the required tools and techniques. Access to different molecular markers based on nuclear and especially cytoplasmic DNAs that emerged after 1980 refined the strategies required for precise and unequivocal conclusions regarding these aspects. Illustrations of these classical and recent tools have been included in the chapters.

Positioning genes and defining gene functions required in many cases different cytogenetic stocks, including substitution lines, addition lines, haploids, monoploids and aneuploids, particularly in polyploid crops. These aspects have been dealt in the relevant chapters. Employment of colchiploidy, fluorescent or genomic in situ hybridization and Southern hybridization have reinforced the theoretical and applied studies on these stocks. Chapters on relevant genera/species include details on these cytogenetic stocks.

Wild crop relatives, particularly wild allied species and subspecies, have been used since the birth of genetics in the twentieth century in several instances such as studies of inheritance, linkage, function, transmission and evolution of genes. They have been frequently used in genetic studies since the advent of molecular markers. Their involvement in molecular mapping has facilitated the development of mapping

populations with optimum polymorphism to construct saturated maps and also illuminating the organization, reorganization and functional aspects of genes and genomes. Many phenomena such as genomic duplication, genome reorganization, self-incompatibility, segregation distortion, transgressive segregation and defining genes and their phenotypes have in many cases been made possible due to the utilization of wild species or subspecies. Most of the chapters contain detailed elucidations on these aspects.

The richness of crop relatives with biotic and abiotic stress resistance genes was well recognized and documented with the transfer of several alien genes into their cultivated counterparts through wide or distant hybridization with or without employing embryo-rescue and mutagenesis. However, the amazing revelation that the wild relatives are also a source of yield-related genes is a development of the molecular era. Apomictic genes are another asset of many crop relatives that deserve mention. All of these past and the present factors have led to the realization that the so-called inferior species are highly superior in conserving desirable genes and can serve as a goldmine for breeding elite plant varieties. This is particularly true at a point when natural genetic variability has been depleted or exhausted in most of the major crop species, particularly due to growing and promoting only a handful of so-called high-yielding varieties while disregarding the traditional cultivars and landraces. In the era of molecular breeding, we can map desirable genes and polygenes, identify their donors and utilize tightly linked markers for gene introgression, mitigating the constraint of linkage drag, and even pyramid genes from multiple sources, cultivated or wild taxa. The evaluation of primary, secondary and tertiary gene pools and utilization of their novel genes is one of the leading strategies in present-day plant breeding. It is obvious that many wide hybridizations will never be easy and involve near-impossible constraints such as complete or partial sterility. In such cases gene cloning and gene discovery, complemented by intransgenic breeding, will hopefully pave the way for success. The utilization of wild relatives through traditional and molecular breeding has been thoroughly enumerated over the chapters throughout this series.

Enormous genomic resources have been developed in the model crop relatives, for example *Arabidopsis thaliana* and *Medicago truncatula*. BAC, cDNA and EST libraries have also been developed in some other crop relatives. Transcriptomes and metabolomes have also been dissected in some of them. However, similar genomic resources are yet to be constructed in many crop relatives. Hence this section has been included only in chapters on the relevant genera.

In this book series, we have included a section on recommendations for future steps to create awareness about the wealth of wild crop relatives in society at large and also for concerns for their alarmingly rapid decrease due to genetic erosion. The authors of the chapters have also emphasized on the imperative requirement of their conservation, envisaging the importance of biodiversity. The importance of intellectual property rights and also farmers' rights as owners of local landraces, botanical varieties, wild species and subspecies has also been dealt in many of the chapters.

I feel satisfied that the authors of the chapters in this series have deliberated on all the crucial aspects relevant to a particular genus in their chapters.

I am also very pleased to present many chapters in this series authored by a large number of globally reputed leading scientists, many of whom have contributed to the development of novel concepts, strategies and tools of genetics, genomics and breeding and/or pioneered the elucidation and improvement of particular plant

genomes using both traditional and molecular tools. Many of them have already retired or will be retiring soon, leaving behind their legacies and philosophies for us to follow and practice. I am saddened that a few of them have passed away during preparation of the manuscripts for this series. At the same time, I feel blessed that all of these stalwarts shared equally with me the wealth of crop relatives and contributed to their recognition and promotion through this endeavor.

I would also like to be candid with regard to my own limitations. Initially I planned for about 150 chapters devoted to the essential genera of wild crop relatives. However, I had to exclude some of them either due to insignificant progress made on them during the preparation of this series, my failure to identify interested authors willing to produce acceptable manuscripts in time or authors' backing out in the last minute, leaving no time to find replacements. I console myself for this lapse with the rationale that it is simply too large a series to achieve complete satisfaction on the contents. Still I was able to arrange about 125 chapters in the ten volumes, contributed by nearly 400 authors from over 40 countries of the world. I extend my heartfelt thanks to all these scientists, who have cooperated with me since the inception of this series not only with their contributions, but also in some cases by suggesting suitable authors for chapters on other genera. As happens with a mega-series, a few authors had delays for personal or professional reasons, and in a few cases, for no reason at all. This caused delays in the publication of some of the volumes and forced the remaining authors to update their manuscripts and wait too long to see their manuscripts in published form. I do shoulder all the responsibilities for this myself and tender my sincere apologies.

Another unique feature of this series is that the authors of chapters dedicated to some genera have dedicated their chapters to scientists who pioneered the exploration, description and utilization of the wild species of those genera. We have duly honored their sincere decision with equal respect for the scientists they rightly reminded us to commemorate.

Editing this series was, to be honest, very taxing and painstaking, as my own expertise is limited to a few cereal, oilseed, pulse, vegetable, and fruit crops, and some medicinal and aromatic plants. I spent innumerable nights studying to attain the minimum eligibility to edit the manuscripts authored by experts with even life-time contributions on the concerned genera or species. However, this indirectly awakened the "student-for-life" within me and enriched my arsenal with so many new concepts, strategies, tools, techniques and even new terminologies! Above all, this helped me to realize that individually we know almost nothing about the plants on this planet! And this realization strikingly reminded me of the affectionate and sincere advice of Dr. Norman Borlaug to keep abreast with what is happening in the crop sciences, which he used to do himself even when he had been advised to strictly limit himself to bed rest. He was always enthusiastic about this series and inspired me to take up this huge task. This is one of the personal and professional reasons I dedicated this book series to him with a hope that the present and future generations of plant scientists will share the similar feelings of love and respect for all plants around us for the sake of meeting our never-ending needs for food, shelter, clothing, medicines, and all other items used for our basic requirements and comfort. I am also grateful to his granddaughter, Julie Borlaug, for kindly extending her permission to dedicate this series to him.

I started editing books with the 7-volume series on Genome Mapping and Molecular Breeding in Plants with Springer way back in 2005, and I have since

edited many other book series with Springer. I always feel proud and satisfied to be a member of the Springer family, particularly because of my warm and enriching working relationship with Dr. Sabine Schwarz and Dr. Jutta Lindenborn, with whom I have been working all along. My special thanks go out to them for publishing this "dream series" in an elegant form and also for appreciating my difficulties and accommodating many of my last-minute changes and updates.

I would be remiss in my duties if I failed to mention the contributions of Phullara – my wife, friend, philosopher and guide – who has always shared with me a love of the collection, conservation, evaluation, and utilization of wild crop relatives and has enormously supported me in the translation of these priorities in my own research endeavors – for her assistance in formulating the contents of this series, for monitoring its progress and above all for taking care of all the domestic and personal responsibilities I am supposed to shoulder. I feel myself alien to the digital world that is the sine qua non today for maintaining constant communication and ensuring the preparation of manuscripts in a desirable format. Our son Sourav and daughter Devleena made my life easier by balancing out my limitations and also by willingly sacrificing the spare amount of time I ought to spend with them. Editing of this series would not be possible without their unwavering support.

I take the responsibility for any lapses in content, format and approach of the series and individual volumes and also for any other errors, either scientific or linguistic, and will look forward to receiving readers' corrections or suggestions for improvement.

As I mentioned earlier this series consists of ten volumes. These volumes are dedicated to wild relatives of Cereals, Millets and Grasses, Oilseeds, Legume Crops and Forages, Vegetables, Temperate Fruits, Tropical and Subtropical Fruits, Industrial Crops, Plantation and Ornamental Crops, and Forest Trees.

This volume "Wild Crop Relatives – Genomic and Breeding Resources: Cereals" includes 11 chapters dedicated to *Aegilops*, *Agropyron* and *Psathyrostachys*, *Avena*, *Dasypyrum*, *Fagopyron*, *Hordeum*, *Oryza*, *Secale*, *Sorghum*, *Triticum*, and *Zea*. The chapters of this volume were authored by 43 scientists from 11 countries of the world, namely Australia, China, Germany, India, Indonesia, Israel, Italy, Philippines, Russia, Turkey, and the USA.

It is my sincere hope that this volume and the series as a whole will serve the requirements of students, scientists and industries involved in studies, teaching, research and the extension of Cereals with an intention of serving science and society.

Clemson, USA Chittaranjan Kole

Contents

Abbreviations

AB-QTL	Advanced backcross QTL
ACO	Aconitate hydrotase
Adh	Alcohol dehydrogenase (gene)
AFLP	Amplified fragment length polymorphism
AIL	Alien introgression line
Al	Altitude
ARN	Average repeat number
ARS	Agriculture Research Service (USDA)
BA or Ba	Basalt soil
BAC	Bacterial artificial chromosome
BARE-1	Barley retroelement 1
Bgt	*Blumeria graminis* f sp. *tritici*
BLAST	Basic alignment search tool
BSA	Bulked segregant analysis
BYDV	Barley yellow dwarf virus
CAAS	Chinese Academy of Agricultural Sciences
CAB	Chlorophyll-a/b binding protein
CAPS	Cleaved amplified polymorphic sequence
CBD	Convention on Biological Diversity
ccSSR	Consensus chloroplast simple sequence repeat
CGIAR	Consultative Group on International Agricultural Research
CIM	Composite interval mapping
CINAU	Cytogenetics Institute, Nanjing Agricultural University, China
cpDNA	Chloroplast DNA
CSSL	Chromosome segmental substutution line
CSTT	Catastrophic Sexual Transmutation Theory
CT	Chlorotolurone (herbicide)
DA	Disomic chromosome addition line
DArT	Diversity arrays technology
Db	*Dasypyrum breviaristatum*
DIA	Diaphorase
DS	Disomic chromosome substitution line
Dv	*Dasypyrum villosum*
Dw	Mean number of dew nights in summer
EADB	European *Avena* Database
ECP/GR	European Cooperative Program for Genetic Resources

ELISA	Enzyme-linked immunosorbent assay
EMS	Ethylmethane sulphonate
En/Spm	Enhancer/suppressor mutator
Est	Esterase (gene)
EST	Expressed sequence tag
EURISCO	European Network of ex situ National Inventories
Ev	Mean annual evaporation
FAL	Federal Agriculture Research Centre (Germany)
FAO	Food and Agriculture Organization (of the United Nation)
FHB	Fusarium head blight
FISH	Fluorescence in situ hybridization
G3pdh	Glyceraldehyde 3-phosphate dehydrogenase
GBIF	Global Biodiversity Information Facility
GBSS1	Granule bound starch synthase
GCA	General combining ability
GE	Genotype-environment interaction
GIS	Geographical information system
GISH	Genomic in situ hybridization
GM	Genetic modification
GMO	Genetically-modified organism
Got/GOT	Glutamate oxaloacetate transaminase
GPI	Glucose phosphate isomerase
GRASSIUS	Grass Regulatory Information Services
GRIN	Germplasm Resources Information Network (USA)
GSI	Gametophytic self-incompatibility
GSS	Genome survey sequences
GWM	Gatersleben wheat microsatellite (marker)
He	Gene diversity
Hk	Hexokinase (gene)
HMW	High molecular weight
Hu	Mean humidity
IAP	Inhibition of alien pollen
IBPGR	International Board for Plant Genetic Resources
ICARDA	International Center for Agricultural Research in the Dry Areas
IM	Interval mapping
INIBAP	International Network for the Improvement of Banana and Plantain
IPGRI	International Plant Genetic Recourses Institute
Ipol	Indophenol oxidase
IRG	International Rice Gene Bank
ISSR	Inter-simple sequence repeat
IT	International Treaty
ITS	Internal transcribed spacer
LD	Linkage disequilibrium
LMW	Low molecular weight
Ln	Longitude
LSR	Long sequence repeats
Lt	Latitude

LtTddRv	Latitude, day-night temperature, and mean relative variability of rainfall
MAAL	Monosomic alien addition line
MAS	Marker-assisted selection
MBC	Map-based cloning
Mdh/MDH	Malate dehydrogenase
MGP	Multilocus genotype pattern
MR	Multiple regression analysis
MX	Metoxuron (herbicide)
Mya	Million years ago
N5BT5D	Wheat line that is nullisomic for 5B and tetrasomic for 5D chromosomes
NAC	NAC transcription factor
NARES	National Agricultural Research and Extension Systems
NGRP	National Genetic Resources Program (USA)
NPGS	National Plant Germplasm System (USA)
NSGC	National Small Grains Collection (USA)
NSSL	National Seed Storage Laboratory (USA)
OMAP	*Oryza* Map Alignment Project
P	Proportion of polymorphic loci
PAGE	Polyacrylamide gel electrophoresis
Pc	*Puccinia coronata* Cda. f. sp. *avenae* Fraser et Led.
PCA	Principal component analysis
PCR	Polymerase chain reaction
Pept	Peptidase
Pg	*Puccinia graminis*
PGD	6-Phosphogluconate dehydrogenase
PGM	Phosphoglucomutase
PGR	Plant genetic resource
PGRC	Plant Gene Resources of Canada
Ph^I	Ph inhibitor line
POOL	Name of Pedigree of Oat Lines Database
Ppd-H1	Psuedo-response regulator
Pst	*Puccinia striiformis* f sp. *tritici*
Pt	*Puccinia triticina*
QTL	Quantitative trait loci
R^2	Coefficient of multiple determination
RAPD	Random(ly) amplified polymorphic DNA
Rd	Mean number of rainy days
RDA	Representational difference analysis
rDNA	Ribosomal DNA
Ren	Rendzina soil
RFLP	Restriction fragment length polymorphism
RIL	Recombinant inbred line
Rn	Mean annual rainfall
Rr	Mean relative interannual variability of rainfall
r_s	Spearman's rank order correlation

Rst	Inter- vs. Intra-population degree of gene differentiation among populations
RUL	Mean values for ^{22}Na uptake in *T. dicoccoides* relative to ^{22}Na uptake in Langdon control
Rv	Mean interannual rainfall variability of rainfall
SBCMV	Soil-borne cereal mosaic virus
SCAR	Sequence characterized amplified region
SDS	Sodium dodecyl sulfate
SEM	Scanning electron microscopy
SESTO	Regional (Northern) PGR Documentation System
SNB	*Stagonospora nodorum* blotch
SNP	Single nucleotide polymorphism
So	Soil type
SSR	Simple sequence repeat
STLT	Sexual Translocation Theory
STS	Sequence tagged site
Ta	Mean August temperature
TARGeT	Tree analysis of related genes and transposons
Td	Seasonal temperature difference
Tdd	Day–night temperature difference
TE	Transposable elements
TF	Transcriptional factor
TILLING	Targeting induced local lesions in genomes
Tj	Mean January temperature
Tm	Mean annual temperature
Tr/TR	Terra rossa soil
Trd	Mean number of tropical days
Tt	*Tilletia tritici*
UPGMA	Unweighted pair group method with arithmetic average
USDA	United States Department of Agriculture
VIR	N. I. Vavilov Institute of Plant Industry (Russia)
WBDC	Wild barley diversity collection
WCM	Wheat curly mite
WIEWS	World Information and Early Warning System (FAO)
WMAI	Wheat monomeric alpha amylase inhibitor gene
WSBMV	Wheat soil-borne mosaic virus
WSMV	Wheat streak mosaic virus
WSSMV	Wheat spindle streak mosaic virus
WUE	Water use efficiency
Yr	Yellow rust

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Chapter 1
Aegilops

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1.1 Introduction

The genus *Aegilops* L. belongs to the tribe Triticeae within the Pooideae subfamily of the grass family Poaceae. The tribe includes six major genera (*http://www.k-state.edu./wgrc/Taxonomy/Triticeaetax.html*), among which the important crop genera are *Triticum* (wheat), *Hordeum* (barley), and *Secale* (rye).

The phylogenetic relationships and evolution within the Triticeae are of great interest due to potentially favorable alleles to be discovered in wild wheat relatives and to be transferred to bread wheat. However, especially the relationships within and between *Aegilops* and *Triticum* in the subtribe Triticinae are a matter of ongoing discussion, and the relationships among the taxa are far from being completely understood. This is also documented in various classification systems, as presented for *Aegilops* in Table 1.1. Most researchers currently follow the latest monograph of van Slageren (1994). However, new data have been recently produced and some aspects have to be reconsidered. New molecular data are urgently needed to provide more insights in the Triticeae phylogeny.

Tables 1.1 and 1.2 report the *Aegilops* taxa considered in this chapter. The genus *Aegilops* comprises 23 annual species, of which 11 are diploids and 12 are allopolyploids (see Table 1.2 and the species descriptions for synonyms).

Some *Aegilops* species participated in wheat evolution and played a major role in wheat domestication. Thus, the genus *Aegilops* represents the largest part of the secondary gene pool of wheat, and several species have been used in crop improvement programs.

The latest revision of *Aegilops* by van Slageren (1994) is based on morphological studies. Van Slageren conducted field trips in the years 1988–1994 and examined about 20,000 herbarium specimens representing, in van Slageren's opinion, an estimated 75–85% of all *Aegilops* material available. In this chapter, we therefore refer to van Slageren (1994) and Hammer (1980a, b) for morphological descriptions and history of the genera *Aegilops* and nomenclature.

For wheat, the latest comprehensive, systematic overview was completed in 1979 by Dorofeev and colleagues. In this chapter, the nomenclature and the genome formula given for *Triticum* by Dorofeev et al. (1979) is mainly followed (exception e.g., *T. dicoccon* Schrank). Other concise comparisons of the main wheat classifications are also available (Hanelt 2001; Mac Key 2005; Hammer et al. 2011).

This chapter is an introduction and an overview on *Aegilops*. A key and a brief botanical description are presented, together with ear morphology and distribution maps, and cytological and molecular data for each species.

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"For the sake of future generations, we MUST collect and study wild and weedy relatives of our cultivated plants as well as the domesticated races. These sources of germplasm have been dangerously neglected in the past, but the future may not be so tolerant. In the plant breeding programs of tomorrow we cannot afford to ignore *any* source of useable genes." Harlan (1970)

C. Kole (ed.), *Wild Crop Relatives: Genomic and Breeding Resources, Cereals*,
DOI 10.1007/978-3-642-14228-4_1,

Table 1.1 Overview of selected *Aegilops* classifications

This chapter	van Slageren (1994)	Kimber et Sears (1983)	Whitcombe (1983)	Hammer (1980a, b)	Kihara (1954)	Eig (1929)	Zhukovsky (1928)
Section *Aegilops*							
1 *Ae. biuncialis* Vis.	*Ae. biuncialis* Vis.	*Triticum macrochaetum* (Shuttl. et Huet) Richter	*Ae. lorentii* Hochst.	*Ae. lorentii* Hochst.	*Ae. biuncialis* Vis.	*Ae. biuncialis* Vis.	*Ae. biuncialis* Vis.
2 *Ae. columnaris* Zhuk.	*Ae. columnaris* Zhuk.	*Triticum columnare* (Zhuk.) Morris et Sears	*Ae. columnaris* Zhuk.	*Ae. columnaris* Zhuk.	*Ae. columnaris* Zhuk.	*Ae. columnaris* Zhuk.	*Ae. columnaris* Zhuk.
3 *Ae. geniculata* Roth	*Ae. geniculata* Roth	*Triticum ovatum* (L.) Raspail	*Ae. ovata* L.	*Ae. geniculata* Roth	*Ae. ovata* L.	*Ae. ovata* L.	*Ae. ovata* L.
ssp. *geniculata*							
ssp. *gibberosa* (Zhuk.) Hammer							
4 *Ae. kotschyi* Boiss.	*Ae. kotschyi* Boiss.	*Triticum kotschyi* (Boiss.) Bowden	*Ae. kotschyi* Boiss.	*Ae. kotschyi* Boiss.	*Ae. kotschyi* Boiss.	*Ae. kotschyi* Boiss.	
5 *Ae. neglecta* Req. ex Bertol.	*Ae. neglecta* Req. ex Bertol. (4x and 6x)	*Triticum triaristatum* (Willd.) Godr. et Gren. (4x and 6x)	*Ae. triaristata* Willd. (4x and 6x)	*Ae. neglecta* Req. ex Bertol.	*Ae. triaristata* Willd. (4x and 6x)	*Ae. triaristata* Willd. (4x and 6x)	*Ae. triaristata* Willd. (4x and 6x)
ssp. *neglecta* (4x)				ssp. *neglecta* (4x)			
ssp. *recta* (Zhuk.) Hammer (6x)				ssp. *recta* (Zhuk.) Hammer (6x)			
6 *Ae. peregrina* (Hackel) Maire et Weiller	*Ae. peregrina* (Hack. in J.Fraser) Maire et Weiller	*Triticum kotschyi* (Boiss) Bowden	*Ae. peregrina* (Hackel) Maire et Weiller	*Ae. peregrina* (Hackel) Maire et Weiller	*Ae. variabilis* Eig	*Ae. variabilis* Eig	
ssp. *peregrina*	var. *peregrina*			ssp. *peregrina*			
	var. *brachyathera* (Boiss.) Maire et Weiller			ssp. *cylindrostachys* (Eig et Feinbrun) Maire et Weiller			
7 *Ae. triuncialis* L.	*Ae. triuncialis* L.	*Triticum triunciale* (L.) Raspail	*Ae. triuncialis* L.	*Ae. triuncialis* L.	*Ae. triuncialis* L.	*Ae. triuncialis* L.	*Ae. triuncialis* L.
ssp. *triuncialis*	var. *triuncialis*			ssp. *triuncialis*			
ssp. p*ersica* (Boiss.) Zhuk.	var. *persica* (Boiss.) Eig			ssp. *persica* (Boiss.) Zhuk.			
8 *Ae. umbellulata* Zhuk.	*Ae. umbellulata* Zhuk.	*Triticum umbellulatum* (Zhuk.) Bowden	*Ae. umbellulata* Zhuk.	*Ae. umbellulata* Zhuk.	*Ae.*		*umbellulata* Zhuk.
Ae. umbellulata Zhuk.	*Ae. umbellulata* Zhuk.						
ssp. *umbellulata*							
ssp. *transcaucasia* Dorof. et Migusch.				ssp. *transcaucasia* Dorof. et Migusch.			

(*continued*)

Table 1.1 (continued)

This chapter	van Slageren (1994)	Kimber et Sears (1983)	Whitcombe (1983)	Hammer (1980a, b)	Kihara (1954)	Eig (1929)	Zhukovsky (1928)
Section *Comopyrum*							
9 *Ae. comosa* Sibth. et Sm.	*Ae. comosa* Sm. in Sibth. et Sm.	*Triticum comosum* (Sibth. et Sm.) Richter	*Ae. comosa* Sibth. et Sm.	*Ae. comosa* Sibth. et Sm.	*Ae. comosa* Sibth. et Sm.	*Ae. comosa* Sibth. et Sm.	*Ae. comosa* Sibth. et Sm.
ssp. *comosa*	var. *comosa*			ssp. comosa			
ssp. *heldreichii* (Boiss.) Eig (syn.: var. *subventricosa* Boiss.)	var. *subventricosa* Boiss.			ssp. *heldreichii* (Boiss.) Eig		ssp. *heldreichii* (Holzm.) Eig	
10 *Ae. uniaristata* Vis.	*Ae. uniaristata* Vis.	*Triticum uniaristatum* (Vis.) Richter	*Ae. uniaristata* Vis.	*Ae. uniaristata* Vis.	*Ae. uniaristata* Vis.	*Ae. uniaristata* Vis.	*Ae. uniaristata* Vis.
Section *Cylindropyrum*							
11 *Ae. cylindrica* Host	*Ae. cylindrica* Host	*Triticum cylindricum* Ces.	*Ae. cylindrica* Host	*Ae. cylindrica* Host	*Ae. cylindrica* Host	*Ae. cylindrica* Host	*Ae. cylindrica* Host
12 *Ae. markgrafii* (Greuter) Hammer	*Ae. caudata* L.	*Triticum dichasians* (Zhuk.) Bowden	*Ae. caudata* L.	*Ae. markgrafii* (Greuter) Hammer	*Ae. caudata* L.	*Ae. caudata* L.	*Ae. caudata* L.
Section *Sitopsis*							
13 *Ae. bicornis* (Forssk.) Jaub. et Sp.	*Ae. bicornis* (Forssk.) Jaub. et Spach	*Triticum bicorne* Forssk.	*Ae. bicornis* (Forssk.) Jaub. et Spach	*Ae. bicornis* (Forssk.) Jaub. et Spach	*Ae. bicornis* (Forssk.) Jaub. et Spach	*Ae. bicornis* (Forssk.) Jaub. et Spach	*Ae. bicornis* (Forssk.) Jaub. et Spach
	var. *bicornis*						
	var. *anathera* Eig						
14 *Ae. longissima* Schweinf. et Muschl.	*Ae. longissima* Schweinf. et Muschl.	*Triticum longissimum* (Schweinf. et Muschl.) Bowden	*Ae. longissima* Schweinf. et Muschl.	*Ae. longissima* Schweinf. et Muschl. emend. Eig s.l.	*Ae. longissima* Schweinf. et Muschl.	*Ae. longissima* Schweinf. et Muschl.	*Ae. longissima* (Schweinf. et Muschl.) Eig
				ssp. *longissima*			
15 *Ae. sharonensis* Eig	*Ae. sharonensis* Eig		*Ae. sharonensis* Eig	ssp. *sharonensis* (Eig) Hammer		*Ae.*	*sharonensis* Eig
16 *Ae. searsii* Feldman et Kislev ex Hammer	*Ae. searsii* Feldman et Kislev ex Hammer	*Triticum searsii* (Feldman et Kislev) Feldman	*Ae. searsii* Feldman et Kislev	*Ae. searsii* Feldman et Kislev ex Hammer			
17 *Ae. speltoides* Tausch	*Ae. speltoides* Tausch	*Triticum speltoides* (Tausch) Gren. ex Richter	*Ae. speltoides* Tausch	*Ae. speltoides* Tausch	*Ae. speltoides* Tausch	*Ae. speltoides* Tausch	*Ae. speltoides* Tausch
ssp. *speltoides*	var. *speltoides*			ssp. *speltoides*			
ssp. *ligustica* (Savign.) Zhuk.	var. *ligustica* (Savign.) Fiori		*Ae. ligustica* (Savign.) Coss.	ssp. *ligustica* (Savign.) Zhuk.		*Ae. ligustica* (Savign.) Coss.	

(*continued*)

Table 1.1 (continued)

This chapter	van Slageren (1994)	Kimber et Sears (1983)	Whitcombe (1983)	Hammer (1980a, b)	Kihara (1954)	Eig (1929)	Zhukovsky (1928)
Section *Vertebrata*							
18 *Ae. crassa* Boiss. (4x and 6x)	*Ae. crassa* Boiss. (4x and 6x)	*Triticum crassum* (Boiss.) Aitch. et Hemsl. (4x and 6x)	*Ae. crassa* Boiss. (4x and 6x)	*Ae. crassa* Boiss. (4x and 6x)	*Ae. crassa* Boiss. (4x and 6x)	*Ae. crassa* Boiss. (4x and 6x)	*Ae. crassa* Boiss. (4x and 6x)
				ssp. *crassa*			
19 *Ae. vavilovii* (Zhuk.) Chennav. (6x)	*Ae. vavilovii* (Zhuk.) Chennav.	*Triticum syriacum* Bowden	*Ae. vavilovii* (Zhuk.) Chennav.	ssp. *vavilovii* Zhuk. (6x)			
20 *Ae. juvenalis* (Thell.) Eig	*Ae. juvenalis* (Thell.) Eig	*Triticum juvenale* Thell.	*Ae. juvenalis* (Thell.) Eig	*Ae. juvenalis* (Thell.) Eig	*Ae. juvenalis* (Thell.) Eig	*Ae. juvenalis* (Thell.) Eig	*Ae. turcomanica* Roshev.
				Ae. turcomanica Roshev.			
21 *Ae. tauschii* Coss.	*Ae. tauschii* Coss.	*Triticum tauschii* (Coss.) Schmalh.	*Ae. squarrosa* L.	*Ae. tauschii* Coss.	*Ae. squarrosa* L.	*Ae. squarrosa* L.	*Ae. squarrosa* L.
ssp. *tauschii*							
ssp. *strangulata* (Eig) Tzvel.							
22 *Ae. ventricosa* Tausch	*Ae. ventricosa* Ces.	*Triticum ventricosum* Tausch	*Ae. ventricosa* Tausch	*Ae. ventricosa* Tausch	*Ae. ventricosa* Tausch	*Ae. ventricosa* Tausch	*Ae. ventricosa* Tausch
Subgenus *Ambylopyrum*							
23 *Ae. mutica* Boiss.	*Ambylopyrum muticum* (Boiss.) Eig	*Triticum tripsacoides* (Jaub. et Spach) Bowden	*Ae. mutica* Boiss.	*Ae. mutica* Boiss.	*Ae. mutica* Boiss.	*Ae. mutica* Boiss.	*Ae. mutica* Boiss.
ssp. *mutica*	var. *muticum*			var. *muticum*			
ssp. *loliacea* (Jaub et Spach) Zhuk.	var. *loliaceum* (Jaub. et Spach) Eig			var. *loliacea* (Jaub. et Spach) Eig			

Table 1.2 Sections and species of *Aegilops*. Genomic formulas of tetraploids and hexaploids are cited as "female × male parent." Underlining indicates modification of the same genome as present in the diploid species. Genome (G) and cytoplasm (C) symbols are according to Kimber and Tsunewaki (1988). Mean nuclear DNA content (Mean 1C) in pg of *Aegilops* species according to Eilam et al. (2007, 2008). nd – not determined

Aegilops section	Diploid				Tetraploid				Hexaploid			
	Species	G	C	Mean 1C	Species	G	C	Mean 1C	Species	G	C	Mean 1C
Aegilops L.	*Ae. umbellulata*	U	U	5.38	*Ae. biuncialis*	UM	U	10.37				
					Ae. columnaris	UM	U^2	10.86				
					Ae. geniculata	MU	M^o	10.29				
					Ae. kotschyi	SU	S^2	12.64				
					Ae. neglecta ssp. *neglecta*	UM	U	10.64	*Ae. neglecta* ssp. *recta*	UMN	U	16.22
					Ae. peregrina	SU	S^s	12.52				
					Ae. triuncialis	UC, CU	C,U	9.93				
Comopyrum (Jaub. et Spach) Zhuk.	*Ae. comosa*	M	M	5.53								
	Ae. uniaristata	N	N	5.82								
Cylindropyron (Jaub. et Spach) Zhuk.	*Ae. markgrafii*	C	C	4.84	*Ae. cylindrica*	DC	D	9.59				
Sitopsis (Jaub. et Spach) Zhuk	*Ae. bicornis*	S^b	S^b	6.84								
	Ae. longissima	S^l	S^l	7.48								
	Ae. sharonensis	S^{sh}	S^l	7.52								
	Ae. searsii	S^s	S^s	6.65								
	Ae. speltoides	S	S	5.81								
Vertebrata Zhuk. emend. Kihara	*Ae. tauschii*	D	D	5.17	*Ae. crassa* ssp. *crassa* (4x)	DM	D^2	10.86	*Ae. crassa* ssp. *crassa* (6x)	DDM	D^2	nd
									Ae. vavilovii	DMS	D^2	17.13
					Ae. ventricosa	DN	D	10.64	*Ae. juvenalis*	DMU	D^2	nd
Subgenus *Amblyopyrum*	*Ae. mutica*	T	nd	nd								

1.2 Botany of the Genus *Aegilops*

1.2.1 *Geographical Distribution and Ecology of* Aegilops

Aegilops is a Mediterranean–western Asiatic element comprising species that occur in both the Mediterranean and Irano-Turanian regions (Hedge et al. 2002). The genus occurs from 10° West to 82° East and from 24° to 47° North (introductions outside the natural distribution are not considered). *Aegilops* grow in Mediterranean Europe and southern Ukraine, the Crimea; as well as Cis- and Transcaucasia; in Africa, north of the Sahara; in western and Central Asia, the region bordered by the deserts of the Arabian peninsula in the south and by the Tian Shan mountains in the east. Several *Aegilops* species have been introduced in the US, of which *Ae. cylindrica* is now widespread. Several species are adventive in northern and northwestern Europe and in the Canary Islands. The altitudinal distribution of the genus varies from −400 m up to 2,700 m, but it differs greatly among the species (Hodgkin et al. 1992; van Slageren 1994). Geographical information system (GIS) distribution maps of *Aegilops* species developed for this chapter are based on van Slageren's (1994) and on our own observations. Adventive locations outside the natural species distribution range are not considered.

According to van Slageren (1994), the largest diversity of the genus *Aegilops* can be found in the Fertile Crescent ranging from Palestine/Israel – Lebanon – Syria – Southeast Turkey – North Iraq to Northwest Iran. Within this area, the central part of the Fertile Crescent between Euphrates and Tigris, where the southern slopes of the Taurus mountain range meet the lowlands and steppes, has the largest diversity. Mapping of *Aegilops* species richness identifies Northwest Jordan, Israel, Lebanon, western Syria, Iraq, and Turkey as areas with more than nine *Aegilops* species. Two main hotspots were found with 12–14 *Aegilops* species (1) western Syria – Northeast Lebanon and (2) northern Iraq (van Slageren 1994; Maxted et al. 2008). According to Hammer (1980a), the origin of *Aegilops* can be sought in the Transcaucasian area, from which diploid species migrated in western and southwestern directions. Later, groups of tetraploid species due to their adaptation capacity spread again both west and southwest around the Mediterranean basin, as well as east into Central Asia.

Aegilops show adaptation to disturbed environments, such as pastures, roadsides, garrigue, and maquis, various types of park-forest, and in edges of and within cultivation, thus both in ruderal and segetal environments. *Aegilops* species grow intermingled with other grasses (including other *Aegilops* and *Triticum* species) and with shrubs. They rarely dominate the vegetation. Ecological descriptions in this chapter are based on van Slageren (1994).

The annual growth habit of *Aegilops* and the predominance of self-pollination are advantageous life strategies in a region with seasonal rainfall and hot summer. Several *Aegilops* species are nevertheless (partially) outcrossing: *Ae. mutica* and *Ae. speltoides* (Hammer 1980a; Sakamoto 1982).

Aegilops shows morphological diversification of the spike and variation in seed dispersal. Three types of spike disarticulation are recognized: wedge, barrel, and whole-spike. Cultivated or otherwise improved forms of *Aegilops* species do not exist (van Slageren 1994).

1.2.2 *Description of the Genus* Aegilops

Family: **Poaceae** Barnhart, Bull. Torrey Bot. Club 22:7 (1895), *nom. cons.* – Alternative name: *Gramineae* Juss., Gen. pl. 28 (1789), *nom. cons.* – Type genus: *Poa* L.

Subfamily: **Pooideae**. Autonym; used for the first time by A.C.H. Braun in Ascherson, Fl. Brandenburg 1(2):810 (1864, "*Poeideae* R. Br.")

Tribe: **Triticeae** Dumort., Observ. Gramin. belg. 84 (1824) – Type genus: *Triticum*

Subtribe: **Triticinae** Griseb., Spic. fl. rumel. 2 (5/6):422 (1846).

The conspectus of *Aegilops* follows Hammer (1980b) with some additions/corrections mainly from Hammer (1987) and van Slageren (1994). For the description of species, the following main sources have been consulted: Hammer (1980b), van Slageren (1994), the classical work on *Aegilops* of Zhukovsky (1928), and Eig (1929). Specific information has been obtained from Bor (1968), Tutin and Humphries (1980), and Davis (1985). For vernacular names, uses, etymology, etc., see van Slageren (1994). An index to scientific names used is presented at the end of the chapter (see Table in Appendix).

1.2.2.1 *Aegilops* L.

Typus: *Aegilops triuncialis* L. designated by Hammer (1980a), sustained by Jarvis (1992), see also van Slageren (1994).

Aegilops can be distinguished from *Triticum* by the absence of a well-developed keel on the glumes, which causes the sharp angle in the glume outline of both the wild and cultivated *Triticum* taxa (van Slageren 1994). A key to the wild taxa of *Aegilops* and *Triticum* can be found in van Slageren (1994).

The genus *Aegilops* has been divided into five sections (Zhukovsky 1928; van Slageren 1994). Three of the sections, namely ***Aegilops*** (species with U-genome and combinations of other genomes with U), ***Cylindropyrum*** (C- and DC- genomes), and ***Vertebrata*** (D-genome and combinations of other genomes with D) consist of both diploid and polyploid species, and the two other sections ***Comopyrum*** (M- or N-genomes) and ***Sitopsis*** (S-genome) have only diploid members (Table 1.2). The key to the sections of *Aegilops* can be found in van Slageren (1994).

Genome: $x = n = 7$. The genus *Aegilops* consists of 11 diploid ($2n = 14$) and 12 polyploid species (tetraploid: $2n = 28$; hexaploid $2n = 42$). *Ae. crassa* and *Ae. neglecta* have both tetraploid and hexaploid races (Table 1.2). For genome formula, cytoplasm, and genome size, see Table 1.2.

1.2.3 Determination and Species Key of the Genus Aegilops

The key of Hammer (1982) allows determining species and few of the infraspecific taxa (see also Hammer 1980b; Table 1.3). Originally, the former key was published in German (Hammer 1980b), followed by an English version (Hammer 1982). A modern key is provided by van Slageren (1994), which contains only few infraspecific races. We include *Aegilops mutica* in the genus *Aegilops* (Tables 1.1 and 1.2).

Nielsen (1981) found that the length of the rachis internode of the lowest fertile spikelet is a good additional character for the distinction of some difficult species, e.g., *Ae. triuncialis* and *Ae. kotschyi* or *Ae. geniculata* and *Ae. umbellulata*. He reported the following data:

Ae. biuncialis: 2–5 mm; *Ae. columnaris*: 1–2 mm; *Ae. comosa* ssp. *heldreichii*: 4–6 mm; *Ae. geniculata*:

Table 1.3 Species key of the genus *Aegilops*. = *species key*"

1	Spikes long, more than 10 times as long as wide (excluding awns), if somewhat less, than 5 or more spikelets per spike	2
1′	Spikes shorter (by shortening of the rachis internodes or by reduction of the number of spikelets), less than 8 times as long as wide (excluding awns), if somewhat more, than 3 or less spikelets per spike	18
2	Spike completely without awns or teeth (examine the terminal spikelet!), lemma rounded at the apex, glumes truncate, spikelets glabrous (*Agropyron* like) or with white erect bristles (ventricose in appearance) Subgenus *Amblyopyrum* ***Aegilops mutica*** Boiss. with ssp. *mutica* and ssp. *loliacea* (Jaub. et Sp.) Zhuk.	
2′	Spike with awns or teeth (examine the terminal spikelet!)	3
3	Glumes without awns, sometimes with teeth or one short thin awn	4
3′	Glumes at least of the terminal spikelets with longer awns	13
4	Spike long, often distinctly two-rowed, at least apical lemma awned (1 awn), lemma in the upper part ± flat, (forms with many awns similar to diploid wild *Triticum* species), glumes often conspicuously keeled, without awns Subgenus *Sitopsis*	5
4′	Spike cylindrical or somewhat tapering towards the apex	8
5	Glumes with little lateral tooth and emarginate upper rim, often with skinny lateral rim ***Ae. speltoides*** Tausch with ssp. *speltoides* and ssp. *ligustica* (Savign.) Zhuk.	
5′	Glumes with 2 ± distinct lateral teeth, separated by a ± obtuse angle, upper rim of the glume flat	6
6	Spike relatively small, delicate, internodes of the rachis short, spikelets 5.5–8.5 mm long, awns present, teeth absent, plants small, up to 20 cm	7
6′	Spike bigger, internodes of the rachis elongated, spikelets 8 – 13 mm long, awns and 1 or 2 teeth present, plants more than 20 cm ***Ae. longissima*** Schweinf. et Muschl. (only apical spikelets extending into awns) and ***Ae. sharonensis*** Eig (awns throughout spike)	
7	All spikelets with awns of more or less equal length ***Ae. bicornis*** (Forssk.) Jaub. et Sp.	
7′	Only terminal spikelets with long awns, one awn curved, very long ***Ae. searsii*** Feldman et Kislev ex K. Hammer	
8	Spikelets ventricose, spike moniliform, glumes with 2 marginal teeth, mostly glabrous Subgenus *Aegilops* ***Ae. ventricosa*** Tausch	
8′	Spikelets not or scarcely ventricose, spike not or scarcely moniliform	9

(*continued*)

Table 1.3 (continued)

9	Spikes and spikelets relatively slender (awnless forms of awned species)	10
9′	Spikes and spikelets relatively stout	11
10	Teeth of glumes and lemma relatively broad at the base ***Ae. peregrina*** (Hackel) Maire et Weiller ssp. *brachyathera* (Boiss.) Maire et Weiller (ssp. *cylindrostachys* (Eig et Feinbrun) Maire et Weiller, nom. illeg.)	
10′	Teeth of glumes and lemma not broad at the base ***Ae. triuncialis*** L. var. *anathera* Hausskn. et Bornm.	
11	Glumes hairy	12
11′	Glumes glabrous, without teeth (very seldom with little teeth or 1 little short awn) ***Ae. tauschii*** Coss. with ssp. *tauschii* and ssp. *strangulata* (Eig) Tzvel.	
12	Glumes with long teeth, mostly 6 or less spikelets per spike ***Ae. juvenalis*** (Thell.) Eig	
12′	Glumes with 1–4 short teeth, mostly 7 or more spikelets per spike ***Ae. crassa*** Boiss. (4x and 6x) and ***Ae. vavilovii*** (Zhuk.) Chennav. (6x)	
13	Upper spikelets sterile, 3 fertile and 3 sterile spikelets per spike, vestigial basal spikelets (2)–3 –(4), lemma awned, at least with some spikelets, glumes more than 11 mm long, mostly with 2 awns, one with broad base, this awn often ± divided ***Ae. columnaris*** Zhuk.	
13′	Upper spikelets fertile	14
14	Glumes with 1 awn, often only at the upper spikelets	15
14′	Glumes with more than 1 awn, often 2 or 3	16
15	Glumes of the terminal spikelets with 1 awn and 2 teeth (very seldom without awn), spike cylindrical, scarcely tapering towards the apex ***Ae. cylindrica*** Host	
15′	Glumes of the terminal spikelets without teeth, spike tapering markedly towards the apex ***Ae. markgrafii*** (Greuter) K. Hammer	
16	Spikes relatively small, delicate, veins of the glumes parallel ***Ae. kotschyi*** Boiss.	
16′	Spikes bigger, veins not parallel	17
17	Awn base in relation to awn length very broad ***Ae. peregrina*** (Hackel) Maire et Weiller	
17′	Awn base not very broad, 5–6 fertile spikelets per spike, vestigial basal spikelets mostly 3, lemma awns mostly absent ***Ae. triuncialis*** L. with ssp. *triuncialis* and ssp. *persica* (Boiss.) Zhuk.	
18	Upper spikelets sterile, conspicuously smaller in size than lower spikelets	19
18′	Upper spikelets fertile, sometimes little smaller in size than lower spikelets, terminal spikelet always with grain	20

(*continued*)

Table 1.3 (continued)

19	Glumes with 2 or 3 awns, glumes less than 11 mm in length (cf. *Ae. columnaris*) ***Ae. neglecta*** Req. ex Bertol. with ssp. *neglecta* (4x) and ssp. *recta* (Zhuk.) K. Hammer (6x)	
19′	Glumes with more than 3 awns ***Ae. umbellulata*** Zhuk. with ssp. *umbellulata* and ssp. *transcaucasia* Dorof. et Migusch.	
20	Glumes with only 1 awn, mostly 2 spikelets per spike ***Ae. uniaristata*** Vis.	
20′	Glumes with more than 1 awn	21
21	At least 1 glume of the terminal spikelet with 2 awns ***Ae. comosa*** Sibth. et Sm. with ssp. *comosa* and ssp. *heldreichii* (Boiss.) Eig (syn.: var. *subventricosa* Boiss.)	
21′	Glumes of all spikelets mostly with more than 2 awns	22
22	Glumes with mostly more than 3 awns ***Ae. geniculata*** Roth with ssp. *geniculata* and ssp. *gibberosa* (Zhuk.) K. Hammer	
22′	Glumes with (2)–3 awns	23
23	Awns of the glumes relatively to awn length very broad ***Ae. peregrina*** (Hackel) Maire et Weiller (ssp. *peregrina*)	
23′	Awns of the glumes not very broad at the base	24
24	Glumes with 2–(3) awns, glumes with conspicuous non-parallel veins, spikelets relatively big, 2–(3) spikelets per spike ***Ae. biuncialis*** Vis.	
24′	Glumes with (2) – 3 awns, glumes with parallel veins, spikelets relatively small, more than 3 spikelets per spike ***Ae. kotschyi*** Boiss.	

4–8 mm; *Ae. juvenalis*: 4–6 mm; *Ae. kotschyi*: 1–2 mm; *Ae. neglecta*: 2–3 mm; *Ae. peregrina*: 2–4 mm; *Ae. umbellulata*: 1–3 mm; *Ae. triuncialis*: 3–6 mm.

These are good key characters, at least for material from the eastern Mediterranean region.

1.2.4 *Brief Description of the* Aegilops *Species*

For more details, see Hammer (1980a, b) and van Slageren (1994).

Aegilops mutica

Aegilops mutica Boiss. – *Amblyopyrum muticum* (Boiss.) Eig (Figs. 1.1 and 1.2)[1]

Annual, outcrossing with long slender spikes. Plants 30–90 cm tall. Spikes not awned, 5–13 cm long, upper florets more or less reduced. Spikelets 5–8 flowered. Glumes 4–9 mm long, hairy (common variant, ssp. *mutica*), or glabrous (rare variant, ssp. *loliacea* (Jaub. et Sp.) Zhuk.). Mixed populations occur locally. Hammer (1980b) differentiates the variants on the var. level. Van Slageren (1994) also prefers the var. level but in the separate genus *Amblyopyrum*. Van Slageren (1994) distinguished *Amblyopyrum* from *Aegilops* by several morphological characters, e.g., awnless linear spikes. Genome: **T**.

Aegilops mutica occurs only in central Turkey and Armenia, frequently on roadsides, the edge of cultivation, dry hillsides, and grassy steppes. Uncommon.

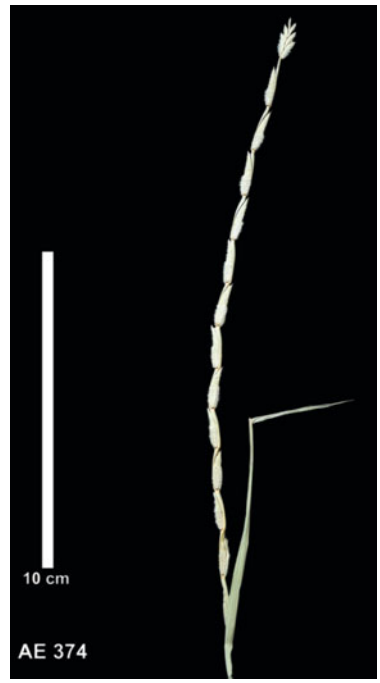

Fig. 1.1 Spike morphology of *Aegilops mutica* (AE374 – IPK Genebank accession number AE 374)

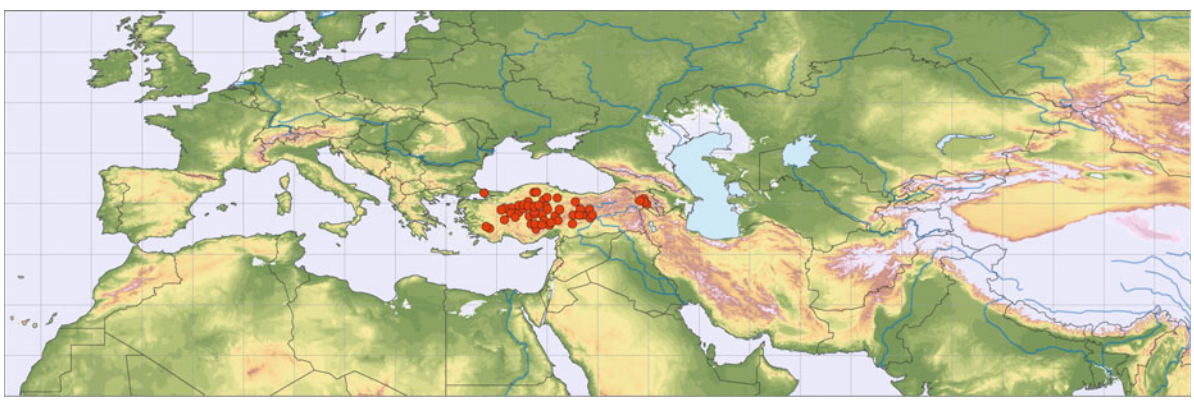

Fig. 1.2 Distribution of *Aegilops mutica*

[1]AE numbers refer to IPK Genebank *Aegilops* accession numbers. GIS distribution maps are based on van Slageren (1994) and our own observations. More information about the accessions can be obtained from *http://gbis.ipk-gatersleben.de/gbis_i/*.

Aegilops speltoides

Aegilops speltoides Tausch – *Sitopsis speltoides* (Tausch) Löve, *Triticum speltoides* (Tausch) Gen. et Richt (Figs. 1.3–1.6).

Annual, outcrossing with long slender spikes. Plants 20–70 cm tall. Spikes 3–25 cm long (excluding awns), with 6–13 spikelets. Glumes 5–9 mm long. Florets 4, muticous or 1-awned. Uppermost spikelet with 2 lower lemmas, each with a setaceous 2–12 cm

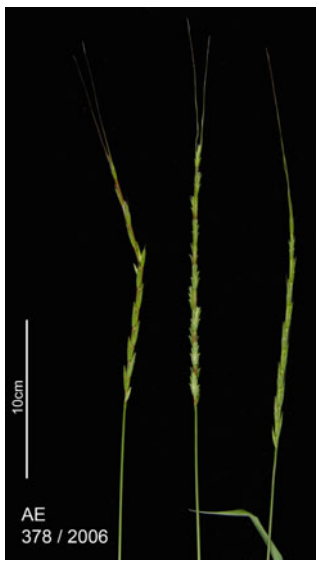

Fig. 1.3 Ear morphology of *Aegilops speltoides* ssp. *speltoides* (AE 378)

Fig. 1.5 Ear morphology of *Aegilops speltoides* ssp. *ligustica* (AE 1611)

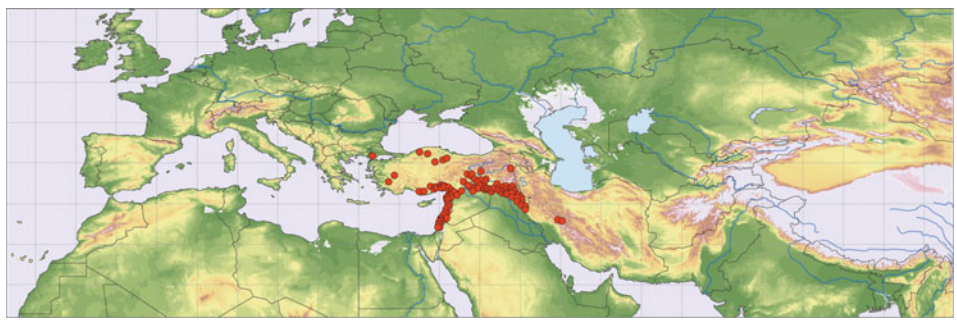

Fig. 1.4 Distribution of *Aegilops speltoides* ssp. *speltoides*

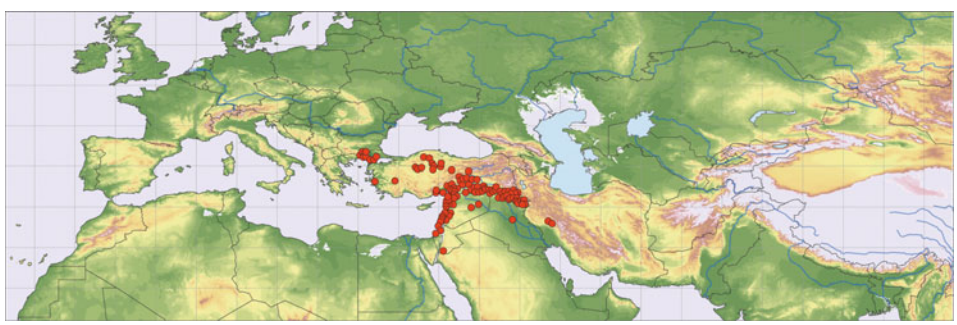

Fig. 1.6 Distribution of *Aegilops speltoides* ssp. *ligustica*

long awn. Lateral spikelets either lacking awns, axis persistent in fruit (**ssp. *speltoides***) or with awns, axis disarticulating in fruit [**ssp. *ligustica*** (Savign.) Zhuk.]. Both variants are interfertile and sympatric, except towards the limit of the species' distribution. Separated on the var. level by van Slageren (1994): var. *speltoides* and var. *ligustica* (Savign.) Fiori. For both subspecies, two botanical varieties each are indicated by Hammer (1980b). Genome: **S**.

The northern and eastern parts of the Fertile Crescent are the major area of occurrence. The species is less common in central and western Turkey. Several adventive sites exist outside the Fertile Crescent. The species is mainly found in grasslands and moderately disturbed sites. Also found in open *Pinus* and *Quercus* forests. Soil texture: clay and loams, as well as more pure sands. Wide variation for soil type has been observed.

Recorded rainfall data are between 450 and 1,450 mm annually. Massive stands on basaltic slopes in open oak park-forest along the foothills of the Taurus and Zagros mountains. From sea level up to 2,000 m.

Aegilops longissima

Aegilops longissima Schweinf. et Muschl. – *Triticum longissimum* (Schweinf. et Muschl.) Bowden, *Sitopsis longissima* (Schweinf. et Muschl.) Löve (Figs. 1.7 and 1.8).

Annual with long slender spikes. Plants erect, 30–70 cm tall (excluding spikes). Spikes 10–20 cm long (excluding awns), with 8–17 spikelets. Apical spikelets extending into a 6–13 cm long awn. Glumes 6–8 mm long. Apex of lower lemmas in apical spikelet extending (each) into 6–13 long diverging awns, with lateral spikelets lacking. Ankory and Zohary (1962) report hybrids between *Ae. longissima* and *Ae. sharonensis*. Genome: $\mathbf{S}^{l}$.

Distributed in coastal Egypt, Israel/Palestine and northwestern Jordan. Uncommon to rare. On sandy soils and sandstones. Present in sand, sandstone, sandy loams; edges of cultivation and roadsides, often together with *Ae. sharonensis*. In Jordan on

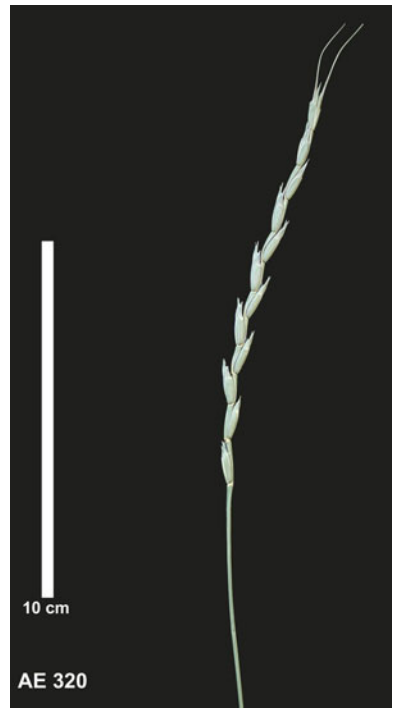

Fig. 1.7 Ear morphology of *Aegilops longissima* (AE 320)

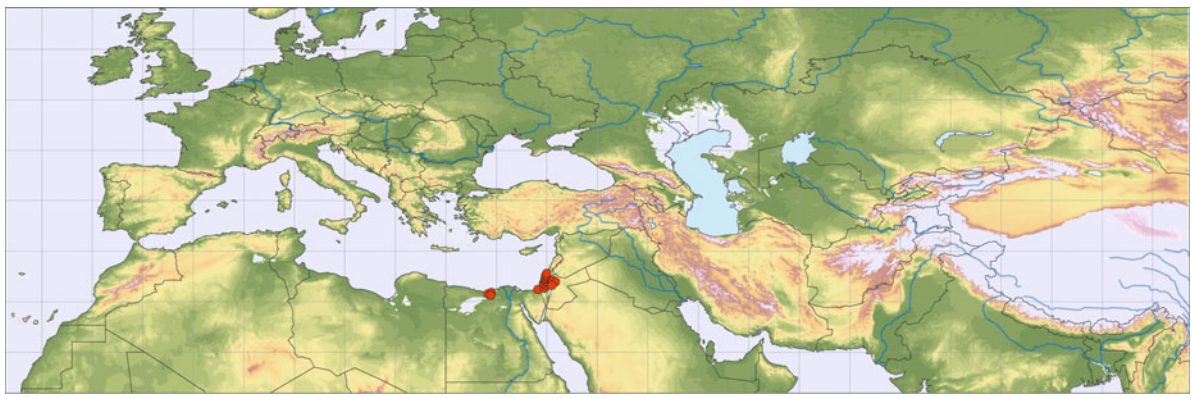

Fig. 1.8 Distribution of *Aegilops longissima*

limestone with terra rossa. It is also known from dry grasslands and abandoned fields. Annual rainfall from 250 to 400 mm. From −200 m up to 600 m.

Aegilops sharonensis

Aegilops sharonensis Eig – *Triticum sharonense* (Eig) Feldman et Sears, *Ae. longissima* Schweinf. et Muschl. ssp. *sharonensis* (Eig) Chennav. *Sitopsis sharonensis* (Eig) Löve (Figs. 1.9 and 1.10).

Fig. 1.9 Ear morphology of *Aegilops sharonensis* (AE 315)

Annual with long slender spikes. Plants erect, 30–70 cm tall (excluding spikes). Spikes 10–20 cm long (excluding awns), with 8–17 spikelets. Apical spikelets extending into a 6–13 cm long awn. Glumes 6–8 mm long. Apex extending into awns up to 9 cm at the apical spikelet, lateral spikelets in short awns in base of spike, rapidly increasing upward to 3–7 cm. Genome: $\mathbf{S^{sh}}$.

Endemic to the coastal plains of Israel and south Lebanon. Very limited distribution (200 × 15 km). In its area, however, it is reported to be common and occurring sometimes in dense stands. Found on dry open grassland and consolidated sand dunes. On sand, sandy loam or marine diluvium bedrock. From sea level up to about 100 m.

Fig. 1.10 Distribution of *Aegilops sharonensis*

Aegilops bicornis

Aegilops bicornis (Forssk.) Jaub. et Sp. – *Triticum bicorne* Forssk., *Sitopsis bicornis* (Forssk.) Löve (Figs. 1.11 and 1.12).

Annual with slender spikes. Plants erect, 20–40 cm tall (excl. spikes). Spikes 4–7.5 cm long (excluding awns), with 8–15 (−19) spikelets. Apex of lemmas of fertile florets with 0.3–0.4 cm long awn in basal spikelets, in the upper part of the spike up to 4.5–6 cm in length (var. *bicornis*). In var. *anathera* Eig [var. *mutica* (Aschers.) Eig], only the lemmas of the upper 5–7 spikelets are awned. Genome: $\mathbf{S^b}$.

Distributed on the coastal regions of Cyprus, Libya, Egypt, and Israel/Palestine, some parts of southern Jordan. A few locations in inland Syria.

It is mainly found in grasslands, under palms, pines, in plantations, and on edges of wheat fields. In the coastal areas, it is found on lowland areas of sandy loams and stabilized sand dunes.

On light sandy, sandy calcareous, or sandstones, average rainfall 75–275 mm annually. At the altitude of 200 m from sea level, but in Jordan found up to the level of 700–900 m.

Fig. 1.11 Ear morphology of *Aegilops bicornis* (AE 1079)

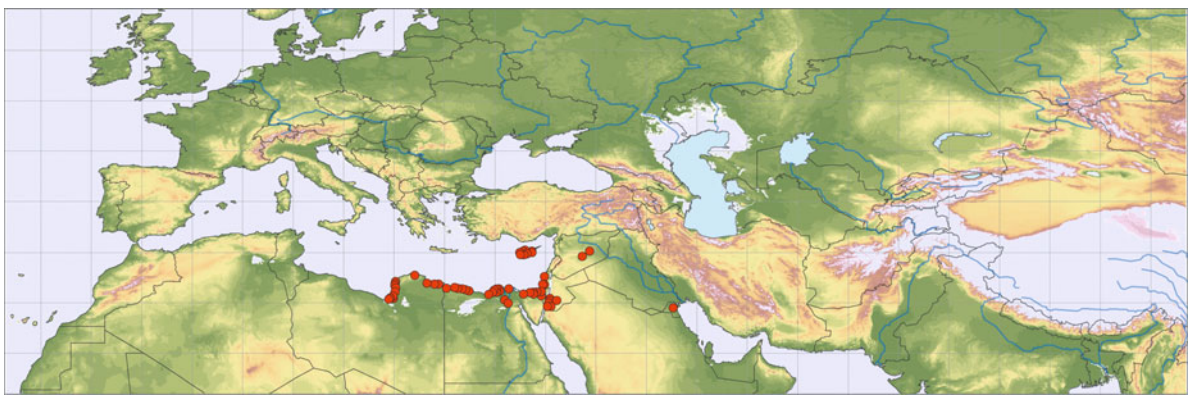

Fig. 1.12 Distribution of *Aegilops bicornis*

Aegilops searsii

Aegilops searsii Feldman et Kislev ex K. Hammer – *Sitopsis searsii* (Feldman et Kislev ex K. Hammer) Löve, *Triticum searsii* (Feldman et Kislev) Feldman et Kislev, nom. inval (Figs. 1.13 and 1.14).

Annual with slender spikes. Plants (10−) 15–35 cm long (excluding spikes). Spikes 6.5–13.5 cm long (excluding awns), with 7–10 (−14) spikelets. Glumes 6–8 mm long. Florets 2–4. Apex of the 2 lower, apical lemmas flat, extending into distinctly different awns, one of the fertile floret well developed, 5–13 cm long, the one of the sterile floret only 1.5–7 cm long. Genome $\mathbf{S^s}$

Limited to Israel/Palestine, Syria, Jordan, and Lebanon. Uncommon throughout its range. A species of hills and mountainous regions.

In dry open grasslands, steppe, ruderal fields, and roadsides. Almost exclusively on limestone, only rarely on basalt or sandstone. Soil texture includes clay and loam, more rarely sand and terra rossa. Annual rainfall data of 150–300 (−500) mm. From 450 to 1,600 m in Jordan, Lebanon and Syria. In Israel/Palestine just reaching the eastern end of the coastal plain and occurring lower to around 100 m.

Fig. 1.13 Ear morphology of *Aegilops searsii* (AE 1071)

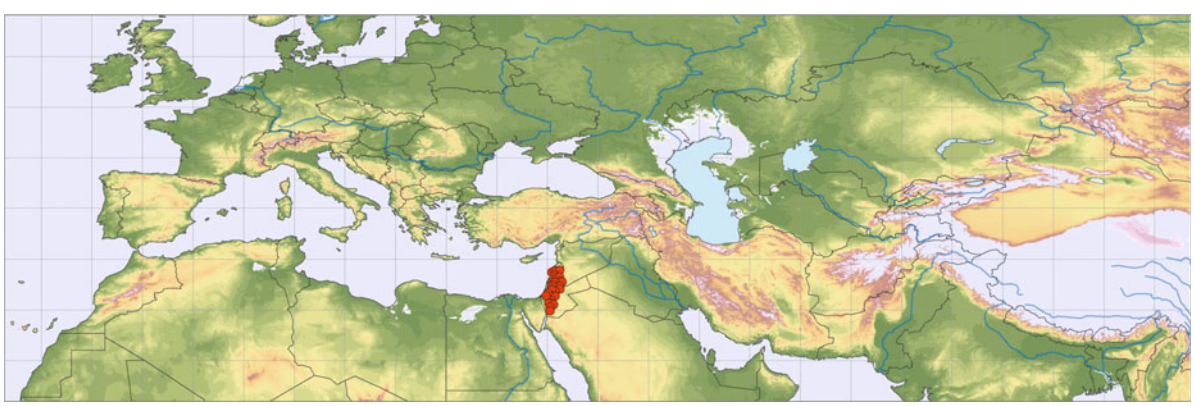

Fig. 1.14 Distribution of *Aegilops searsii*

Aegilops markgrafii

Aegilops markgrafii (Greuter) K. Hammer – *Aegilops caudata* auct. non L., *Triticum markgrafii* Greuter. The proposal of Scholz and van Slageren (1994) to conserve *Ae. caudata* failed (Figs. 1.15 and 1.16).

Annual with slender spikes. Plants 25–50 cm tall. Spikes 4–11 cm long (excluding awns), with 3–9 spikelets. Glumes 9–11 mm long. At the uppermost spikelets glumes each tapering into divergent awn, 4–10 cm long (1–1.5 mm broad at base), lacking basal teeth. Vestigial spikelets 2. Lateral spikelets toothed (var. *markgrafii*) or one of the teeth setaceous awned [var. *polyathera* (Boiss.) Hammer]. Morphological variation limited. Genome: **C**.

Naturally occurring mainly in Aegean and western Turkey, where growth can be abundant. Less common and more sporadic in inland Turkey and along the Fertile Crescent.

Found on roadsides, grassland including steppe, edges of and within fields of cultivation, various forest types. Often on dry, rocky, slopes of limestone, more rarely on shales, schist, sandstone or granite. Soil texture pre-dominantly clay and sandy loams. *Aegilops markgrafii* can form dense stands, often together with other *Aegilops* species. Collected annual rainfall data indicate a range of 300–700 mm. From sea level up to 1,850 m.

Fig. 1.15 Ear morphology of *Aegilops markgrafii* (AE 1399)

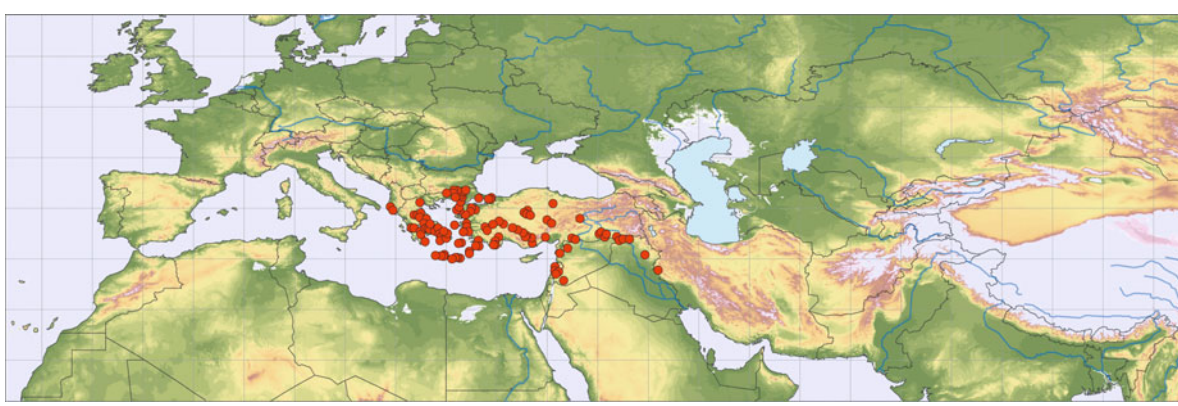

Fig. 1.16 Distribution of *Aegilops markgrafii*

Aegilops biuncialis

Aegilops biuncialis Vis. – *Triticum biunciale* (Vis.) Richter, nom. illeg., *Aegilops lorentii* Hochst., *Triticum lorentii* (Hochst.) Zeven, *Triticum macrochaetum* (Shuttlew. et A. Huet. ex Duval-Jouve) Richter (Figs. 1.17 and 1.18).

Many-tillered annual with short spikes. Plants 10–40 cm tall (excluding spikes). Spikes 1.5–3.5 cm long (excluding awns), with 2 (–3) fertile and 1 (–2) rudimentary spikelets. Glumes 8–10 mm long. Spikes breaking off as a unit. Apical spikelet always with 3 awns, up to 4–7 cm long. Some variation, e.g., in the pubescence of glumes (see Hammer 1980a). Genome: **UM**.

Mainly in the Aegean, also Turkey, Bulgaria, Cyprus, the western part of Fertile Crescent, in the eastern Cis-, and Transcaucasia, and in the southern part of the Crimea and adjacent parts of Russia and the Ukraine. Generally growing on dry areas, roadsides, edges of cultivation and various forest types. Also in grasslands, maquis, steppe and dry, rocky mountain slopes. Extensive populations have been observed, e.g., in Daghestan. This species mainly grows on limestone and also on schists, shales, basalt, granite, and pillow lavas.

Soil texture: mainly clay or sandy loam, or clay. Annual rainfall data indicate some drought tolerance, but the species also occurs in areas with as much as 1,250 mm. From 200 m in Jordan up to 1,750 m.

Fig. 1.17 Ear morphology of *Aegilops biuncialis* (AE 1430)

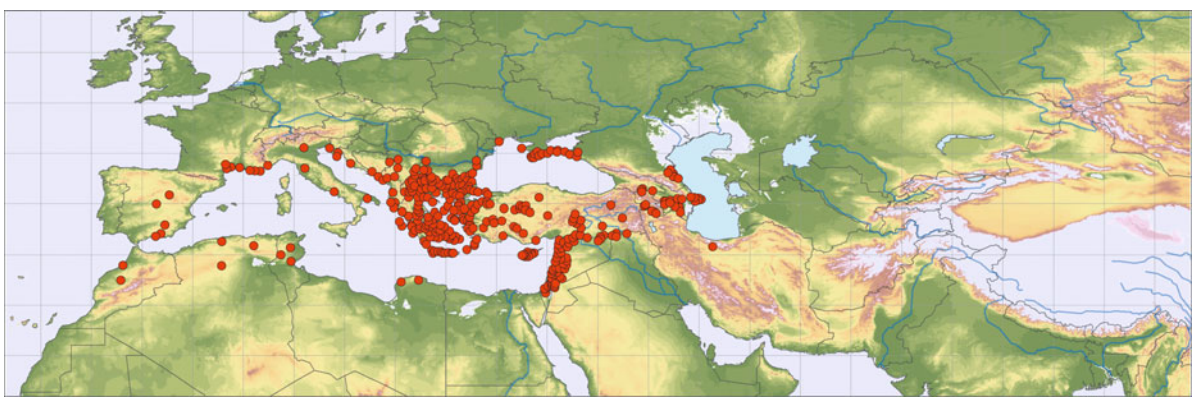

Fig. 1.18 Distribution of *Aegilops biuncialis*

Aegilops kotschyi

Aegilops kotschyi Boiss. – *Triticum kotschyi* (Boiss.) Bowden, *Aegilemma kotschyi* (Boiss.) Löve (Figs. 1.19 and 1.20).

Multitillered annual. Plants 10–30 cm tall (excluding spikes). Spike 0.5–4 cm long (excluding awns), with 2–6 fertile, and 2–3 vestigial spikelets. Spikes breaking off as a unit. Glumes of lower spikelet ovate-oblong, 4–5 mm long, with 3 (sometimes 2) awns, 2.5–4.5 cm long. Lemma awn half as long as those of glumes.

Fig. 1.19 Ear morphology of *Aegilops kotschyi* (AE 1215)

Limited variation. Hammer (1980a) indicates five botanical varieties. Some forms are difficult to differentiate from *Ae. peregrina* (see key for both species, van Slageren 1994). Genome: **SU**.

Along the coast of eastern North Africa and the western part of the Fertile Crescent. Locally common in this range. Extending into the Saharo-Arabian region to Kuwait and eastern Saudi Arabia. Usually in scattered populations, but sometimes in large stands.

In dry wadis and sand dunes. Also known from steppe, wastelands, roadsides and dry grasslands, fields, plantations and woodlands. Predominantly on sandy soils but also on loss, gravel, sandy clays and loams, and light clayloams. Mainly on sandstone and limestone, less frequently on alluvium or maritime sands and occasionally basalt. Growing under 100–425 mm annual rainfall. From −300 m up to 1,550 m.

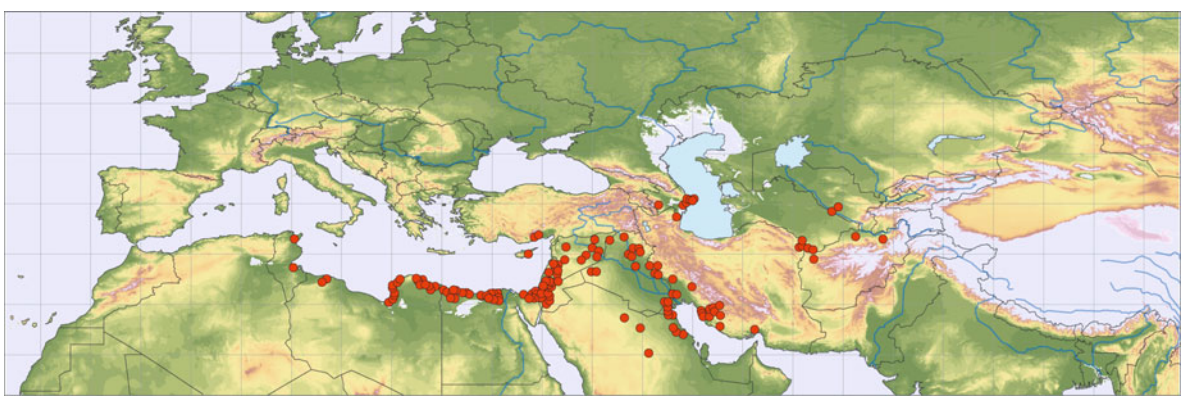

Fig. 1.20 Distribution of *Aegilops kotschyi*

Aegilops triuncialis

Aegilops triuncialis L. – *Triticum triunciale* (L.) Raspail, *Aegilopodes triuncialis* (L.) Löve (Figs. 1.21 and 1.22).

Multitillered annual with subcylindrical spikes. Plants 20–30 cm tall (excluding spikes). Spike 2.5–6 cm long (excluding awns), with 3–5 fertile and (2–) 3 rudimentary spikelets. Spikes breaking off as a unit. Glumes of lateral spikelets with 2–3 awns, 1.5–6 cm long. Central awn of apical glumes 5–8 cm long, lateral awns 1–3 cm long (ssp. *triuncialis*). In ssp. *persica* (Boiss.) Zhuk. the lateral awns are reduced to teeth or short awns. Genome: **UC**.

Fig. 1.21 Ear morphology of *Aegilops triuncialis* (AE 1652)

Occurring all over southern Europe and the Near East, extending eastwards into Central Asia. Well-represented along the entire Fertile Crescent. Also found in Cyprus and the southern Crimea as well as in Cis-Caucasia. Common throughout its range. Introduced in the US and a weed on rangeland. In Europe found as an adventive in several countries.

Locally abundant in generally dry, somewhat disturbed habitats, and dry rocky slopes of hills and mountains. Also present on edges of and within cultivation. Vegetation types include garrigue, maquis, grassland, shrub- and wood-lands, (open) forests. Also found in the steppe up to the margin of the desert but more rarely also in humid pastures, river terraces, and even at the seaside. Predominately on limestone and basalt, but also on shales, pillow lava, silicate, terra rossa, karsts, schist, and sandstone. Soil texture also varies widely, but the species grows mainly on clay- and sandy loam.

As a typical colonizer, the species can be found in massive stands and dominate the vegetation. Together with *Ae. geniculata*, this is the most widespread species of the genus *Aegilops* and grows under a similarly wide annual rainfall amplitude, varying from 125 mm up to 1,400 mm. From sea level up to 2,700 m in Morocco.

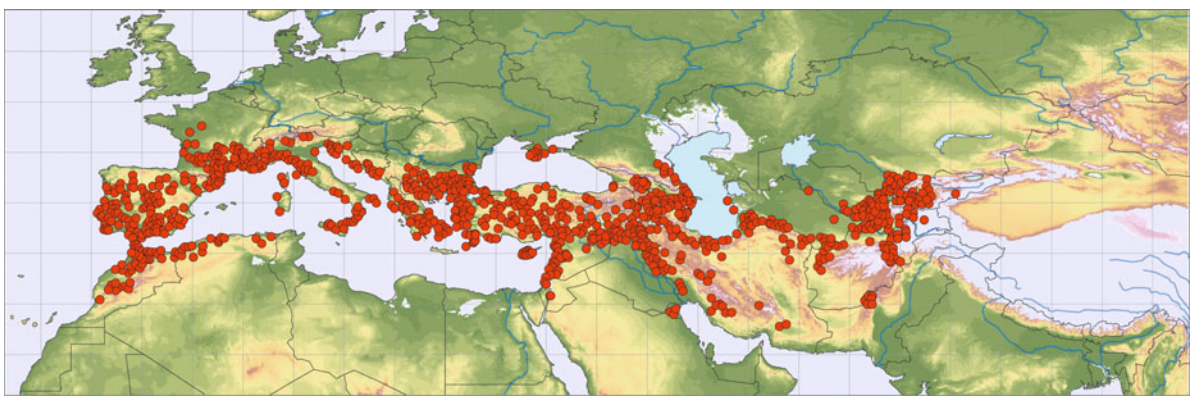

Fig. 1.22 Distribution of *Aegilops triuncialis*

Aegilops cylindrica

Aegilops cylindrica Host – *Triticum cylindricum* Ces., Pass. et Gib., *Cylindropyrum cylindricum* (Host) Löve (Figs. 1.23 and 1.24).

Annual with slender spikes. Plants 20–40 (–80) cm tall. Spikes 5–7 (–10) cm long (excluding awns), with 5–7 (–9) spikelets. Glumes 8–9 mm long. Uppermost spikelet with 3–4 small awns arising from glumes and lemmas. Outer glume 2-toothed at base of awn. Spike falling entire or mostly disarticulating into cylindrical spikelets. Vestigial spikelets 1–2. Four botanical varieties are indicated by Hammer (1980b), most on the basis of presence/absence of awns. Genome: **DC**.

Widespread, with tendency to weedy behavior. Occurring mainly westward from Turkey into Bulgaria, Romania, the Balkans, and into Hungary, northward into the Caucasus region and along the Black Sea coast, and eastward into Central Asia. In the Fertile Crescent, mainly present in the northern part. Introduced in the US and present in many states.

A species from ruderal and disturbed sites, dry hill- and mountain slopes, grasslands, and close by or within cultivation. Soil bedrock is mainly calcareous and basaltic, less frequently on sands. Soil textures include clay, clayloams, but also on more pure sands. Annual rainfall data from 450 to 800 mm. *Aegilops cylindrica* may grow in large stands after recent disturbances. From −28 m up to about 2,000 m.

Fig. 1.23 Ear morphology of *Aegilops cylindrica* (AE 1614)

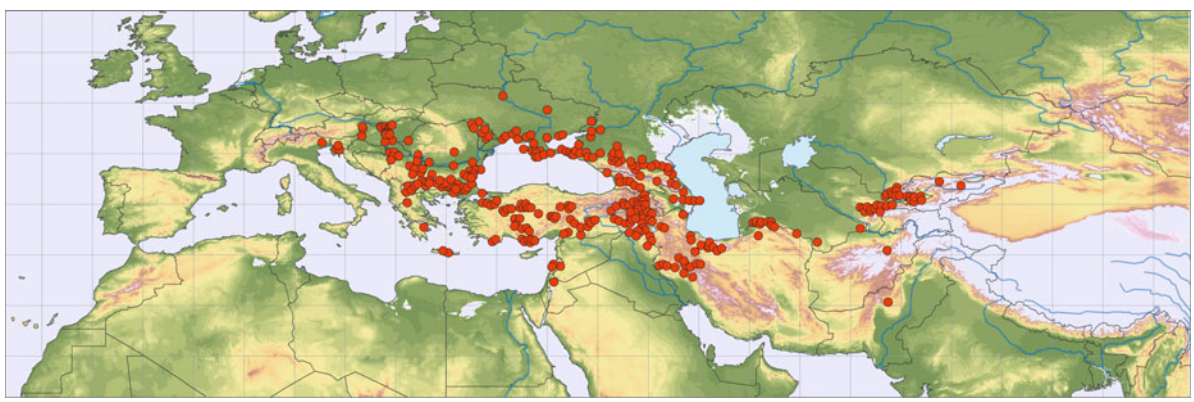

Fig. 1.24 Distribution of *Aegilops cylindrica*

Aegilops tauschii

Aegilops tauschii Coss. – *Triticum tauschii* (Coss.) Schmalh., *Aegilops squarrosa* auct. non L., *Patropyrum tauschii* (Coss.) Löve (Figs. 1.25–1.27).

Annual with slender spikes. Plants 30–40 cm tall. Spikes 5–7 cm long (excluding awns), with 9–11 spikelets. Lateral spikelets barrel-shaped, usually disarticulating. Uppermost spikelet with unawned glumes. Lower lemmas bearing slender awn up to 3–4 cm long. Spikes slightly constricted between the barrel-shaped spikelets (ssp. *tauschii*) or constricted between the ventricose spikelets (ssp. *strangulata*). A number of morphological variants are indicated by Hammer (1980b). Genome: **D**.

Almost exclusively east of the 40° longitude. Its center of distribution is along the southern shores of the Caspian Sea and in Azerbaijan. This species is the

Fig. 1.25 Ear morphology of *Aegilops tauschii* ssp. *tauschii* (AE 1650)

Fig. 1.26 Ear morphology of *Aegilops tauschii* ssp. *strangulata* (AE 1605)

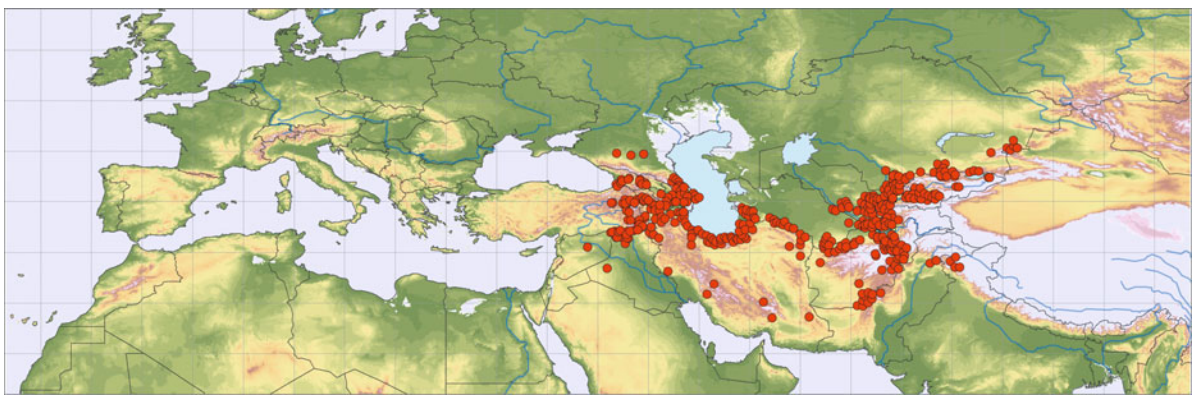

Fig. 1.27 Distribution of *Aegilops tauschii* (both subspecies)

only diploid *Aegilops* that has spread mainly eastwards from the center of origin of the genus. *Aegilops tauschii* is the only species known with certainty from China.

A species of wide ecological amplitude, known from dry grasslands, fallow, steppes, and moderately disturbed sites, roadsides, and edges of and within cultivation. But also found in woodlands and marginal forests, degraded forests, on stony slopes, in irrigated fields and river valleys, and in humid forests along the southern belt of the Caspian Sea. Predominantly on basalt, the soil texture is mainly loam, sandy loam, clayloam, or sandy clay. *Aegilops tauschii* is able to grow on silty soils. Rainfall data indicated a range of 150–350 mm annually, sometimes even below 100 mm. From sea level up to 2,700 m.

Aegilops crassa

Aegilops crassa Boiss. – *Triticum crassum* (Boiss.) Aitch. et Hemsl., *Gastropyrum crassum* (Boiss.) Löve (Figs. 1.28 and 1.29).

Annual with long slender spike. Plants 30–50 cm tall. Spikes 6–9 cm long, adpressed velutinous, disarticulating. Glumes 8–10 × 4–5 mm, subtruncate. Lemmas 2-toothed, those of upper spikelets often broadly awned. Uppermost spikelet narrower than lateral one. Glumes short toothed. It is difficult to differentiate *Ae. crassa* (tetraploid and few hexaploid races) with moniliform spikes from the hexaploid *Ae. vavilovii* (Zhuk.) Chennav. with narrowly cylindrical spikes. Both species occur sympatric and are robust and drought-tolerant. Three varieties of ssp. *crassa* are indicated by Hammer (1980b). Genome: *Ae. crassa* ssp. *crassa* (4x) **DM**; *Ae. crassa* ssp. *crassa* (6x) **DDM**.

Occurring in central, western, and northwestern Iran, in central and northern Iraq, northern Afghanistan,

Fig. 1.28 Ear morphology of *Aegilops crassa* (AE 244)

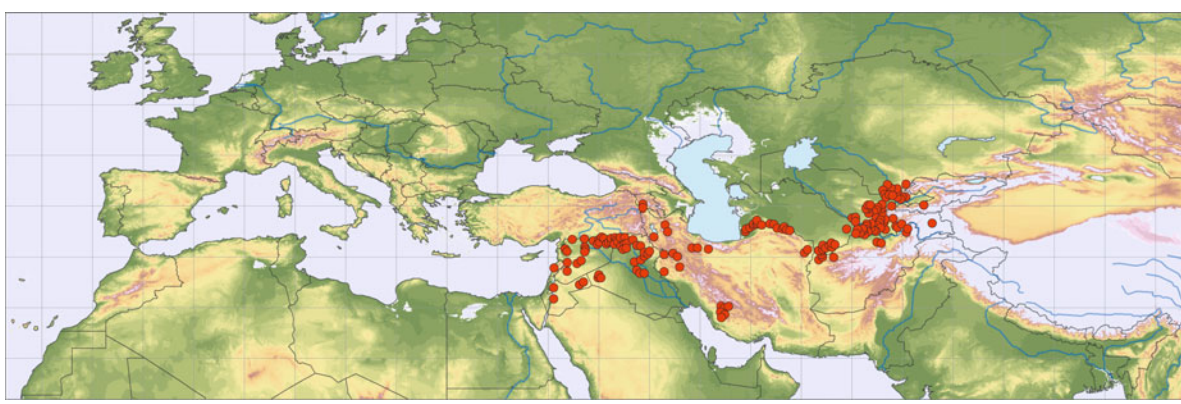

Fig. 1.29 Distribution of *Aegilops crassa*

southernmost parts of Kazakhstan, western Kyrgyzstan, southern Turkmenistan and Uzbekistan, northern Tajikistan, as well as in northern and northeastern Syria and southern Turkey. Records from Jordan and Lebanon.

A drought-tolerant species that grows under 150–350 mm annual rainfall in steppe, fallow, arid grasslands, along roadsides, within as well as in margins of cultivation and on rocky slopes. Mainly found on limestone, more rarely on basalt. Soil textures including clay and sandy loam and sand. From 260 m up to 1,650 m.

Aegilops vavilovii

Aegilops vavilovii (Zhuk.) Chennav. – *Triticum syriacum* Bowden, *Gastropyrum vavilovii* (Zhuk.) Löve, *Ae. crassa* var. *palaestina* Eig, *Ae. crassa* ssp. *vavilovii Zhuk* (Figs. 1.30 and 1.31).

Annual with long slender spike. Plants 20–60 (–75) cm tall excluding spikes. Spikes narrowly cylindrical, tapering in upper half. Spikes (7.5–) 10–15 cm long excluding awns, with (5–) 7–10 spikelets. Rudimentary spikelets absent, rarely 1–2. Glumes adpressed-velutinous, the apex with 2–3 teeth. Lateral lemma apex with sharp tooth at the keel. Caryopsis adherent. Genome: **DMS** (**DM** × **S**).

Predominantly occurring in Jordan, Palestine/Israel, and Lebanon. Also found in southern Turkey and in Iraq. Uncommon throughout its range and rare in Turkey and Iraq. Few isolated sites are known from Saudi Arabia, but they are probably resulting from introduction.

A species of roadsides, fallow, grasslands, and edges of and within cultivated areas. Populations of *Ae. vavilovii* can predominantly be found on limestone, more occasionally also on basalt, flint, or sandstone. The majority of rainfall data in the range of 100–275 mm, but up to 550 mm is recorded in some higher locations. From 275 m up to 1,550 m.

Fig. 1.30 Ear morphology of *Aegilops vavilovii* (AE 1581)

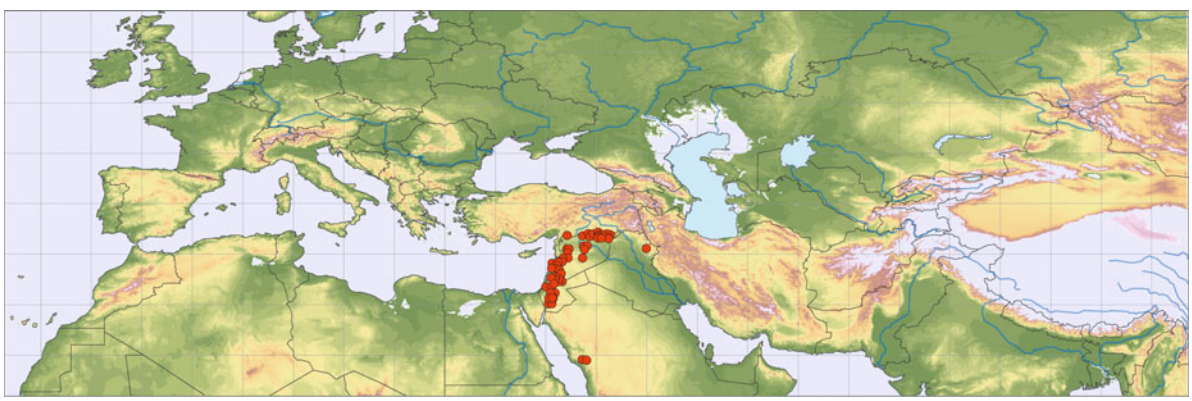

Fig. 1.31 Distribution of *Aegilops vavilovii*

Aegilops ventricosa

Aegilops ventricosa Tausch – *Triticum ventricosum* (Tausch) Ces., Pass. et Gib., *Gastropyrum ventricosum* (Tausch) Löve (Figs. 1.32 and 1.33).

Annual with long slender spike. Plants 40 (–65) cm tall (excluding spikes). Spikes 5–12 cm long (excluding awns), with (3–) 6–11 spikelets. Spikes distinctly moniliform. Glumes 7–8 mm long. Apical spikelets extending into a 4 cm long awn. Spikelets of barrel type, disarticulating at maturity. Hammer (1980b) accepts three morphological races on the level of botanical variety. Van Slageren (1994) calls them "former subvar." Genome: **DN**.

Occurring north and northwest of the Sahara, in the Iberian Peninsula, and in southern France, Corsica, Sardinia, and some other parts of southern Italy (Pignone et al. 1992). Uncommon throughout its range.

A species of grasslands, roadsides, sandy wadis, and edges of and within cultivation. Also found in shrubs of *Pistacia* and *Juniperus* and oak forests. This species can be found predominantly on soils with a limestone bedrock, far less on basalt or sandstone. Recorded soil textures include mainly clay and sandy loams. Growth on very poor, stony soils, as well as on saline locations, and even marshy riversides reported. Rainfall data vary widely: from less than 100 mm up to 600 mm. From sea level up to 1,850 m.

Fig. 1.32 Ear morphology of *Aegilops ventricosa* (AE 1568)

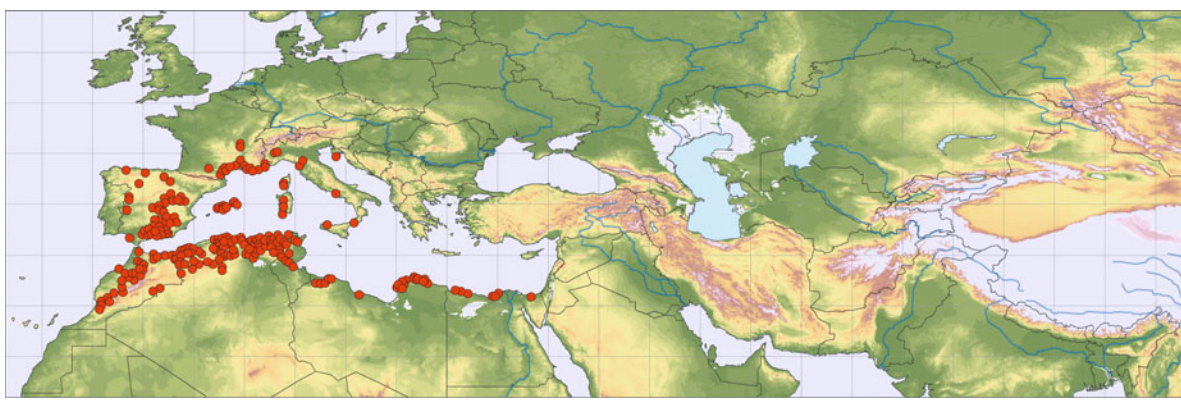

Fig. 1.33 Distribution of *Aegilops ventricosa*

Aegilops juvenalis

Aegilops juvenalis (Thell.) Eig – *Triticum juvenale* Thell., *Aegilonearum juvenale* (Thell.) Löve (Figs. 1.34 and 1.35).

Annual with medium long spikes. Plants 10–35 cm tall (excluding spikes). Spikes 3–7 cm long (excluding awns), with 3–6 spikelets, cylindrical to slightly moniliform. Glumes adpressed velutinous, lateral glumes with 2 widely spaced awns. Spikelets cylindrical to urceolate, 8–13 mm long. Glumes 2-toothed, lemmas with a long awn flanked at the base by 2 teeth or short awns. Hammer (1980b:235) maintained *Ae. turcomanica* Roshev. as a separate species based on Bowden's (1959) remark that *Ae. turcomanica* should be tetraploid and, therefore, different from the hexaploid *Ae. juvenalis*. So far, no new tetraploid material has been found to reestablish *Ae. turcomanica* (see also van Slageren 1994). Genome: **DMU** (**DM** × **U**).

Occurring at rather dispersed locations in Central Asia (Turkmenistan, Uzbekistan) as well as in Azerbaijan and in the northern part of the Fertile Crescent. Rare throughout its range. In steppe, dry roadsides, grasslands, cultivated fields, and hillsides. Found on clay but probably also on other soil textures. Recorded annual rainfall from only 250–350 mm. From 140 m up to 1,000 m.

Fig. 1.34 Ear morphology of *Aegilops juvenalis* (AE 91)

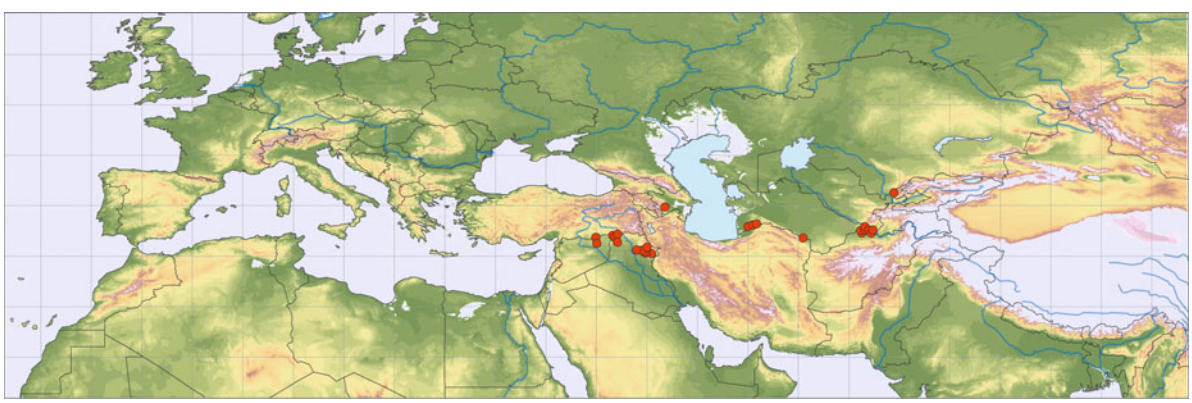

Fig. 1.35 Distribution of *Aegilops juvenalis*

Aegilops comosa

Aegilops comosa Sm. in Sibth. et Sm. – *Triticum comosum* (Sm. in Sibth. et Sm.) K. Richt., *Comopyrum comosum* (Sm. in Sibth. et Sm.) Löve (Figs. 1.36 and 1.37).

Annual, slender plants, 15–40 cm tall (excluding spikes). Spikes cylindrical, 1.5–3 cm long (excluding awns). 1–3 lateral spikelets, only top spikelet long-awned. Glumes of uppermost spikelet each with 3 awns, the middle one 3–10 cm long, the lateral ones shorter. Spikes narrowly cylindrical, glumes with slender, parallel veins (ssp. *comosa* var. *comosa* according to van Slageren 1994). Spikes stout, glumes with prominent veins, bowed outwards [ssp. *heldreichii* (Holzm. ex Boiss.) Eig, according to van Slageren (1994) var. *subventricosa* Boiss.)]. For both subspecies, 4 botanical varieties each are indicated by Hammer (1980b). Genome: **M**.

Occurring in coastal regions of the former Yugoslavia, Albania, and coastal and inland Greece. Rare throughout its range but more common in Greece.

On roadsides, grasslands, and hillsides, in garrigue, and sometimes in cultivated fields. Mainly on limestone with clayloam soil texture. Rarely on saline soils. From sea level up to 500 m, rarely to 800 m.

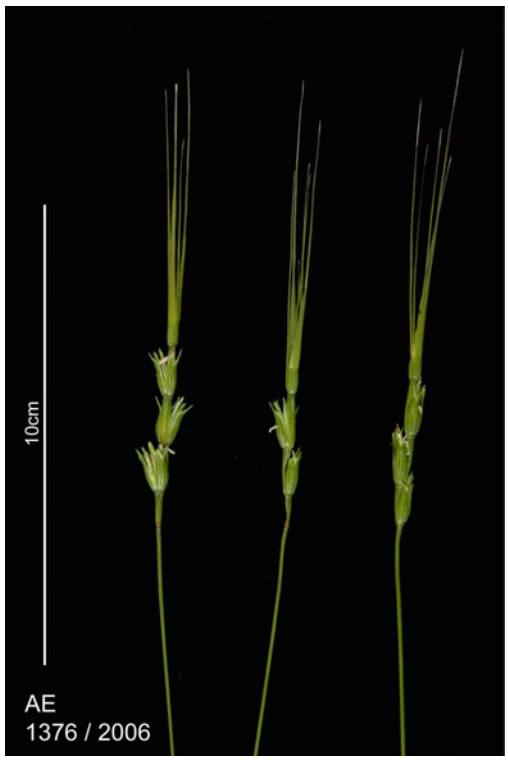

Fig. 1.36 Ear morphology of *Aegilops comosa* (AE 1376)

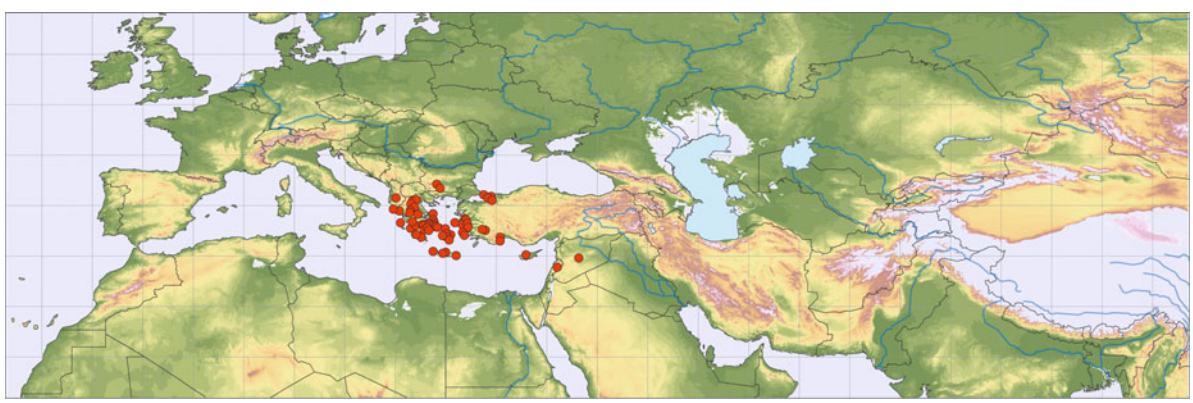

Fig. 1.37 Distribution of *Aegilops comosa*

Aegilops uniaristata

Aegilops uniaristata Vis. – *Triticum uniaristatum* (Vis.) K. Richter, *Chennapyrum uniaristatum* (Vis.) Löve (Figs. 1.38 and 1.39).

Annual with medium long spikes. Plants 20–40 cm tall (without spikes). Spikes 1–2.5 cm long (excluding awns), with 3–4 spikelets. Each glume of uppermost spikelet with 3–4 cm long awn and 2 small lateral teeth at base. Lateral spikelets with prominent veins. The whole spike breaking off as a unit. Limited morphological variation. Genome: **N**.

Occurring in coastal Croatia, Greece, Albania, and rarely in Italy. Uncommon to rare throughout its range. Van Slageren (1994) considered the species as extinct or extremely rare in Turkey.

In dry grasslands and bushy slopes, mainly on rocky, calcareous soils, more rarely on sandstone. From sea level up to about 750 m.

Fig. 1.38 Ear morphology of *Aegilops uniaristata* (AE 576)

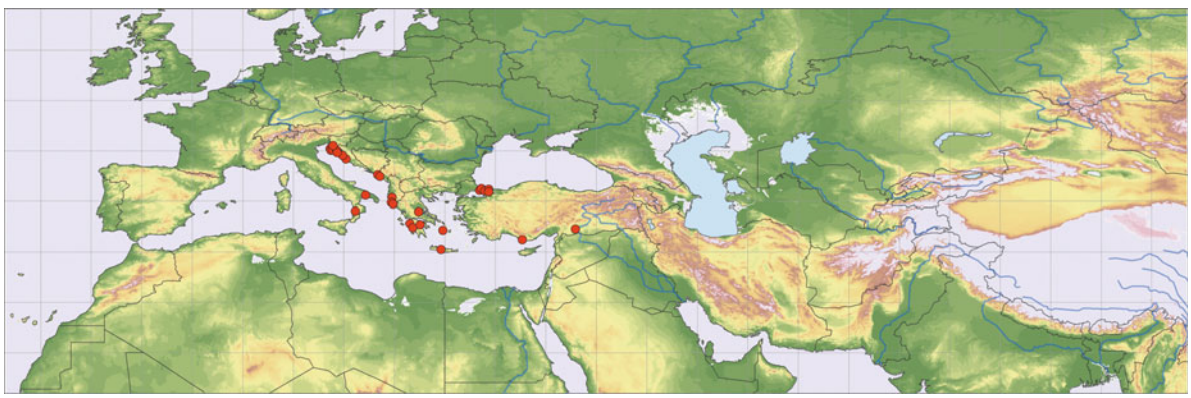

Fig. 1.39 Distribution of *Aegilops uniaristata*

Aegilops umbellulata

Aegilops umbellulata Zhuk. – *Triticum umbellulatum* (Zhuk.) Bowden, *Kiharapyrum umbellulatum* (Zhuk.) Löve (Figs. 1.40 and 1.41).

Annual with short spikes. Plants 10–30 cm tall (without spikes). Spikes 3–5 cm long (without awns), with 2 fertile spikelets. Spike breaking off as a unit. Vestigial spikelets 3. Glumes with 3–7 setaceous, 20–35 mm long awns. Two lower lemmas excerted from glumes, bearing awns. Glumes with 5–7 awns (ssp. *umbellulata*), glumes with 3–4 awns (ssp. *transcaucasica* Dorof. et Migusch.). A special key for distinguishing this species from *Ae. geniculata* is provided by van Slageren (1994). Genome: **U**.

Predominantly occurring in Turkey but also present along most of the Fertile Crescent, in Transcaucasia and Iran. Uncommon throughout its range.

A species of fallow, grasslands, roadsides, margins of cultivation and of edges, and within forests and plantations. In Greece found at the seaside on sandy dunes. This species mainly grows on shallow, rocky soils with bedrock consisting predominantly of limestone or basalt. Soil textures recorded are clay and sandy loams, with more pure clay and loam, terra rossa and alluvium, gravel. Annual rainfall data vary between 350 and 700 mm. From sea level up to 1,800 m.

Fig. 1.40 Ear morphology of *Aegilops umbellulata* (AE 1616)

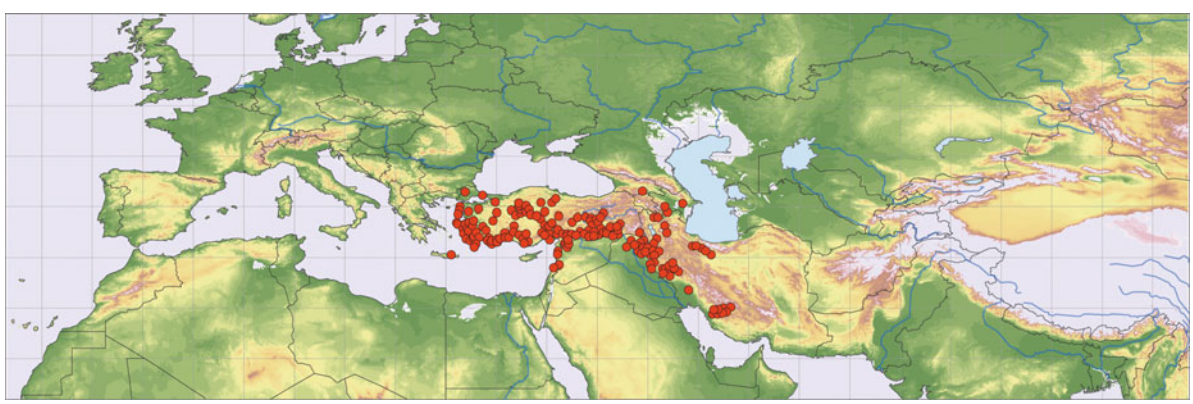

Fig. 1.41 Distribution of *Aegilops umbellulata*

Aegilops peregrina

Aegilops peregrina (Hackel) Maire et Weiller – *Aegilops variabilis* Eig, *Triticum peregrinum* Hackel, *Aegilemma peregrina* (Hackel) Löve (Figs. 1.42 and 1.43).

Annual with medium long spikes. Plants 15–40 cm tall (excl. spikes). Spikes 1.5–5 cm long (excluding awns), with (2–) 3–5 fertile and (2–) 3 rudimentary spikelets. Spikes breaking off as a unit, rudimentary spikelets remaining attached to the culm. Glumes 3–5 mm, with veins somewhat unequally spaced and 2–3 unequally wide and long awns. Lemma apex with 1–2 awns and 1–2 uneven teeth. A race with cylindrical spike (five and more spikelets per spike) and mostly without awns is var. *brachyathera* (Boiss.) Maire et Weiller, considered by Hammer (1980a) as a subspecies [ssp. *cylindrostachys* (Eig et Feinbrun) Maire et Weiller, nom. illeg. = var. brachyathera]. The typical ssp. *peregrina* has ovoid spikes and mostly awns. Ten races of the variety level are indicated by Hammer (1980b) for this very variable species. Genome: **SU**.

Occurring abundantly in Israel/Palestine, western Jordan, Lebanon, and western Syria. Uncommon to rare in Turkey, some Greek islands, Iraq, Azerbaijan, coastal Egypt, and Cyprus. Extending eastward into Iran.

A species from dry, ruderal sites in coastal areas and hill and mountain slopes. In garrigues, semi-steppe, open *Quercus* and *Pinus* forests, as well as plantations. The predominant bedrock is limestone. Rainfall data in the range of 150–350 mm only in annual rainfall varying from 300 to 800 mm. *Ae. peregrina* is also reported from mountainous locations in Lebanon, Syria, and Turkey that receive as much as 1,300 mm annually. From −380 up to 1,600 m.

Fig. 1.42 Ear morphology of *Aegilops peregrina* (AE 1610)

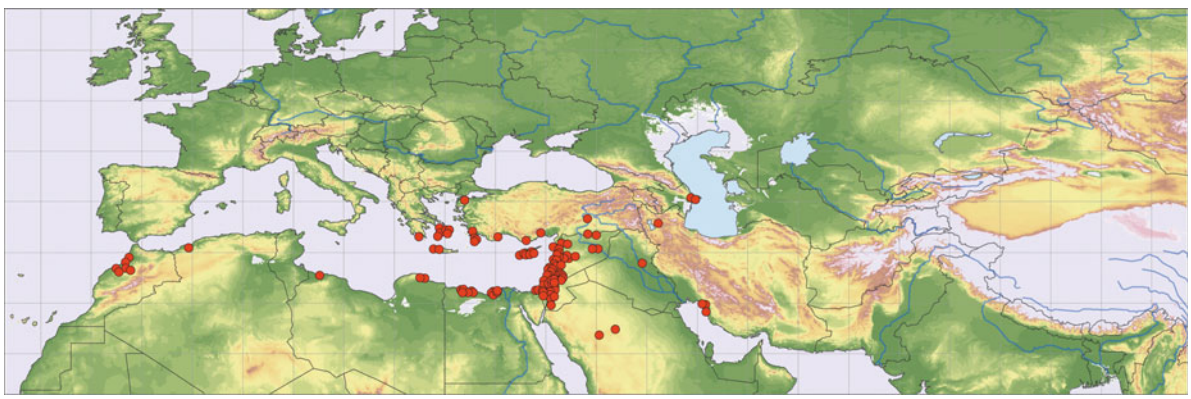

Fig. 1.43 Distribution of *Aegilops peregrina*

Aegilops columnaris

Aegilops columnaris Zhuk. – *Triticum columnare* (Zhuk.) Morris et Sears, comb. inval (Figs. 1.44 and 1.45).

Multitillered annual. Plants 20–50 cm tall (excluding spikes). Spike 2–4 cm long (excluding awns). Lower 2–3 spikelets fertile, upper 2 ones sterile, smaller. Spikes breaking off as a unit. Vestigial spikelets 3. Glumes of fertile spikelet 7–11 mm long, with 2–3 awns. Lemmas with 2–3 setulose awns, more slender and shorter than the glume awns.

The species shows a limited morphological variation. Sometimes, it is difficult to be distinguished from *Ae. neglecta*. For a special key, see van Slageren (1994). Genome: **UM**.

Occurring mainly in Turkey and the western Fertile Crescent, but scattered in the eastern part of the arc as well. The area of distribution extends westwards to Crete, eastwards to Armenia and Azerbaijan, rare in Iran. Uncommon throughout its range.

In dry open fields, road- and hillsides, more rarely in forests. Mainly found on limestone, less frequently on basalt. Soil textures are predominantly stony, with additional clay, clay loam and occasionally sand. The range of annual rainfall data 450–1,250 mm. From 450 m up to 1,990 m, only occasionally lower than 450 m but also found at sea level.

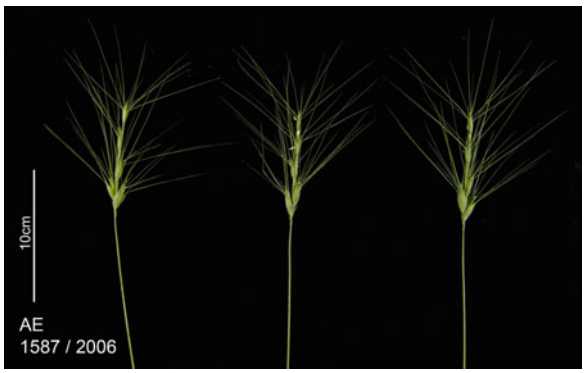

Fig. 1.44 Ear morphology of *Aegilops columnaris* (AE 1587)

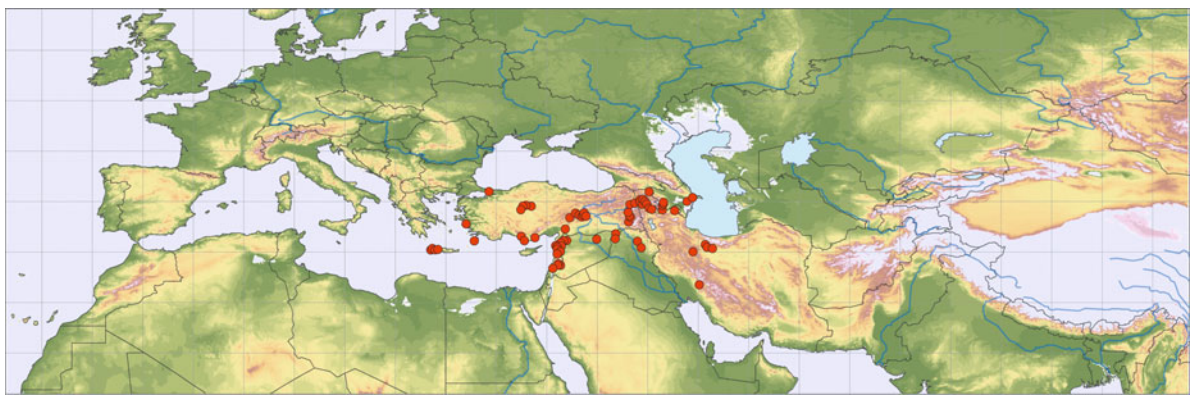

Fig. 1.45 Distribution of *Aegilops columnaris*

Aegilops neglecta

Aegilops neglecta Req. ex Bertol. – *Aegilops triaristata* Willd., nom. illeg., *Triticum neglectum* (Req. ex Bertol.) Greuter (Figs. 1.46–1.48).

Multitillered annual. Plants 25–35 cm tall (excluding spikes). Spike 3–6 cm long (including erecto-patent awns), with 2 fertile and 1–2 upper sterile spikelets. Spikes breaking off as a unit. Vestigial spikelets (2–) 3. Glumes of lower spikelets ovate-elliptic, 9–10 cm long. Awns 2–3, 2–5 cm long, broad at base, setaceous above.

Fig. 1.46 Ear morphology of *Aegilops neglecta* ssp. *neglecta* (AE 369)

The tetraploid (ssp. *neglecta*) and hexaploid races [ssp. *recta* (Zhuk.) Hammer] are difficult to distinguish (van Slageren 1994). But there are some indications, e.g., Hammer (1980b), Kimber and Feldman (1987). More variation on the tetraploid level is described by Hammer (1980b).

Genome: *Ae. neglecta* ssp. neglecta: **UM**; *Ae. neglecta* ssp. recta: **UMN**.

Occurring from Portugal to Turkey and into Turkmenistan.

Generally in dry, somewhat disturbed habitats and vegetation types such as fallow, grasslands, roadsides, stony fields, and hillslopes, maquis, garrigue, in forests or scrubs, as well as within and on the edges of cultivation. Occasionally found on river banks and generally more humid habitats. The parent rock is mainly limestone. Recorded soil textures include mainly loam, clayloam, and sandy loam. Collected

Fig. 1.47 Ear morphology of *Aegilops neglecta* ssp. *recta* (AE 1648)

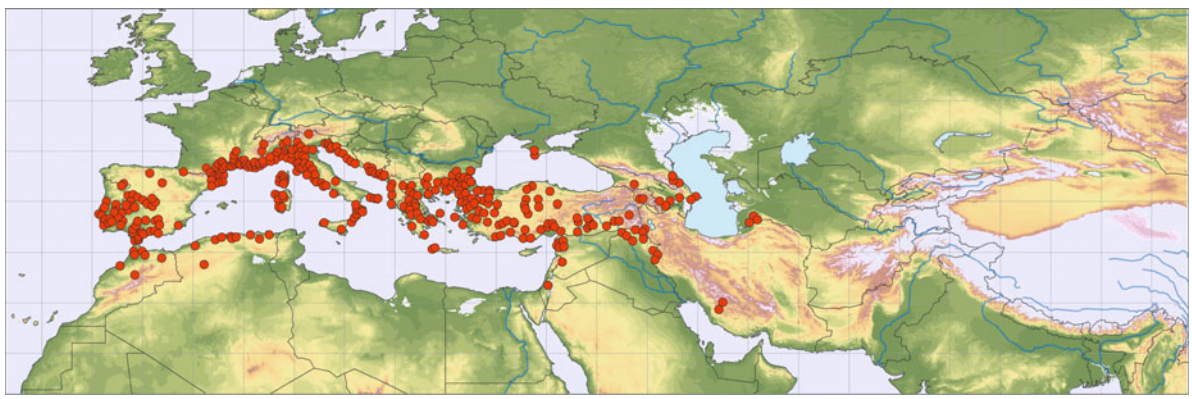

Fig. 1.48 Distribution of *Aegilops neglecta*, both subspecies

rainfall data vary from 450 to 750 mm, and in some sites it can be as high as 1,400 mm. From −200 m up to 2,000 m.

Aegilops geniculata

Aegilops geniculata Roth – *Aegilops ovata* auct. non L., *Triticum ovatum* auct. non Rasp., no valid name of this species exists under *Triticum* (van Slageren 1994) (Figs. 1.49 and 1.50).

Multitillered annual. Plants 10–30 cm tall (excluding spikes). Spike 1.2–1.8 cm long (excluding awns), with 2 (–3) lower fertile spikelets and 1 uppermost sterile spikelet. Vestigial spikelets 1 (–2). Spikes breaking off as a unit. Glumes subventricose with (3–) 4–5 setaceous awns, 1.5–2.5 cm long. Lemma awns as long as those of glumes.

Ssp. *geniculata* has more lax spikes and often more than 3 spikelets per spike, whereas ssp. *gibberosa* Zhuk. has very dense spikes and not more than 3 spikelets per spike. A number of botanical varieties of this variable species has been described (Hammer 1980b). Genome: **MU**.

Common in southern Europe and the Aegaean but relatively rare in Turkey and Transcaucasia. Introduced in the Canary Islands. Adventive in parts of central and northeastern Europe.

Locally abundant in generally dry, somewhat disturbed habitat such as fallow, wastelands, roadsides, and dry rocky slopes of hills and mountains. Also on edges of and within cultivation. Vegetation types include mainly garigue, maquis, grassland, shrub- and woodlands, forests and scrub and steppe. Bedrock is predominantly limestone. Soil texture also varies widely. *Ae. geniculata* can grow on very stony and rocky soils. As a typical colonizer, the species can be found in massive stands, especially at regularly disturbed places. Together with *Ae. triuncialis*, this is the most widespread species of the genus and grows under a similarly wide annual rainfall amplitude, varying from less than 100 mm up to 1,100 mm. Mainly from −300 m to 1,750 m.

Fig. 1.49 Ear morphology of *Aegilops geniculata* (AE 1653)

1.3 Evolution of *Aegilops* and *Triticum*

Studies on *Aegilops* and *Triticum* evolution have attracted large attention over more than 150 years and have produced a large amount of literature. Selected studies in *Aegilops–Triticum* based on morphological, cytogenetical, and molecular studies are summarized in Table 1.4. Recent molecular studies on

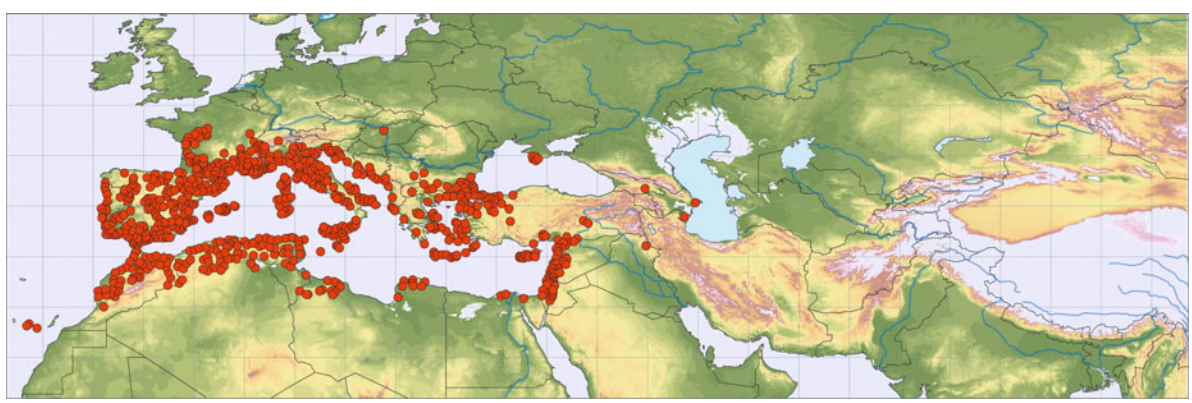

Fig. 1.50 Distribution of *Aegilops geniculata*

Table 1.4 Summary of published phylogenetic and diversity studies

Field of interest	References
Morphology	
Morphology including hybrid fertility; pollen fertility	Zhukovsky (1928); Eig (1929); Sarkar and Stebbins (1956); Kihara (1954); Johnson (1975); Johnson and Dhaliwal (1976); Hammer (1980a, b); Kimber and Sears (1987); van Slageren (1994); Ohta (2000); Panajiotidis et al. (2000); Seberg and Frederiksen (2001); Matsuoka and Takumi (2007)
Cytogenetics	
GISH	Raskina et al. (2002)
Fluorescence in situ hybridization	Raskina et al. (2004); Salina et al. (2006)
N-banding and genomic in situ hybridization	Gill and Chen (1987)
C-banding	Gill and Kimber (1974); Friebe et al. (1992); Badaeva et al. (2002, 2004)
Nuclear DNA content with flow cytometry	Özkan et al. (2003, 2009)
Chloroplast markers and genes	
Plasmon	Tsunewaki et al. (1996, 2002)
Allozyme variation and variation in chloroplast DNA, 2D-protein patterns, SS and 18-268 rRNA gene and spacer nucleotide sequences, and variation in repeated nucleotide sequences	Dvorak and Zhang (1992)
cp microsatellite	Provan et al. (2004); Gandhi et al. (2005); Matsuoka et al. (2005, 2008); Matsuoka and Takumi (2007)
Sequences	Gielly and Taberlet (1994); Miyashita et al. (1994); Kellogg et al. (1996); Vanichanon et al. (2003); Yamane and Kawahara (2005); Golovnina et al. (2007); Kilian et al. (2007a); Haider and Nabulsi (2008)
Nuclear markers and genes	
Isozymes	Guadagnolo et al. (2001b); Kawahara (2002)
Electrophoretic analysis of alpha gliadins	Masci et al. (1992)
Intergenic spacer nor loci	Sallares and Brown (1999)
5′ External transcribed spacer	Sallares and Brown (2004)
SSCP	Ohsako et al. (1996)
RAPD	Guadagnolo et al. (2001a, b); Khlestkina (2001); Goryunova et al. (2004)
RFLP	Takumi et al. (1993); Gielly and Taberlet (1994); Mori et al. (1995); Sasanuma et al. (1996); Dvorak et al. (1998b); Galili et al. (2000)
AFLP	Heun et al. (1997); Monte et al. (2001); Sasanuma et al. (2002, 2004); Kilian et al. (2007a)
EST unigenes	Dvorak and Akhunov (2005)
genome specific amplified length and CAPS polymorphisms	Kadosumi et al. (2005)
SSR and EST-SSR	Lelley et al. (2000); Pestsova et al. (2000); Guadagnolo et al. (2001a, b); Adonina et al. (2005); Gandhi et al. (2005); Zhang et al. (2006)
Inter-retroelement amplified polymorphism (IRAP) markers	Saeidi et al. (2008)
Sequences	Kellogg et al. (1996); Wang et al. (2000); Allaby and Brown (2001); Faris et al. (2001); Huang et al. (2002); Vakhitov et al. (2003); Goryunova et al. (2005); Mason-Gamer (2005); Petersen et al. (2006); Baum et al. (2009) Kilian et al. (2007a); Massa and Morris (2006); Chalupska et al. (2008); Salse et al. (2008)
Sequences Ha locus only	Massa et al. (2004); Chantret et al. (2005); Massa and Morris (2006); Li et al. (2008a, b); Bhave and Morris (2008a, b)
Sequences LMW and HMW only	Gu et al. (2004, 2006); Long et al. (2005); Li et al. (2008a, b); Jiang et al. (2008)

phylogenetic relationships of *Aegilops* and *Triticum* species review also selected previous literature (Yamane and Kawahara 2005; Petersen et al. 2006).

Morphological studies (including monographs) and collection expeditions by Zhukovsky (1928), Eig (1929), Kihara (1954), Hammer (1980a, b), Kimber and Sears (1987), and van Slageren (1994), among others, have provided detailed knowledge and various classification systems (Table 1.1). The great morphological variation displayed within many species in the *Aegilops–Triticum* group has been the cause for extensive recognition of taxa below the species rank and led to various opinions on their relationships (Hammer 1980a, b; van Slageren 1994). These studies also provided knowledge on the geographic distribution of the species and on their ecological requirements.

The latest monograph on *Aegilops* has been published by van Slageren (1994). He summarized the current knowledge on morphological traits and concluded that *Aegilops*, *Amblyopyrum* and *Triticum* are separate genera. Van Slageren (1994) described 22 *Aegilops*, 1 *Amblyopyrum* and 4 *Triticum* species.

Sakamura (1918), Sax and Sax (1924), and Kihara (1924) used cytogenetic methods and recognized that wheat species fall into three groups based upon their ploidy level (1) diploid $2n = 14$ = einkorn wheat; (2) tetraploid $4n = 28$ = emmer wheats; and (3) hexaploid $6n = 42$ = bread wheats (Fig. 1.51). Those cytogenetic studies led to the genome definition: A, B, C, D, G, M, N, S, T, U types are still used today in wheat/*Aegilops* research. These studies provided knowledge on genome structure, crossability, genome size, and phylogeny. The genome formulas are summarized in Table 1.2.

For more than two decades now, the use of molecular markers has provided new information on genetic diversity of *Aegilops/Triticum* species, their relationships (Kellogg et al. 1996; Huang et al. 2002; Provan et al. 2004; Sallares and Brown 2004; Yamane and Kawahara 2005; Petersen et al. 2006), centers of domestication (Heun et al. 1997; Ozkan et al. 2002, 2005; Mori et al. 2003; Haudry et al. 2007; Luo et al. 2007; Kilian et al. 2007b), time frame of evolution (Huang et al. 2002; Dvorak and Akhunov 2005;

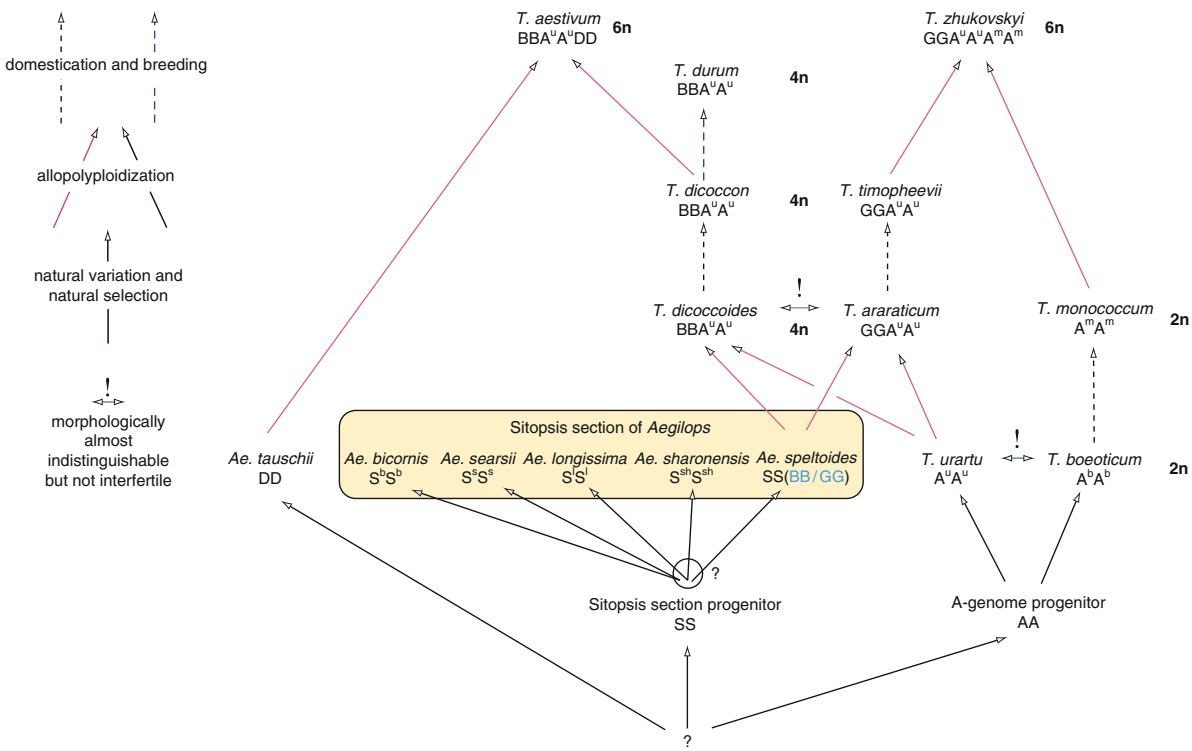

Fig. 1.51 Overview of wheat evolution and events. One similar figure has been originally published in Kilian et al. 2007a (SI). Published with permission from Oxford University Press

Chalupska et al. 2008), the domestication process (Tanno and Willcox 2006; Weiss et al. 2006; Kilian et al. 2007b), and the existence of specific alleles supporting domesticated traits and alleles to be considered for crop improvement (Komatsuda et al. 2007). The connection between molecular markers and domestication geography in *Triticum* took root in the paper by Heun et al. (1997), who found that on the basis of amplified fragment length polymorphism (AFLP) markers, the closest wild relatives of domesticated einkorn (*Triticum monococcum* L., diploid) occur in a very restricted area within the Karacadag mountain range in South-East Turkey. From that, they concluded, not unreasonably, that this area is the site where humans first domesticated einkorn.

Importantly and uniquely among the cereals, hexaploid bread wheat (*Triticum aestivum* L.) has no direct hexaploid wild progenitor (Fig. 1.51). It possesses three sets of homoeologous chromosomes, designated as BBA^uA^uDD (first position of B chromosomes indicate that the cytoplasm was provided by the B-genome donor), whose origins have differing degrees of certainty. The superscript "u" in the A^u-genome designation indicates that the A-genome is of the type found in *Triticum urartu* Thum. ex Gandil.

The D chromosomes stem from wild diploid *Aegilops tauschii* through alloploidization with the domesticated tetraploid *T. dicoccon* Schrank (emmer, BBA^uA^u) that was domesticated from wild *T. dicoccoides* (Körn. ex Aschers. & Graebn.) Schweinf.; (wild emmer wheat, BBA^uA^u). See also Kihara (1944), McFadden and Sears (1944, 1946) or Huang et al. (2002) for more details. The A^u and B chromosomes derive from the hybridization between the wild A^uA^u diploid *T. urartu* and a wild diploid B-genome donor (Dvorak 1976; Nishikawa 1983; Dvorak et al. 1988, 1993; Huang et al. 2002; Kilian et al. 2007a), frequently reported to belong to the *Sitopsis* section of *Aegilops*, which includes five species.

Strong evidence points to the outcrossing *Aegilops speltoides* (SS) (or an unknown similar species) as the female parent of all wild tetraploid wheats (Sarkar and Stebbins 1956; Riley et al. 1958; Tsunewaki and Ogihara1983; Dvorak and Zhang 1990; Wang et al. 1997; Khlestkina and Salina 2001; Petersen et al. 2006; Kilian et al. 2007a; Chalupska et al. 2008) and to *T. urartu* (A^uA^u) as the male parent (Dvorak and Zhang 1990; Huang et al. 2002; Zhang et al. 2002; Kilian et al. 2007a). Kilian et al. (2007a) used a 4-tiered approach. Using 70 AFLP loci, they sampled molecular diversity among 480 wheat lines from their natural habitats encompassing all S-genome *Aegilops* (*Sitopsis* section), to learn which are the putative donors of wheat B-genome and the G-genome of the second wild tetraploid wheat species *T. araraticum* Jakubz. (Araratian wheat, GGA^uA^u) and the hexaploid domesticated wheat *T. zhukovskyi* Menabde et Ericzjan (Zhukovskyi's wheat, $GGA^mA^mA^uA^u$). Fifty-nine *Aegilops* representatives for S-genome diversity were compared at 375 AFLP loci with diploid, tetraploid, and 11 nulli-tetrasomic *T. aestivum* cv. Chinese Spring lines produced by Sears (1954). B-genome-specific markers allowed pinning the origin of the B-genome to S chromosomes of *Ae. speltoides*, while excluding other *Aegilops* section *Sitopsis* species. The outbreeding nature of *Ae. speltoides* influences its molecular diversity and bears upon inferences of B and G-genome origins. Haplotypes at nuclear and chloroplast loci *ACC1*, *G6PDH*, *GPT*, *PGK1*, *Q*, *VRN1*, and *ndhF* for ~70 *Aegilops* and *Triticum* lines (0.73 Mb sequenced) reveal both B- and G-genomes of polyploid wheats as unique samples of *Ae. speltoides* haplotype diversity. These have been sequestered by the *T. dicoccoides* and *T. araraticum* [wild progenitor of *T. timopheevii* (Zhuk.) Zhuk. Timopheev's wheat, GGA^uA^u] lineages during their independent origins.

The hybridization, which generated the BBA^uA^u wheats, may have taken place between 0.25 and 1.3 MYA according to some estimates (Mori et al. 1995; Huang et al. 2002; Dvorak and Akhunov 2005), while the event that led to the GGA^uA^u wheats likely occurred later (Huang et al. 2002). The distinctly reticulate evolutionary relationships between wheats, with different ploidy levels tracing to hybridization events, are shown in Fig. 1.51.

However, despite intensive research, the origin of the B-genome of wheat still remains in great obscure (Huang et al. 2002; Yamane and Kawahara 2005; Petersen et al. 2006). Of all other genomes analyzed, the *Ae. speltoides* genome is the most closely related to the wheat B-genome. Yet, molecular studies, as well as genome size analysis (Eilam et al. 2007), suggest that *Ae. speltoides* in its present form could serve as the donor of the wheat B-genome. The most common opinion is that B-genome was donated by another related species, yet not found, or extinct, or that the B-genome of wheat is a recombinant genome that combines the genetic contribution of several

diploid species (Zohary and Feldman 1962; Johnson 1975; Feldman 1978).

Also, the recent study of Salse et al. (2008) on the evolutionary relationships at the *Storage Protein Activator* (*SPA*) locus region could not gain more conclusions. Four bacterial artificial chromosome (BAC) clones, spanning the *SPA* locus of, respectively, the A-, B-, D-, and S-genomes, were isolated, sequenced, and compared. On the basis of conserved sequence length as well as identity of the shared nontransposable element regions and the *SPA* coding sequence, *Ae. speltoides* appears to be more evolutionary related to the B-genome of *T. aestivum* than the A- and D-genomes. However, the differential insertions of transposable elements (TE), none of which are conserved between the two genomes, led to the conclusion that the S-genome of *Ae. speltoides* has diverged very early from the progenitor of the B-genome, which remains to be identified.

It is accepted that *T. aestivum* originated from a cross (crosses) between domesticated hulled tetraploid emmer *T. dicoccon* (or the free-threshing hard wheat *T. durum,* or the free-threshing archeological *T. parvicoccum* Kislev) and the goat grass *Aegilops tauschii* (DD) (Kihara 1944; McFadden and Sears 1946; Kerber 1964; Kislev 1980; Dvorak et al. 1998a, b; Matsuoka and Nasuda 2004). This cross should have taken place after emmer (or hard wheat cultivation) spread east from the Fertile Crescent into the natural distribution area of *Ae. tauschii*. The cross occurred most probably south or west of the Caspian Sea about 8,000 years ago (Hammer 1980b; Nesbitt and Samuel 1996; Salamini et al. 2002; Giles and Brown 2006). *Aegilops tauschii* encompasses several morphological varieties that are roughly grouped into *Ae. tauschii* ssp. *tauschii* and *Ae. tauschii* ssp. *strangulata* (Kihara et al. 1965; Hammer 1978; Jaaska 1995; Dvorak et al. 1998a). Several studies show that *Ae. tauschii* ssp. *strangulata* provided the wheat D-genome (at least twice), but contributions from both subspecies are also discussed (Nishikawa et al. 1980; Jaaska 1981; Dvorak et al. 1998b; Talbert et al. 1998). If only a few *Ae. tauschii* genotypes participated in the synthesis of *T. aestivum*, this polyploidization should have been accompanied by the reduction of wheat diversity (Haudry et al. 2007). However, high mutation rates, together with buffering effects caused by polyploidy, enabled hexaploid wheat to enhance their diversity (Dubcovsky and Dvorak 2007).

1.3.1 Distribution of the S-Genome Species of Aegilops

The geographical distribution of all *Aegilops* Section *Sitopsis* species is of interest as they may have contributed to the B-genome of wheat. All five species are diploids and mostly have limited distribution (Table 1.2; Figs. 1.3–1.14). *Ae. bicornis*, *Ae. longissima*, *Ae. sharonensis*, and *Ae. searsii* all occur within a very limited area in the Near East. Only *Ae. speltoides* can be found along the Fertile Crescent with both races often occurring sympatrically and without ecological tendencies (Hammer 1980a). The question of tetraploid wheat evolution now arises. Where did wild emmer (*T. dicoccoides*, genome-BBA^{u}A^{u}) and wild *T. araraticum* (genome-GGA^{u}A^{u}) originate? Which *Sitopsis* species donated genomes-B and G to wheat? If *Ae. speltoides* was the donor of the wheat B-genome (especially the ssp. *ligustica*; Sarkar and Stebbins 1956), then this may have happened over a large area, comprising almost the entire Fertile Crescent and large parts of Turkey. If, on the other hand, any of the other *Sitopsis* species was involved, the most likely region must have been the Jordan River valley.

1.3.2 Phylogenetic Relationships within and between Aegilops–Triticum

Wheat genome donors are of great interest, and efforts to study their genomes have been undertaken and are currently underway. Research focuses mainly on *Aegilops* section *Sitopsis* species as the potential wheat B-genome donors; on *Ae. tauschii* as the wheat D-genome donor; and on *Triticum urartu* as the A-genome donor.

Studies on phylogenetic relationships within and between *Aegilops–Triticum* have been based on various methods (Table 1.4). Recent molecular studies, however, still suffer from limited and biased taxon sampling. Rarely, all diploid species of *Aegilops* and *Triticum* are included in such comparative analyses. For instance, no one has considered all 23 *Aegilops* and all the four wild *Triticum* (diploid: *T. urartu*, *T. boeoticum* Boiss.; tetraploid: *T. dicoccoides* and *T. araraticum*) species.

The most complete molecular studies in terms of taxon sampling, suitable outgroups, and loci considered

have been published by Yamane and Kawahara (2005) and by Petersen et al. (2006). These authors, in addition, summarize the recent literature on the subject.

Petersen et al. (2006) resequenced two nuclear single-copy genes (*disrupted meiotic cDNA*, DMC1; and *translation elongation factor G*, EF-G) and the chloroplast locus *ndhF*. The authors found strong evidence that the wheat D-genome was derived from *Ae. tauschii*, the wheat A-genome was derived from *T. urartu*, and the wheat B-genome was derived from *Ae. speltoides*. Their phylogenetic analysis suggests that *Triticum*, *Aegilops*, or *Triticum* plus *Aegilops* are not monophyletic. *Ae. speltoides* clusters with all other wheat B-genomes at a basal position of the tree and therefore violates the monophyly of the *Aegilops–Triticum* group. They also discussed the option that monophyly could be restored by the exclusion of *Ae. speltoides*. In this case, *Ae. speltoides* should be removed from *Aegilops* and transferred to the genus *Sitopsis* (Jaub. & Spach) Löve.

Yamane and Kawahara (2005) studied the intra- and interspecific relationships among diploid *Aegilops– Triticum* species. Their analysis focused on four noncoding chloroplast genome regions (*trnC–rpoB* intergenic region, the *trnF–ndhJ* intergenic region, the *ndhF–rpl32* intergenic region, and the *atpI–atpH* intergenic region). The authors concatenated the sequence data for each accession (2,740 bp without indels, microsatellites, and inversions), studied DNA sequence variation, and detected 62 haplotypes in 115 accessions of 13 diploid species. Yamane and Kawahara (2005) found evidence that *Aegilops* and *Triticum* species should be included in one genus only; if *Aegilops* and *Triticum* are retained as separate genera, then *Ae. speltoides* must be placed in a new genus. Several accessions of each species were analyzed. This allowed them to conclude that several *Aegilops* species including the *Sitopsis* section of *Aegilops* (except *Ae. speltoides*) underwent speciation. The authors point out that *Ae. mutica* does not occupy a basal position in the *Aegilops–Triticum* tree and may have originated relatively recently. Therefore, the authors include *Ae. mutica* in *Aegilops*. In their study, *Ae. speltoides* clustered at a basal position and differed *significantly* from other *Sitopsis* species. *Ae. markgrafii* seems to be polyphyletic and the section *Comopyrum* paraphyletic.

1.3.3 Case Study: Grain Hardness – A Trait Modified by Domestication

Grain hardness or texture is an important quality trait because of its connection with the susceptibility to damage during milling and the amount of water uptake during baking. Compared to *T. aestivum*, *T. durum* Desf. (hard wheat) has hard endosperm.

A single locus influencing hardness (i.e., conferring the "soft" phenotype) has been named *Ha* (hardness) and mapped to the short arm of chromosome 5D (Sourdille et al. 1996). The locus encodes mainly friabilins, proteins included in the prolamin superfamily: puroindoline a (*Pina*), puroindoline b (*Pinb*), and grain softness protein (*Gsp-1*) (Gautier et al. 1994; Rahman et al. 1994; Kan et al. 2006). The three genes are tightly linked (Sourdille et al. 1996; Giroux and Morris 1998; Giroux et al. 2000; Chantret et al. 2004). The three genes have been investigated in BAC clones of several *Triticum* and *Aegilops* species (Tranquilli et al. 1999; Turnbull et al. 2003; Chantret et al. 2004, 2005; Li et al. 2008a, b). The molecular basis and the evolutionary events at this locus were considered. The variation at the *Ha* locus arose from gene losses after polyploidization. Genomic rearrangements, such as transposable element insertions, deletions, duplications, and inversions contributed to the differences between species of different ploidy levels (Chantret et al. 2005; Massa and Morris 2006). *Pina* and *Pinb* genes are conserved in all diploid *Triticum*, *Aegilops*, and closely related *Pooideae* but are deleted from the A^u and B chromosomes in tetraploid BBA^uA^u wheats (Gautier et al. 2000; Li et al. 2008a, b). The *Pina* and *Pinb* gene losses in BBA^uA^u tetraploids were caused by a large genomic deletion, probably of independent origin in the A^u and B progenitor genomes (Li et al. 2008a, b). *Pina* and *Pinb* genes are nevertheless present on chromosome 5D in hexaploid wheats, because they were donated by *Ae. tauschii*, at the time of the emergence of the allohexaploid bread wheat. Recently, Li et al. (2008a, b) have shown that *Pina* and *Pinb* were eliminated from the G-genome but maintained in the A^u-genome of tetraploid GGA^uA^u wheats. This supports the independent polyploidization events leading to BBA^uA^u and GGA^uA^u wheats.

The *Gsp-1* genes are conserved in genomes A^u-, B-, D-, and G- at all ploidy levels. They constitute a multigene family and may be functionally important, particularly in *T. durum* where they have major roles in plant defense and only a minor influence on grain texture (Gollan et al. 2007).

After the acquisition of *Ha* by hexaploid wheats via the D-genome, the spreading of the hexaploid species to the north forced breeders to select hexaploid hard wheat cultivars, and thus the selection pressure on *Ha* increased. Hexaploid hard wheats have a mutation in either *Pina* or *Pinb* but not in *Gsp-1* (Giroux and Morris 1998). In addition, other genetic factors unlinked to the *Ha* locus may contribute to modify grain hardness (Perretant et al. 2000).

Massa and Morris (2006) documented the complete coding sequence for *Pina*, *Pinb*, and *Gsp-1* genes in the Tribe Triticeae. Maximum likelihood analyses performed on Bayesian phylogenetic trees showed distinct evolutionary patterns among *Pina*, *Pinb*, and *Gsp-1*. Results revealed positive selection at *Pina* and detected amino acid residues along the mature *Pina* protein with a high probability (>95%) of having evolved as a response to better adaptation. Massa and Morris (2006) hypothesized that positive selection at the *Pina* region is congruent with its role as a plant defense gene.

The recent knowledge on puroindolines is summarized in Morris (2002) and Bhave and Morris (2008a, b).

1.4 Cytogenetic Studies and Karyotype

1.4.1 *Wheat–*Aegilops *Genetic Relationships by Chromosome Pairing*

Hybrids between wheat and different *Aegilops* species have been produced and documented by different researchers (Kihara 1937; Knobloch 1968; Kimber and Abu Bakar 1979; Sharma and Gill 1983; Table 1.5). Kimber and Abu Bakar (1979) developed an important database on chromosome pairing during meiosis in hybrids between wheat and its relatives. This database has been used for assessing genome affinity and determining species relationships (Kimberet al. 1981; Mujeeb-Kazi and Kimber 1985; Gill and Chen 1987). Based on chromosome pairing information, numerical methods of assessing genome affinity or similarity of the A-, B-, and D-genomes of *T. aestivum* to related genomes of other species have been developed (Kimber and Hulse 1978; Driscoll et al. 1979; Alonso and Kimber 1981; Kimber and Alonso 1981; Kimber et al. 1981). Genomic affinity of individual chromosomes can also be determined by sequential banding and genomic in situ hybridization (GISH) (Jiang and Gill 1993, 1994). Staining techniques were used to develop a cytogenetic karyotype of wheat and to analyze cereal chromosomes (Gill and Kimber 1974; Gill et al. 1991b). Nonisotopic methods of mapping DNA sequences in situ on chromosomes were used to construct a molecular karyotype of wheat (Rayburn and Gill 1985; Jiang and Gill 1994). These methods had greatly facilitated cytogenetic analysis in wheat and related species; and vertical transfers of chromosomes or of their segments among or within species (Friebe et al. 1991, 1996a, b). Data obtained from these different approaches allowed the description and a better understanding of the relationships between *Aegilops* and wheat genomes.

1.4.2 *Genome Size in* Aegilops–Triticum

Genome size is a constant feature of a species of any organism. However, a number of evolutionary processes affect genome size, including polyploidization, fixation of accessory chromosomes, formation of large duplications, and expansions of satellite DNA, or the dynamics of transposable elements (SanMiguel et al. 1998). Recently, Eilam et al. (2007, 2008) determined genome size in *Aegilops* species (Table 1.2). In these diploid species, using flow cytometry, they detected a very reduced intraspecific variation. However, in contrast to the situation found at the intraspecific level, at the interspecific level, they report that the DNA amount per haploid nucleus ranges from 9.59 pg in *Ae. cylindrica* to 12.64 pg in *Ae. kotschyi*. Allotetraploid species with either the S-, G-, or B-genome, namely *Ae. kotschyi* and *Ae. peregrina*, have the largest amount of DNA, and those with the C-genome, that is, *Ae. cylindrica* and *Ae. triuncialis*, have the smallest. According to the cited authors, the difference between the intraspecific and the interspecific values should reflect interspecific changes in DNA amount that occurred during speciation.

Table 1.5 Available information on *Aegilops* interspecific hybrids (based on Kimber and Feldman 1987). + Positive results, *nd* Not determined

	Male ♂	*Aegilops bicornis*	*Aegilops biuncialis*	*Aegilops columnaris*	*Aegilops comosa*	*Aegilops crassa*	*Aegilops cylindrica*	*Aegilops geniculata*	*Aegilops juvenalis*	*Aegilops kotschyi*	*Aegilops longissima*	*Aegilops markgrafii*	*Aegilops mutica*	*Aegilops peregrina?*	*Aegilops searsii?*	*Aegilops sharonensis*	*Aegilops neglecta*	*Aegilops speltoides*	*Aegilops tauschii*	*Aegilops triuncialis*	*Aegilops umbellulata*	*Aegilops uniaristata*	*Aegilops vavilovii*	*Aegilops ventricosa*	*Triticum aestivum*	*Triticum monococcum*	*Triticum timopheevi*	*Triticum turgidum*	*Triticum zhukovskyi*
Female ♀	Genome	S^b	UM	UM	M	DM/DDM /DMS	CD	MU	DMU	SU	S^l, S^h	C	T	SU	S^S		UM/ UMN	S	D	UC	U	N		DN	ABD	Am	GA	BA	GAA
Aegilops bicornis	S^b	−	−	−	−	−	−	−	−	−	−	−	+	nd	nd	nd	−	−	+	−	+	+	nd	−	+	+	−	−	−
Aegilops biuncialis	UM	+	−	+	+	+	−	+	−	+	+	+	−	nd	nd	nd	+	+	−	+	−	−	nd	−	−	−	−	−	−
Aegilops columnaris	UM	+	+	−	+	+	−	−	−	+	−	+	−	nd	nd	nd	−	+	−	+	+	+	nd	−	−	−	−	−	−
Aegilops comosa	M	−	−	−	+	−	−	−	−	−	+	−	+	nd	nd	nd	−	−	+	−	−	+	nd	−	+	−	−	+	−
Aegilops crassa	DM/DDM /DMS	+	−	+	−	+	+	+	−	+	+	+	+	nd	nd	nd	−	+	+	+	+	+	nd	+	+	−	−	+	−
Aegilops cylindrica	CD	−	+	−	−	+	−	+	+	−	−	+	−	nd	nd	nd	+	−	+	+	−	−	nd	+	−	+	−	+	−
Aegilops geniculata	MU	−	−	+	+	−	−	−	−	+	+	+	−	nd	nd	nd	+	+	+	+	+	+	nd	+	−	+	+	+	−
Aegilops juvenalis	DMU	−	−	+	−	+	+	−	−	−	+	+	+	nd	nd	nd	−	+	+	+	+	+	nd	+	+	−	−	−	−
Aegilops kotschyi	SU	−	+	+	−	−	−	+	−	+	+	+	−	nd	nd	nd	−	+	−	−	+	−	nd	−	+	−	+	+	−
Aegilops longissima	S^l	+	−	−	+	−	+	−	−	+	+	+	+	nd	nd	nd	−	+	−	−	+	+	nd	−	+	+	+	+	−
Aegilops markgrafii	C	+	+	−	+	−	+	−	−	−	+	−	+	nd	nd	nd	−	+	+	−	+	+	nd	−	−	−	+	+	−
Aegilops mutica	T	−	−	−	−	−	−	−	−	−	−	−	−	nd	nd	nd	−	−	−	−	−	−	nd	−	−	−	−	−	−
Aegilops neglecta	UM/UMN	−	+	+	−	+	+	−	−	+	−	+	−	nd	nd	nd	+	+	−	+	−	+	nd	+	−	−	−	+	−
Aegilops peregrina	SU	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Aegilops searsii	S^S	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Aegilops sharonensis	S^{sh}	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Aegilops speltoides	S	+	−	−	+	−	−	−	−	−	+	+	+	nd	nd	nd	−	−	+	−	−	−	nd	−	−	+	−	+	−
Aegilops tauschii	D	+	−	−	−	−	+	−	−	−	−	−	+	nd	nd	nd	−	−	−	−	−	+	nd	+	+	−	−	−	−
Aegilops triuncialis	UC	−	−	−	−	−	−	−	−	+	−	+	−	nd	nd	nd	+	−	−	−	−	−	nd	+	+	−	−	+	−
Aegilops umbellulata	U	+	−	−	+	−	+	−	−	−	−	+	+	nd	nd	nd	−	+	+	−	−	+	nd	+	−	−	−	−	−
Aegilops uniaristata	N	+	−	−	+	−	−	−	−	−	−	+	+	nd	nd	nd	−	−	−	−	+	−	nd	−	−	−	−	−	−
Aegilops vavilovii	DMS	−	−	−	−	−	+	−	−	−	−	−	−	nd	nd	−	−	−	+	−	−	−	nd	+	−	−	−	−	−
Aegilops ventricosa	DN	−	−	−	−	+	+	+	−	−	−	+	−	nd	nd	nd	−	+	+	−	−	+	nd	−	+	+	+	+	−
Triticum aestivum	ABD	−	+	+	+	+	+	+	+	+	+	+	+	nd	nd	nd	+	+	+	+	+	−	nd	+	+	+	+	+	+
Triticum monococcum	Am	+	−	−	−	−	−	−	−	−	−	−	+	nd	nd	nd	−	+	+	−	−	+	nd	−	−	+	−	+	−
Triticum timopheevii	GA	+	−	−	+	−	+	−	−	−	+	+	−	nd	nd	nd	−	+	+	+	+	+	nd	+	+	+	+	+	−
Triticum turgidum	BA	+	−	−	+	−	+	+	−	−	+	+	+	nd	nd	nd	+	+	+	+	+	+	nd	+	+	+	+	+	−
Triticum zhukovskyi	GAA	−	−	−	−	−	−	−	−	−	−	−	−	nd	nd	nd	−	−	−	−	−	−	nd	−	+	−	+	−	−

1.4.3 Gene Flow Between Aegilops and Triticum

Hybridization and introgression are natural processes occurring particularly among closely related species. Cultivated plants, wheats included, hybridize naturally with their wild relatives (De Candolle 1883; Ellstrand et al. 1999). Also, the natural hybridization between wheat and some of the *Aegilops* generates sometimes viable seeds (van Slageren 1994). It is, therefore, not surprising that gene flow from wheat to *Aegilops* may have negative consequences for the wild species, such as formation of a super-weed and transfer of herbicide resistance. Natural gene transfer from wild to cultivated species requires their occurrence in the same field, pollen shedding by the donor, stigma receptivity of the recipient species, and viability of the derived progenies.

All wild relatives of cultivated wheat have the potential to hybridize with wheat (van Slageren 1994). These *Aegilops* and *Triticum* species are annual, predominantly self-pollinating species well adapted to the seasonal rainfall of their geographic distribution area (Calder 1966). The flowering time of *Aegilops* and other wheat wild relatives is from April–May until June–July, depending on the species and on the location (van Slageren 1994). This period coincides with the flowering of cultivated wheat in the Mediterranean environments (Azzi 1954).

The spike of *Aegilops* and bread wheat require 3 to 5 days to complete flowering (Peterson 1965; Boguslavsky 1979), the duration is longer in *Aegilops* than in wheat, due to asynchronous tillering (van Slageren 1994). This enables wild species to better adapt to the environment and to ensure seed production. The continuous flowering period of *Aegilops* species increases their chance of cross pollination with cultivated wheat. In addition, the success of gene flow depends on pollination mode (Hamrick et al. 1979; Govindaraju 1988), flower structure, and pollen dispersal (Waines and Hegde 2003).

Most *Aegilops* species are autogamous (self fertilizing) (Peterson 1965; Hammer 1980b), whereas *Ae. speltoides* is allogamous (cross pollinating) and *Ae. markgrafii* facultative allogamous (Hammer 1980a; Hammer 1987). Hybridization and gene transfer depend, in part, on the degree and duration of glumes opening at anthesis, on anther size, and on pollen production and viability (Hucl 1996; Waines and Hegde 2003). The rate of outcrossing of autogamous *Aegilops* species is not well documented, but a large variation of traits affecting floral ecology was found in 114 races of the autogamous *Aegilops tauschii* (Hammer 1978). Pollen dispersal depends on anther extrusion and on the amount of pollen produced per anther (Joppa et al. 1968). Viability of pollen ranges between 15 and 30 min, depending on environmental conditions (de Vries 1971), and this time period is sufficient to allow cross-pollination (Hedge and Waines 2004). For instance, wheat pollen has the capacity to move at distances more than 60 m (Khan et al. 1973). No published information exists on pollen transport in *Aegilops* species.

1.4.4 Spontaneous Hybridizations Between Wild Wheat Relatives and Bread Wheat

The occurrence of natural hybridizations between bread wheat and its wild relatives has been documented (Kimber and Feldman 1987; van Slageren 1994; Zaharieva and Monneveux 2006). For instance, spontaneous hybrids between *Ae. geniculata* and bread wheat were reported by Requien in 1825 and Fabre in 1838 (Dondlinger 1916; van Slageren 1994); natural hybrids between *Ae. neglecta* and bread wheat and between *Ae. triuncialis* and bread wheat were described by Godron (1854) and Lange (1860), respectively. The first *Aegilops cylindrica* × wheat hybrid was described in 1917 by von Degen (van Slageren 1994). Van Slageren (1994) listed natural hybrids between bread wheat and various *Aegilops* species in Europe. Kimber and Feldman (1987) provided an overview on *Triticum* × *Aegilops* hybrids. Guadagnolo et al. (2001a, b) reported that under field conditions in Switzerland, the hybridization rate between *Ae. cylindrica* and wheat was 3%. The authors described that natural hybrids were limited to tetraploid *Aegilops* species and wheat. The hybrids were observed along roadsides, adjacent to wheat fields, or next to the fields where wheat had been cultivated in the previous year. Most natural hybrids between *Aegilops* and *Triticum* were sterile, but seeds were occasionally produced. Snyder et al. (2000) observed that the hybrid fertility between *Ae.*

cylindrica and wheat ranged from 2 to 4% and that viable seeds can be produced by hybrids with either jointed goatgrass (*Ae. cylindrica*) or wheat as a pollen donor.

It can be concluded that the transfer of a transgene from genetically modified wheat to *Aegilops* spp. could take place. The distribution patterns of wild wheat relatives indicate that ecological situations exist with a higher likelihood of interspecific hybridization and gene transfer between wheat and wild relatives.

Hybrid seed set between tetraploid *Aegilops* and wheat shows a great variation. The highest value is found when an *Aegilops* species is the female parent. Moreover, hybrids sterility can be overcome by backcrossing the hybrid to a parent or by spontaneous chromosome doubling. The close relationship between the bread wheat D-genome and most of the constitutive genomes of the tetraploid *Aegilops* suggest a higher probability of transfer when the trait is located on a D-genome chromosome. Gene introgression from wheat is expected to occur more frequently in *Ae. cylindrica* and *Ae. ventricosa*, given the high recombination between homologous D-genome of the wheat and the *Aegilops* chromosomes. Attention should focus on the tetraploid species, which are more abundant and intercross more frequently with cultivated wheat.

1.5 Role in Development of Cytogenetic Stocks and Their Utility

1.5.1 *Substitution and Addition Lines of* Aegilops *in* Triticum *Background*

Since bread wheat is an allohexaploid species with A, B, and D-genomes, each of the chromosomes has two closely related chromosomes. Therefore, most genes have homoeoalleles (one pair per genome), which can functionally compensate for one another. Because of this genetic structure, bread wheat easily tolerates addition or deletion of a pair of chromosomes, or of a single chromosome, or of part of a chromosome. It is, in this sense, well known that wheat tolerates addition of chromosomes from *Aegilops* or substitution of a chromosome with a homoeolog of *Aegilops*. *Aegilops* addition and substitution lines allow to study the genetic effects of individual alien chromosomes in the background of hexaploid wheat, and to understand the homoeologous relationship between chromosomes of different species (Miller and Reader 1987), besides locating genes on a particular chromosome (Riley et al. 1966). Up to date, 18 *Aegilops* species have been used to produce complete or partial sets of addition lines (Table 1.6) and 11 *Aegilops* species to produce complete or partial set of substitution lines (Table 1.7).

Aegilops addition and substitution lines are the starting materials of prebreeding programs aimed at the transfer of alien traits to cultivated varieties (Feldman and Sears 1981). Knowing the characters of *Aegilops* species, it is possible to identify *Aegilops* chromosomes or their segments based on plant morphology, chromosome banding, and biochemical and molecular markers.

The first step in production of an addition line is to generate a wheat–*Aegilops* amphiploid. This objective is achieved by doubling the chromosomes of a wheat–*Aegilops* hybrid by colchicine treatment. The production method of an addition line in wheat background is presented in Fig. 1.52. In few cases, including hybrids of wheat with *Ae. tauschii*, *Ae. comosa*, *Ae. longissima* or *Ae. kotschyi*, unreduced 2*n* gametes were formed, resulting in spontaneous formation of amphiploids (Feldman 1983; Ozkan et al. 2001). Amphiploids are genetically stable and allow the evaluation of the alien germplasm in a common genetic background. The presence of a whole wild genome in the amphiploid hybrids renders them unsuitable for a direct agronomic use. Alien addition lines are produced by backcrossing recurrently the amphiploid to a wheat parent (Feldman and Sears 1981; Sears 1981). Progenies with the whole wheat chromosome complement and an extra chromosome are cytologically selected. These are evaluated by morphological, cytological, biochemical, and molecular markers, and a series of lines, each having a different single alien chromosome, are selected. Self-pollination of these progenies yields disomic addition lines, which are selected cytologically. In some cases, the development of addition and substitution lines can be problematic because the chromosomes of some *Aegilops* species carry gametocidal genes (Endo and Tsunewaki 1975; Maan 1975; Endo and Katayama 1978).

Disomic addition lines are genetically stable, free of a large portion of the original alien chromatin, and allow the evaluation of the alien genes present in a single chromosome. Knowing the homoeologous group assignment of any added chromosome, it is possible to substitute it for any of its wheat homoeologs.

Table 1.6 List of major stocks of wheat–*Aegilops* chromosome addition lines

Aegilops species	Chromosome disomic (monosomic) additions	Telosome additions	Method of chromosome identification	Reference
Ae. bicornis	$3S^b$, $7S^b$			Shepherd and Islam (1988)
Ae. biuncialis	Five (2M,3M,7M,3U,5U)		GISH, FISH	Schneider et al. (2005)
Ae. comosa	2M			Riley et al. (1968)
Ae. cylindrica	Two lines (one is 4C)		RFLP	Bai et al. (1995)
Ae. geniculata	13 lines + 1 mono	Nine + two monotelo	C-band	Friebe et al. (1999)
	Four lines		Not identified	Mettin et al. (1977)
	6U			Stoilova and Spetsov (2006)
Ae. longissima	Two complete sets	All ($6S^lL$ mono)	C-band	Feldman (1975); Friebe et al. (1993)
Ae. markgrafii	Six lines (A missing)		C-band	Friebe et al. (1992)
			SSR	Peil et al. (1998); Schubert and Blüthner (1995)
Ae. mutica	Four lines (A, C, E, F)			Dover (1973); Friebe et al. (1996c)
Ae. neglecta ssp. *recta*	Three lines (homoeologous groups 5, 2, 7)		RFLP	Bai et al. (1994)
Ae. peregrina	11 lines			Driscoll (1974)
	Complete set		RFLP, isozyme	Yang et al. (1996)
	Unidentified lines		C-band	Friebe et al. (1996a); Spetsov et al. (1997)
Ae. searsii	Complete set	Complete set	C-band	Pietro et al. (1988); Friebe et al. (1995a)
Ae. sharonensis	$2S^{sh}$			Endo unpublished
				Miller and Reader unpublished
	Complete set			Zhang et al. (2001)
Ae. speltoides	All seven (3S and 6S are monosomics)	Seven lines	RFLP	Friebe et al. (2000)
Ae. tauschii	3 lines (hexaploids)		C-band	Dhaliwal et al. (1990)
Ae. tauschii	Complete monosomic set[a] in durum cv. PBW114			
Ae. umbellulata	Complete set	Nine lines	C-band, GISH	Kimber (1967)
	(not all are pure)			Friebe et al. (1995b)
Ae. uniaristata	Five lines, 2N not complete (6N missing)		RFLP, RAPD, SSR	Miller et al. (1997); Iqbal et al. (2000)
Ae. ventricosa	Seven lines			Dosba et al. (1978); Dosba (1982)

[a]Some added chromosomes are intertranslocated

A substitution line is produced from an addition line by crossing the latter with a selected wheat line that is either monosomic, monotelosomic, or nullisomic for the chromosome to be substituted for. The selection of desired genotypes from the F_2 progeny can be helped by cytological and marker analyses. Production of substitution lines in wheat is illustrated in Fig. 1.53. In most combinations, the alien chromosome compensates for the missing wheat homoeolog, and plant vigor and fertility are not harmed. Yet, alien undesirable chromatin still exists in the substitution lines, rendering them unsuitable for direct agronomic use.

1.6 Role in Crop Improvement

1.6.1 Traits Modified by Domestication

Domesticated cereal crops differ from their wild relatives in several traits, some of them apparently consciously selected by humans. The most important Triticeae traits modified during domestication are the free-threshing state and brittle rachis. Additional modifications taking place during domestication and subsequent breeding concern seed size, kernel row

Table 1.7 Overview on major stocks of wheat–*Aegilops* chromosome substitution lines

Aegilops species	Chromosome disomic substitutions	Telosome substitutions	Method of identification	Reference
Ae. comosa	2M(2A), 2M(2B), 2M(D)			Riley et al. (1966)
Ae. geniculata	5M(5D)			Friebe et al. (1999); Dhaliwal and Harjit-Singh (2002)
Ae. longissima	Complete set		C-band	Friebe et al. (1993)
	5S(5A), 5S(5B), 5S(5D)		Isozymes	Millet et al. (1988)
	A, C, D			Netzle and Zeller (1984)
Ae. markgrafii	5C(5A), 5C(5D)			Muramatsu (1973); Friebe et al. (1992)
Ae. peregrina	Unidentified lines			Spetsov et al. (1997)
	G (2A), G (2B), E (5B)			Shepherd and Islam (1988)
Ae. searsii	Complete set	31 lines	C-band	Friebe et al. (1995a)
Ae. sharonensis	4S(4A), 4S(4B), 4S(4D)			Miller (1983)
Ae. speltoides	Complete set		RFLP	Friebe et al. (2000)
Ae. tauschii	Complete set in durum cv. Langdon			Joppa and Williams (1988)
Ae. umbellulata	1U, 2U, 5U, 7U for their wheat homoeologues			Riley et al. (1971, 1973);
	2 addition unknown chromosomes			Chapman and Riley (1970); Shepherd and Islam (1988); Makino (1976, 1981)
Ae. uniaristata	3N(3A), 3N(3B), 3N(3D)			Miller et al. (1995)

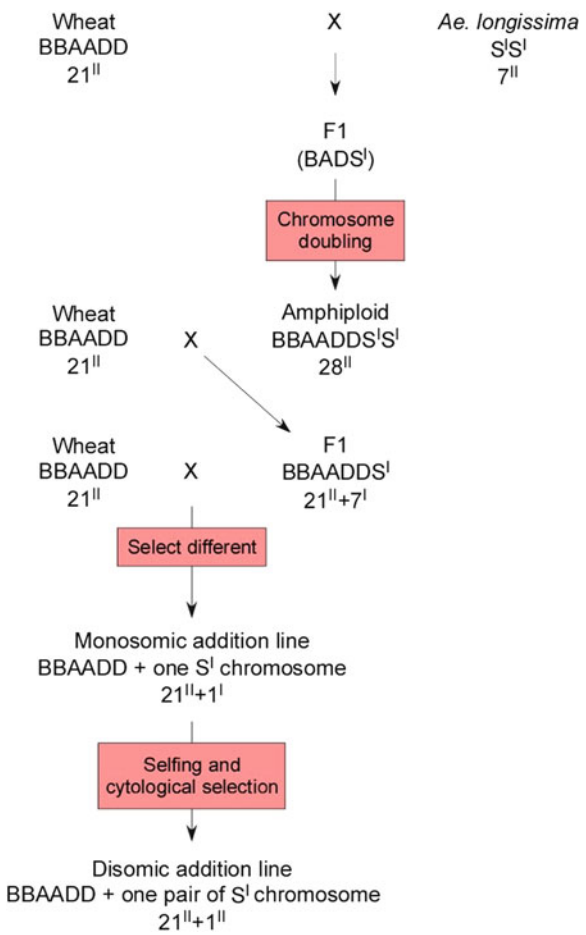

Fig. 1.52 Schematic overview on production of *Aegilops longissima* disomic addition lines

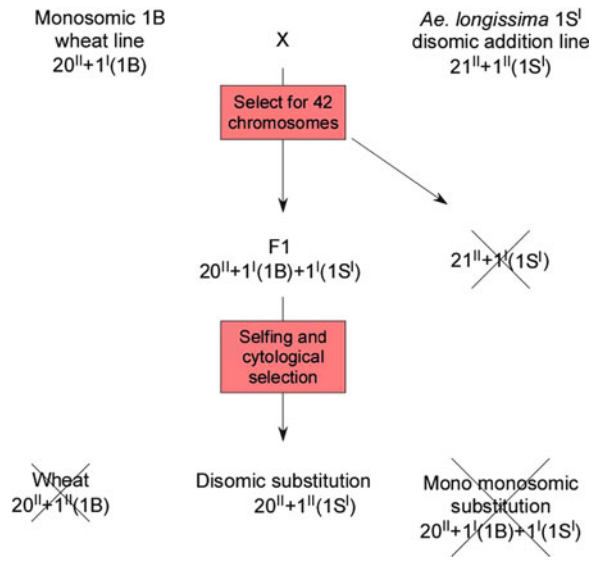

Fig. 1.53 Schematic overview on production of $1S^1$ (1B) *Aegilops longissima* disomic substitution lines

type, plant height, grain hardness, tillering, seed dormancy, photoperiod, vernalization, and heading date. In addition, the spread of the domesticated cereals out of the Fertile Crescent required the adaptation to new environments, supported by newly arisen favorable alleles at critical genetic loci. Studies on traits modified by domestication also provide data for phylogenetic relationships. Recent studies mainly focus on few crop model species such as rice, maize,

and barley. Synteny between grass species, however, will allow collecting useful results for the much more complex wheat genome.

1.6.2 Case Study: Control of Flowering Time

The control of flowering time is central to reproductive success and has a major impact on grain yield in Triticeae. Wild progenitors of domesticated cereals are well adapted to the prevailing environmental conditions in the Fertile Crescent. These species use the rainfall to establish their vegetative structures before winter, and vernalization will delay flowering until the winter has passed. In spring, soil moisture can be used for grain filling, and an early flowering in response to long days (LDs) allows the plant to complete its growing cycle ahead of the hot summer (Cockram et al. 2007).

The first cereals domesticated in the Fertile Crescent most probably had the photoperiodic (response to day length) and vernalization (response to winter temperature) phenotypes of their progenitors. During the domestication process and during the spread of agriculture out of the Fertile Crescent, novel adaptive traits suited for the new environments were selected. One key event was the selection of spring types that can be also sown after winter. These spring types lack vernalization requirement and show different response to LDs. Reduced photoperiod response is important in Europe and North America, where growing seasons are long (Turner et al. 2005).

Wheat, *Aegilops*, barley, and *Arabidopsis* show similar flowering responses to photoperiod and vernalization, in contrast to rice and soybean, which flower in response to short days and show no response to vernalization. We present an overview on photoperiod and vernalization research in the Triticeae. Only few studies on flowering time-related topics have been published in *Aegilops*, mostly focusing on *Ae. tauschii* and *Ae. cylindrica* (Shindo and Sasakuma 2001; Fandrich et al. 2008).

1.6.2.1 Photoperiod

The major loci affecting the photoperiod response (*Ppd* genes) are in a colinear position on the short arm of the group 2 chromosomes in wheat and barley. In barley, dominant *Ppd-H1* alleles confer early flowering under LDs but have no effect under short days (SD). Plants carrying the mutated, recessive *ppd-H1* allele are late-flowering. The *Ppd-H1* is a pseudoresponse regulator (PRR) most similar to *PRR7* in *Arabidopsis* and was positionally cloned in barley by Turner et al. (2005). These authors provided evidence that a single point mutation within the conserved CCT domain of PRR results in an amino acid change, leading to insensitivity under LDs. In wheat, the allelic series of *Ppd* loci has decreasing potency from *Ppd-D1* to *Ppd-B1* to *Ppd* to *A1* (Worland 1996). The *Ppd-H2* gene has been mapped in barley on chromosome 1H (Laurie et al. 1995). The winter allele delays flowering under short days. No equivalent gene has been identified in wheat yet.

Further, major photoperiod-related genes/gene families appear to be conserved between barley and *Arabidopsis*, involving the *GIGANTEA* (*GI*), *CONSTANS* (*CO*), and *FLOWERING LOCUS T* (*FT*) genes in *Arabidopsis* and their orthologs in barley *HvGI*, *HvCO*, and *HvFT* (Griffiths et al. 2003; Dunford et al. 2005; Cockram et al. 2007; Faure et al. 2007). None of the grass quantitative trait loci (QTLs) associated with flowering time cosegregate with orthologous *Arabidopsis* "flowering" genes, that is, different major determinants of photoperiod most probably have been selected in the Triticeae (Börner et al. 1998; reviewed in Griffiths et al. 2003).

At the moment, *Ppd-H1* is the only locus conferring natural genetic variation in photoperiod response in barley. Jones et al. (2008) studied molecular diversity at the *Ppd-H1* locus in a comprehensive barley collection. The mutated nonresponsive, late-flowering allele *ppd-H1* was present in wild barley from Iran, suggesting that the nonresponsive mutation originated in wild barley in the eastern part of the Fertile Crescent. Evidence is still missing on the botanical status of these wild accessions. These wild barleys could have received introgressions from cultivated barleys.

Recently, Matsuoka et al. (2008) studied the timing of flowering in 200 lines of *Ae. tauschii*. Such studies on the ecological and geographical framework are needed for our understanding of wild wheats and their hidden potential for wheat improvement.

1.6.2.2 Vernalization

In the Triticeae, two major genes, with epistatic interactions, control the vernalization (*VRN*) pathway: *VRN1* and *VRN2* (Yan et al. 2003, 2004a, b; von Zitzewitz et al. 2005; Dubcovsky et al. 2006). The *VRN1* gene on chromosome 5 in wheat and barley is similar to the *Arabidopsis* MADS-box transcription factor *Apetala 1 (AP1)*, which initiates the transition of the apical meristem from the vegetative to the reproductive state (Yan et al. 2003). Most probably the same gene should map in a paralogous location also in species of the genus *Aegilops*. Mutations in regulatory regions (promoter and the first intron of this gene) are associated with a dominant spring growth habit (*Vrn1*) (Yan et al. 2003, 2004a; Fu et al. 2005; von Zitzewitz et al. 2005). The second vernalization gene, *VRN2* (*ZCCT* in barley), is a zinc finger CCT domain transcription factor that blocks the photoperiod pathway through direct or indirect downregulation of *VRN1* and *VRN3* (Fu et al. 2005; Karsai et al. 2005; Koti et al. 2006). *VRN2* transcription is repressed by vernalization and by short days. Loss-of-function mutations (*vrn2a*), or complete gene deletion (*vrn2b*), are associated with a recessive spring habit that does not require vernalization to flower (Yan et al. 2004b; Dubcovsky et al. 2005).

It was recently shown that a third vernalization locus in barley is located on chromosome 7H, *VRN-H3*, colinear with *VRN-B3* in bread wheat and encoding an ortholog of the *Arabidopsis* floral pathway integrator, *FT1*, and colinear with *OsFT* of rice (Yan et al. 2006; Faure et al. 2007). The dominant, early-flowering wheat *Vrn3* allele is associated with the insertion of a retroelement in the promoter, while in barley, a mutation in the first intron is associated with the early allele (Yan et al. 2006). This study provides evidence that differences in flowering time are associated with *FT1* allelic variation. The link between photoperiod and vernalization is evident, even at the molecular level, if one considers that *VRN2* is expressed only when photoperiod-responsive plants are grown under long-day photoperiod (Trevaskis et al. 2006). There is probably a second *VRN1* repressor, and a candidate may be *VRT2* (*vegetative to reproductive transition-1*), a MADS-box gene (Danyluk et al. 2003; Kane et al. 2005; Szücs et al. 2006, 2007).

No molecular studies on vernalization have been carried out in wheat relatives, yet. However, Kilian et al. (2007a, b) included one *VRN1* gene fragment in their phylogenetic (multilocus) analysis of einkorn wild and domesticated lines.

1.7 Role in Crop Improvement Through Traditional and Advanced Tools

Of all genera of the Triticeae tribe, *Aegilops* is the most closely related to *Triticum*. Moreover, a whole *Ae. tauschii* genome is a part of the bread wheat genome. Cultivated wheat suffers from inadequate genetic diversity within its primary gene pool because modern plant breeding has strengthened the tendency to reduce variability (Hammer and Laghetti 2005; Fu and Somers 2009). This situation is even more critical when undefeated resistance genes are necessary to control a newly emerged pest that threatens global wheat production (this was the case of leaf rust resistance from *Ae. umbellulata* (Sears 1956) and of resistance to the stem rust highly virulent race Ug99 (Singh et al. 2006)). *Aegilops*, on the other hand, exhibits wide diversity for various desirable attributes and, as such, serves as invaluable genetic reservoir for wheat improvement. It is, therefore, unsurprising that many attempts have been made to assess the breeding potential of different *Aegilops* species and to exploit them to improve genetically wheat production. In the following paragraphs, we evaluate *Aegilops* species as members of the gene pool available for wheat improvement.

Hybrids between hexaploid wheat and, for example, *Ae. tauschii* have seven bivalents at the meiotic first metaphase. This indicates that each chromosome of *Ae. tauschii* is homologous to its respective chromosome of the wheat D-genome and readily pair with it at meiosis. Indeed, *Ae. tauschii* is considered by Harlan (1992) as a member of the primary gene pool of wheat. Gene transfer from D-genome of *Aegilops* species is straightforward and requires standard breeding methods as described below.

Each one of the three wheat genomes, A-, B-, and D-, is homoeologous to the diploid genomes of *Aegilops* other than *Ae. tauschii*, namely genomes C-, M-, N-, S-, U-, and T-. The chromosomes of these genomes will only rarely pair and recombine with wheat chromosomes at meiosis. According to Harlan (1992), *Aegilops* species with genomes other than D are

included in the secondary gene pool of wheat, and gene transfer from these species into wheat requires cytogenetic manipulations to enhance the recombination between alien and wheat homoeologous chromosomes.

The polyploid nature of wheat allows it to tolerate addition of *Aegilops* chromosomes or substitution (see above) of its native chromosomes for their *Aegilops* homoeologs without a major effect on plant vigor or fertility. By the use of such addition and substitution lines, the identification of desirable genes and their transfer to wheat cultivars are facilitated. Different genetic wheat stocks with a duplicated chromosome or deficient for a chromosome have been prepared and used in the process of gene transfer.

Genetic resources of *Aegilops* in genebanks have been systematically evaluated for useful traits (e.g., Hammer 1985; Valkoun et al. 1985). *Aegilops* species have been used as forage plants (van Slageren 1994) and introduced into other countries. Taxa of other genera have been used to create hybrid species. (I) × *Aegilotriticum* Fourn. (hybrid between *Aegilops* species and *Triticum* species) is a hybrid genus with potential value for breeding purposes; (II) *Aegilops* also participated in the important hybrid genus × *Triticosecale* Wittmack. ('Triticale', hybrid between *Triticum* and *Secale*) and (III) in the prospective × *Tritordeum* Asch. et Graebn. (hybrid between *Triticum* and *Hordeum*).

1.7.1 Methods of Gene Transfer

The ultimate goal in gene transfer is the introgression of a small alien chromosome segment carrying the desired gene without its flanking genes, which may be deleterious for wheat performance. This goal can be achieved when a gene is transferred from the D-genome of an *Aegilops* species, as detailed below. However, in the case of gene introgression from *Aegilops* species with genomes homoeologous to the wheat genomes, the low level of homoeologous pairing results in transfer of a chromosome segment, which is long enough to carry undesired alien genes in addition to the target gene (Feldman 1988), a phenomenon called genetic drag. Different cytogenetic manipulations were proposed to reduce the amount of the alien chromatin.

1.7.1.1 Gene Transfer from *Aegilops* Species with D-Genome

When the desired gene resides in the D-genome of *Ae. tauschii*, or of another polyploid *Aegilops* from section *Vertebrata* or of *Ae. cylindrica*, homologous pairing is expected between the donor and the recipient bread wheat chromosome (of the D-genome) and no pairing induction is required. Yet, as a result of ploidy difference and nonhomology between the A and B wheat genomes and the *Aegilops* genomes other than D, such wheat–*Aegilops* hybrids have a high level of sterility.

One method that facilitates gene transfer from *Ae. tauschii* to bread wheat and also overcomes the difficulty due to ploidy difference includes the production of a synthetic allopolyploid wheat line prior to gene transfer. In this case, tetraploid wheat with BA-genome is hybridized with *Ae. tauschii*, and the chromosomes of the F_1 hybrid are doubled using colchicine treatment. The product is a fertile synthetic hexaploid BAD genotype fully homologous to bread wheat. Hexaploid wheat homologous chromosomes will readily recombine in the hybrid. Such synthetic lines serve as a gene pool derived from *Ae. tauschii* that is ready for screening for any desired trait and allow for an easy transfer of the responsible gene. Indeed, this method is suitable for gene transfer from *Ae. tauschii* to wheat (Table 1.8) and has been adopted by a larger number of wheat breeders (e.g., synthetic lines of CIMMYT; Trethowan et al. 2003).

When the gene donor is a polyploid species (e.g., *Ae. ventricosa*; genome-DN), a tetraploid wheat genotype (genome-BBA^uA^u) can be used to bridge between the donor and the recipient hexaploid wheat (Table 1.8). The initial hybrid with the BBA^uA^uDDNN-genome, when hybridized to bread wheat (BBA^uA^uDD), generates fertile derivatives where only chromosomes of the N-genome remain unpaired. In such hybrids, translocations may occur mainly between chromosomes of the D-genomes (Delibes et al. 1987). In addition, desired alien translocations can be obtained also between N chromosomes and other wheat chromosomes (Jahier et al. 2001). Attempts to directly hybridize *Ae. ventricosa* and bread wheat were unsuccessful, and the only hybrid obtained was male sterile (Doussinault et al. 1983).

Table 1.8 Pest and disease resistance genes transferred from *Aegilops tauschii* to wheat

Resistance to pests and diseases	Gene(s) provided	Method of transfer: Production of synthetic 6x line with 4x line or direct cross with 6x wheat	Reference
Cereal cyst nematode	*Cre3*	4x	Eastwood et al. (1993)
Greenbug	*Gb3, Gb4, Gb7, Gba*,[a] *Gbb*,[a] *Gbc*,[a] *Gbd*,[a] *Gbx*,[a] *Gbz*[a]	4x cvs.	Martin et al. (1982); Hollenhorst and Joppa (1983); Flinn et al. (2001); Weng and Lazar (2002); Smith and Starkey (2003); Zhu et al. (2004); Weng et al. (2005); Zhu et al. (2005)
Hessian fly	*H26*	6x cv. Karl	Cox and Hatchett (1994)
Leaf rust	*Lr21*	4x Tetra Canthatch[b]	Kerber and Dyck (1969)
	Lr22a	4x Tetra Canthatch[b]	Dyck and Kerber (1970)
	Lr32	4x Tetra Canthatch[b]	Kerber (1987)
	Lr39	6x cv. Witchita	Raupp et al. (2001)
	Lr41	6x cv. TAM107	Cox et al. (1994)
	Lr42	6x cv. TAM107	Cox et al. (1994)
	Lr43	6x cv. TAM107	Cox et al. (1994)
Powdery mildew	*Pm2*	4x durum cv.	Lutz et al. (1994)
	Pm19	4x durum cv.	Lutz et al. (1995)
Root knot nematode	*Rkn*	4x durum	McIntosh et al. (2008)
Russian wheat aphid	*Dn3*	4x *T. turgidum*	Nkongolo et al. (1991)
Septoria nodorum	*Snb3*	4x	McIntosh et al. (2008)
Septoria tritici	*Stb5*	4x *T. dicoccoides*	Arraiano et al. (2001)
Stripe rust	*Yr28*	4x durum cv. Altar84	McIntosh (1988); Singh et al. (2000)
Stem rust	*Sr33*	4x Tetra Canthatch[b]	Kerber and Dyck (1979)
	Sr45		McIntosh (1981)

[a]Allelic or linked to *Gb3*
[b]Extracted tetraploid

1.7.1.2 Gene Transfer from Genomes of Different *Aegilops* Species Other than D

A controlled scheme for evaluation and exploitation of *Aegilops* for wheat improvement was proposed by Feldman (1983) and reviewed by Qi et al. (2007). This scheme includes three steps:

1. Addition of the whole alien genome by production of a wheat–*Aegilops* amphiploid (see above).
2. Production of a pair of alien chromosome addition and substitution lines (see above).
3. Production of an *Aegilops*–wheat recombinant chromosome with the desired gene.

This procedure is also known as chromosome engineering (Sears 1981). Methods to carry out this step are described below (Sect. 1.7.1.2.1–1.7.1.2.3). The procedure requires knowledge of the size and location of the transferred segment. If the introgressed segment is too long, or a genetic drag is manifested, the genetic material cannot be used directly in wheat breeding programs and requires prebreeding to delete undesired alien chromatin. A method to reduce the size of an alien segment was proposed by Sears (1981, 1983) and requires identification of the transferred segments and hybridization between transfers with recombination points at either sides of the target gene. Pairing of two translocated chromosomes, one recombined distally to the gene and the other proximally to it, will result in an interstitial alien segment. Further reduction in the size of the alien segment may be obtained by additional induction of homoeologous pairing (see Sect. 1.7.1.2.1) (Sears 1981, 1983). Ceoloni et al. (2005) combined these methods and used cytogenetic and molecular tools in their attempts to reduce the size of *Agropyron elongatum* (Host) P. Beauv. chromosomal segment transferred to durum wheat.

While stepping forward, a smaller proportion of the alien genetic material is included in the progenies. Evaluation is carried out at every step in order to assess the potential of alien gene introgression into the wheat genetic background. This scheme is, however, time- and labor-consuming. Therefore, attempts invested in the production of translocations by homoeologous pairing would better pay back if the translocations are evaluated for additional desirable traits.

Indeed in a number of cases, the initial material that was made available was used by other scientists to exploit the initiative. An example is the series of 70 *Ae. ventricosa* translocation lines prepared by M.A. Peña in Cuenca, Spain (Delibes et al. 1993), and used by different cytologists to select seven different gene transfers (Table 1.9). Other laboratories started their gene transfer from alien addition (Ceoloni et al. 1988) or substitution lines (Aghaee-Sarbarzeh et al. 2002; Kuraparthy et al. 2007a) that were prepared by others. In several cases, spontaneous rather than homoeologous pairing was exploited to obtain translocations (Table 1.9).

Induction of Homoeologous Pairing

The preferred method for gene transfer is chromosome pairing induction between the donor and the recipient chromosomes. Since under induction, pairing may occur among all homoeologs (including chromosomes of the wheat genomes). This step should be followed by a series of backcrosses to the wheat parent in order to recover the original wheat genomes and accompanied with selection for the desired *Aegilops* segment bearing the target trait.

The most popular method to enhance pairing between homoeologous chromosomes is the use of the recessive mutant of the homoeologous pairing suppressor *Ph1*, located on the long arm of chromosome 5B (Okamoto 1957; Riley and Chapman 1958). The mutant *ph1b* corresponds to a deficiency of the *Ph1* locus of common wheat (Sears 1977) and the mutant *ph1c* of *Ph1* in durum wheat (Giorgi 1983). Another homoeologous pairing suppressor, termed *Ph2*, with much weaker expression, has been located on 3DS of wheat (Mello-Sampayo and Canas 1973), but it was rarely used to enhance pairing, except by Ceoloni and Donini (1993). In the absence of *Ph1*, the rate of chromosome pairing in hybrids between wheat and any *Aegilops* species with homoeologous genome is greatly enhanced (Riley 1966).

A second method to promote homoeologous pairing is based on genes of *Ae. speltoides*. In this species, most lines enhance homoeologous pairing with wheat chromosomes by suppressing the action of the *Ph1* gene due to the presence of the *Ph1* suppressor genes *Su1-Ph1* and *Su2-Ph1* (Kimber and Athwal 1972; Dvorak et al. 2006). These lines (termed high pairing type) can be directly used to pollinate a desired wheat cultivar for gene transfer and the hybrid backcrossed to the recurrent cultivar. Early generations (F_1 and BC_1) are highly sterile but will produce some seeds when pollinated by the wheat cultivar parent (Feldman and Millet unpublished). Moreover, high pairing *Ae. speltoides* can be used as additional player in a cross of wheat with another alien species to promote pairing and recombination between chromosomes of the two alien species, and wheat homoeologs, as in Riley et al. (1968), for *Ae. comosa* (genome-M) and wheat. In addition, certain lines of *Ae. longissima* can partially suppress the *Ph1* gene effect, thereby inducing homoeologous pairing (Mello-Sampayo 1971). Genotypes were found also in *Ae. peregrina* and *Ae. kotschyi* that promote homoeologous pairing (Fernandez-Calvin and Orellana 1991).

Few years ago, *Ph* suppressor genes (*Su-Ph1*, are termed also Ph^I) were transferred from *Ae. speltoides* to a bread wheat line (Chen et al. 1994). This line is an efficient inducer of homoeologous pairing since Ph^I genes are dominant and epistatic to the wheat *Ph*; thus the Ph^I gene acts in the hybrids also as heterozygote. This method has been successfully exercised to transfer leaf rust and stripe rust resistance genes from *Ae. umbellulata* (Chhuneja et al. 2008a) and from *Ae. triuncialis* and *Ae. geniculata* to bread wheat (Aghaee-Sarbarzeh et al. 2002).

Recovering the original wheat genome is usually obtained through a series of backcrosses of the wheat–*Aegilops* hybrid to a wheat cultivar as a recurrent parent. Since alien and wheat chromosomes do not pair in the presence of the *Ph* wild type allele, alien chromosomes and chromosome segments will be eliminated in few backcross generations. Maintenance of the desired gene requires an easy identification of the gene product (resistance, protein, etc.) or a marker adjacent to the gene to be selected for.

Induction of Alien Translocations by Ionizing Irradiation

One drawback associated with pairing induction between the donor and the recipient chromosomes is the reduced number of chiasmata (1–2) per pair of chromosomes and the predominant occurrence of chiasmata in the terminal and subterminal chromosome

Table 1.9 Methods used in different disease- and pest-resistance gene transfers from *Aegilops* species other than *Ae. tauschii* to wheat

Donor species	Gene(s) transferred[a]	Method of gene transfer			Reference
		Induction of translocation	Alien addition/ substitution	Bridging species	
Ae. comosa	*Sr34*[b]; *Yr8*[b]	*Ph*^{I} in *Ae. speltoides*	Resistant monosomic addition 2M	*T. aestivum*	Riley et al. (1968); McIntosh et al. (1982)
Ae. geniculata	*Lr57*; *Yr40*	*Ph*^{I} in Chinese Spring	Disomic substitution 5M^{g}(5D)	*T. aestivum*	Aghaee-Sarbarzeh et al. (2002); Dhaliwal and Harjit-Singh (2002); Chhuneja et al. (2007); Kuraparthy et al. (2007a)
Ae. longissima	*Pm13*[c]	*ph1b* induced homoeologous pairing	Ditelosomic 3S^{l}S addition line		Ceoloni et al. (1988); Donini et al. (1995); Cenci et al. (1999)
Ae. peregrina	*Rkn-mn1*				Yu et al. (1990); Barloy et al. (2000)
Ae. sharonensis	*Lr56, Yr38*	Spontaneuos	None		Marais et al. (2006)
Ae. speltoides	*Lr28*[c]	*Ph*^{I} in *Ae. speltoides*			McIntosh et al. (1982)
	Lr35[b] *Sr39*[b]		None	*T. monococcum*	Kerber and Dyck (1990)
	Lr36[b]; *Lr51*				Dvorak (1977); Dvorak and Knott (1990); Naik et al. (1998); Seyfarth et al. (1999); Helguera et al. (2005)
	Pm12/Pm32				Dvorak (1977); Miller et al. (1987); Dvorak and Knott (1990); Jia (1996); Hsam et al. (2003); Song et al. (2007)
	Lr47	Initial large translocation obtained by fast neutron irradiation	none		Wells et al. (1982)
	Gb5[b]	was reduced using *ph1b*			Lukaszewski (1995); Dubcovsky et al. (1998)
	Sr32[b]	*ph1b* induced homoeologous pairing			McIntosh (1991); Friebe et al. (1996b)
Ae. triuncialis	*Lrtr; Lr58*	*Ph*^{I} in Chinese Spring	Disomic substitution 5Ut(5A)	*T. aestivum*	Aghaee-Sarbarzeh et al. (2002); Kuraparthy et al. (2007b)
	Cre7; H30	Spontaneous	None	*T. turgidum*	Romero et al. (1998); Martin-Sanchez et al. (2003); Montes et al. (2008)
Ae. umbellulata	*Lr9*[c]	X-ray irradiation	Resistant monosomic	*T. turgidum*	Sears (1956)
			addition, then monosomic	*T. turgidum*	
			isochromosome addition	*T. turgidum*	
	Yr , Lr	*Ph*^{I} in Chinese Spring	none	*T. durum*	Chhuneja et al. (2007, 2008a)
	Lr, Sr	Spontaneous	none	*T. durum*	Özgen et al. (2004)
Ae. ventricosa	*Cre5*[c]; *Pm*[c]	Spontaneous	none	*T. turgidum*	Delibes et al. (1987); Jahier et al. (1996); Jahier et al. (2001)
	Pch1[c]	Spontaneous	None	*T. turgidum*	Garcia-Olmedo et al. (1984); Jahier et al. (1978); Doussinault et al. (1983)
Ae. ventricosa	*Lr37*,[c] *Sr38*,[c] *Yr17*[c]; *H27*				Bariana and Mcintosh (1993, 1994); Delibes et al. (1997); Seah et al. (2001); Helguera et al. (2003); Blaszczyk et al. (2004)
	Cre2; Cre6				Delibes et al. (1993, 1997); Ogbonnaya et al. (2001)

[a]Resistance gene symbols: *Yr* Stripe rust, *Sr* Stem rust, *Lr* Leaf rust, *Pch* Eyespot, *Pm* Powdery mildew, *Gb* Green bug, *Cre* Cereal cyst nematode, *Rkn* Root knot nematode, *H* Hessian fly

[b]Not used in agriculture (no information for unmarked genes)

[c]Used in agriculture

regions. Genes located in the more proximal regions of the chromosomes will not be included in the translocated segment or will be transferred together with a large alien chromosome segment. To overcome this drawback, X-ray or gamma ionizing irradiation treatments may be used to break chromosomes with the target to obtain alien chromatin transfer between the donor and the recipient genomes (Sears and Gustafson 1993). Alien and wheat chromosome segments reunite spontaneously to form alien segments embedded within the wheat chromosomes. This method was used by Sears (1956) to transfer leaf rust resistance from *Ae. umbellulata* (U-genome) to wheat.

Irradiation results in random chromatin translocation between chromosomes. Irradiation of a genotype monosomic for both the *Aegilops* chromosome and one of its wheat homoeologs increases the probability for pollen selection against noncompensating translocations (Sears and Gustafson 1993). Irradiation of alien wheat hybrids in the absence of *Ph1* may increase the number of compensating translocations (Sears and Gustafson 1993).

A method to reduce a whole arm translocation into small segments was presented by Chen et al. (2008) in support of wheat – *Dasypyrum villosum* (L.) Candargy, syn. *Haynaldia villosa* (L.) Schur introgressions. A 6VS/6AL translocation line was gamma-irradiated and immediately pollinated by wheat, giving rise to an impressive number of small terminal and interstitial alien translocations.

Gametocidal Induction of Alien Translocations

Another method to induce transfer of small alien segments to wheat is based on the capacity of gametocidal genes (Sect. 1.7.2) to break chromosomes. This method proved effective in producing wheat–rye translocations but has never been used to induce wheat–*Aegilops* translocations. Chromosome 3C of *Ae. triuncialis* was introduced into wheat along with the chromosome 1R of rye (Masoudi-Nejad et al. 2002). Many small segments of 1R were translocated to different wheat chromosomes as a result of chromosome breakage caused by the gametocidal gene located on 3C.

1.7.2 Obstacles for Successful Gene Transfer from Aegilops Species to Wheat

Exploitation of alien species for wheat improvement is hampered by several biological and genetic obstacles, such as reduced crossability between parental lines (Claesson et al. 1990; Koba and Shimada 1993) and low seed setting in the hybrid (Claesson et al. 1990). Shriveled F_1 endosperms would require the usage of embryo rescue techniques (Miller et al. 1987).

Wheat–*Aegilops* hybrids may suffer from abnormal morphology, such as lethal or semilethal necrosis, as was found by Nishikawa (1962) in hybrids between certain tetraploid wheat lines and synthetic hexaploid wheat.

A different obstacle to gene transfer from *Aegilops* to wheat is represented by gametocidal genes (*Gc*) (Tsujimoto 2005), a group of selfish genes that induce chromosome breakage in gametes not having them. This mechanism prevents the transmission of these gametes and ensures that only gametes containing the *Gc* genes are transmitted. Consequently, the *Gc*-carrying *Aegilops* chromosome is included in the genome of every offspring deriving from self-pollinating or backcrossing of the wheat–*Aegilops* hybrid. *Gc* genes were detected on chromosome 3C of certain lines of *Ae. markgrafii*, and *Ae. triuncialis* (Endo and Tsunewaki 1975), on chromosome 2C of *Ae cylindrica* (Endo 1979), on chromosome 4M of *Ae. geniculata* (Kynast et al. 2000), on chromosomes $2S^{l}$ and $4S^{l}$ of *Ae. longissima*, on $2S^{sh}$ and $4S^{sh}$ of *Ae. sharonensis* (Maan 1975; Endo 1985), and on 2S and 6S of *Ae. speltoides* (Tsujimoto and Tsunewaki 1984, 1988; Kota and Dvorak 1988). Selection for a genotype lacking *Gc* is proposed to ensure successful gene transfer from any of the named species. An "antigametocidal" mutant wheat line that suppresses the action of *Ae. sharonensis Gc2* gene was generated by Friebe et al. (2003). This line has a translocated 4BL·4BS-$4S^{sh}$S chromosome (4B with a terminal $4S^{sh}$S segment) carrying a *Gc2* gene that was mutated by EMS and is designated $Gc2^{mut}$. Normal transmission of mutant and wild type alleles was observed in heterozygotes $Gc2/Gc2^{mut}$ rather than preferential transmission of *Gc2*. This mutant, which allows for exclusion of the undesired $4S^{sh}$ chromosome from

wheat lines having introgression from *Ae. sharonensis*, was used by Millet et al. (2007) in their attempt to introgress leaf rust and stripe rust resistance from *Ae. sharonensis* to wheat.

1.7.3 Exploitation of the Aegilops Gene Pool for Wheat Improvement

Conspicuous diversity in a wide variety of attributes is found in different *Aegilops* species (Table 1.10). These attributes include resistance to biotic stresses (different diseases and pests; Tables 1.8–1.10), tolerance to abiotic stresses such as drought, extreme temperature, soil mineral toxicity, and deficiency, and improvement of nutritional value (protein content) and product quality (protein and starch attributes; Table 1.10). Nevertheless, as evident from Table 1.8 (D-genome introgressions) and Table 1.9 (introgressions from other genomes), the only genes that have been transferred from *Aegilops* to wheat are those controlling resistance to biotic stresses. This may be explained as the outcome of three main reasons:

1. The urgent need to combat diseases and other pests. Past and current resistances may be defeated by the pathogen and new resources are needed to save the crop.
2. The predominant simple inheritance (usually monogenic) of the resistance, when it is compared to more complex traits such as drought, heat, or salt tolerance, or protein composition.
3. The ease of using the resistance/susceptibility symptoms as a direct selection marker (there is no need to develop and use molecular or other markers).

Moreover, even when dealing with pest resistance genes, only a small portion of the transfers were actually used in agriculture (McIntosh et al. 1995; Friebe et al. 1996b), perhaps because of the genetic drag resulting from a transfer of the long segments of *Aegilops* chromosomes, which negatively affect the crop (Ortelli et al. 1996).

To conclude, the pioneering work of Sears (1956) is still the method of choice used to transfer genes from *Aegilops* to wheat. The improvement of chromosome engineering methodologies and the public objection to genetically modified organisms (GMO) indicate that chromosome engineering may remain the preferred method in the near future. Modern molecular tools now facilitate the transfer of chromosomes and allow selecting for tiny translocations, which have an expected minimal deleterious effect on wheat performance. The underutilized gene pool of *Aegilops* is the best genetic resource for wheat improvement. A greater demand for useful variation in response to diverse and changing environment and in product quality should further promote the exploitation of the *Aegilops* gene pool.

1.7.4 More Uses of Aegilops Species

1.7.4.1 *Aegilops* Cytoplasms as Male Sterility Inducer in Wheat

Already Kihara (1951) showed that nucleus substitution in *Ae. markgrafii* cytoplasm by bread wheat produces male sterile alloplasmic wheat lines. This nucleus substitution was carried out by pollinating an *Ae. markgrafii* genotype by a bread wheat cultivar and backcrossing by the wheat parent as a male. Usually, 4 to 7 backcrosses were sufficient to eliminate the *Aegilops* chromosomes (Panayotov 1980), and the process can be accelerated by cytological selection of the offspring. A long-term study showed that between 5 and 40 backcross generations, these alloplasmic wheat lines remained unchanged, that is, they are male sterile and female fertile (Tsunewaki 1993). In a review on genome–plasmon interactions, Tsunewaki (1993) clarified that the combinations of 12 wheat varieties as nucleus donors with different *Aegilops* cytoplasms resulted in different levels of male sterility. Similar results were obtained by Panayotov (1980) using two bread wheat cultivars with 17 *Aegilops* cytoplasms. Consequently, cytoplasms from *Ae. biuncialis*, *Ae. markgrafii*, *Ae. columnaris*, *Ae. comosa*, *Ae. geniculata*, *Ae. neglecta*, *Ae. speltoides*, and *Ae. triuncialis* were shown to completely male-sterilize at least one cultivar.

Fertility restoration genes (*Rf*) against sterilizing cytoplasms were found in wheat (Tsunewaki 1982) but are naturally present in the genome of the cytoplasm donor species. Moreover, an *Rf* gene against the sterilizing cytoplasm of *T. timopheevii* was transferred from *Ae. umbellulata* to wheat (Yinhuai et al. 1991).

A system was proposed, based on alloplasmic female lines and a restorer line, to produce hybrid

Table 1.10 Exploitable traits from *Aegilops* species (excluding traits that have been already transferred – see Tables 1.8 and 1.9)

	Donor species	Gene(s) studied and chromosome positions, the genome of bread wheat	Reference
(a) Resistance to pests and diseases			
Barley yellow dwarf	*Ae. biuncialis, markgrafii, neglecta, triuncialis*		Makkouk et al. (1994)
Cereal aphid	*Ae. searsii, columnaris, neglecta, umbellulata*		Holubec and Havlíčková (1994)
Cereal cyst nematode (*Heterodera avenae*)	*Ae. peregrina*	*CreX; CreY*	Jahier et al. (1998)
Eyespot (*Tapesia yallundae*)	*Ae. ventricosa*		Chao et al. (1989); Huguet-Robert et al. (2001); Groenewald et al. (2003)
Fusarium head blight	*Ae. speltoides*		Fedak et al. (2004)
Greenbug (*Schizaphis graminum*)	*Ae. speltoides*	*Gb*	Friebe et al. 1991; Fedak et al. (2004)
Hessian fly (Mayetiola destructor)	*Ae. geniculata, longissima, speltoides, tauschii*		El Bouhssini et al. (2008)
	Ae. tauschii	*H13* (6D); *H22* (1DS); *H23* (6D); *H24*	Gill et al. (1987); Ma et al. (1993); Raupp et al. (1993); Liu et al. (2005); Wang et al. (2006)
Leaf rust (*Puccinia recondita*)	*Ae. kotschyi*	*Lr54*	Marais et al. (2005)
	Ae. markgrafii	*Lr* (5D)	Chhuneja et al. (2008b)
	Ae. peregrina	*Lr59*	Marais et al. (2008)
	Ae. tauschii	*Lr40* (1DS)	Rowland and Kerber (1974); Raupp et al. (1983); Cox et al. (1992); Hussien et al. (1997); Huang and Gill (2001); Huang et al. (2003); Singh et al. (2004); Hiebert et al. (2007)
	Ae. umbellulata		Athwal and Kimber (1972)
	Ae. searsii, umbellulata		Buloichik et al. (2008)
Powdery mildew (*Erysiphe graminis*)	*Ae. comosa*		Bennett (1984)
	Ae. geniculata	*Pm29* (7D)	Zeller et al. (2002); Stoilova and Spetsov (2006)
	Ae. sharonensis		Olivera et al. (2007)
	Ae. tauschii	*Pm34*; *Pm35* (5DL)	Miranda et al. (2006); Qiu et al. (2006); Miranda et al. (2007)
	Ae. umbellulata	*Pm*(39)	Zhu et al. (2006)
	Ae. longissima, searsii, umbellulata		Buloichik et al. (2008)
Septoria tritici	*Ae. speltoides*		McKendry and Henke (1994)
Soil borne mosaic virus	*Ae. tauschii*	*Sbm1*	Narasimhamoorthy et al. (2006)
Spot blotch	*Ae. sharonensis*		Olivera et al. (2007)
Stem rust (*Puccinia graminis*)	*Ae. speltoides*		Gold et al. (1999); Faris et al. (2008)
	Ae. tauschii	*Sr46*	Mihova (1988); McIntosh et al. (2008); Sambasivam et al. (2008)
Stripe rust (*Puccinia striiformis*)	*Ae. kotschyi*	*Yr37*	Marais et al. (2005)
	Ae. speltoides, uniaristata, umbellulata		Mihova (1988)
Tan Spot	*Ae. bicornis, biuncialis, columnaris, crassa, cylindrica, geniculata, neglecta, speltoides, tauschii*		Alam and Gustafson (1988)
	Ae. sharonensis		Olivera et al. (2007)

(*continued*)

Table 1.10 (continued)

	Donor species	Gene(s) studied and chromosome positions, the genome of bread wheat	Reference
	Ae. tauschii	*Tsr3*	Tadesse et al. (2006)
Wheat curl mite	*Ae. tauschii*	*Cmc1; Cmc4*	Conner et al. (1991); Malik et al. (2003)
Powdery mildew, leaf rust, eyespot, glume blotch	489 accesions of 19 species tested		Börner et al. (2006)
(b) Other traits investigated			
Aconitase	*Ae. longissima*	*Aco-S* (6S)	Chenicek and Hart (1987)
	Ae. umbellulata	*beta-Amy-U1*	Ainsworth et al. (1987)
Aluminum tolerance	*Ae. uniaristata*		Miller et al. (1995, 1997)
Drought tolerance	*Ae. columnaris, geniculata, kotschyi, longissima, peregrina, sharonensis, tauschii, triuncialis, umbellulata*		Damania et al. (1992); Waines et al. (1993); Rekika et al. (1998); Monneveux et al. (2000); Farooq and Azam (2001)
Endopeptidase	*Ae. bicornis*		Koebner et al. (1988)
	Ae. longissima	*Ep-S* (7S)	Hart and Tuleen (1983)
	Ae. umbellulata	*Ep-U1* (1U)	Koebner et al. (1988)
Frost tolerance	*Ae. cylindrica, neglecta, tauschii, triuncialis, umbellulata*		Limin and Fowler (1981); Monneveux et al. (2000)
Grain protein percentage	*Ae. longissima*		Levy et al. (1985)
Grain hardness protein	*Ae. bicornis, longissima, searsii, sharonensis, speltoides*	*Pina* and *PinbS*^{1}, *Sb*^{1}, *Sl*^{1}, *S*^{s}, *S*^{sh}	Morris et al. (2001); Lillemo et al. (2002); Massa et al. (2004)
Grain softness protein	*Ae. tauschii*	*GspD1*	Massa et al. (2004)
Grain weight	*Ae. longissima*		Millet et al. (1988)
Heat tolerance (vegetative phase)	*Ae. longissima, searsii, speltoides*		Waines (1994)
HMW and LMW, Gliadin subunits	*Ae. tauschii, longissima, umbellulata*	*GluD1, GluS*^{1}, *GluU, GliD, GliS*^{1}, *GliU*	Shepherd (1973); Brown et al. (1979); Lagudah and Halloran (1988); Gupta and Shepherd (1990); Hueros et al. (1991); William et al. (1993); Rodriguez-Quijano and Carrillo (1996);Rodriguez-Quijano et al. (1996) Gianibelli et al. (2001, 2002a, b)
Nucleolus organizer regions	*Ae. umbellulata*	*Nor-U1* (1U)	Martini et al. (1982)
	Ae. umbellulata	*Nor-U3* (5U)	Martini et al. (1982)
	Ae. speltoides	*Nor-2* (6S)	Dvorak et al. (1984)
Peroxidase	*Ae. longissima*	*Per-S* (2S)	Liu and Gale (1990)
Salt tolerance	*Ae. comosa, crassa, cylindrica, geniculata, neglecta, juvenalis, kotschyi, tauschii, triuncialis, umbellulata, vavilovii*		Farooq et al. (1989); Rekika et al. (1998); Monneveux et al. (2000); Farooq and Azam (2001)
Subtilisin inhibitor	*Ae. longissima*	*Si-S* (1S)	Koebner (1990)
	Ae. umbellulata	*Si-U2* (1U)	Koebner (1990)
Tolerance to Mn toxicity	*Ae. speltoides*		Dinev and Netcheva (1995)
Tolerance to zinc defficiency	*Aegilops* species with U-genome		Cakmak et al. (1999)
Trypsin inhibitor	*Ae. mutica*	*Ti-M* (5M)	Koebner (1987)
	Ae. sharonensis	*Ti-S* (5S)	Koebner (1987)

wheat seeds. According to this, the female line is an alloplasmic wheat cultivar in a sterilizing cytoplasm and the male parent is another wheat cultivar having the restorer gene(s), natural or transferred to it from an *Aegilops* species. The female line is maintained by a male fertile euplasmic wheat line that is genomically identical to the female line. Such a system has been realized with *T. timopheevii* cytoplasm but not with *Aegilops* cytoplasm. Attempts were carried out to produce such an alloplasmic female line from a wheat cultivar having the 1BL/1RS translocation from rye and *Ae. peregrina* (S^s) cytoplasm (Feldman and Millet unpublished). Since the *Rf* genes for this cytoplasm are located on 1BS, which is missing in this cultivar, the alloplasmic plant is male sterile and its hybrid is restored by a normal (non 1B/1R) wheat cultivar. However, this alloplasmic female, when cross pollinated, produced twin and embryoless seeds (Tsunewaki et al. 1974), and fertility restoration was incomplete. Moreover, a decrease in chlorophyll content and grain yield in similar fertile alloplasmic cultivars renders this cytoplasm unsuitable for hybrid production (Feldman and Millet unpublished)

1.7.4.2 Induction of Haploid Production in Wheat

Six *Aegilops* cytoplasms induced haploids at the considerable rate of 11–56% in alloplasmic wheat with a translocated 1BL/1RS chromosome pollinated by the wheat parent (Kihara and Tsunewaki 1962; Tsunewaki et al. 1974). Cytoplasms of these species, namely *Ae. columnaris*, *Ae. kotschyi*, *Ae. markgrafii*, *Ae. peregrina*, *Ae. umbellulata*, and *Ae. triuncialis*, which possess either cytoplasm type U^2, S^2, C, S^s, or U (see Table 1.2), induced haploid production as a result of a high frequency of parthenogenesis in the egg cells (Tsunewaki et al. 1974). Haploid production may be used to accelerate breeding programs by obtaining homozygous double haploids.

1.7.4.3 Production of Deletion Lines by Gametocidal Genes

It has been shown by Endo (1988) that a monosomic addition of chromosome 2C of *Ae. cylindrica* causes chromosome breakage in progenies not having it. This is ascribed to the gametocidal effect of this chromosome (Endo 1990). More chromosomes of other *Aegilops* species are also described. This feature has been used to produce deletion stocks of common wheat (430 lines). These lines involve all the 21 wheat chromosomes, with terminal deletions of various lengths (Endo and Gill 1996). Three *Aegilops* species with gametocidal capacity, namely *Ae. cylindrica*, *Ae. triuncialis*, and *Ae. speltoides*, were used to produce these stocks. The deletion stocks show phenotypic variations depending on the size of the chromosomal deficiency and serve as a tool for physical mapping of wheat chromosomes.

1.8 Genomic Resources

A number of genomic resources have been developed or are being developed for wheat and the Triticeae by international cooperation efforts. Due to the large (17 Gb) and complex hexaploid wheat genome, at the moment the diploid barley (*Hordeum vulgare* L.) is considered the Triticeae model species. Barley genome research develops cutting-edge tools that can be used for wheat genomics. It can be expected that these efforts will include, in the near future, *Aegilops* as well. Selected examples for these organizations are (1) **ITMI** (International Triticeae Mapping Initiative, *http://wheat.pw.usda.gov/ITMI/*). ITMI was established to provide support in the coordination of research efforts in molecular genetics, genomics, and genetic analysis of the Triticeae. Coordinated topics also include *Aegilops*, genetics of abiotic stress resistance, and Triticeae informatics. (2) **ETGI** (European Triticeae Genomics Initiative, *http://pgrc.ipk-gatersleben.de/etgi/*). ETGI is a platform for the coordination and representation of Triticeae Genomics Research at the European level and serves as a link to the international research community represented by ITMI. (3) **IGROW** (International Genome Research on Wheat, *http://www.k-state.edu/igrow/IGROW_history.html*). (4) **IWGSC** (International Wheat Genome Sequencing Consortium, *http://www.wheatgenome.org/*).

1.8.1 Databases

Several databases and resources have been established that aim to curate the increasing amount of genomic information. Selected databases are:

1. **GrainGenes**, *http://www.graingenes.org*. This is the international database for the wheat, barley, rye, and oat genomes. For these species, it is the primary repository for information on genetic maps, mapping probes and primers, genes, alleles, and QTLs. Documentation includes primer sequences, polymorphism descriptions, genotype and trait scoring data, experimental protocols, photographs of marker polymorphisms, disease symptoms, and mutant phenotypes. These data are integrated with sequence and bibliographic records selected from external databases and results of BLAST searches of the ESTs.
2. **BLAST** databases. These databases are maintained locally and serve local research activities. Custom databases are set up to screen against Triticeae sequences, assemblies, cDNA libraries, and other specialty formatted databases (*http://blast.ncbi.nlm.nih.gov/Blast.cgi*).
3. **cerealsDB.uk.net** (*http://www.cerealsdb.uk.net/index.htm*). Maintained at the University of Bristol for investigating gene functions in cereals.
4. **wEST-SQL database** (*http://wheat.pw.usda.gov/wEST/*). This is a USDA-ARS sponsored server of nucleic acid sequence data for Triticeae-associated research projects.
5. **Gramene** (*http://www.gramene.org/*). It is a curated data resource for comparative genome analysis in the grasses.
6. **TIGR Wheat genome database** (*http://www.tigr.org/tdb/e2k1/tae1/*). A bioinformatics resource for annotating and analyzing the wheat genome.
7. **HarvEST** is mainly an expressed sequence tag (EST) database-viewing software that emphasizes gene function oriented to comparative genomics and the design of oligonucleotides, in support of activities such as microarray content design, function annotation, and physical and genetic mapping (*http://harvest.ucr.edu/*).
8. **TREP**, the Triticeae Repeat Sequence Database. It contains a collection of repetitive DNA sequences from different Triticeae species (*http://wheat.pw.usda.gov/ITMI/Repeats/index.shtml*).
9. **Wheat D-Genome by Physical Mapping** (*http://wheat.pw.usda.gov/PhysicalMapping/*). The database has been developed to assess gene distribution across the D-genome chromosomes. The *Aegilops tauschii* genome is homologous with the D-genome of wheat. Physical maps of each of the seven *Ae. tauschii* chromosomes are constructed and a large number of gene loci are placed on the physical maps. The physical maps will be integrated with the wheat linkage and deletion maps.
10. **TriAnnot** Project (*http://urgi.versailles.inra.fr/projects/TriAnnot/*). Aims at deciphering the chromosomal location and biological function of wheat genes. The aim of TriAnnot pipeline is to provide a wheat and barley automated annotation.
11. **TriMEDB**, Triticeae Mapped EST database (*http://trimedb.psc.riken.jp/index.pl*). It provides information on mapped cDNA markers that are related between barley and wheat, along with various annotations.

1.8.2 Linkage Maps

Molecular linkage maps are under development in almost all crop species. To explore the genetic relationships in the Triticeae and to facilitate the transfer of agronomically important genes, genetic maps as well as linkage maps have been developed also for *Aegilops*. Currently, six diploid wheat genetic maps are available (Table 1.11), which have been developed to map disease-resistant genes.

The first genetic restriction fragment length polymorphism (RFLP)-based linkage map of *Aegilops tauschii* (DD) was developed by Gill et al. (1991b) to study the relationship of the D-genome of bread wheat with *Ae. tauschii*. This linkage map contains 178 markers. Thirty-five loci were mapped by aneuploid analysis in *T. aestivum*. One hundred and fifty-two loci, including 143 RFLPs, eight proteins, and one leaf rust resistance gene, were mapped in an F_2 population (60 plants) of *Ae. tauschii*. One hundred and twenty-seven loci were placed in linkage groups belonging to seven D-genome chromosomes of *Ae. tauschii*.

A more saturated linkage map was developed for *Ae. tauschii* with 546 loci and 164 agronomically

Table 1.11 Genetic linkage maps available for *Aegilops* species

Species	Year	Parent (♀)	Parent (♂)	Marker type	No. of marker	Maps	Reference
Ae. tauschii	1991	TA1691	TA1704	RFLP	291	1D–7D	Gill et al. (1991a, 1992)
Ae. tauschii	2000	AUS 18913	CPI 110856	RFLP	24	1D	Spielmeyer et al. (2000a)
Ae. tauschii	2000	AUS 18913	CPI 110856	RFLP	17	1D	Spielmeyer et al. (2000b)
Ae. tauschii				RFLP	123	1D–7D	*http://wheat.pw.usda.gov/cgi-bin/graingenes/report.cgi?class=mapdata&name=T.tauschii%2C%20Appels*
Ae. longissima, Y × Y	2001	Y154-1	Y431-1	RFLP	74	1S–7S	Zhang et al. (2001)
Ae. umbellulata, A × C	1998	JIC2010001	JIC2010003	RFLP	89	1U–7U	Zhang et al. (1998)

important genes (Boyko et al. 1999). This map was compared with *T. aestivum*, and discrepancies were found between the orders of the markers on chromosomes 4D, 5D, and 7D of *Ae. tauschii* with the genetic and physical map of *T. aestivum*.

Another detailed RFLP map was constructed by Spielmeyer et al. (2000a) for the distal end of the short arm of chromosome 1D of *Ae. tauschii* (AUS18911, carrying *Lr21* and *Sr45*) and wheat (CPI 110799, carrying the stem rust resistance gene *Sr33*). Large DNA fragments isolated from a BAC library of *Ae. tauschii* were used by Spielmeyer et al. (2000b) to determine the relationship between physical and genetic distance at seed storage protein loci located at the distal end of chromosome 1DS. Highly recombinogenic regions were identified where the ratio of physical to genetic distance was estimated to be smaller than 20 kb/cM. Fifty-eight F_2 individuals were sufficient to identify recombinants between most of the mapped RFLP markers. A higher recombination frequency was observed between and within *Glu-D3* and *Gli-D1* in *Ae. tauschii*, as compared to previous studies in the corresponding loci of the D-genome of the hexaploid wheat. Variation was reported at seed storage protein loci among *Ae. tauschii* accessions from diverse geographical origins (Lagudah and Halloran 1988). Most of the 79 accessions were distinguished on the basis of unique gliadin (*Gli-D1*) haplotypes. Such high levels of diagnostic haplotypes occurring in *Ae. tauschii* could be accounted for by the relatively high recombination frequency among the *Gli-D1* loci.

A comparative genetic map of the *Ae. longissima* genome was developed by Zhang et al. (2001) using RFLP probes with known locations in wheat. A high degree of conserved colinearity was observed between the wild diploid *Ae. longissima* and the wheat genome. Chromosomes $1S^l$, $2S^l$, $3S^l$, $5S^l$, and $6S^l$ were colinear with wheat chromosomes 1D, 2D, 3D, 5D, and 6D, respectively. The analysis confirmed that chromosomes $4S^l$ and $7S^l$ were translocated relative to wheat. The short arms and large part of the long arms were homoeologous to most of wheat chromosomes 4D and 7D, respectively, but the distal segment of 7D was translocated from $7S^lL$ to the distal region of $4S^lL$. The map and RFLP markers were used to analyze a set of "Chinese Spring" (CS)/*Ae. longissima* chromosome addition lines. The availability of disomic CS/*Ae. longissima* addition lines for chromosomes $1S^l$, $2S^l$, $3S^l$, $4S^l$, and $5S^l$ was evident. A set of *Ae. sharonensis* chromosome addition lines was also available for analysis. Due to the gametocidal nature of *Ae. sharonensis* chromosomes $2S^l$ and $4S^l$, additions $1S^l$, $3S^l$, $5S^l$, $6S^l$, and $7S^l$ were produced in a (4D) $4S^l$ background, and $2S^l$ and $4S^l$ in a euploid wheat background. The 4/7 translocation found in *Ae. longissima* was also evident for *Ae. sharonensis*.

Zhang et al. (1998) developed a comparative genetic map of *Ae. umbellulata* using probes previously mapped in hexaploid wheat. All seven *Ae. umbellulata* chromosomes displayed one or more rearrangements relative to wheat. These structural changes were consistent with the subterminal morphology of chromosomes 2U, 3U, 6U, and 7U. Comparison of the chromosomal locations assigned by mapping and those obtained by hybridization to wheat/*Ae. umbellulata* single chromosome addition lines supported the identification of the added *Ae. umbellulata* chromosomes and indicated that no cytological rearrangements had taken place during the production of the alien wheat aneuploid lines.

1.8.3 BAC Libraries

The availability of a large-insert genomic library is considered an absolute prerequisite for positional gene cloning. Large-insert libraries are also indispensable for studies of genome structure. Bacterial artificial chromosomes (BACs) have proven to be the most versatile vectors for the construction of such libraries (Shizuya et al. 1992). They are easy to maintain and reproduce, have low levels of chimerism, and are easy to screen by DNA hybridization.

Massive efforts are underway to develop physical maps of bread wheat. BAC clone-based physical maps have been developed already for wheat chromosome 3B (Paux et al. 2008). These efforts ultimately will lead to whole wheat genome sequencing. Seven BAC libraries are available for *Aegilops tauschii* (wheat D-genome donor) and one BAC library for *Ae. speltoides* (wheat B-genome donor) (Table 1.12; Moullet et al. 1999; Akhunov et al. 2005).

Moullet et al. (1999) have used the *Ae. tauschii* accession Aus18913 for the construction and characterization of a large DNA insert library from the D-genome of wheat. The library consists of 144,000 clones with an average insert size of 119 kb. The accession Aus18913 contains several genes of economic importance, among which the cereal cyst nematode-resistant gene *Cre3* (syn. *CcnD1*), the HMW glutenin subunits *Dx2* and *DyT2*, and an unusual HMW gliadin-designated *T1* (Lagudah and Halloran 1988), as well as resistance to stem, leaf, and stripe rusts.

Akhunov et al. (2005) reported the construction and characterization of large-insert BAC libraries for *Triticum urartu*, *Ae. speltoides*, and *Ae. tauschii*. The libraries are equivalent to 3.7, 5.4, and 4.1 of the *T. urartu*, *Ae. speltoides*, and *Ae. tauschii* genomes, respectively. The libraries were used to estimate the proportion of known repeated nucleotide sequences and gene content in each genome by BAC-end sequencing. Repeated sequence families previously detected in Triticeae accounted for 57%, 61%, and 57% of the *T. urartu*, *Ae. speltoides*, and *Ae. tauschii* genomes, and coding regions accounted for 5.8%, 4.5%, and 4.8%, respectively. The estimated average size of inserts in *T. urartu*, *Ae. speltoides*, and *Ae. tauschii* libraries was 110 kb, 115 kb, and 115 kb, respectively (Lijavetzky et al. 1999; Yu et al. 2000; Allouis et al. 2003; Cenci et al. 2003; Nilmalgoda et al. 2003). The successful recovery of all single-copy genes used for hybridization indicated that these three BAC libraries are valuable tools for genomic studies and gene isolation. BAC clone information were essential for marker development in the *Lr34* region (Bossolini et al. 2006), which led to the cloning of the *Lr34* gene (Krattinger et al. 2009). The *Ae. tauschii* accession AS75 was chosen as the parent of a high resolution mapping population used for anchoring AL8/78 BAC contigs on the *Ae. tauschii* genetic map (Dvorak et al. 1998b; Luo et al. 2009). *Ae. speltoides* is an outcrosser, and the F_4 family from the cross *Ae. speltoides* 2-12-4-8-1-1-1 × *Ae. speltoides* PI36909-12-II was used for the construction of the *Ae. speltoides* library. The family was homozygous for two *Ph1* suppressor genes (Chen and Dvorak 1984).

Salse et al. (2008) screened BAC libraries from the bread wheat cultivar *T. aestivum* cv. Renan and from *Ae. speltoides* with PCR markers for the *Storage Protein Activator* (*SPA*) locus. They sequenced the longest BAC clones from genomes A-, B-, D-, and S-. Non-shared transposable elements (TE) insertions within the *SPA* orthologous gene locus were selected for evolutionary studies (see above).

Table 1.12 BAC resources available for *Aegilops* species (*http://www.wheatgenome.org/projects.php*; *http://www.plantsciences.ucdavis.edu/Dubcovsky/BAC-library/ITMIbac/ITMIBAC.htm*)

Species	Accession	Vector	Restriction site	No. of clones	Clone size (kb)	Coverage	Curator
Ae. tauschii	AL8/78	pECBAC1	EcoR I	54,000	167	2.2X	H.B. Zhang
Ae. tauschii	AL8/78	pECBAC1	Hind III	59,000	189	2.2X	H.B. Zhang
Ae. tauschii	AL8/78	pCLDO4541	Hind III	52,000	190	3.2X	H.B. Zhang
Ae. tauschii	AL8/78	pECBAC1	BamH I	59,000	149	2.8X	H.B. Zhang
Ae. tauschii	AL8/78	pCLDO4541	BamH I	76,000	174	2.4X	H.B. Zhang
Ae. tauschii	Aus 18913	pCLDO4541	Hind III	144,000	120	4.2X	E. Lagudah
Ae. tauschii	AS75	pECBAC1	BamH I	181,248	115	4.1X	J. Dvorak
Ae. speltoides	2-12-4-8-1-1-1	pECBAC1	BamH I	237,312	115	5.4X	J. Dvorak

Current techniques of screening BAC libraries of large eukaryotic genomes with molecular markers during the construction of physical maps are slow and laborious. Recently however, Luo et al. (2009) reported a new high-throughput strategy for screening BAC libraries and anchoring of clones on a genetic map based on single nucleotide polymorphisms (SNPs).

1.8.4 Sequences Available

Three types of studies have produced sequence data of *Aegilops*: Phylogenetic studies, the search for new disease-resistant alleles and, more recently, activities dedicated to the sequencing of the whole bread wheat genome. An overview of *Aegilops* sequences released at GenBank database (*http://www.ncbi.nlm.nih.gov/Genbank/*) is shown in Table 1.13. It is evident that few sequences have been released for most *Aegilops* species. Furthermore, in the database, the ratio chloroplast to nuclear sequences indicates that still relatively few nuclear loci have been sequenced in *Aegilops*. The chloroplast sequences have been mainly used for phylogenetic studies. Compared to other *Aegilops* species, more sequence information is available for *Ae. speltoides* and *Ae. tauschii* as they are the species most related to the B- and D-genomes of bread wheat, respectively. Genome sequencing projects are ongoing in *Ae. tauschii* and *Ae. umbellulata*.

1.9 Conservation Initiatives

Aegilops species have hidden and unexplored potential for wheat improvement. On the other hand, there has been a considerable loss of *Aegilops* germplasm due to an increase of urbanization, as well as loss of natural habitat (Anikster and Noy-Meir 1991; Hawkes et al. 2000). There is only one report of endangered *Ae. sharonensis* populations that were rescued just before the destruction of their habitats (Millet 2006). *Aegilops* species need to be conserved in situ in their natural habitats if genetic diversity has to be preserved.

Greater emphasis on in situ conservation of genetic diversity is required, employing also ex situ conservation in genebanks as a safety back-up (Hammer 1980b). Maxted et al. (2008) discuss *Aegilops* conservation based on a compiled dataset of 9,866 unique germplasm accessions described in four datasets (duplicate observations and observations outside the natural distribution range were removed, and only 36% of the original accessions were considered). *Aegilops* datasets were from (I) "Global Database of Wheat Wild Relatives" (at ICARDA; 12,476 accessions), (II) "CGIAR system-wide information network for genetic resources" (SINGER – *http://singer.grinfo.net*; 3,569 accessions), (III) "European Plant Genetic Resources" (EURISCO– *http://eurisco.ecpgr.org/index.php*; 7,684 accessions), and (IV) "Germplasm Resources Information Network" (GRIN – *http://www.ars-grin.gov/cgi-bin/npgs/html/index.pl*; 3,626 accessions). The combined, corrected dataset of *Aegilops* germplasm accessions was then analyzed by ex situ conservation gap analysis. The ecogeographic analysis of *Aegilops* species found that all 21 species are represented in ex situ collections, but the number of accessions varies considerably. Potential undersampled areas were identified and the size and range of these predicted conservation gaps varied considerably between species. This process highlighted ex situ conservation priorities for *Aegilops* species in Cyprus, Egypt, Greece, Iran, Israel, Libya, Spain, Syria, Tajikistan, Tunisia, Turkey, Turkmenistan, and Uzbekistan, as well the priority in conservation for *Ae. bicornis*, *Ae. comosa*, *Ae. vavilovii*, *Ae. juvenalis*, *Ae. kotschyi*, *Ae. peregrina*, *Ae. sharonensis*, and *Ae. uniaristata*. The most recent detailed overview of *Aegilops* genetic resources stored in ex situ genebank collections can be found in Knüpffer (2009).

Genetic diversity studies have demonstrated extensive genetic diversity in the original populations of the wild gene pool in the natural habitat. This diversity, however, is impossible to preserve by standard ex situ collection procedures (Valkoun and Damania 1992). In addition, it is an accepted rule that during field collection trips only few plants per site are sampled. On the other hand, under in situ conservation, a much larger and continuously evolving genetic diversity is preserved. Germplasm could be repeatedly collected from in situ conservation sites for evaluations. Long-term monitoring of biodiversity at in situ conservation sites is essential, and the sites should be actively managed to limit grazing.

In situ conservation of cereals has been of interest and studied since several decades, and few protected

Table 1.13 Overview of *Aegilops* sequences released in GenBank database (*http://www.ncbi.nlm.nih.gov/*)

	Nucleotide total[a]	cp[b]	mi[c]	*n*[d]	Nucleotide EST[e]	Nucleotide GSS[f]	Popset[g]	UniSTS[h]	Protein	Genome projects ongoing
Aegilops bicornis	117	38		79			24		77	
Aegilops biuncialis	75	70		5			7		31	
Aegilops columnaris	79	56		23			8		34	
Aegilops comosa	144	84		60			20		45	
Aegilops crassa	62	42	1	19			9		33	
Aegilops cylindrica	84	72		12			8		38	
Aegilops geniculata	106	77		29			8		44	
Aegilops juvenalis	30	11		19			11		12	
Aegilops kotschyi	74	50		24			10		38	
Aegilops longissima	228	43		185		1	23		113	
Aegilops sharonensis	125	33		92			25		80	
Aegilops markgrafii	202	146		56			18	42	64	
Aegilops mutica	59	46		13			12		16	
Aegilops neglecta	92	70		22			8		33	
Aegilops peregrina	86	71		15			7		35	
Aegilops searsii	141	40		101			21		94	
Aegilops speltoides	547	102		445	4,315		90		300	
Aegilops tauschii	1,327	75	1	1,251	116	5,055	49	4	510	Dvorak et al.
Aegilops triuncialis	303	287		16			10		123	
Aegilops umbellulata	165	115		50	15		15		20	Gill et al.
Aegilops uniaristata	67	38		29			17		1	
Aegilops vavilovii	12	0		12			3		29	
Aegilops ventricosa	52	30		22			8			

State: Early January 2009

Note: [a]Total nucleotide sequences (NCBI); [b]Chloroplast sequences (NCBI); [c]Mitochondrial sequences (NCBI); [d]Nucleotide sequences excluding 2 and 3; [e]Single read cDNA sequences (annotated biological features not included); [f]Genome Survey Sequences first single-read genomic sequences (rarely include biological features); [g]Population study data sets sequences collected to study the evolutionary relatedness of populations. Different members of same the species or from different species; [h]Markers or sequence tagged sites

sites have been established (Damania 1994; Stolton et al. 2006; Maxted et al. 2008). (1) Ammiad in Galilee/Israel (Anikster and Noy-Meir 1991; Horovitz and Feldman 1991; Noy-Meir et al. 1991; Anikster et al. 1997; Kaplan 2008); (2) the Erebuni Nature Reserve in Armenia (Vavilov 1951); and (3) the Ceylanpinar State Farm in southeastern Turkey (Karagöz 1998).

The spatial analysis of *Aegilops* species diversity by Maxted et al. (2008) identified five potential areas for the optimal locations of further in situ conservation reserves for *Aegilops* species. The regions identified were located in (1) central Israel; (2) northern Lebanon and Syria; (3) northwestern Turkey; (4) Turkmenistan; and (5) southern France. Maxted et al. (2008) recommended that at least one genetic reserve should be created in each of the regions identified.

1.10 Conclusions and Final Considerations

Several aspects concerning the genus *Aegilops* L. have been reviewed in this chapter. We have considered 21 annual species and followed in their classification the monographs of Hammer (1980a, b) and van Slageren (1994). We have shown that *Aegilops* species have been closely involved in wheat evolution, played a major role in wheat domestication and will play a critical role in future wheat improvement.

The keys to obtain deeper insights to *Aegilops* genetic diversity, *Aegilops–Triticum* molecular biological relationships, and to harvest and preserve suitable alleles for future wheat improvement are (1) a

comprehensive germplasm collection covering the whole distribution area of each species; (2) the comparison of several accessions for each species considering all ploidy levels; (3) the use of new molecular fingerprinting techniques and the access to high-throughput sequencing technologies (Goldberg et al. 2006; Wicker et al. 2006); and (4) the improvement of analytical methods capable of treating various issues based on mathematical and statistical models (Pluzhnikov and Donnelly 1996; Thuillet et al. 2005; Haudry et al. 2007).

New genomic resources for future plant breeding have to be developed. International consortia, such as the International Triticeae Mapping Initiative (ITMI) and the International Wheat Genome Sequencing Consortium (IWGSC), will lead to accelerated gene discovery and will shed new light on mechanisms that have shaped the wheat and *Aegilops* genomes during their evolution.

Archeological excavation campaigns should also consider studies on *Aegilops* species. This will provide new information on ancient *Aegilops* distribution and their ecology. See Kilian et al. (2009) for a review on Triticeae domestication.

In spite of more than 200 years of botanical exploration of the Orient, resulting in many herbaria and germplasm collections, sequence data, and transferred alleles, our knowledge on the genus *Aegilops* is far from complete. We urgently need detailed studies for each species dealing with natural distribution range, ecology, soil, geomorphology, molecular resources, and genome sequences.

There is an urgent need for an active in situ conservation to protect *Aegilops* species in their natural habitats. The human urbanization pressure increases, and the landscape, together with the farming practices, is rapidly changing. Already the establishment of new protected areas would be a success, while the existing reserves have to be enlarged in order to protect larger populations.

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Appendix

Index to scientific names used for this chapter = Register of species names

Name
Aegilemma kotschyi
Aegilemma peregrina
Aegilonearum juvenale
Aegilopodes triuncialis
Aegilops turcomanica
Aegilops bicornis
Aegilops bicornis var. *anathera*
Aegilops bicornis var. *bicornis*
Aegilops bicornis var. *mutica*
Aegilops biuncialis
Aegilops caudata
Aegilops columnaris
Aegilops comosa
Aegilops comosa ssp. *comosa*
Aegilops comosa var. *comosa*
Aegilops comosa ssp. *comosa* var. *comosa*
Aegilops comosa ssp. *heldreichii*
Aegilops comosa var. *subventricosa*
Aegilops crassa
Aegilops crassa ssp. *crassa*
Aegilops crassa ssp. *vavilovii*
Aegilops crassa var. *palaestina*
Aegilops cylindrica
Aegilops geniculata
Aegilops geniculata ssp. *geniculata*
Aegilops geniculata ssp. *gibberosa*
Aegilops juvenalis
Aegilops kotschyi
Aegilops ligustica
Aegilops longissima
Aegilops longissima ssp. *longissima*
Aegilops longissima ssp. *sharonensis*
Aegilops lorentii
Aegilops markgrafii
Aegilops markgrafii var. *markgrafii*
Aegilops markgrafii var. *polyathera*
Aegilops mutica
Aegilops mutica ssp. *loliacea*
Aegilops mutica var. *loliacea*
Aegilops mutica ssp. *mutica*
Aegilops mutica var. *mutica*

(*continued*)

Name
Aegilops neglecta
Aegilops neglecta ssp. *neglecta*
Aegilops neglecta ssp. *recta*
Aegilops ovata
Aegilops peregrina
Aegilops peregrina ssp. *cylindrostachys*
Aegilops peregrina ssp. *peregrina*
Aegilops peregrina var. *peregrina*
Aegilops peregrina var. *brachyathera*
Aegilops searsii
Aegilops sharonensis
Aegilops speltoides
Aegilops speltoides ssp. *ligustica*
Aegilops speltoides var. *ligustica*
Aegilops speltoides ssp. *speltoides*
Aegilops speltoides var. *speltoides*
Aegilops squarrosa
Aegilops tauschii
Aegilops tauschii ssp. *strangulata*
Aegilops tauschii ssp. *tauschii*
Aegilops triaristata
Aegilops triuncialis
Aegilops triuncialis ssp. *persica*
Aegilops triuncialis ssp. *triuncialis*
Aegilops triuncialis ssp. *triuncialis*
Aegilops triuncialis var. *anathera*
Aegilops turcomanica
Aegilops umbellulata
Aegilops umbellulata ssp. *transcaucasica*
Aegilops umbellulata ssp. *umbellulata*
Aegilops uniaristata
Aegilops variabilis
Aegilops vavilovii
Aegilops ventricosa
× *Aegilotriticum*
Agropyron
Amblyopyrum muticum
Amblyopyrum muticum var. *muticum*
Amblyopyrum muticum var. *loliaceum*
Chennapyrum uniaristatum
Comopyrum comosum
Cylindropyrum cylindricum
Gastropyrum crassum
Gastropyrum vavilovii
Gastropyrum ventricosum
Gramineae
Kiharapyrum umbellulatum
Patropyrum tauschii
Poaceae
Pooideae
Section *Aegilops*
Section *Comopyrum*
Section *Cylindropyrum*
Section *Sitopsis*
Section *Vertebrata*
Sitopsis bicornis
Sitopsis longissima
Sitopsis searsii
Sitopsis speltoides
Subgenus *Aegilops*
Subgenus *Amblyopyrum*
Subgenus *Sitopsis*
Triticeae
Triticinae
× *Tritordeum*
× *Triticosecale*
Triticum aestivum
Triticum araraticum
Triticum bicorne
Triticum biunciale
Triticum boeoticum
Triticum carthlicum
Triticum columnare
Triticum comosum
Triticum crassum
Triticum cylindricum
Triticum dichasians
Triticum dicoccoides
Triticum dicoccon
Triticum juvenale
Triticum kotschyi
Triticum longissimum
Triticum lorentii
Triticum macrochaetum
Triticum markgrafii
Triticum monococcum
Triticum neglectum
Triticum ovatum
Triticum parvicoccum
Triticum peregrinum
Triticum searsii
Triticum speltoides
Triticum syriacum
Triticum tauschii
Triticum tripsacoides
Triticum triunciale
Triticum triaristatum
Triticum turanicum
Triticum umbellulatum
Triticum uniaristatum
Triticum urartu
Triticum ventricosum
Triticum zhukovskyi

(*continued*)

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Chapter 2
Agropyron and *Psathyrostachys*

Richard R.-C. Wang

2.1 Introduction

Wheatgrass and wildrye grasses are some of the most important grasses in the temperate regions of the world (Asay and Jensen 1996a, b). These drought-resistant grasses are excellent sources of forage and habitat for livestock and wildlife; and they are valued for weed control, habitat use, soil stabilization, and watershed management. Many of these grasses are related to and have been hybridized with cultivated cereal crops including wheat (*Triticum aestivum* L. and *T. durum* Desf.), barley (*Hordeum vulgare* L.), and rye (*Secale cereale* L.) as genetic sources for disease resistance, salinity tolerance, and other traits. These hybrids were summarized in several early review articles (Sharma and Gill 1983; Dewey 1984; Wang 1989a). Since then, certain specific subjects of alien gene transfer from wild Triticeae into wheat have been extensively reviewed and discussed (Knott 1989; Pienaar 1990; Jiang et al. 1994b; Friebe et al. 1996; Fedak 1999; Jauhar and Chibbar 1999; Repellin et al. 2001; Sahrawat et al. 2003; Jauhar 2006; Qi et al. 2007; Trethowan and Mujeeb-Kazi 2008).

The taxonomy of the wheatgrass and wildrye grasses has been the object of considerable controversy. Even with the advent of molecular phylogenetics, Kellogg (2006) stated that "generic relationships within Triticeae have always been and remain problematic." In North America, the wheatgrasses traditionally have been included in the genus *Agropyron*, and the wildryes have largely been treated as species in the genus *Elymus* (Bowden 1965; Hitchcock 1971). Depending on how the genus *Agropyron* was treated, the number of species in this genus varied. Bentham (1882) included about 20 species in *Agropyron*, whereas Hackel (1887) listed 32 species. More recently, however, taxonomic realignments have been proposed that are based on genomic or biological relationships as well as plant morphology (Tsvelev 1976; Dewey 1984; Yen et al. 2005b). Dewey (1984) proposed that *Agropyron* be restricted to species of the crested wheatgrass complex, a polyploid series based on the **P**-genome. Thus, bluebunch wheatgrass, previously *A. spicatum* (Pursh) Scribner & Smith, and related species based on the **St**-genome are now included in the genus *Pseudoroegneria* A. Löve. Tall wheatgrass and intermediate wheatgrass are now included in the genus *Thinopyrum* A. Löve as *Th. ponticum* (Podp.) Barkworth & D.R. Dewey and *Th. intermedium* (Host) Barkworth & D.R. Dewey, respectively. Species in this genus possess the **J**- or **E**-genome, which Dewey (1984) designated as "**J** = **E**," and sometimes also contains the **St**-genome (Liu and Wang 1993; Kishii et al. 2005). Slender wheatgrass, previously *A. trachycaulum* (Link) Malte ex H.F. Lewis, and its self-fertile caespitose relatives are in the genus *Elymus* along with several wildryes. This genus is based on the **St**-genome, in combination with one or more of **H**-, **Y**-, **W**-, or **P**-genomes (Wang et al. 1995).

Depending on the taxonomic treatment, between 200 and 250 wheatgrass and wildrye species have been described worldwide (Asay and Jensen 1996a, b). More than two-thirds are native to Eurasia and about 22 to 30 are considered native to North America.

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Relatively few species are found in South America, New Zealand, Australia, and Africa. The wheatgrass and wildrye grasses are generally adapted to subhumid to arid climatic conditions in steppe or desert regions. In North America, the wheatgrass and wildrye grasses are most prevalent in the northern Great Plains, as well as on the semi-arid to arid rangelands of the Intermountain and Great Basin Regions. In their natural setting, wheatgrass and wildrye grasses are most often found in association with other grasses, sedges, forbs, and shrubs.

The genus *Agropyron* Gaertner belongs to the tribe Triticeae, which is composed of about 20 genera and 400–500 species (Löve 1884). Although it is now agreed by taxonomists that *Agropyron* should be restricted to *A. cristatum* and its close relatives, for this review, *Agropyron* will be used to include species in the genera *Australopyrum* (Tzvelev) A Löve, *Dasypyrum* (Coss. & Durieu) T. Durand, *Elymus* Linnaeus, *Leymus* Hochstetter, *Pascopyrum* A. Löve, *Pseudoroegneria* (Nevski) A. Löve, and *Thinopyrum* A. Löve, etc. Some of these genera are not widely accepted by taxonomists. There is even less agreement on how their members should be treated. As a result, most Triticeae species had one or more synonyms, indicating that they were placed in the past by taxonomists in the genus *Agropyron* (Table 2.1). On the other hand, *Psathyrostachys* Nevski is a small genus with about 10 species that were formerly treated in genera *Elymus* and *Hordeum* (Quattrocchi 2006; Table 2.2).

Wheatgrasses and wildryes have been mainly used as forage crops (Wang and Jensen 2009). They are also served as the tertiary gene pool for wheat improvement (Dewey 1984; Mujeeb-Kazi and Wang 1995). Because species of various known genome constitutions in the perennial Triticeae (i.e., **P**, **St**, **Ns**, **E**, **ESt**, **StH**, **StY**, **StHY**, **StWY**, and **NsXm**, etc. as designated by Wang et al. 1995) have been successfully crossed with wheat (Jiang et al. 1994b), all species in the genera *Agropyron*, *Pseudoroegneria*, *Psathyrostachys*, *Thinopyrum*, *Elymus*, and *Leymus* are theoretically capable of being hybridized with wheat. Hybrids, natural or man-made, between wheat and these species had been generally called *Agrotricum*. The use of wheatgrasses and wildryes as forage crops had been recently reviewed (Wang and Jensen 2009). Thus, only the contribution of specific wheatgrass and wildrye species to wheat improvement will be reviewed in this chapter.

2.2 Basic Botany

2.2.1 Morphology

Wheatgrass encompasses five genera of Triticeae and wildrye species belong to three genera (Table 2.1); thus their morphological features are highly variable. Brief descriptions of morphology for some important wheatgrass and wildrye grasses were provided in the previous reviews (Asay and Jensen 1996a, b). More detailed morphological descriptions for *Agropyron, Pseudoroegneria, Psathyrostachys, Elymus, Leymus, Thinopyrum*, and *Pascopyrum* can be found in the *Flora of North America, Volume 24* (Barkworth et al. 2007). With the advent of internet, people can now find the required information on these grasses or crops by visiting the websites housing the "Catalogue of New World Grasses (Poaceae)" (Soreng et al. 2000 onwards), "Flora of China, Volume 22" (Wu and Raven 2004), and the "GrassBase – The Online World Grass Flora" (Clayton et al. 2006 onwards). These interactive electronic databases are valuable resources for taxonomic nomenclature, geographical distributions, and other pertinent information of any species of interest to the visitors.

2.2.2 Distribution

Based on "Flora of North America, Volume 24" (Barkworth et al 2007) and "Flora of China, Volume 22" (Wu and Raven 2004), geographic distributions of perennial Triticeae species are presented in Tables 2.3 and 2.5.

2.2.2.1 *Agropyron* Senso Stricta

Crested wheatgrass is indigenous to the Steppe region of European Russia and southwestern Siberia, and it was apparently cultivated initially in the Volga district east of Saratov (Kirk 1932). This genus comprises 10 to 15 species, of which *A. cristatum* and *A. fragilie* have been introduced and widely cultivated in North America and five species (one endemic) are found in China.

The tetraploid form of crested wheatgrass is the most common of the three crested wheatgrass ploidy

Table 2.1 Examples of Triticeae species that had ever been included in the genus *Agropyron* or other genera. Species that had been hybridized with wheat are in bold-face

Current scientific name	Synonym
Agropyron badamense Drob.	*Agropyron desertorum* aucto., non Schultes & Schultes
Agropyron cimmericum Nevski	*Agropyron dasyanthum* subsp. *birjutczense* (Lavr.) Lavr.
***Agropyron cristatum* (L.) Beauv.**	*Bromus cristatus* L.; *B. distichus* Georgi; *Triticum pumilum* L.f.
Agropyron dasyanthum Ledeb.	*Triticum dasyanthum* (Ledeb.) Sprengel
***Agropyron desertorum* (Fischer ex Link) Schultes**	*Triticum desertorum* Fischer ex Link
Agropyron fragile (Roth) Candargy	*Agropyron sibiricum* (Willd.) Beauv.; *Triticum fragile* Roth; *T. sibiricum* Willd.
Agropyron krylovianum Schischk.	*Elytrigia kryloviana* (Schischk.) Nevski
***Agropyron michnoi* Roshevitz**	–
***Agropyron mongolicum* Keng**	–
Agropyron pumilum Candargy	*Triticum pumilum* Steudel, *non* L.
Agropyron tanaiticum Nevski	–
Australopyrum pectinatum (Labillardiere) A. Löve	*Agropyron pectinatum* (Labillardiere) P. Beauv.; *A. brownei* (Kundt) Tzvelev
***Dasypyrum villosum* (L.) Candargy**	*Agropyron villosum* (L.) Link; *Haynaldia villosa* (L.) Schur
Elymus alaskanus (Scribner &Merr.) A. Löve subsp. *alaskanus*	*Agropyron boreale* (Turcz.) Drobov
Elymus alaskanus subsp. latiglumis (Scribner & J.G. Sm.) A. Löve	*Agropyron latiglume* (Scribner & J.G. Sm.) Rydb
Elymus alatavicus (Drobov) A. Löve	*Agropyron alatavicum* Drobov
Elymus angulatus J. Presl in C. Presl	*Agropyron breviaristus* Hitchc.
Elymus antiquus (Nevski) Tzvelev	*Agropyron antiquus* Nevski; *A. microlepis* Bor
Elymus arizonicus (Scribn. & J.G. Smith) Gould	*Agropyron arizonicum* Scribner & Smith
Elymus batalinii (Krasn.) A. Löve	*Agropyron batalinii* (Krasn.) Roshev.
Elymus burchan-buddae (Nevski) Tzvelev	*Agropyron burchan-buddae* Nevski
Elymus canaliculatus (Nevski) Tzvelev	*Agropyron canaliculatum* Nevski
***Elymus caninus* (L.) L.**	*Agropyron caninum* (L.) P. Beauv.; *A. alpinum* Schur.; *A. pauciflorum* Schur.
***Elymus caucasicus* (C. Koch) Tzvelev**	*Agropyron caucasicum* (C. Koch) Grossh.; *A. roegneri* (Griseb.) Boissier
***Elymus ciliaris* (Trin.) Tzvelev**	*Agropyron ciliare* (Trin.) Franch.
***Elymus dolichatherus* (Keng) A. Löve**	*Roegneria dolichatera* Keng
Elymus drobovii (Nevski) Tzvelev	*Agropyron drobovii* Nevski; *A. turkestanum* Drobov
Elymus enysii (Kirk) A. Löve & Connor	*Agropyron enysii* Kirk; *A. aristatum* Cheeseman
***Elymus fibrosus* (Schrenk) Tzvelev**	*Agropyron fibrosum* (Schrenk) Candargy
Elymus formosanus (Honda) A. Löve	*Agropyron formosanum* Honda
Elymus glaucescens Seberg	*Agropyron pubiflorum* (Steudel) Parodi; *A. antarcticum* Parodi
Elymus gmelinii (Ledeb.) Tzvelev	*Agropyron turczaninovii* Drobov
Elymus grandiglumis (Keng) A. Löve	*Agropyron grandiglume* (Keng) Tzvelev
Elymus hyperarcticus (Polunin) Tzvelev	*Agropyron boreale* var. *hyperarcticum* (Polunin) A. Löve & D. Löve
Elymus jacquemontii (Hooker f.) Tzvelev	*Agropyron jacquemontii* Hooker f
Elymus kengii Tzvelev	*Agropyron kengii* Tzvelev
Elymus lanceolatus (Scribner & Smith) Gould	*Agropyron lanceolatum* Scribner & Smith; *A. dasystachyum* (Hook.) Scribner
Elymus longearistatus (Boiss.) Tzvelev	*Agropyron longearistatum* (Boiss.) Boiss.
Elymus mendocinus (Parodi) A. Löve	*Agropyron mendocinum* Parodi
Elymus multiflorus (Banks & Solander ex Hook. f.) A. Löve & Connor	*Agropyron multiflorum* (Banks & Solander ex Hook. f.) Kirk ex Cheeseman
Elymus mutabilis (Drobov) Tzvelev	*Agropyron mutabilis* Drobov
***Elymus nipponicus* Jaaska**	*Agropyron yezoense* Honda
Elymus parodii Seberg & G. Petersen	*Agropyron condensatum* J. Presl
***Elymus rectisetus* (Nees in Lehm.) A. Löve & Connor**	*Agropyron youngii* (Hook. f.) Candargy

(*continued*)

Table 2.1 (continued)

Current scientific name	Synonym
Elymus repens (L.) Gould	*Agropyron repens* (L.) P. Beauv.
Elymus scabriglumis (Hackel) A. Löve	*Agropyron scabriglume* (Hackel) Parodi
***Elymus scabrus* (R. Br.) A. Löve**	*Agropyron scabrum* (R. Br.) P. Beauv.
***Elymus semicostatus* (Nees ex Steud.) A. Löve**	*Agropyron semicostatum* Nees ex Steud.
Elymus stebbinsii Gould	*Agropyron parishii* Scribner J.G. Smith
Elymus subsecundus (Link.) A. Löve & D. Löve	*Agropyron subsecundum* (Link) Hitchc.
Elymus tianschanicus (Drobov) Nevski	*Agropyron tianschanicum* Drobov; *Elymus tianschanigenus* Czerepanov;
***Elymus tibeticus* (Melderis) G. Singh**	*Agropyron tibeticum* Melderis; *Roegneria tibetica* (Melderis) H. L. Yang
***Elymus trachycaulus* (Link) Gould ex Shinners**	*Agropyron tenerum*Vasey; *A. violaceum* (Hornem.) Lange
Elymus transhyrcanus (Nevski) Tzvelev	*Agropyron transhyranicum* (Nevski) Bondartseva
***Elymus tsukushiensis* Honda**	*Agropyron tsukushiensis* (Honda) Ohwi
Festucopsis festucoides (Maire) A. Löve	*Agropyron festucoides* Maire; *A. pseudofestucoides* Emb
Festucopsis serpentini (C.E. Hubbard) Melderis	*Agropyron kosanii* Cernjav. & Soska in Cernjav.
Leymus chinensis (Trin.) Tzvelev	*Agropyron chinense* (Trin.) Ohwi
***Leymus racemosus* (Lam.) Tzvelev**	*Elymus giganteus* Vahl; *Leymus giganteus* (Vahl) Pilger
Leymus ramosus (Trin.) Tzvelev	*Agropyron ramosum* (Trin.) K. Richter
Leymus secalinus (Georgi) Tzvelev	*Agropyron chinorossicum* Ohwi; *A, berezovcanum* Prodan
Pascopyrum smithii (Rydb.) A. Löve	*Agropyron smithii* Rydberg; *A. occidentalis* (Scribner) Scribner
Peridictyon sanctum (Janka) O. Seberg, S. Frederiksen, & C. Baden	*Agropyron sanctum* (Janka) Hackel ex Formanek
Pseudoroegneria cognata (Hackel) A. Löve	*Agropyron cognatum* Hackel; *A. ferganense* Drobov; *A. dsungaricum* Nevski
***Pseudoroegneria geniculata* (Trin.) A. Löve**	*Agropyron geniculatum* (Trin.) C. Koch
Pseudoroegneria pertenuis (C.A May.) A. Löve	*Agropyron pertenue* (C.A May.) Nevsski
Pseudoroegneria spicata (Pursh) A. Löve	*Agropyron spicatum* (Pursh) Scribner & Smith; *A. divergens* (nees) Vasey;
***Pseudoroegneria stipifolia* (Czern. Ex Nevski) A. Löve**	*Agropyron stipifolium* Czern. Ex Nevski
Pseudoroegneria strigosa (M. Bieb.) A. Löve	*Agropyron strigosum* (M. Bieb.) Boiss; *A. microcalyx* (Regel) Candargy
Pseudoroegneria tauri (Boiss. & Bal.) A. Löve	*Agropyron tauri* Boiss. & Bal.
***Thinopyrum bessarabicum* (Savul. & Rayss) A. Löve**	*Agropyron bessarabicum* Savul. & Rayss
Thinopyrum caespitosum (C. Koch) Z.-W. Liu & R.-C. Wang	*Agropyron caespitosum* C. Koch; *Lophopyrum caespitosum* (c. Koch) A. Löve
***Thinopyrum elongatum* (Host) D.R. Dewey**	*Agropyron elongatum* (Host) P. Beauv; *Triticum elongatum* Host; *Lophopyrum elongatum* (Host) A. Löve
***Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey**	*Agropyron intermedium* (Host) P. Beauv; *Elytrigia intermedia* (Host) Nevski
***Thinopyrum junceiforme* (A. Löve & D. Löve) A. Löve**	*Agropyron junceum* ssp. *boreoatlandicum* Simonet & Guinochet
***Thinopyrum junceum* (L.) A. Löve**	*Agropyron junceum* (L.) P. Beauv.
Thinopyrum nodosum (Nevski) D.R. Dewey	*Agropyron nodosum* Nevski; *Lophopyrum nodosum* (Nevski) A. Löve
***Thinopyrum ponticum* (Podp.) Barkworth & D.R. Dewey**	*Agropyron elongatum* (Host) P. Beauv.; *Elytrigia pontica* (Podp.) Holub; *Lophopyrum ponticum* (Podp.) A. Löve
Thinopyrum scirpeum (K. Presl) D.R. Dewey	*Agropyron scirpeum* K. Presl; *Lophopyrum scirpeum* (K. Presl) A. Löve

levels, spanning the natural distribution range of the complex from central Europe and the Middle East across Central Asia to Siberia, China, and Mongolia (Tsvelev 1976; Dewey 1986). The diploids are distributed across the same range; however, they occur much more sporadically. Although the hexaploids are rare, they are known to occur in Turkey, Iran, and Kazakhstan (Dewey and Asay 1975). Diploid, tetraploid, and hexaploid species in this genus are all based on the basic genome-**P**, thus forming a common gene

Table 2.2 Species in the genus *Psathyrostachys*. Species that had been hybridized with wheat are in bold-face

Current scientific name	Synonym	Agricultural status
P. caduca (Boiss.) Melderis	*Elymus caducus* Boiss.	–
P. daghestanica (Alexeenko) Nevski	*Elymus daghestanicus* Alexeenko; *Hordeum daghestanicum* (Alexeenko) Alexeenko	–
P. fragilis* (Boiss.) Nevski subsp. *fragilis	*Hordeum fragile* Boiss.; *Elymus fragilis* (Boiss.) Griseb.	–
P. fragilis subsp. *secaliformis* Tzvelev	*Elymus secaliformis* Trin. ex Steud.	–
P. fragilis subsp. *villosus* C. Baden	*Elymus junceus var. villosus* Drobov	–
***P. huashanica* Keng**	–	Model plant
***P. juncea* (Fisch.) Nevski**	*Elymus junceus* Fischer; *E. altaicus* Sprengel; *E. desertorum* Kar. & Kir.; *E. albertii* Regel	Forage
P. juncea var. *hyalantha* (Rupr.) S.L. Chen	*Elymus hyalanthus* Rupr.	–
P. kronenburgii (Hack.) Nevski	*Hordeum kronenburgii* Hackel; *Elymus kronenburgii* (Hackel) Nikif.	–
P. lanuginosa (Trin.) Nevski	*Elymus lanuginosus* Trin.; *Hordeum lanuginosum* (Trin.) Schenk	Model plant
P. perennis Keng	–	–
P. rupestris (Alexeenko) Nevski	*Hordeum rupestre* Alexeenko	–
P. stoloniformis C. Baden	–	–

Table 2.3 Geographic distribution of some perennial Triticeae species. Species that had been hybridized with wheat are in bold-face

Current scientific name	Distribution	Agricultural status
Agropyron badamense Drob.	Central Asia	–
Agropyron cimmericum Nevski	Endemic to the Black Sea and Caucasus regions	–
***Agropyron cristatum* (L.) Beauv.**	Eastern Europe, western Siberia, eastern Siberia, Far East, Mongolia, China	Forage
Agropyron dasyanthum Ledeb.	Eastern Europe, along the Dniepr and Molochnaya Rivers	–
***Agropyron desertorum* (Fischer ex Link) Schultes**	Eastern Europe, Caucasus. western Siberia, Central Asia, Mongolia, China	Forage
Agropyron fragile (Roth) Candargy	Eastern Europe, Caucasus; western Siberia, Central Asia, Mongolia, China	Forage
Agropyron krylovianum Schischk.	Western Siberia, eastern Siberia, Central Asia	–
***Agropyron michnoi* Roshevitz**	Eastern Siberia, Mongolia, China	–
***Agropyron mongolicum* Keng**	China (Gansu, Nei Mongol, Ningxia, Shaanxi, Shanxi, Xinjiang)	Forage
Agropyron pumilum Candargy	Endemic to the banks of the Enisei River, eastern Siberia	–
Agropyron tanaiticum Nevski	Eastern Europe, endemic to the Volga and Don river basins	–
Australopyrum pectinatum (Labillardiere) A. Löve	Australia, New Zealand	–
***Dasypyrum villosum* (L.) Candargy**	Southern Europe to Turkey, the Crimea, Caucasus	Model plant
Elymus alaskanus (Scribner &Merr.) A. Löve subsp. *alaskanus*	Alaska, Canada (Yukon, Northwest Territories)	–
Elymus alaskanus subsp. latiglumis (Scribner & J.G. Sm.) A. Löve	Canada (Alberta, BC) USA (Alaska, Idaho, Washington, Montana, Wyoming)	–
Elymus alatavicus (Drobov) A. Löve	Kazakhstan, Kyrgyzstan, Tajikistan, Mongolia, China (Gansu, Xinjiang, Tibet)	–
Elymus angulatus J. Presl in C. Presl	Peru south to Tierra del Fuego, east to western Argentina and Bolivia	–
Elymus antiquus (Nevski) Tzvelev	Western and northwestern China, Nepal	–
Elymus arizonicus (Scribn. & J.G. Smith) Gould	USA (AZ, CA, NM, TX), Mexico	–
Elymus batalinii (Krasn.) A. Löve	China (Xinjiang, Tibet), Mongolia, Russia	Model plant

(*continued*)

Table 2.3 (continued)

Current scientific name	Distribution	Agricultural status
Elymus burchan-buddae (Nevski) Tzvelev	China, India, maybe in Nepal	–
Elymus canaliculatus (Nevski) Tzvelev	Alai, Iran, Kashmir, Pakistan, Pamir, Tadzhikistan, Tibet	–
***Elymus caninus* (L.) L.**	China, Kazakhstan, Kyrgyzstan, Russia, Turkmenistan, Uzbekistan; SW Asia, Europe	–
***Elymus caucasicus* (C. Koch) Tzvelev**	Daghestan, Turkmenia, northern Iran	–
***Elymus ciliaris* (Trin.) Tzvelev**	China, Japan, Korea, Mongolia, Russia	Model plant
***Elymus dolichatherus* (Keng) A. Löve**	Southwestern China	–
Elymus drobovii (Nevski) Tzvelev	Asia-temperate: Soviet Middle Asia	–
Elymus enysii (Kirk) A. Löve & Connor	New Zealand	–
***Elymus fibrosus* (Schrenk) Tzvelev**	Finland, Russian Federation	–
Elymus formosanus (Honda) A. Löve	Taiwan	–
Elymus glaucescens Seberg	Southern Chile and Argentina	–
Elymus gmelinii (Ledeb.) Tzvelev	China, Japan, Kazakhstan, Korea, Kyrgyzstan, Mongolia, Russia, Turkmenistan, Uzbekistan	–
Elymus grandiglumis (Keng) A. Löve	China (Qinghai)	–
Elymus hyperarcticus (Polunin) Tzvelev	USA (Alaska); Russia (Arctic)	–
Elymus jacquemontii (Hooker f.) Tzvelev	Western Tibet and the Himalayas, above 3,900 m	–
Elymus kengii Tzvelev	China	–
Elymus lanceolatus (Scribner & Smith) Gould	USA, Canada	–
Elymus longearistatus (Boiss.) Tzvelev	Gissar-Darvaz, Iran, Tadzhikistan	Model plant
Elymus mendocinus (Parodi) A. Löve	Argentina (endemic around Mendoza)	–
Elymus multiflorus (Banks & Solander ex Hook. f.) A. Löve & Connor	New Zealand	–
Elymus mutabilis (Drobov) Tzvelev	Mongolia, Russia; C and SW Asia, Europe, China	–
***Elymus nipponicus* Jaaska**	North and Northeast Asia	–
Elymus parodii Seberg & G. Petersen	Southern Brazil, Uruguay, and northern Argentina	–
***Elymus rectisetus* (Nees in Lehm.) A. Löve & Connor**	Australia	Model plant
Elymus repens (L.) Gould	China, India, Japan, Korea, Mongolia, Russia; C and SW Asia, Europe	Weed
Elymus scabriglumis (Hackel) A. Löve	Argentina	–
***Elymus scabrus* (R. Br.) A. Löve**	Australia	Model plant
***Elymus semicostatus* (Nees ex Steud.) A. Löve**	Afghanistan, India, Nepal, Pakistan	–
Elymus stebbinsii Gould	California	–
Elymus subsecundus (Link.) A. Löve & D. Löve	USA, Canada	–
Elymus tianschanicus (Drobov) Nevski	China, Kazakhstan, Kyrgyzstan, Turkmenistan, Uzbekistan	–
***Elymus tibeticus* (Melderis) G. Singh**	China (Tibet, Yunnan), Bhutan	–
***Elymus trachycaulus* (Link) Gould ex Shinners**	North America (USA, Canada, Mexico)	–
Elymus transhyrcanus (Nevski) Tzvelev	Iran and Central Asia	–
***Elymus tsukushiensis* Honda**	China, Korea, Japan	–
Festucopsis festucoides (Maire) A. Löve	Morocco (High Atlas)	–
Festucopsis serpentini (C.E. Hubbard) Melderis	Albania	–
Leymus chinensis (Trin.) Tzvelev	China, Korea, Mongolia, Russia	Forage
***Leymus racemosus* (Lam.) Tzvelev**	China (Xinjiang), Kazakhstan, Kyrgyzstan, Mongolia, Russia, Turkmenistan, Uzbekistan	Forage
Leymus ramosus (Trin.) Tzvelev	China (Xinjiang), Mongolia, Russia (W Siberia); Europe	–
Leymus secalinus (Georgi) Tzvelev	China, India, Japan, Korea, Kazakhstan, Kyrgyzstan, Mongolia, Russia, Turkmenistan, Uzbekistan	–

(*continued*)

Table 2.3 (continued)

Current scientific name	Distribution	Agricultural status
Pascopyrum smithii (Rydb.) A. Löve	USA	Forage
Peridictyon sanctum (Janka) O. Seberg, S. Frederiksen, & C. Baden	Eastern Greece and southern Bulgaria	–
Pseudoroegneria cognata (Hackel) A. Löve	Russia, Jungaria-Kashgaria, Kashmir, China (Xinjiang, Tibet)	–
***Pseudoroegneria geniculata* (Trin.) A. Löve**	Western Siberia (Altai), eastern Siberia (Agara-Sayan. Mongolia)	–
Pseudoroegneria pertenuis (C.A May.) A. Löve	Northwest Iran and Caucasus	Model plant
Pseudoroegneria spicata (Pursh) A. Löve	Western North America	Forage
***Pseudoroegneria stipifolia* (Czern. Ex Nevski) A. Löve**	Russia, Ukraine, Caucasus	Model plant
Pseudoroegneria strigosa (M. Bieb.) A. Löve	Russia Crimea	Model plant
Pseudoroegneria tauri (Boiss. & Bal.) A. Löve	Turkey, Iraq, Iran, Syria	Model plant
***Thinopyrum bessarabicum* (Savul. & Rayss) A. Löve**	Turkey, Moldova, Ukraine, Bulgaria, Romania [Black Sea shores]	Model plant
Thinopyrum caespitosum (C. Koch) Z.-W. Liu & R.-C. Wang	Iran, Iraq, Syria, Turkey, Armenia; Azerbaijan, Turkmenistan, Pakistan, Ukraine, France	Model plant
***Thinopyrum elongatum* (Host) D.R. Dewey**	Eastern and southern Europe, Caucasus, western Asia, northern Africa	Model plant
***Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey**	Iran, Iraq, Syria, Turkey, Caucasus, Soviet Middle Asia, Pakistan, Europe	Forage
***Thinopyrum junceiforme* (A. Löve & D. Löve) A. Löve**	Europe	Model plant
***Thinopyrum junceum* (L.) A. Löve**	Southern Europe, western Asia, northern Africa, naturalized in North America	–
Thinopyrum nodosum (Nevski) D.R. Dewey	Ukraine	Model plant
***Thinopyrum ponticum* (Podp.) Barkworth & D.R. Dewey**	Eastern and southern Europe, Caucasus, western Asia, northern Africa	Forage
Thinopyrum scirpeum (K. Presl) D.R. Dewey	Southern Europe (Albania; Greece [incl. Crete]; Italy – Sicily; Yugoslavia, Spain), Algeria, Turkey	Model plant

pool in which gene flows occur between the three ploidy levels (Asay and Dewey 1979).

2.2.2.2 *Pseudoroegneria*

This genus consists of about 15 species that are distributed throughout the Northern hemisphere; *Pseudoroegneria spicata* is the only North American species with the remainder indigenous to Eurasia. The genus consists of diploid ($2n = 2x = 14$) and tetraploid ($2n = 4x = 28$) taxa, all of which contain the **St**-genome or some variation of it. The polyploid races of *Pseudoroegneria* are autoploids (**StSt**) or near-autoploids ($\mathbf{St_1St_2}$). Some tetraploid *Pseudoroegneria* species such as *P. tauri* (Bioss. & Bal.) Á. Löve (Wang et al. 1986) and *P. deweyi* K.B. Jensen, S.L. Hatch, & J.K. Wipff (Jensen et al. 1992) contain **St**- and **P**-genomes. These species have subsequently been treated in a newly erected genus *Douglasdeweya* as *D. wangyii* C. Yen, J.L. Yang & B.R. Baum and *D. deweyi* (K. B. Jensen, S.L. Hatch & J.K. Wipff) C. Yen, J.L. Yang & B.R. Baum, respectively (Yen et al. 2005a). Relationships among *Douglasdeweya*, *Pseudoroegneria*, and *Agropyron* have been clearly shown in a study of their 5S ribosome DNA sequences (Baum et al. 2008).

2.2.2.3 *Psathyrostachys*

Species in this genus are indigenous to the steppes and semi-desert regions from western Russia and Turkey eastward to Siberia and China; all *Psathyrostachys* species contain the basic **Ns**-genome. Among 10 *Psathyrostachys* species listed (Table 2.5), only *P. juncea* (Fisch.) Nevski (Russian wildrye) is

cultivated in North America; and six species, of which three are endemic, are found in China. Species of *Psathyrostachys* are predominately diploids with tetraploids reported in the former Soviet Union.

2.2.2.4 *Elymus*

Elymus is by far the largest genus of the Triticeae when defined according to genome content (Dewey 1984). It contains approximately 150 species that have the pivotal **St**-genome in combination with one or more of **H**-, **Y**-, **W**-, or **P**-genomes (Wang et al. 1995). Geographically, these grasses occur in the temperate regions of both hemispheres, mainly in Asia; of these, 88 species (62 endemic) are in China, while 32 species are native and seven are non-native to North America. Yen et al. (2005b) divided *Elymus* into six genera strictly based on genome compositions – *Douglasdeweya* C. Yen, J.L. Yang & B.R. Baum (**PPStSt**), *Roegneria* C. Koch (**StStYY**), *Anthosachne* Steudel (**StStWWYY**), *Kengylia* C. Yen & J.L. Yang (**PPStStYY**), *Campeiostachys* Drob. (**HHStStYY**), and *Elymus* L. (**StStHH**, **StStStHH,** and **StStHHHH**). The **Y**-genome has not been traced to any existing diploid species. However, recent reports suggest that **Y**- and **St**-genomes shared a common ancestral genome (Liu et al. 2006; Okito et al. 2009).

2.2.2.5 *Leymus*

Leymus is a polyploid genus of about 50 species that are found in temperate regions of the northern hemisphere; 24 species (11 endemic) occur in China and 17 species (11 native, four introduced, and two natural hybrids) are present in North America. All *Leymus* species are based on the **Ns**-genome of *Psathyrostachys* and the **Xm**-genome of an unknown origin (Wang and Jensen 1994; Jensen and Wang 1997; Zhang et al. 2006; Liu et al. 2008). Although several reports suggested that tetraploid *Leymus* species are near autopolyploids having the $\mathbf{Ns_1Ns_2}$-genome formula (Zhang and Dvořák 1991; Anamthawat-Jónsson and Bödvarsdóttir 2001; Bödvarsdóttir and Anamthawat-Jónsson 2003), more recent studies support the conclusion that the genomic constitution of tetraploid *Leymus* species is **NsXm** (Zhang et al. 2006; Liu et al. 2008; Fan et al. 2009; Larson et al. 2009). Still further, data obtained by Fan et al. (2009) suggest that the **Xm**-genome might have derived ancestrally from the **P** of *Agropyron* and **F** of *Eremopyrum triticeum* (Gaertn.) Nevski. Because adaptive radiation might have occurred in *Leymus* species (Fan et al. 2009), the rich diversity and ecological adaptation of *Leymus* species observed by Yang et al. (2008) could be accounted for.

2.2.2.6 *Thinopyrum*

Contained within this genus are about 20 species (Dewey 1984). These species are indigenous to Europe, particularly in the Mediterranean region, western Asia, and northern Africa. The most common introduced grasses are intermediate and tall wheatgrass in North America. *Thinopyrum* as treated herein consists of three species complexes: *Th. junceum* (L.) A. Löve, *Th. elongatum* (Host) D.R. Dewey; and *Th. intermedium*. Species in this genus possess the **J**- (or **E**)-genome, which Dewey (1984) designated as "**J** = **E**," and sometimes contains the **St**-genome (Liu and Wang 1993a, b; Kishii et al. 2005). This genus consists of diploids, segmental allotetraploids, segmental allohexaploids, and genomically complex octaploids and decaploids.

2.2.3 Genome Size

The Triticeae tribe is characterized with large chromosomes in groups of seven, i.e., diploid is $2n = 2x = 14$ and decaploid is $2n = 10x = 70$. Each group of seven chromosomes is represented by a genome symbol so that the haplome of a diploid *Agropyron* species is shown as **P** and that of a tetraploid species is **PP**. Genome symbols follow the designations by the International Triticeae Consortium (Wang et al. 1995). The nuclear DNA content of various species (included in Tables 2.4 and 2.5) was reported by Vogel et al. (1999). The mean nuclear DNA content on a diploid basis (DNA pg/2C) were as follows: *Agropyron* (**P**) 13.9 pg, *Pseudoroegneria* (**St**) 8.8 pg, *Psathyrostachys* (**Ns**) 16.7 pg, and *Thinopyrum* genomes ($\mathbf{E^b}$) 14.9 pg

Table 2.4 Chromosome number, genome constitution and DNA content of important perennial Triticeae species including those (in bold) that had been hybridized with wheat

Current scientific name	2*n*	Haplome[a]	DNA (pg/2C)[b]
***Agropyron cristatum* (L.) Beauv.**	14–42	**P to PPP**	13.63
Agropyron dasyanthum Ledeb.	28	**PP**	–
***Agropyron desertorum* (Fischer ex Link) Schultes**	28	**PP**	25.92
Agropyron fragile (Roth) Candargy	14, 28	**P, PP**	–
***Agropyron michnoi* Roshevitz**	28	**PP**	–
***Agropyron mongolicum* Keng**	14	**P**	15.37
Agropyron tanaiticum Nevski	28	**PP**	–
Australopyrum pectinatum (Labillardiere) A. Löve	14	**W**	–
***Dasypyrum villosum* (L.) Candargy**	14	**V**	–
Elymus abolinii (Drob.) Tzvelev	28	**StY**	18.7
Elymus alaskanus (Scribner & Merr.) A. Löve subsp. *alaskanus*	28	**StH**	–
Elymus alaskanus subsp. latiglumis (Scribner & J.G. Sm.) A. Löve	28	**StH**	–
Elymus alatavicus (Drobov) A. Löve	42	**StPY**	30.31
***Elymus altissimus* (Keng) A. Löve**	28	**StY**	–
***Elymus anthosachnoides* (Keng) A. Löve**	28	**StY**	–
Elymus antiquus (Nevski) Tzvelev	28	**StY**	–
Elymus arizonicus (Scribn. & J.G. Smith) Gould	28	**StH**	–
Elymus batalinii (Krasn.) A. Löve	42	**StPY**	–
Elymus burchan–buddae (Nevski) Tzvelev	28	**StY**	–
***Elymus canadensis* L.**	28	**StH**	21.11
Elymus canaliculatus (Nevski) Tzvelev	28	**StY**	–
***Elymus caninus* (L.) L.**	28	**StH**	17.87
***Elymus caucasicus* (C. Koch) Tzvelev**	28	**StY**	–
***Elymus ciliaris* (Trin.) Tzvelev**	28	**StY**	17.33
***Elymus cylindricus* (Franch.) Honda**	42	**StHY**	–
***Elymus dahuricus* Turez ex Griseb**	42	**StHY**	25.79
***Elymus dolichatherus* (Keng) A. Löve**	28	**StY**	–
Elymus drobovii (Nevski) Tzvelev	42	**StHY**	–
Elymus enysii (Kirk) A. Löve & Connor	28	**HW**	–
***Elymus fibrosus* (Schrenk) Tzvelev**	28	**StH**	–
Elymus glaucus Buckl.	28	**StH**	18.48
Elymus gmelinii (Ledeb.) Tzvelev	28	**StY**	–
Elymus grandiglumis (Keng) A. Löve	42	**StPY**	–
Elymus jacquemontii (Hooker f.) Tzvelev	28	**StY**	–
***Elymus kamoji* (Ohwi) S. L. Chen**	42	**StHY**	–
Elymus kengii Tzvelev	42	**StPY**	–
Elymus lanceolatus (Scribner & Smith) Gould	28	**StH**	16.71
Elymus longearistatus (Boiss.) Tzvelev	28	**StY**	–
Elymus multiflorus (Banks & Solander ex Hook. f.) A. Löve & Connor	42	**StWY**	–
Elymus mutabilis (Drobov) Tzvelev	28	**StH**	16.96
***Elymus nipponicus* Jaaska**	28	**StY**	–
***Elymus parviglumis* (Keng) A. Löve**	28	**StY**	–
***Elymus pendulinus* (Nevski) Tzvelev**	28	**StY**	–
***Elymus rectisetus* (Nees in Lehm.) A. Löve & Connor**	42	**StWY**	–
***Elymus repens* (L.) Gould**	42	**StStH**	–
Elymus scabriglumis (Hackel) A. Löve	42	**StStH**	–
***Elymus scabrus* (R. Br.) A. Löve**	42	**StWY**	–
***Elymus semicostatus* (Nees ex Steud.) A. Löve**	28	**StY**	–
***Elymus shandongensis* B. Salomon**	28	**StY**	–
Elymus sibiricus L.	28	**StH**	16.61
Elymus stebbinsii Gould	28	**StH**	–
Elymus subsecundus (Link.) A. Löve & D. Löve	28	**StH**	–

(*continued*)

Table 2.4 (continued)

Current scientific name	2*n*	Haplome[a]	DNA (pg/2C)[b]
Elymus tianschanicus (Drobov) Nevski	28	**StY**	–
***Elymus tibeticus* (Melderis) G. Singh**	28	**StY**	–
***Elymus trachycaulus* (Link) Gould ex Shinners**	28	**StH**	18.14
Elymus transhyrcanus (Nevski) Tzvelev	42	**StStH**	–
***Elymus tschimganicus* (Drobov) Tzvelev**	42	**StStY**	–
***Elymus tsukushiensis* Honda**	42	**StHY**	–
Elytrigia acutum	42	–	–
***Elytrigia varnense* (Velen.) Holub**	42	–	–
***Elytrigia pungens* (Pers.) Tutin**	56	**EStStP or EStLP**	–
Festucopsis festucoides (Maire) A. Löve	14	**L**	–
Festucopsis serpentini (C.E. Hubbard) Melderis	14	**L**	–
***Leymus angustus* (Trin.) Pilger**	56, 84	**NsNsXmXm**	–
Leymus chinensis (Trin.) Tzvelev	28	**NsXm**	19.56
***Leymus cinereus* (Scribn. & Merr.) A. Löve**	28	**NsXm**	–
***Leymus innovatus* (Beal) Pilger**	28	**NsXm**	–
***Leymus mollis* (Trin.) Pilger**	28	**NsXm**	–
***Leymus multicaulis* (Kar. & Kir.) Tzvelev**	28	**NsXm**	–
***Leymus racemosus* (Lam.) Tzvelev**	28	**NsXm**	22.36
Leymus ramosus (Trin.) Tzvelev	28	**NsXm**	20.31
Leymus sabulosus (M. Bieb.) Tzvelev	28	**NsXm**	22.85
Leymus secalinus (Georgi) Tzvelev	28	**NsXm**	21.47
***Leymus triticoides* (Buckl.) Pilger**	28	**NsXm**	21.87
Pascopyrum smithii (Rydb.) A. Löve	56	**StHNsXm**	34.33
Peridictyon sanctum (Janka) O. Seberg, S. Frederiksen, & C. Baden	14	**Xp**	–
Pseudoroegneria cognata (Hackel) A. Löve	14	**St**	–
***Pseudoroegneria geniculata* (Trin.) A. Löve**	28	**StSt**	17.22
Pseudoroegneria libanotica (Hackel D.R. Dewey)	14	**St**	7.91
Pseudoroegneria pertenuis (C.A May.) A. Löve	28	**StP**	–
Pseudoroegneria spicata (Pursh) A. Löve	14	**St**	9.26
***Pseudoroegneria stipifolia* (Czern. Ex Nevski) A. Löve**	14	**St**	8
***Pseudoroegneria strigosa* (M. Bieb.) A. Löve**	14	**St**	9.59
Pseudoroegneria tauri (Boiss. & Bal.) A. Löve	14	**St**	–
***Thinopyrum bessarabicum* (Savul. & Rayss) A. Löve**	14	**J or E^b**	14.92
Thinopyrum caespitosum (C. Koch) Z.-W. Liu & R.-C. Wang	28	**ESt**	19.88
***Thinopyrum curvifolium* (Lange) D.R. Dewey**	28	**EE or J^eJ^e**	–
***Thinopyrum distichum* (Thunb.) A. Löve**	28	**JE or E^bE^e**	–
***Thinopyrum elongatum* (Host) D.R. Dewey**	14	**E or J^e**	11.97
***Thinopyrum gentryi* (Melderis) D.R. Dewey**	42	**$EE^{st}St$ or ESt(V-J-R)**	–
***Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey**	42	**$EE^{st}St$ or ESt(V-J-R)**	26.09
***Thinopyrum junceiforme* (A. Löve & D. Löve) A. Löve**	28	**JE or E^bE^e**	24.79
***Thinopyrum junceum* (L.) A. Löve**	42	**JJE or $E^bE^bE^e$**	–
Thinopyrum nodosum (Nevski) D.R. Dewey	28	**ESt**	–
***Thinopyrum ponticum* (Podp.) Barkworth & D.R. Dewey**	70	**$EEEE^{st}E^{st}$ or EEEStSt**	45.26
***Thinopyrum sartorii* (Boiss. & Heldr.) A. Löve**	28	**JE or E^bE^e**	–
Thinopyrum scirpeum (K. Presl) D.R. Dewey	28	**EE or J^eJ^e**	–
***Elymus repens*/*Agropyron desertorum* amphiploid**	70	**StStHPP**	–

[a]Wang et al. (1995)
[b]Vogel et al. (1999)

and (**E^e**) 12.0 pg (Vogel et al. 1999). Based on the difference, the **Y**-genome size was determined to be 9.3 pg. Thus, Vogel et al. (1999) concluded that gain or loss of nuclear DNA has occurred during the evolution of the perennial Triticeae and was probably part of speciation.

Table 2.5 Geographic distribution, chromosome number, genome constitution and DNA content of *Psathyrostachys* species. Species that had been hybridized with wheat are in bold-face

Current scientific name	Distribution	2*n*	Haplome[a]	DNA (pg/2C)[b]
P. caduca (Boiss.) Melderis	Afghanistan, Asia, Europe	–	–	–
P. daghestanica (Alexeenko) Nevski	Dagestan	14, 28	**Ns, NsNs**	–
P. fragilis* (Boiss.) Nevski subsp. *fragilis	Iran, Russia	14	**Ns**	16.79
P. fragilis subsp. *secaliformis* Tzvelev	Caucasus, Iran, Iraq, Turkey, Russia	28	**Ns,Ns**	–
P. fragilis subsp. *villosus* C. Baden	Turkey, Russia	14	**Ns**	–
***P. huashanica* Keng**	China (Shaanxi)	14	**Ns**	–
***P. juncea* (Fisch.) Nevski**	Afghanistan, Russia, C. Asia, China	14	**Ns**	15.57
P. juncea var. *hyalantha* (Rupr.) S.L. Chen	China (Xinjiang), C. Asia, Russia	–	–	–
P. kronenburgii (Hack.) Nevski	Russia, China (Xinjiang), Kazakhstan, Kyrgyzstan, Tadzhikistan, C. Asia	14	**Ns**	–
P. lanuginosa (Trin.) Nevski	Afghanistan, China (Xinjiang), Kazakhstan, Kyrgyzstan, C. Asia, W. Siberia	14, 28	**Ns, NsNs**	–
P. perennis Keng	China	14	**Ns**	–
P. rupestris (Alexeenko) Nevski	Dagestan, Russia	14, 42	**Ns**	–
P. stoloniformis C. Baden	China (Gansu, Qinghai)	14	**Ns**	17.88

[a]Wang et al. (1995)
[b]Vogel et al. (1999)

2.2.4 Cytology and Karyotype

Karyotypes in 22 diploid species of perennial Triticeae, representing **P**-, **St**-, **J** (=**E**)-, **H**-, **I**-, **Ns**-, **W**-, and **R**-genomes, had been studied (Hsiao et al. 1986). Each basic genome manifests a unique karyotypic pattern for its seven chromosomes. Prior to this, C-banding patterns were studied in 10 diploid species encompassing five basic genomes – **P**, **Ns**-, **J** (=**E**)-, **St**-, and **W**- (designated as C, Ju, J = E, S, and V, respectively, in Endo and Gill 1984). Based on differences in C-banding patterns, Endo and Gill (1984) questioned the equivalence of **J**- and **E**-, which was a viewpoint at that time shared by Dewey (1984) and Dvořák et al. (1984) based on extensive evidence from karyotype and genome analyses (Cauderon and Saigne 1961; Heneen and Runemark 1972; Dvořák 1981; McGuire 1984).

Since then, the close relationship between **J**- and **E**-genome in *Th. bessarabicum* (Savul. and Rayss) A Löve and *Th. elongatum* (Host) D.R. Dewey (= *Lophopyrum elongatum* (Host) A. Löve), respectively, had been revealed by studies using different methodologies, including chromosome pairing (Wang 1985; Pienaar 1988; Forster and Miller 1989; Wang and Hsiao 1989), random amplified polymorphic DNA (RAPD) and sequence tagged site (STS) markers (Wei and Wang 1995; Li et al. 2007), genomic in situ hybridization (GISH) technique (Kosina and Heslop-Harrison 1996; Chen et al. 1998, 2003), chloroplast DNA sequences (Mason-Gamer et al. 2002; Liu et al. 2008), sequences of a gene encoding plastid acetyl-CoA carboxylase (Fan et al. 2007), and nuclear rDNA internal transcribed spacer (ITS) sequences (Hsiao et al. 1995; Liu et al. 2008; Yu et al. 2008). But the use of one basic genome symbol for these two species and/or the merge of them into the same genus were rejected by some researchers (Jauhar 1988, 1990a, b; Jarvie and Barkworth 1992; Hsiao et al. 1995; Jauhar et al. 2004). Only Fan et al. (2009) used the same basic genome symbol while keeping the two genera delimitation.

Forster and Miller (1989) pointed out that the relationship between **J**- and **E**-genomes is similar to that between $\mathbf{R^c}$ and $\mathbf{R^m}$ (genomes of *S. cereale* L. and *S. montanum* Guss., respectively); and they concluded that, on the basis of chromosome pairing in the diploid hybrids reported by Wang (1985), the **J**-genome in *Th. bessarabicum* should be designated $\mathbf{E^b}$, as proposed by Dvořák (1981) and McGuire (1984). At the second International Triticeae Symposium, the Genome Designation Committee (Wang et al. 1995) adopted the symbols $\mathbf{E^e}$ and $\mathbf{E^b}$ for the genome in *Th. elongatum* and *Th. bessarabicum*, respectively. However, some Triticeae workers preferred to use $\mathbf{J^e}$ and $\mathbf{J^b}$ for genomes in these two species (Chen et al. 1998, 2003; Li et al. 2003) while others transferred *Th. bessarabicum* to the genus *Lophopyrum* so that it could have the

symbol $\mathbf{E^b}$ along with $\mathbf{E^e}$ for *L. elongatum* (Yen et al. 2005b; Yu et al. 2008).

2.3 Role in Elucidation of Origin and Evolution of Allied Crop Plants

Perennial Triticeae species are related to important cereal crops, including wheat, barley, rye, and man-made triticale (×*Triticosecale* Wittm. Ex A. Camus). Studies on evolution of these annual crop species often included perennial Triticeae species, or vice versa; thus, the literature, such as the report by Petersen et al. (2006), was useful in understanding the evolution of both groups of species.

The *Thinopyrum bessarabicum/Th. elongatum* amphidiploid was originally synthesized by Wang (Wang and Hsiao 1989; Wang 2006). Based on his analysis on some of these amphidiploid plants, Jauhar (1988) disputed Wang's (1985) conclusion on the close relationship between the two diploid *Thinopyrum* species. Jauhar et al. (2004) further studied the trigeneric hybrids, with and without *Ph1*, of durum wheat with the amphidiploid of *Th. bessarabicum/Th. elongatum*. Their data revealed that without the *Ph1* in the $\mathbf{ABE^bE^e}$ hybrid, there were 3.97 chiasmata between (and among) $\mathbf{E^b}$- and $\mathbf{E^e}$-genome chromosomes, compared to 2.29 chiasmata between (and among) **A**- and **B**-genome chromosomes. Thus, the two **E**-genomes are more closely related to each other than **A** and **B** are to each other. Interestingly, the average *c* value (mean arm-pairing frequency; Alonso and Kimber 1981) for the diploid **AB** hybrids was 0.319 (cited in Wang 1990), so that for $\mathbf{E^bE^e}$ hybrids should be 0.553 ($= 0.319 \times 3.97 \div 2.29$). The observed *c* value for $\mathbf{E^bE^e}$ hybrids was 0.552 (Wang 1985) to 0.597 (Jauhar 1988). As demonstrated in many studies, chromosome pairing in diploid hybrids does reveal the true genome relationships (Forster and Miller 1989; Wang 1989b, 1992).

Using genomic hybridization (both Southern and in situ hybridization), Liu et al. (2007) demonstrated that the **St** and **E**-genomes are closely related and also that the two are more closely related to the **D**-genome than to the **A**- and **B**-genomes. Among the three wheat genomes, **A**- and **D**-genomes are closer with each other than either one is to **B**-genome. These conclusions are in agreement with those based on chromosome pairing data obtained from intergenomic diploid hybrids and their derivatives (Wang 1990, 1992, 1993). The closeness between **St** and **E** was also supported by the study based on the sequences of a gene encoding plastid acetyl-CoA carboxylase (Fan et al. 2007) and 5S rDNA (Shang et al. 2007). The **St** and **J** (=**E**) were also more closely related to the **R**-genome of *Secale* than to the **V**-genome of *Dasypyrum* (Shang et al. 2007). Based on ITS of the nuclear rDNA sequences, Hsiao et al. (1995) reported that **E** (=$\mathbf{E^e}$) and **J** (=$\mathbf{E^b}$) jointly clustered with **A**, **B**, and **D**; but genomes **B**- and **D**- were grouped more closely together than were **A**- and **D**-genomes.

Because of these genome relationships among **St**-, **E**-, and **A**-, **B**-, and **D**-genomes, more cross-hybridization occurred on wheat chromosomes when DNA from the **E**-genome (either $\mathbf{E^b}$ or $\mathbf{E^e}$) was used as the probe in genomic or fluorescence in situ hybridization (GISH or FISH) of wheat–*Thinopyrum* hybrid derivatives. These cross-hybridization signals were often misinterpreted as translocated alien segments in wheat chromosomes. To avoid such interpretation, an adequate probe/block DNA ratio must be selected based on a preliminary test of the negative control using the same wheat line used for alien gene transfer. Using the total genomic DNA of *Th. intermedium* [$2n = 6x = 42$; $\mathbf{E^bE^eSt}$ (Liu and Wang 1993a); or $\mathbf{E^eSt(V\text{-}J\text{-}R)}$ (Kishii et al. 2005)] or *Th. ponticum* [$2n = 10x = 70$; $\mathbf{EEEE^{st}E^{st}}$ (Chen et al. 1998); or $\mathbf{E^bE^eE^xStSt}$ (Liu et al. 2007)] as probes for GISH or FISH would often lead to false-positives from cross-hybridizations between **E**-genome probing DNA and **D**- or **A**-genome chromosomes. Therefore, it is advised to use **St** genomic DNA as the probe in GISH or FISH so that only the **St**- and **E**-genome chromosomes or chromosomal segments will be showing hybridization signals (Wang and Zhang 1996; Zhang et al. 1996).

2.4 Role in Development of Cytogenetic Stocks and Their Utility

To utilize alien genes for wheat improvement, the first step is crossing wheat with the alien species followed by the production of amphidiploids through chromosome doubling. Partial amphiploids usually contain a varying combination of chromosomes originating

Table 2.6 Depositories of wheat alien addition, substitution, and translocation lines

Location and contact	Alien species	Amphiploids	Addition lines	Substitution lines	Translocation lines
Wheat Genetic and Genomic Resources Center, Kansan State University, Manhattan, Kansas, USA; wgrc@k-state.edu	*Thinopyrum elongatum*	cv. Chinese Spring/*Th. elongatum*	Chinese Spring disomic addition lines (CS-DA): 7 ($1E^e - 7E^e$); Chinese Spring ditelosomic addition lines (CS-DtA): 5 ($1E^eS$, $3E^eS$, $3E^eL$, $6E^eS$, $6E^eL$); miscellaneous addition lines: 1	Miscellaneous substitution lines: 3 (7E/AD)	–
	Thinopyrum scirpeum	cv. Chinese Spring/*Th. scirpeum*	–	–	–
	Thinopyrum ponticum	cv. PWM206/*Th. ponticum* Partial Amphiploid; cv. PWM206/*Th. ponticum* Partial Amphiploid; cv. PWMIII/*Th. ponticum* Partial Amphiploid; cv. PWM706/*Th. ponticum* Partial Amphiploid; cv. OK7211542/*Th. ponticum* Partial Amphiploid; Triticum aestivum subsp. aestivum/*Th. ponticum* Partial Amphiploid.	–	–	Disomic substitutions in Chinese Spring = 37 (3B/3Ag, 3D/3Ag, and 7D/7Ag translocations from 21 different *Th. elongatum* accessions, two interstitial translocations Ti3D·3Ag; all produced by ER Sears); disomic substitutions in other cultivars = 2 (OK65C77-6 resistant to WSMV, T4B·4E)
	Thinopyrum bessarabicum	Triticum aestivum subsp. *aestivum*/*Th. bessarabicum*	–	–	–
	Thinopyrum distichum	Triticum aestivum subsp. aestivum/*Th. distichum*	–	–	–
	Thinopyrum intermedium	cv. Vilmorin 27/*Th. intermedium* #1; cv. Zhong/*Agropyron intermedium:* 9 lines; cv. Summer/*Th. intermedium:* 2 lines; cv. OK7211542/*Th. intermedium*; cv. Otrastajusca 38/*Th. intermedium*; cv. Otrastajusca 38/*Th. intermedium* Partial Amphiploid; Triticum turdigum subsp. durum cv. Nodak/*Th. intrmedium* Partial Amphiploid: MT1, MT2; Triticum turdigum subsp. durum cv. Nodak/*Th. intrmedium* Partial Amphiploid.	CS-DA: 4 (unknown); Vilmorin 27 disomic addition lines (V27-DA): 6 (1Ai, 3Ai – 7Ai) V27-DtA: 1 (7AiS); Zhong 8423 disomic addition lines (Z8423-DA): 7 (unknown); miscellaneous addition lines: 4	Miscellaneous substitution lines: 6	Disomic substitutions in Sunstar = 7 (1BS/7Ai#1S·7Ai#1L, T7DS-7DL·7Ai#1L, 7DS/7Ai#1S·7Ai#1L (5 lines)); disomic substitutions in Heine IV = 6 (T1DS·1DL-7Ai#2L, T2AS·2AL-7Ai#2L (2 lines), T6DS·6DL-7Ai#2L, T7Ai#2L-3DS·3DL, T7Ai#2L-5AS·5AL); disomic substitutions in other cultivars = 4 (T7DS-7Ai#1L·7Ai#1S and one unknown in Chinese Spring); 6AL·4Ai#2S, T6AS·4Ai#2L, T4DL·4Ai#2S, T7AS-7St#1S·7St#1L in Centurk.

(*continued*)

Table 2.6 (continued)

Location and contact	Alien species	Amphiploids	Addition lines	Substitution lines	Translocation lines
	Elymus ciliaris	*Elymus ciliaris*/*Triticum aestivum* subsp. *aestivum* cv. Inayama-komugi	CS-DA: 9 (1St, 1Y, 2St, 3St, 5Y, 7St; 1St + 5Y = dDA; 1 unknown); CS-DtA: 2 (1YS, 2StL)	–	Elymus ciliaris disomic substitution/addition in Chinese Spring = 2 ($1Y^{c}S$)
	Elymus trachycaulus	–	CS-DA: 7 (1H, 1St, 5H–7H; 1H + 4H = dMA; 5St = MA); CS-DtA: 9 (1HS, 1HL, 1StL, 5HS, 5HL, 7HS, 7HL; 5StS, 5StL = MA)	–	Elymus trachycaulus·E. trachycaulus disomic additions in Chinese Spring = 6 ($1H^{t}S{\cdot}2H^{t}S$, $1H^{t}S{\cdot}5H^{t}L$, $1H^{t}S{\cdot}6H^{t}L$, $1H^{t}S{\cdot}7H^{t}L$, $1St^{t}L{\cdot}7St^{t}L$, $2H^{t}S{\cdot}7H^{t}S$); E. trachycaulus·E. trachycaulus monosomic additions in Chinese Spring = 1 ($1H^{t}L{\cdot}3St^{t}L$); E. trachycaulus disomic substitutions in Chinese Spring = 3 ($1H^{t}S$, $1St^{t}S$, zebra chromosome (z5A)); E. trachycaulus isosomic translocations in Chinese Spirng = 3 ($1H^{t}S{\cdot}1H^{t}S$, $5H^{t}L{\cdot}5H^{t}L$)
	Elymus tsukushiense	–	Miscellaneous addition lines: 3	Miscellaneous substitution lines: 1	–
	Leymus racemosus	–	CS-DA: 7 (2Lr, 5Lr–7Lr; 1Lr 5Lr, 3Lr 7Lr = dDA); CS-DtA: 2 (2LrS, 7LrS)	Miscellaneous substitution lines: 1	–
	Leymus arenarius	*Triticum* sp./*Leymus arenarius*	–	–	–
	Critesion bogdanii	*Triticum timopheevii* subsp. *timopheevii*/*Critesion bogdanii*	–	–	–
	Critesion californicum	*Critesion californicu*/cv. Chinese Spring	–	–	–
	Dasypyrum villosum	*Triticum aestivum*/*D. villosum*	CS-DA: 16 (#1: 1V, 2V, 4V – 7V; #2 3V – 5V; #3: 1V – 7V)	–	–
Tottori Alien Chromosome Bank of Wheat (TACBOW), University of Tottori, 4-101 Koyama-Minami, Tottori 680-8550 Japan; PHONE: 0857-31-5352; FAX: 0857-31-5347	*Leymus racemosus*	–	15 (A, C, E, F, H, I, J, K, L, N, 2Lr, 5Lr, 7Lr, ?)	2 (H, 2Lr)	–
	Leymus mollis	–	3 (A, G, H)	–	–
	Elymus trachycaulus	–	DA: 6 (1H, 1H + 4H, 1St, 5H, 5St, 6H); DtA: 8 (1HS, 1HL, 1StL, 5HS, 5HL, 5StS, 7HS, 7StL); 10 (complex translocation additions)	1 (20″ + T7AL 1AS-1StS); 1 (19″ + 1″ [T7AL 1AS-1StS (7A)] + 1″ [T5DL 7AS (5D)])	1 (20″ + 1″ 1HS.1BL)

	Elymus ciliaris	–	7 (1St, 1Y, 1YS, 2St, 3St, 1St + 5Y, ?Y)	–	–	
	Psathyrostachys huashanica	–	5 (A, B, C, D, E)	–	–	
	Thinopyrum elongatum	*Triticum durum*/*Th. elongatum*	DA: 7 ($1E^e$–$7E^e$); DtA: 6 ($1E^eS$, $3E^eS$, $3E^eL$, $6E^eS$, $6E^eL$, $7E^eL$)	–	–	
	Thinopyrum intermedium	*Triticum durum*/*Th. intermedium*; *Triticum aestivum*/*Th. intermedium*	12 (1Ai, 3Ai, 4Ai, 5Ai, 6Ai, 7Ai, B, C, D, E, F, G)	–	–	
	Dasypyrum villosum	*Triticum durum*/*D. villosum*	7 (1V– 7V)	–	–	
	Hordeum chilense		5 (2H, 4H, 5H, 6H, 7H)	1 (1H)	–	
Forage & Range Research Laboratory (FRRL), USDA-ARS, Logan, Utah, USA; Richard.Wang@ars.usda.gov	*Thinopyrum junceum*	*Triticum aestivum* cv. CS/*Th. junceum* partial amphiploids: 9 ($2n = 56 = 21'' + 7''$)	CS-DA: 13 ($1E^b$-$1E^e$, $2E^b$-$2E^e$, $5E^b$-$5E^e$, $6E^b$-$6E^e$, $7E^b$-$7E^e$, and complex translocations)	–	–	
	Thinopyrum bessarabicum	–	CS-DA: 7 ($1E^b$–$7E^b$)	–	–	
	Thinopyrum elongatum	cv. Chinese Spring/*Th. elongatum*	Chinese Spring disomic addition lines (CS-DA): 7 ($1E^e$–$7E^e$)	–	–	
	Elymus rectisetus	–	*Triticum aestivum* cv. Fukuhokomugi DA: 9 (?W, ?W, 18″ABD + 1″?St + 1″?St +1″?W + 1″?Y, etc.)	Miscellaneous substitution lines: 6 [18″ABD+ ″?St + 1″?St + 1″?W		(2D + 3B + ?A), unknown etc.]
	–					
Dasypyrum villosum	*Triticum aestivum*/*D. villosum*	CS-DA: 7 (1V–7V)	–	–		

from the alien species (Banks et al. 1993; Fedak et al. 2001). Therefore, crossing different partial amphiploids is not advised. Then through a series of backcrosses with wheat, addition, substitution, or translocation lines are developed and subsequently phenotyped for the desired traits. These cytogenetic stocks (Table 2.6) provide essential tools for chromosome engineering in wheat.

Chromosome translocations can result from either spontaneous or induced recombination. Various methods have been used to induce chromosomal recombination: irradiation (Sears 1956), tissue culture (Banks et al. 1995), and homeologous pairing (Wang et al. 1977, 1980, 2003b; Kibirige-Sebunya and Knott 1983; Koebner and Shepherd 1985; Islam and Shepherd 1992; Aghaee-Sarbarzeh et al. 2002). The last method involves the use of N5BT5D (Sears 1966), *Ph1b* deletion mutant (Sears 1977), or Ph inhibitor (Ph^I) line (Chen et al. 1994) to promote homeologous recombination by removing or suppressing the effect of *Ph1* gene on the long arm of chromosome 5B. The latter two wheat lines are more advantageous than the 5B nullisomy in producing translocation lines (Qi et al. 2007).

Wheat translocation lines involving "*Agropyron*" have been characterized and listed for their utilization in agriculture (WGGRC, *http://www.k-state.edu/wgrc/Germplasm/Stocks/Agropyron_translocations.html*; accessed on 29 Oct 2009, 16:19 GMT). Due to deleterious traits associated with transferred alien chromosome segments, i.e., linkage drags, most alien transfers had not led to acceptable wheat cultivars. The majority of alien genes transferred and utilized in wheat cultivars are single genes for disease resistance, especially those to the rusts and viruses.

Marais et al. (2001) used Sears' *ph1b* mutant to induce allosyndetic recombination for further shortening of the *Lr19* translocation segment in line *Lr19*-149, which lacked the deleterious yellow endosperm pigmentation gene. The shortest alien chromosome segment was obtained in the recombinant line *Lr19*-149-299 but still retained the segregation distortion gene *Sd2*.

Dundas et al. (2007) reported the advancements made over the past two decades on improving the usefulness of translocation lines with alien genes for stem rust resistance by eliminating linkage drags. Using *ph1bph1b*-induced homeologous recombination between the alien chromosome segments and normal wheat chromosomes, lines with shortened alien chromatin were identified by dissociation patterns of molecular-based markers. New lines of bread wheat were developed containing 1RS segment with rust resistance gene *SrR* (*S. cereale* L.), 6Ae#1L chromosome segments with *Sr26* (*Th. ponticum*), 2S#1 chromosome segments with *Sr32* and a previously unnamed gene, 2S#2 chromosome segment with *Sr39* (*Triticum speltoides*), 4G#1 chromosome segments with *Sr37*, and 2G#2 chromosome segments with *Sr40* (*T. timopheevii*).

Recently, a disomic addition line ($2n = 44$) derived from *T. aestivum* cv. Fukuhokomugi × *Elymus rectisetus* (Nees in Lehm.) A. Löve & Connor hybrids, which were made aiming to transfer apomixis (see under Sect. 2.6 later), had been identified to have resistance to both tan spot (caused by *Pyrenophora tritici-repentis*) and *Stagonospora nodorum* blotch (SNB; caused by *S. nodorum* Castellani and Germano) (Oliver et al. 2008). Furthermore, Xu et al. (2009) identified seven wheat–*Th. intermedium* amphiploids, one wheat–*Th. ponticum* amphiploid, six durum–*Aegilops speltoides* amphiploids, one wheat–*Th. junceum* disomic addition line, two wheat–*Ae. caudata* disomic addition lines, and a wheat–*Th. bessarabicum* 7J disomic addition line that might carry novel genes for resistance to the stem rust race TTKSK (commonly known as Ug99 or TTKS).

Resistance to barley yellow dwarf virus (BYDV) in *Th. intermedium* is located on two group-7 chromosomes (7**St** and 7**E**) and one group-2 chromosome 2Ai-2 (an **E-St** translocation) in addition lines L1, P107, and Z6, respectively (Banks et al. 1995; Sharma et al. 1995; Wang and Zhang 1996; Lin et al. 2007). Subsequently, genes for BYDV resistance *Bdv2* and *Bdv3* were identified based on translocation lines derived from L1 and P107, respectively (see Table 2.7 in Sect. 2.6 later; Banks et al. 1995; Hohmann et al. 1996; Francki et al. 1997; Crasta et al. 2000; Zhang et al. 2000, 2009; Ayala et al. 2001; Xin et al. 2001; Larkin et al. 2002; Ohm et al. 2005). In addition, BYDV resistance was reported in the disomic addition line Tai-27 (Jiang et al. 2009), which appears to be similar to lines Z1 and Z6 (Larkin et al. 1995; Barloy et al. 2003; Han et al. 2003; Lin et al. 2006), all of which contain the group-2 **St** chromosome in the wheat background. Although BYDV resistance gene *Bdv4* on the group-2 **St** chromosome has not yet been transferred to a wheat chromosome, expressed

Table 2.7 Alien resistance genes for biotic stress transferred to wheat

Diseases or pests	Gene symbol	Alien species	Alien chromosome	Wheat chromosome	Germplasm or cultivars	Molecular markers	References
Leaf rust (*Puccinia recondita* f.sp. *tritici*)	*Lr19*	*Thinopyrum ponticum and Th. distichum*	7Ae#1L	7DL = T7DS.7DL-7Ae#1L in Agatha and Sears trasfer 7D-7Ag no.1; 7DL = T7DS.7DL-7Ae#1-7DL in Mutant 235; 7AL = T7A-7Ae#1 in Sears' 7A-7Ag No.12	Agatha; Indis; L503; L513; Mutant 28; Mutant 235; Sunnan; Oasis 86; Lr19-I49-299; I-22; I-23; I-96	The gene order: Sd-1–Xpsr105 -7D- Xpsr129–7D- Lr19 -Wsp- D1- Sr25–Y; Cent–Sd1–Xpsr165–7D–Xpsr105–7D -Xpsr129–7D–XcslH81-1–Xwg380–7D–Xmwg2062–7D–Lr19–Wsp-D1–Sr25/Y; RAPD, SCAR and SSR markers co-inciding with, or flanking, Lr19 in a derivative of Knott's Agatha Mutant 28 (C80.1) were reported; An STS marker closely linked and distal to Lr19 was developed from an AFLP	Eizenga (1987); Friebe et al. (1994); Autrique et al. (1995); Prins and Marais (1998); Prins et al. (1996, 2001); Zhang et al. (2005); Gupta et al. (2006)
	Lr24	*Thinopyrum ponticum*	3Ae#1L	3DL in Agent, Cody, Osage, Payne; 1BL = T1BL.1BS-3Ae#1L in Amigo, Teewon	Cody; Osage; Payne; SST 23; SST 44 = T4R; Timpaw; Torres; Wanken; Australian genotypes; Blueboy II; Fox; Lockett; Parker 76; Siouxland	Always present with Sr24; cosegregation with RAPD marker that was converted to a SCAR; Linked with SCAR marker SCS73719 earlier thought to tag Lr19; A PCR marker, Sr24#12, was confirmed across all sources of Lr24	McIntosh et al. (1977); Jiang et al. (1994a); Autrique et al. (1995); Dedryver et al. (1996); Prabhu et al. (2004); Mago et al. (2005)
	Lr29	*Thinopyrum ponticum*	7Ae#1	7DS = 7DL-7Ae#1L.7Ae#1S.	Sears' CS 7D/Ag#11; RL6080 = Thatcher*6/ Sears' 7D/Ag#11	Cosegregation with two RAPDs	Procunier et al. (1995); Friebe et al. (1996)
	Lr38	*Th. intermedium*	7Ai#2L	1DL = T1DS.1DL-7Ai#2L. v: T25; 2AL = 2AS.2AL-7Ai#2L. v: W49 = T33; 3DS = 3DL.3DS-7Ai#2L. v: T4; 5AS = 5AL.5AS-7Ai#2L. v: T24; 6DL = 6DS.6DL-7Ai#2L. v: T7	T25; W49 = T33; T4; T24; T7; RL6097 = Thatcher*6/ T7		Friebe et al. (1992, 1993, 1996)

(*continued*)

Table 2.7 (continued)

Diseases or pests	Gene symbol	Alien species	Alien chromosome	Wheat chromosome	Germplasm or cultivars	Molecular markers	References
	Lr55	*Elymus trachycaulis*	1HS	1B = 1BL.1H^{t}S	KS04WGRC45 = Heyne*3/TA5586		Friebe et al. (2005)
Stem rust (*Puccinia graminis*)	*Sr24*	*Thinopyrum ponticum*	3Ae#1L	3DL = T3DS.3DL-3Ae#1L in Sears' 3D/Ag translocations; T1BL = 1BS-3Ae#1L in Amigo	Agent; Blueboy II; Collin; Cloud; Cody; Fox; Gamka; Karee; Kinko; Palmiet; Sage; SST 23; SST 25; SST 44 = T4R; SST 102; Torres; Wilga; Siouxland; Australian genotypes; T1BL = 1BS-3Ae#1L Amigo; Teewon	Sr24 is completely linked in coupling with Lr24 and often with red grain color	Sears (1973); McIntosh et al. (1977); The et al. (1992); Jiang et al. (1994a); Autrique et al. (1995); Friebe et al. (1996)
	Sr25	*Thinopyrum ponticum*	7Ae#1L	7DL = T7DS.7DL-7Ae#1L in Sears' CS 7D/7Ag translocations; 7AL = T7A-7Ae#1L in Sears' 7A/7Ae#1L No. 12	Sears' CS 7D/7Ag translocations except #11; Agatha = T4; Mutant 28; Sears' 7A/7Ae#1L No. 12; Indis	Sr25/Lr19 often show complete linkage; Sears' 7D/7Ag#11 carries neither Sr25 nor Lr19	McIntosh et al. (1977); Eizenga (1987); Kim et al. (1993); Friebe et al. (1994)
	Sr26	*Thinopyrum ponticum*	6Ae#1L	6AL = T6AS.6AL-6Ae#1L.	Avocet; Flinders; Harrier; Jabiru; King; Kite; Knott's 6A-6Ae#1L translocation to Thatcher; Takari; Bass; Eagle	A PCR marker, Sr26#43 was reported	Friebe et al. (1994, 1996); Mago et al. (2005)
	Sr43	*Thinopyrum ponticum*	7Ae#2L	T7DL-7Ae#2L.7Ae#2S or T7DS.7Ae#2L	KS10-2; KS23-9; KS24-1; KS24-2		Kim et al. (1993); Friebe et al. (1996)
	Sr44	*Thinopyrum intermedium*	7Ai#1L	T7DS-7Ai#1L.7Ai#S; Several 7A-7Ai#1L translocations	Line 86.187; Several 7A-7Ai#1L translocations		Friebe et al. (1996); Khan (2000)
Colonization by curl mites (*Eriophyes tulipa*)	*Cmc2*	*Thinopyrum ponticum*	6Ae#2S	T6AS.6Ae#2S; T5BL.6Ae#2S; 6D = T6DL.6Ae#2S	875-94-2; Rescue derivative		Whelan (1988); Whelan and Hart (1988); Friebe et al. (1996)
Wheat Streak Mosaic Virus (WSMV)	*Wsm1*	*Thinopyrum intermedium*	4ES = 4Ai#2S in CI 15092 ($2n = 42$)	4A = T4AL.4Ai#2S; 4D = T4DL.4Ai#2S; T6AS.4Ai#2L + T6AL-4Ai#2S	CI 17766 = B-6-37-1; CI 17884; KS90H445; KS90H450; CI 17883	*Wsm1* cosegregated with a STS amplified by the primer set STSJ15	Liang et al. (1979); Wang et al. (1980); Friebe et al. (1991); Talbert et al. (1996)

Barley yellow dwarf virus (BYDV)	*Bdv2*	*Thinopyrum intermedium*	7St = 7Ai#1L in L1 ($2n = 44$)	T7DS.7DL-7Ai#1L in TC14 (Distal 10% of 7DL), translocation point between RFLP markers Xpsr680 and Xpsr965; T7DS-7Ai#1S.7Ai#1L in TC5, TC6, TC8, TC9, TC10; 1B = T1BS-7A#1S.7Ai#1L in TC7	Glover (with TC6); Mackellar = LH64C (from tissue culture); TC14*2/Hartog; TC14*2/Spear; TC14*2/Tatiara; Yw243, Yw443, Yw642 and Yw1029 (derived by ph1 induced recombination)	Complete association with Xpsr129-7D, Xpsr548-7D, XksuD2-7D, XcslH81-7D, and Xgwm37-7D selected as a diagnostic marker; Two RGAP and 1 RAPD markers developed for the Yw series also effective for at least TC14	Banks et al. (1995); Larkin et al. (1995); Hohmann et al. (1996); Wang and Zhang (1996); Zhang et al. (2000, 2004)
	Bdv3	*Thinopyrum intermedium cv. Ohahe*	7E in P107 ($2n = 44$) and P29 ($2n = 42$)	7DS.7DL-7EL	PI 634825 = P961341	RFLP and GISH; Three SSRs (gdm67, wmc121, and gdm46) and two STSs (BE442572, S253737) for bin 3; one SSR (barc172) and two STSs (BF293181, BE442755) for bin 4	Sharma et al. (1995); Francki et al. (1997); Crasta et al. (2000); Ohm et al. (2005); Ayala-Navarrete et al. (2009)
	Bdv4	*Thinopyrum intermedium*	2Ai#2	2Ai#2(2D); 2Ai#2(2B)	Addition lines TAI-27, DH549 and DH554 ($2n = 44$); substitution in N431, N452, HG295, and Yi4212; substitution in N420 and N439; translocation line Y5579	EST-based PCR marker BF145935	Lin et al. (2006, 2007); Ayala-Navarrete et al. (2007); Zhang et al. (2009)
Scab, Head blight (*Fusarium graminearum*)	*Fhb3* confers type 2 resistance similar to Sumai 3	*Leymus racemosus*	7Lr#1S	7A = T7AL.7Lr#1S in T09; T4BS·4BL-7Lr#1S + T4BL-7Lr#1S·5Lr#1S in T01; T6BS·6BL-7Lr#1S + T6BL·5Lr#1S in T14	NAU502 DA7Lr#1 ($2n = 44$); NAU601 T4BS.4BL-7Lr#1S ($2n = 42$); NAU615 T4BS.4BL-7Lr#1S-1 ($2n = n = 42$); NAU617 T6AL.7Lr#1S ($2n = 42$); NAU635 T1BL.7Lr#1S ($2n = 42$); T09 ($2n = 42$); T01 ($2n = 44$); T14 ($2n = 44$)	Three PCR-based markers, BE586744-STS, BE404728-STS, and BE586111-STS, specific for 7Lr#1S were developed	Cai et al. (2005); Chen et al. (2005); Qi et al. (1997, 2008)
Powdery mildew (*Blumeria graminis* DC)	*PmP*	*Agropyron cristatum*	Unknown	Unknown	Xiaobing	Two AFLP marker loci, XM55P66 and XM55P37, flanked the locus with a distance of 0.8 cM and 2.4 cM, respectively, from the locus	Zhou et al. (2005)

sequence tag (EST) sequences for the resistance had been isolated (Jiang et al. 2004, 2005, 2009). These DNA sequences will be useful in furthering our understanding of the BYDV resistance genes.

Thinopyrum species, especially *Th. ponticum*, are highly tolerant to salinity (Zhang et al. 2005; Colmer et al. 2006). Tolerance to abiotic stresses, such as drought, cold, or salinity, is usually controlled by complex physiological processes that involve the action of many independent genes. Thus, these traits are generally quantitatively inherited, e.g., salinity tolerance of *Th. elongatum* is governed by several genes located on different chromosomes of the $\mathbf{E}^{e}$-genome (Dvořák et al. 1988; Dubcovsky et al. 1994; Zhong and Dvořák 1995). As a result, the single-chromosome disomic addition lines are always less tolerant to abiotic stresses or certain diseases than the amphidiploid or partial amphidiploid, exemplified by the salinity tolerance in the wheat–*Thinopyrum junceum* derivatives (Wang et al. 2003b) and the wheat scab (or Fusarium head blight, FHB) resistance in wheat × *Leymus racemosus* (Lam.) Tzvelev derivatives (Cai et al. 2005; Chen et al. 2005). In these cases, gene(s) from each disomic addition line carrying a different alien chromosome must be independently transferred to a homeologous wheat chromosome before separate translocation lines are subsequently crossed to achieve gene pyramiding. Despite this, partial salt tolerance of *Th. junceum* and *Th. ponticum* have been transferred into wheat giving their hybrid derivatives increased salt tolerance over the recipient wheat lines (Wang et al. 2003b; Chen et al. 2004).

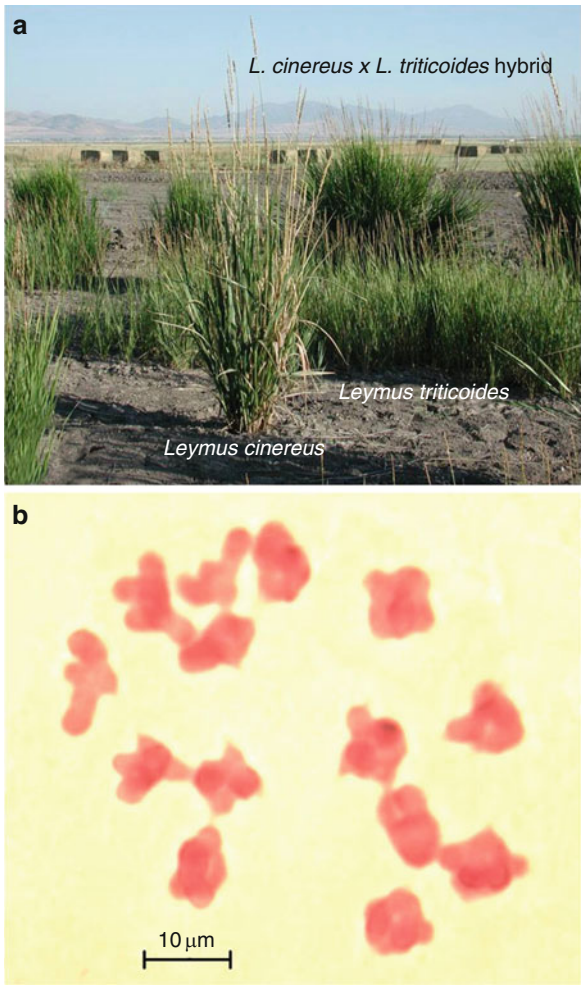

Fig. 2.1 *Leymus* hybrid derivatives used as mapping populations for the **NsXm**-genome. (**a**) Plants of *Leymus cinereus* (*front*), *L. triticoides* (*middle*), and their F_1 hybrid (*back*). (**b**) Chromosome pairing at meiotic metaphase I in the *L. cinereus* × *L. triticoides* F_1 hybrid. Photo 1A is provided by Steve R. Larson, USDA-ARS FRRL

2.5 Role in Classical and Molecular Genetic Studies

After the salt tolerance genes from AJDAj5 (a wheat–*Th. junceum* disomic addition line; Charpentier 1992) and Ph^I (the line carrying Ph^I gene allele from *Ae. speltoides*; Chen et al. 1994) were transferred into two translocation lines W4909 and W4910 (Wang et al. 2003a), a microarray study was carried out to trace transcriptome changes (or gene actions) to the two parents (Mott and Wang 2007). Because Chinese Spring (CS) wheat was the common background of the two parental lines, CS was included in the microarray study. By comparing the gene expression in the five lines, Mott and Wang (2007) were able to demonstrate that the combination of genes from AJDAj5 and Ph^I resulted in a higher salinity tolerance in W4909 and W4910 than the two parental lines, which were more salt-tolerant than CS. It is also clear that W4909 and W4910 inherited from Ph^I the mechanism that allowed the plants to tolerate high Na^+ concentrations in their leaves. While many other genes might be involved to account for their salt tolerance, a gene for tonoplast aquaporin from Ph^I and a gene for putative potassium channel protein attributable to AJDAj5 were identified

as candidate genes for the tissue salt tolerance in W4909 and W4910.

Efforts to map important agronomic traits have been conducted with mapping populations derived from the hybrid between *Leymus cinereus* (*Scribn. & Merr.*) *A. Löve* and *L. triticoides* (Buckl.) Pilger (Wu et al. 2003; Hu et al. 2005; Larson et al. 2006; Larson and Mayland 2007). *L. cinereus* and *L. triticoides* are tall caespitose and short rhizomatous perennial Triticeae grasses, respectively (Fig. 2.1a); but the interspecific hybrid had complete chromosome pairing (Fig. 2.1b) and was highly fertile to produce progenies that are suitable for mapping molecular markers of many contrasting characteristics in the two species. Quantitative trait loci (QTLs) for circumference of rhizome spreading, proportion of bolting culms, anthesis date, and plant height were mapped in one study (Larson et al. 2006) and those for neutral detergent fiber, acid detergent fiber, crude protein, and 14 minerals content in another (Larson and Mayland 2007). These studies contribute valuable data benefiting the comparative genomics of monocotyledon grasses that include rice, maize, wheat, barley, rye, and sorghum.

2.6 Role in Crop Improvement Through Traditional and Advanced Tools

Table 2.7 lists the transferred genes for resistance to disease and insect pests that originated from perennial Triticeae species. These genes were introgressed into wheat through chromosome engineering by aforementioned methods in Sect. 2.4. All except three cases (those for *Lr55*, *Fhb3*, and *PmP*) originated from *Thinopyrum* species. This can be attributed to the fact that among the genomes of the perennial Triticeae, the **E**-genome in *Thinopyrum* species is most closely related to the **ABD**-genome of wheat (Hsiao et al. 1995; Liu et al. 2007).

The short arm of chromosome 1H carrying *Lr55* for leaf rust resistance in *Elymus trachycaulis* was fused by centromere to 1B long arm of wheat in the germplasm KS04WGRC45 (Friebe et al. 2005). Scab resistance gene *Fhb3* from *L. racemosus* was transferred to wheat by recombinations involving 4**B**L, 6**A**L, and 1**B**L with 7Lr#1S (=either 7**Ns**S or 7**Xm**S) in several germplasm lines (Chen et al. 2005; Qi et al. 1997, 2008). According to Zhou et al. (2005), the Chinese wheat "Xiaobing" is a derivative of *T. aestivum*/*Agropyron cristatum* that carries a dominant powdery mildew resistance gene, temporarily named *PmP*. These three genes are the only transferred alien genes that originated from a genome other than **E** of the perennial Triticeae.

Ahmad and Comeau (1991) aimed to transfer apomixes into wheat by producing interspecific hybrids between *T. aestivum* and *E. scabrus* (R. Br.) A. Löve. Unfortunately, *E. scabrus* is not apomictic and they were unsuccessful in obtaining backcross progeny from hybrid plants. Later, transfering apomixis into wheat was attempted with the synthesis of interspecific hybrids between *T. aestivum* and apomicitc *E. rectisetus* (Carman and Wang 1992; Wang et al. 1993; Liu et al. 1994; Peel et al. 1997). However, the trait was not fully expressed in the hybrids probably due to the suppressive action of genes for sexual reproduction in wheat. Thus, all addition lines derived from the backcross progenies of original hybrids of this combination (Xue and Wang 1999) exhibited no sign of apomixis, except in one 46-chromosome addition line that was able to repeatedly produce a 23-chromosome offspring, through a very low frequency of parthenogenesis, among other sexual progenies (Fig. 2.2; Wang unpublished). This suggests that even the expression of parthenogenesis, one of processes for apomictic reproduction, requires more than two *E. rectisetus* chromosomes, let alone the

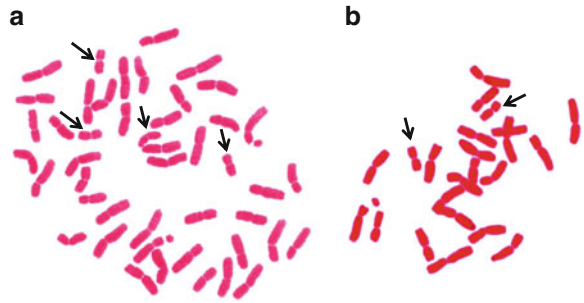

Fig. 2.2 A backcross derivative of *Triticum aestivum* cv. Fukuhokomugi × *Elymus rectisetus* had the capability of producing seed through parthenogenesis, even though at a very low frequency. The 46-chromosome addition line (**a**), which had two pairs of alien chromosomes (*arrowed*) in the wheat genome, produced a 23-chromosome offspring (**b**) apparently through parthenogenesis, i.e., a $n = 23$ female gamete in the 46-chromosome plant developed into the functional embryo, without being fertilized by a male gamete in the pollen grain, in a seed resulting in the 23-chromosome offspring

more complex apomixis. Today, apomictic wheat remains a dream for wheat researchers.

The most successful utilization of alien genetic resources for wheat cultivar development is exemplified by the Chinese cultivar "Xiaoyan 6" that has been grown on more than 10 million ha. in China since 1980 (Xueyong Zhang personal communication). Xiaoyan 6, a derivative of hybrids between wheat and *Thinopyrum ponticum*, has also been used as a core parent for wheat breeding in China in the past 20 years. Pedigree analysis of the Chinese wheat cultivars showed that there are more than 50 wheat varieties derived from crosses involving Xiaoyan 6. These derivative varieties have been grown on more than 20 million ha. and increased the total wheat grain production by 7.5 billion kg. Xiaoyan 6 has set an unparalleled example in the development and application of wheat varieties through wide hybridization and chromosomal engineering in China. The positive contribution of Xiaoyan 6 to wheat production and breeding in China is possibly due to the possession of three valuable characteristics (1) wide spectrum and durable resistance to stripe rust; (2) tolerance to high temperature, strong light, and hot-dry wind; and (3) superior grain quality suitable for making traditional Chinese wheat food products. For the enormous contribution of Xiaoyan 6 in food production, Chinese Government granted its State Scientific and Technological Award for 2006 to scientist Prof. Li Zhensheng, who developed the cultivar in 1979 and is the tenth Chinese scientist to win this top award. Prof. Li has also introduced chromosome engineering to the breeding of wheat strains with a "nullisomic backcross method," which has reduced the duration of wheat breeding through distant hybridization to 3.5 years, whereas the previous breeding of "Xiaoyan" took 20 years.

2.7 Development of Genomics Resources

Molecular markers had been developed for chromosomes of diploid *Thinopyrum bessarabicum* and *Th. elongatum*. William and Mujeeb-Kazi (1995) found six diagnostic protein isozymes for the **J** (=$\mathbf{E^b}$)-genome chromosomes in the wheat background. Then, Zhang et al. (2002) identified amplified fragment length polymorphism (AFLP) and RAPD markers for five of the seven $\mathbf{E^b}$-genome chromosomes. Markers that were present in the wheat × *Th. bessarabicum* amphidiploid but absent in the five disomic addition lines would be markers for the two missing chromosomes 3$\mathbf{E^b}$ and 6$\mathbf{E^b}$. You et al. (2004) developed specific simple sequence repeat (SSR) marker for $\mathbf{E^e}$-genome of *Thinopyrum* spp. using wheat microsatellites.

Collections of annual Triticeae ESTs were available for *Hordeum vulgare*, *S. cereale*, *T. aestivum*, and *Triticum monococcum* (*http://www.plantgdb.org/prj/ESTCluster/progress.php*; accessed on 29 Oct 2009, 16:21 GMT). Barley EST primer sets had been used to find polymorphic markers for identifying alien Triticeae chromosomes in wheat addition lines (Hagras et al. 2005). The number of polymorphic markers distinguishing wheat from some perennial Triticeae ranged from 78 to 572. Again, the **E**-genome in *Th. elongatum* was grouped with **ABD** of wheat by having the lowest number of polymorphic markers between their genomes. The dendrogram from their data did not largely deviate from those based on other molecular phylogenies (Hsiao et al. 1995; Peterson and Seberg 2002; Peterson et al. 2006).

Only recently, EST libraries of *L. cinereus*/*L. triticoides* hybrid, a mixture of *Elymus wawawaiensis* J.R. Carlson & Barkworth and *E. lanceolatus* (Scribn. & J. G. Sm.) Gould, and *Pseudoroegneria spicata* (Pursh) A. Löve had become available (Bushman et al. 2008). Sequences were obtained in both directions for 16,128 clones of the *Pseudoroegneria* library, 15,360 clones of the *Leymus* library, and 10,368 clones of the *Elymus* library. The EST sequences were assembled into 8,780 unigenes for *P. spicata*, 11,281 unigenes for *Leymus*, and 7,212 unigenes for *Elymus*. The three library databases are available for searching on the ESTIMA Web site (*http://titan.biotec.uiuc.edu/triticeae/*).

For the *Leymus* interspecific hybrid, a bacterial artificial chromosome (BAC) library consisting of over 400,000 clones, resulted from independent digestions of genomic DNA by two restriction enzymes *Bam*HI and *Mbo*I, had been constructed (Larson et al. 2007). This library is being utilized to map agronomically important traits for gene discovery (Larson et al. 2009).

ESTs have also been obtained from wheat alien addition lines using chromosome microdissection techniques in combination with several molecular methods (Jiang et al. 2004, 2005, 2009; Zhou et al. 2008). The alien chromosomes in these addition lines, which had been either treated (e.g., infected by a

pathogen) or untreated (used as the control), were first dissected under an inverted microscope and collected into microcentrifuge tubes for molecular manipulations to clone desired genes. ESTs for disease resistance genes, especially those for BYDV, have been isolated from chromosomes of *Th. intermedium* and then sequenced (Jiang et al. 2004, 2005, 2009). These genomic resources will be useful to develop molecular markers for the application of marker-assisted selection (MAS) by breeders.

2.8 Scope for Domestication and Commercialization

2.8.1 Perennial Wheat

Perennial wheat was another dreamed goal for wheat scientists because it would save money for wheat farmers and reduce soil erosion due to tillage, wind, and water runoff, a concern for soil conservationists. Again, introducing genes for perenniality into wheat by chromosome engineering would be as difficult as making apomictic wheat because these traits are believed to be controlled by many genes present on different chromosomes. This belief was the result of numerous observations that some partial amphidiploids between wheat and perennial Triticeae, especially *Thinopyrum* species, exhibited perenniality (Lyubimova 1991; Cai et al. 2001; Scheinost et al. 2001). After many years of cessation of perennial wheat research in the former Soviet Union as well as United States (Tsitsin 1960; Suneson et al. 1963), the hope was rekindled by the report that gene or genes on a single-chromosome 4**E** of *Th. elongatum* confers a polycarpic perennial habit to annual wheat (Lammer et al. 2004). Research on perennial wheat is being actively carried on by Prof. Stephen S. Jones and his colleagues at the Washington State University (Cox et al. 2002a, b; Murphy et al. 2007).

2.8.2 Domestication of Perennial Triticeae Species for Bread Making

Because perennial wheat is not yet a reality, several perennial Triticeae species have been exploited and/or improved for using their grains to make bread (Cox et al. 2002a, b). Rodale Institute Research Center (a division of Rodale Press, Inc.) in Kutztown, PA, had devoted many years of effort to perennial grain development by adapting *Th. intermedium* as a new grain crop called Wild Triga (Wagoner 1990, 1995). Having higher levels of protein (20.8%), fat (3.21%), and ash (2.64%) than wheat, seeds of Wild Triga are used for human consumption. Wild Triga has higher levels than wheat of all essential amino acids except lysine. Naked grain that lost its hulls can be ground into flour to make baked product or cooked whole like rice. Because of their good seed yield and quality and the fact that they lose their hulls somewhat easily as compared to some of the other cultivars, "Oahe" and "Luna" intermediate wheatgrass are the cultivars most suitable for perennial grain production.

The Land Institute in Kansas has evaluated almost 1,500 accessions representing 85 species of *Agropyron*, *Thinopyrum*, *Elymus*, and *Leymus*, along with 2,630 accessions of other species, between 1979 and 1987 (Jackson and Jackson 1999). The species having the greatest potential for domestication was *L. racemosus* (giant or mammoth wildrye). However, among 16 accessions evaluated over 2 years, yields did not exceed 830 kg/ha (Piper 1993), and yield declined rapidly in the following generations. Thus, there is no current breeding program for grain yield in *L. racemosus* and, until selection is undertaken, no conclusions can be drawn regarding its potential for direct domestication as a perennial grain crop.

Leymus arenarius (L.) Hochst. (Lyme grass or beach wildrye) has been used as a food grain since the time of the Vikings (Griffin and Rowlett 1981). Lately, it has been studied as a potential grain crop in Iceland (Anamthawat-Jónsson 1996). This northern European species ($2n = 8x = 56$) is more closely related to *L. racemosus* ($2n = 4x = 28$, southeastern European and central Asian) than to *L. mollis* (Trin.) Pilger (tetraploid, $2n = 4x = 28$, northern American/Pacific) (Anamthawat-Jónsson and Bödvarsdóttir 2001). These three *Leymus* species had been hybridized with wheat to produce amphidiploids (Anamthawat-Jónsson et al. 1997; Anamthawat-Jónsson 1999). But these three perennial Triticeae species have not been fully domesticated to have a significant impact on production of perennial grain crops. It is partly due to "relatively small efforts at domestication, which are within the capabilities of non-profit organizations such as the Rodale Institute or Land Institute, must be expanded to a much larger scale by university, government, or corporate breeding programs if wholly

new perennial grain crops are to be developed" (Cox et al. 2002b).

2.9 Some Dark Sides and Their Addressing

Elymus repens (L.) Gould, commonly named quackgrass, is an introduced noxious weed with aggressively strong rhizomatous growth and complex genomic origins (Fahleson et al. 2008). This weed grass has an almost worldwide distribution and is easily hybridized with species in many genera of Triticeae in nature (Dewey 1984). Thus, it is subjected to strict regulations on seed certification and movements. The cultivar Newhy (Asay et al. 1991), which was developed from derivatives of the cross between hexaploid quackgrass (*E. repens*; **StStStStHH**) and tetraploid bluebunch wheatgrass (*Pseudoroegneria spicata*; **StStStSt**), has seeds so similar to those of quackgrass that growers of this cultivar often had difficulty to get their seed certified on the seed purity.

Although *E. repens* does not pose a great threat of genetic contamination to wheat, barley, and rye, many perennial Triticeae species are capable of producing unreduced gametes and hybridizing with other species naturally. The best example is that of *Pascopyrum smithii* (Rydb.) A. Löve ($2n = 8x = 56$; **StStHHNsNsXmXm**), which originated from the natural hybridization between an *Elymus* species (**StStHH**) and a *Leymus* species (**NsNsXmXm**). The *Elymus* species arose from a hybrid between *Pseudoroegneria* (**StSt**) and *Hordeum* (**HH**), whereas *Leymus* arose from that between *Psathyrostachys* (**NsNs**) and an unknown (**XmXm**) species (Jones et al. 2000; Redinbaugh et al. 2000). Therefore, transgenics for herbicide resistance in perennial Triticeae species should not be pursued due to the concern of gene flow from wild Triticeae to annual cereals (Wang and Jensen 2009).

2.10 Conservation Initiatives

Loss of wilderness from human activities (such as home building, road construction, and recreation, etc.) had led to extinction of many species including plants and animals. For example, China has put *Psathyrostachys huashanica* Keng on the list of endangered species. Seed of this species are either not available in gene banks or have lost germinability. Because this species has a unique karyotype (Hsiao et al. 1986) for the **Ns**-genome, preserving it both in situ and ex situ shall be a very important conservation issue in China.

Wheatgrasses and wildryes are being preserved as seed in many Gene Banks, with the US National Plant Germplasm System (NPGS) located in Pullman, Washington, holding the largest number of accessions (Wang and Jensen 2009). NPGS lists approximately 610 accessions of *Agropyron* (3 species), 1,000 *Elymus* accessions (over 300 species/hybrids), 229 accessions of *Pseudoroegneria* (8 species), 534 accessions of *Thinopyrum* (12 species), 17 accessions (3 species) of *Elytrigia*, 450 accessions of *Leymus* (28 species), 31 accessions of *Pascopyrum* (1 species), and 86 accessions of *Psathyrostachys* (4 species).

From this review, it is obvious that *Th. intermedium* and *Th. ponticum* had been the two most valuable wild relatives contributing a wide range of desirable traits to wheat cultivar development (Table 2.7). Firstly, it is due to the fact that these two species have genes for resistance to many diseases and pests including leaf rust, stem rust, stripe rust, common root rot, wheat scab, wheat streak mosaic virus (WSMV), BYDV, greenbug, and wheat curl mite (Friebe et al. 1991, 1992; Chen et al. 2003; Li et al. 2003, 2004, 2005; Shen and Ohm 2007; Jiang et al. 2009; Xu et al. 2009), tolerance to abiotic stresses such as drought, high temperature, and salinity (Chen et al. 2004; Trethowan and Mujeeb-Kazi 2008), perennial growth habit (Cai et al. 2001), and grain quality traits such as high protein content (Feng et al. 2004; Chen et al. 2007). Secondly, it can be attributed to the fact that these two species contain the basic genomes **E**- (or **J**-) and **St**- that are closely related to **A** and **D** of bread wheat (see Sect. 2.3). *Th. intermedium* is rhizomatous, whereas *Th. ponticum* is caespitose. Both of them are native to Europe and western Asia but were introduced and well established in North America. Because of their polyploidy nature, these two species could have multiple origins involving different progenitor species in different geographic areas at different time scales. Therefore, there should be a great wealth of genetic variability and molecular polymorphism (García et al. 2002) worthy of being exploited by breeders and

biologists, respectively. Collection, characterization, and preservation of these and other *Thinopyrum* species should be emphasized by government and non-government organizations that are concerned about genetic diversity and natural heritage.

2.11 Recommendations for Future Actions

Perennial Triticeae species such as wheatgrasses (*Agropyron*) and wildryes (*Psathyrostachys*) are important grasses that serve not only as forage crops but also as tertiary gene pools for wheat improvement. Many desirable genes that are absent in wheat, particularly those for resistance or tolerance to biotic and abiotic stresses, could be found in these wild grasses. As the environmental conditions deteriorate, shortage of clean fresh water will make drought- and salt-tolerant wheat cultivars necessities for keeping mankind from hunger. Similarly, the appearance of new races or biotypes of wheat pests requires the continuing search and incorporation of new disease/insect resistance genes into wheat cultivars. These genes are oftentime present in wild grasses related to wheat and can be introgressed into wheat through chromosome engineering and new molecular tools. Furthermore, some perennial Triticeae grasses also carry desirable genes for wheat grain quality that may improve bread-making or nutritional values (Feng et al 2004; Chen et al. 2007). This prospect needs more future attention to fully exploit the potential of the tertiary gene pool.

With climate change and energy shortage becoming hard-pressing problems for the whole world, perennial wheat and biomass production from perennial wheatgrasses and wildryes are two prospects that may contribute to the solution of these problems. The former can reduce the use of energy by eliminating the tillage and seeding operations. The latter can produce alternative biofuels to supplement the fossil fuel. These two areas of research certainly will receive more emphasis from this moment on.

Conservation of these wheat-relative grasses is pivotal to ensure continuing success in wheat improvement for sustainable food production. Both in situ and ex situ conservation of the wild germplasm are needed. As ex situ conservation effort intensified, cryopreservation of DNA samples representing each species should be undertaken to supplement the seed storage method. Planning collecting expeditions of new accessions or species should take into consideration targeted traits and the favorable environmental conditions for such traits to select likely geographic regions to find plants carrying desirable genes. For example, resistance to Fusarium head blight (wheat scab, a destructive disease in the warm and humid wheat-growing areas of the world) was found in many perennial Triticeae species including *L. racemosus* (syn. *Elymus giganteus* Vahl.), *Roegneria kamoji* (Ohwi) Ohwi ex Keng (syn. *E. tsukushiensis Honda)*, *R. ciliaris* (Trin.) Nevski [syn. *E. ciliaris* (Trin.) Tzvelev], and *Dasypyrum villosa* L. (Oliver et al. 2005) that are all growing in humid regions (Cai et al. 2005). Characterization of wild Triticeae grasses for desirable traits still needs to be strengthened. A global network of regional gene banks holding seed and/or DNA samples of these wheat relatives must also encourage the exchanges of materials and information by making them freely available to bona fide scientists to use in wheat germplasm enhancement projects.

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Chapter 3
Avena

Igor G. Loskutov and Howard W. Rines

Devoted to Ken Frey – Oat Breeder

3.1 Introduction

Oat is one of the most important cereal crops in the world. The genus *Avena* L. includes cultivated species with different ploidy levels and a number of wild species reflecting a wide range of botanical and ecological diversity. A majority of these forms came from the centers of origin, which by definition shows great diversity of *Avena* species. With this in view, oat species became the subject of investigations to specify the complex organization of the *Avena* genus and indicate aspects of its evolution and phylogenetic links between the species. Further search for agronomical traits and utilization of new oat breeding sources is very important for breeding purposes.

3.1.1 Basic Botany of the Species

3.1.1.1 Taxonomic Position of *Avena* Species

Investigation of species diversity using morphological traits is very important for their systematization and determining taxonomic position. Taxonomic and morphological descriptions of species of the genus *Avena* L. was started as far back as the sixteenth century (Tournefort 1700) and continues till today. A great number of species had been described by a number of authors since establishment of the system of binary nomenclature proposed by C. Linnaeus (Linneaus 1753) (Table 3.1). In most cases, characterization of a species was initially performed on the basis of morphological traits.

An example of one of the first descriptions of the diploid species is the characterization of cultivated *Avena strigosa* (Schreber 1771). Later, this species was described as *A. hispanica* Ard. (Malzev 1930). Another wild diploid species *A. pilosa* was first described during examination of the material collected in the Transcaucasus (Marshall Bieberstein 1819). During processing of the Algerian materials, the same species was described under the name *A. eriantha* Durieu. Another original species was described under the name *A. clauda* (Durieu de Maisonneuve 1845). These two species are morphologically similar: the only distinction is that *A. clauda* disarticulates single florets instead of spikelets, like *A. pilosa*, due to the presence of a callus only in the lowest floret, governed by one or two genes (Rajhathy and Thomas 1967). According to the opinion supported by Malzev (1930), disarticulation of spikelets is an advanced way of seed distribution. With the help of two awns in a spikelet, seeds can move and twist into the soil surface more efficiently than with only one awn of a single floret. Moreover, with a spikelet, several seeds, instead of just one, bury into the soil simultaneously.

There are two more closely related species, one described under the name of *A. ventricosa* (Balansa 1854) and the other as *A. bruhnsiana* (Gruner 1867). Along with morphological and physiological similarities, and thanks to the elongated callus, their seeds can screw into the toughest stony soil even under the strongest drought in the steppe, deserted areas, or on ranges. Along with the above-mentioned species, *A. longiglumis* was initially described by the author in the course of processing the materials from Algeria

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Table 3.1 The history of description *Avena* species

Species	Ploidy	Year of description	Reference
A. fatua L.	6	1753	Linneaus (1753)
A. sativa L.	6	1753	Linneaus (1753)
A. sterilis L.	6	1762	Linneaus (1762)
A. strigosa Schreb.	2	1771	Schreber (1771)
A. barbata Pott ex Link	4	1799	Pott (1799)
A. hirtula Lag.	2	1816	Lagasca (1816)
A. pilosa M. Bieb.	2	1819	Marshall Bieberstein (1819)
A. clauda Durieu	2	1845	Durieu de Maisonneuve (1845)
A. longiglumis Durieu	2	1845	Durieu de Maisonneuve (1845)
A. occidentalis Durieu	6	1845	Durieu de Maisonneuve (1845)
A. byzantina C. Koch	6	1848	Koch (1848)
A. abyssinica Hochst.	4	1851	Hochstetter (1852)
A. macrostachya Balansa et Durieu	4	1854	Balansa and Durieu Maisonneuve (1854)
A. ventricosa Balansa	2	1854	Balansa (1854)
A. wiestii Steud.	2	1855	Steudel von (1855)
A. ludoviciana Durieu	6	1855	Cosson and Durie de Maisonneuve (1855)
A. bruhnsiana Gruner	2	1867	Gruner (1867)
A. vaviloviana (Malz.) Mordv.	4	1927	Malzev (1930)
A. magna Murphy et Terrell	4	1964	Rajhathy et al. (1966)
A. murphyi Ladizinsky	4	1971	Ladizinsky (1971a)
A. prostrata Ladizinsky	2	1971	Ladizinsky (1971b)
A. damascena Rajhathy et Baum	2	1972	Rajhathy and Baum (1972)
A. canariensis Baum, Raihathy et Sampson.	2	1973	Baum et al. (1973)
A. atlantica Baum et Fedak	2	1985	Baum and Fedak (1985a)
A. agadiriana Baum et Fedak	4	1985	Baum and Fedak (1985b)
A. insularis Ladizinsky	4	1996	Ladizinsky (1998)

(Durieu de Maisonneuve 1845). According to Malzev (1930), all the mentioned species are primitive ones, and this view was later supported by other researchers (Rajhathy and Thomas 1974).

The diploid oat *A. prostrata* was described during exploration of the southwestern coast of Spain by a collecting mission. The most typical characters of the species are the minimal size and some other traits related to the grains as well as the prostrate juvenile growth of the plant (Ladizinsky 1971b). The species *A. damascena*, characterized by the semi-prostrate juvenile growth, was described for the first time during an expedition to the Syrian territory (Rajhathy and Baum 1972). The diploid species *A. canariensis* collected on Fuerteventura Island (Spain, the Canaries) disarticulates spikelets and has bidentate lemma tips (Baum et al. 1973), the latter trait not characteristic of the diploid species. The lodicules and epiblast of the species are of the hexaploid cultivated type, though it is not characteristic in all of the diploid species. *A. wiestii* was described on the basis of seed samples and herbarium materials collected in Egypt (Steudel von 1855). The species *A. hirtula* was initially described in Spain (Lagasca 1816) and *A. atlantica* – during a collecting mission to the Atlantic coast of Morocco (Baum and Fedak 1985a). Morphologically, the latter species is similar to *A. hirtula*, with the only difference that *A. atlantica* disarticulates spikelets instead of single florets.

The tetraploid species *A. barbata* was initially described in Portuguese material (Pott 1799). Later, this species was described as *A. hirsuta* Roth (Malzev 1930), though according to Baum (1977), *A. hirsuta* is a synonym of *A. matritensis* Baum. Romero Zarco (1990) subdivides *A. barbata* into *A. barbata* subsp. *barbata* proper and *A. barbata* subsp. *lusitanica*, while Baum (1977) assigns the species rank to the latter subspecies and proposes the diploid species *A. lusitanica* Baum. The ploidy level of the latter species has been questioned recently (Markhand and Leggett 1996). The species *A. vaviloviana* has been found by Vavilov (1965b) in Ethiopia and initially described as a subspecies of *A. strigosa* subsp. *vaviloviana* Malz., but later it was promoted to a species level

(Mordvinkina 1936). Malzev (1930) believed this species to be a wild parent form of *A. abyssinica* (Hochstetter 1852) with which it shared the same natural habitat that differed from that of other tetraploid species. According to Vavilov, the African group of oats, studied by Trabut (1909), had to be subjected to a special study, as there was no reason for uniting it with the European and Asian species (Vavilov 1965a). At present, this group of species is considered independent from the others (Baum 1977; Loskutov 2008).

The tetraploid species *A. agadiriana* was first described from the material collected in Morocco on the Atlantic and Mediterranean coasts. Morphologically, this species resembles *A. barbata* (Baum and Fedak 1985b), but it sharply differs in that *A. agadiriana* disarticulate spikelets and bidentate lemma tips. The species *A. magna* was described during explorations of the Mediterranean coast in Morocco (Rajhathy et al. 1966). It is believed that this species had been initially described as *A. moroccana* Gdgr.; however, no direct proof of that has been found. The discovery of *A. magna* has "filled the last cell" concerning traits homology in the tetraploid species, as it was the species with only the first lower floret to have a callus. This type of joint had been previously described only for the diploid and hexaploid species. Another species, *A. murphyi*, has been discovered and described from southern Spain (Ladizinsky 1971a). A series of morphological traits makes this species similar to *A. magna*, and therefore it was attributed to the *Pachycarpa* Baum section (Baum 1977) characterized by bidentate lemma tips. The species *A. insularis* was discovered and described in Italy, on Sicily. Morphologically, it occupies an intermediate position between *A. murphyi* and *A. sterilis* (Ladizinsky 1998).

The outcrossing tetraploid perennial species *A. macrostachya* was discovered and described in Algeria (Balansa and Durieu de Maisonneuve 1854), though it is believed that initially it had been described under the name of *Helictotrichon macrostachyum* Holub (Holub 1958). Many researchers, due to morphological characteristics, regarded this species as a primitive representative of the genus *Avena* (Malzev 1930; Baum and Rajhathy 1976; Baum 1977; Loskutov 2007).

Although the hexaploid *A. fatua* was one of the first described species (Linneaus 1753), a unified hypothesis has not been developed yet on the origin of this species regardless of the magnitude of research. The difficulty in identifying the progenitor of this species relates to its very wide polymorphism, which, according to Vavilov, is linked with its natural broad habitat (Vavilov 1965a). The species *A. occidentalis* has been described in the Algerian materials (Durieu de Maisonneuve 1845). This species greatly resembles *A. fatua* but has some differences concerning disease resistance and vegetative period duration. Other wild (*A. sterilis* – Linneaus 1762 and *A. ludoviciana* – Cosson and Durie de Maisonneuve 1855) and cultivated (*A. sativa* – Linneaus 1753 and *A. byzantina* – Koch 1848) hexaploid species have been described long ago and a broad spectrum of subsequent research has been devoted to their taxonomic affinity (Malzev 1930; Rajhathy and Thomas 1974; Baum 1977; Loskutov 2007).

Thus, the taxonomic position of *Avena* species is not always unambiguous. In recent years, numerous collecting missions targeted on oat species have been carried out with new species being subjected to thorough investigation.

3.1.1.2 Geographical Locations of Genetic Diversity and Morphology of *Avena* Wild Species

Natural and ecological conditions under which oat species grow differ considerably in terms of rainfall, temperature regime, altitude, and soil and subsoil types. The richest occurrence and highest genetic diversity of wild oat species in the Old World is observed in a narrow territory between latitude 20 and 40° N. It stretches mainly through basins of the Mediterranean, Black, and Caspian seas with very diverse ecological conditions.

Morphological traits used for oat species characterization relate to the vegetative part of a plant; however, the main taxonomic characters are the detailed morphological features related to the structure of generative organs.

Diploid Species

Avena clauda Durieu, 1845, Rev. Bot., 1: 360, excl. syn.; Coss. et Durieu, 1855, Expl. sc. Alger., 2: 111, t. 41, f. 2; Steud., 1855, Syn. Pl. gram., 1: 234; Hausskn., 1894, Mitteil. Thur. Bot. Ver., 6: 42, 45; idem, 1899,

Mittel. Thur. bot., XIII, XIV: 47, excl. var. *solida* Hausskn.; Batt. et Trabut, 1895, Fl. Alger. Monocot.: 180; idem. 1902, Fl. anal. Alger.: 370; Malzev 1930, Ovs. & Ov.,: 230; Roshevitz 1934, Fl. USSR, II: 260; Baum 1974, Canad. Jour. Bot., 52: 2243; Tzvelev, 1976, Poaceae USSR,: 242; Rodionova et al. 1994, Cult. Fl.,: 100–102.

Annual. Juvenile growth prostrate to semi-prostrate. Flowering stem erect, 60–100 cm high. Panicum unilateral. Spikelets awned, and containing 3–5 florets. Glumes very unequal, lower glume one-half of upper one with 7 veins. Lemma tips biaristulate to bisubulate. All florets disarticulate at maturity. Awn inserted at 1/3 of the lemma. Lemmas small or medium sized, glabrous, or pubescent; pubescence of white color. Callus elongated, linear, about 3 mm long.

A. clauda can be found together with *A. pilosa*. It grows in Bulgaria, Greece, throughout Asia Minor, in Turkish, Iranian, and Iraqi Kurdistan, in Uzbekistan, Azerbaijan, high-altitudinal areas of Jordan, in Israel, Lebanon, Syria, Algeria, and Morocco. The species grows under conditions of the steppe climate with up to 350–500 mm annual rainfall, in mountainous semi-deserts and deserts, on coastal sands, on gray-brown, gray-meadow, alluvial meadow, chestnut and podzolic black soils, as well as on brownish and yellowish-brown stony soils (Malzev 1930; Roshevitz 1934; Ladizinsky 1971c; Baum et al. 1972a; Baum 1977; Kanan and Jaradat 1996; Leggett et al. 1992; Sheidai et al. 2002; Loskutov 2007).

Avena pilosa M.Bieb., 1819, Fl. Taur.-cauc., III Suppl.: 84; Griseb., 1844, Spicil. Fl. rumel.: 452; Koch C., 1848, in Linnaea, XXI: 392; Ledeb., 1853, Fl. Ross., 4: 413; Steud., 1855, Syn. Pl. gram.: 231; Coss. et Durieu, 1855, Expl. Sc. Alger., II: 109; Batt. et Trabut, 1895, Fl. Alger. Monocot.: 179; idem. 1902, Fl. anal. Alger.: 370; Malzev 1930, Ovs. & Ov.,: 233; Roshevitz 1934, Fl. USSR, II: 261; Rodionova et al. 1994, Cult. Fl.,: 102–105. – ***A. eriantha*** Durieu, 1845, Rev. Bot., 1: 360; Coss. et Durieu, 1855, Expl. sc. Alger., 2: 109; Nevski 1934, Work. Asie. Univ., ser. 8в, 17: 3; Baum 1974, Canad. Jour. Bot., 52: 2243; Tzvelev, 1976, Poaceae USSR,: 243; Romero Zarco 1996, Lagascal., 18, 2: 171–198. – *A. clauda* var. *solida* Hausskn., 1885, Mitt. geogr. Ges., 3: 237–239.

Annual. Juvenile growth prostrate to semi-prostrate. Flowering stems erect, 55–85 cm high. Panicle unilateral. Spikelets awned medium sized and containing 2–3 florets. Glumes very unequal, lower glume one-half of upper one with 7 veins. Glumes medium sized, glabrous, or pubescent; pubescence of white color. Lemma tips biaristulate to bisubulate. Only the lower floret disarticulates at maturity. Awn inserted at 1/2 of the lemma. Callus linear elongated, about 3–4 mm long.

A. pilosa (syn. *A. eriantha* Durieu) is usually found in a mixture with *A. clauda*. It occurs in Spain, Greece, Bulgaria, Ukraine (Crimea), Russia (Dagestan), Iranian, Turkish and Iraqi Kurdistan, Uzbekistan, Azerbaijan, Turkmenistan, Morocco, Algeria, Lebanon, Syria, Jordan, and Israel. It grows on poor soils, e.g., gray-brown, gray-meadow, alluvial meadow, chestnut and podzolic black soils, as well as on limestone slopes, stony gray soils and on pebbly heavy loamy soils (Baum 1977; Baum et al. 1972a; Kanan and Jaradat 1996; Ladizinsky 1971c; Leggett et al. 1992; Malzev 1930; Musaev et al. 1976; Romero Zarco 1996; Roshevitz 1934; Sheidai et al. 2002).

Avena ventricosa Balansa. ex Coss., 1854, Bull. Soc. Bot. Franc., 1: 14; Coss. et Durieu, 1855, Expl. sc. Alger., 2: 109; Malzev 1930, Ovs. & Ov.,: 240, excl. subsp. *bruhnsiana* (Grun.) Malz.; Ladiz. et Zohary, 1971, Euphyt.,: 385–387; Baum 1974, Canad. Jour. Bot., 52: 2243; Tzvelev, 1976, Poaceae USSR,: 243; Rodionova et al. 1994, Cult. Fl.,: 110.

Annual. Juvenile growth prostrate. Flowering stems erect, about 65 cm high. Panicle unilateral. Spikelets awned medium sized, and containing 2 florets. Glumes nearly unequal, about 27–30 mm long with 7 veins. Lemmas small sized, glabrous. Lemma tips bisubulate. Only the lower floret disarticulates at maturity. Awn inserted at 1/2 of the lemma. Callus very long awl-shaped, about 5 mm.

A. ventricosa occurs on Cyprus as an adventive species. The primary focus of its distribution is located in Algeria and the secondary one, according to the unconfirmed reports, in Iran. Its typical habitats are stony soils with a surface of hard loamy crust, as well as a range of soils from limy to sandy desert ones (Malzev 1930; Bor 1968; Musaev and Isaev 1971; Baum et al. 1972a).

Avena bruhnsiana Gruner 1867, Bull. Soc. Nat. Moscou, 40, 4: 458, t. IXB; Roshevitz 1934, Fl. USSR, II: 261; Musaev et Isaev, 1971, Work. AN AzSSR, 27: 64–65; Rodinova et al., 1994, Cult. Fl.,: 111. – *A. ventricosa* subsp. *bruhnsiana* (Grun.) Malz., 1930, Ovs & Ov.: 242.

Annual. Juvenile growth prostrate. Flowering stems erect, 70–110 cm high. Panicle unilateral. Spikelets awned medium sized and containing 2 florets. Glumes nearly unequal, about 40 mm long with 7 veins. Lemmas medium sized, pubescent; pubescence of white color. Lemma tips bisubulate. Only the lower floret disarticulates at maturity. Awn inserted at 1/2 of the lemma. Callus awl-shaped very long, about 10 mm.

A. bruhnsiana is an endemic species that used to grow in abundance throughout the Apsheron Peninsula in Azerbaijan in dry sandy and loamy places. At present, this species can evidently be found only in its northeastern part near the coastal sands on gray-brown soils (Malzev 1930; Roshevitz 1934; Musaev 1969; Musaev and Isaev 1971; Loskutov 2007).

Avena longiglumis Durieu., 1845, Rev, Bot., 1: 359; Coss. et Durieu, 1855, Expl. Sc. Alger., 2: 110; Steud., 1855, Syn. Pl. gram.: 233; Batt. et Trabut, 1895, Fl. Alger. Monocot.: 179; idem. 1902, Fl. anal. Alger.: 370; Malzev 1930, Ovs & Ov.,: 238; Ladiz. et Zohary, 1971, Euphyt.,: 385–387; Baum 1974, Canad. Jour. Bot., 52: 2243; Rodionova et al. 1994, Cult. Fl.,: 113–115 Romero Zarco 1996, Lagascal., 18, 2: 171–198. – *A. barbata* var. *longiglumis* Hausskn., 1899, Mittel. Thur. bot., XIII, XIV: 48.

Annual. Juvenile growth semi-erect to prostrate with nodes covered by hairs. Flowering stems erect, 50–180 cm high. Panicle unilateral to equilateral. Spikelets large and containing 2–3 florets. Glumes nearly unequal, much longer than lemmas, 40 mm long with 9–10 veins. Lemmas large, pubescent, nearly equal. All florets awned. Lemma tips biaristulate 12 mm long. All florets disarticulate at maturity. Awn inserted at 1/2 of the lemma. Callus very long awl-shaped, about 10 mm.

A. longiglumis can be found in the western Mediterranean, southern Spain, Portugal, Greece, Italy, Syria, Libya, Morocco, Algeria, Israel, and Jordan. This species grows on more dense substrates, on neutral red sandy soils, and prefers light sandy soils with a varying moisture content – from fertile coastal to poor sandy desert soils (Malzev 1930; Ladizinsky 1971c; Baum et al. 1972a; Rajhathy and Thomas 1974; Leggett et al. 1992; Kanan and Jaradat 1996; Romero Zarco 1996; Perez de la Vega et al. 1998).

Avena damascena Rajhathy and Baum 1972, Canad. Jour. Genet., 14: 645–654; 1989, Rep. Work. Gr. Avena,: 19–32; Rodionova et al. 1994, Cult. Fl.,: 115.

Annual. Juvenile growth prostrate. Flowering stem geniculate, 70–80 cm high. Panicle equilateral. Spikelets awned small and containing 3 florets. Glumes equal, about 20 mm long with 7 veins. Lemma tips biaristulate. All florets disarticulate at maturity. Awn inserted at lower 1/3 of the lemma. Callus rounded-elliptical.

A. damascena was first discovered in Syria and then in Morocco. The species grows under dry stony desert conditions on alluvial-pebbly grounds as well as on red-brown dry loamy sands within the 700–1,650 m altitudinal range (Rajhathy and Baum 1972; Baum 1977; Leggett et al. 1992).

Avena prostrata Ladizinsky, 1971, Israel Jour. Bot., 20: 297–301; Baum 1974, Canad. Jour. Bot., 52: 2243; 1989.

Annual. Juvenile growth prostrate. Flowering stem geniculate, 50–60 cm high. Panicle unilateral. Spikelets awned small and containing 2–3 florets. Glumes equal, 12–15 mm long with 9–10 veins. Lemmas small, pubescent. Lemma tips biaristulate. All florets disarticulate at maturity. Awn inserted at lower 1/3 of the lemma. Callus rounded.

A. prostrata was discovered on the southwestern coast of Spain on poor loess deposits on limy and metamorphic rocks as well as in stony habitats. Also, the species has been found in the coastal part of Morocco on fertile red-brown soils (Ladizinsky 1971b; Leggett et al. 1992).

Avena canariensis Baum, Raihathy et Sampson, 1973, Canad. Jour. Bot., 51, 4: 759–762; Ladiz., 1989, Rep. Work. Gr. Avena,: 19–32; Rodionova et al. 1994, Cult. Fl.,: 116.

Annual. Juvenile growth prostrate. Flowering stems erect, 50–75 cm high. Panicum unilateral. Spikelets awned small and containing 2–3 florets. Glumes equal, 18–20 mm long with 9–10 veins. Lemmas small, pubescent. Lemma tips bidentate. Only the lower floret disarticulates at maturity. Awn inserted at 1/2 of the lemma. Callus elliptic.

A. canariensis is an endemic species from the Canary Islands (Spain), where it occurs on Fuerteventura and Lanzarote islands. It grows within the 200–500 m range on piles of basalt stones, volcanic ash, or well-structured dry volcanic soils (Baum et al. 1973; Baum 1977).

Avena wiestii Steud., 1855, Syn. Pl. gram.: 231; Roshevitz 1934, Fl. USSR, II: 265; Mordvinkina 1936, Cult. Fl. USSR,: 423; Baum 1974, Canad. Jour. Bot.,

52: 2243; Rodionova et al. 1994, Cult. Fl.,: 105–108. – *A. barbata* var. *caspica* Hausskn., 1894, Mitteil. Thur. Bot. Ver., 6: 41, 45. – *A. strigosa* subsp. *wiestii* (Steud.) Thell., 1911, Veirt. Natur. Ges., LVI: 333; idem, 1928, Rec. trav. bot. neer., XXVa: 435; Malzev 1930, Ovs. & Ov.,: 276; Ladiz. et Zohary, 1971, Euphyt.,: 385–387. – *A. barbata* subsp. *wiestii* (Steud.) Mansf., 1959, Kulturpflanze Beih., 2: 479; Tzvelev, 1976, Poaceae USSR,: 242; Romero Zarco 1996, Lagascal., 18, 2: 171–198.

Annual. Juvenile growth semi-erect to prostrate. Flowering stems erect, 75–140 cm high. Panicle equilateral. Spikelets awned small and containing 2 florets. Glumes nearly equal, 10–20 mm long with 7–9 veins. Lemma tips biaristulate 3–6 mm long with 2 denticula. All florets disarticulate at maturity. Awn inserted at lower 1/3 of the lemma. Callus oval, about 2 mm long.

A. wiestii can most often be found in Spain, eastern Transcaucasia, Azerbaijan, Turkey, Iraq, Iran, Syria, Jordan, Israel, Algeria, Egypt, in northern Sahara, and in the Arabian Peninsula. The species grows in the arid zone with 50–250 mm annual rainfall on sandy loess soils, limy slopes, gray-brown, gray-meadow and alluvial meadow soils, as well as on loamy sands in deserts and volcanic soils (Malzev 1930; Roshevitz 1934; Baum et al. 1972a; Romero Zarco 1984, 1996; Loskutov 2007).

Avena hirtula Lag., 1816, Gen. Sp. Nov.: 4; Steud., 1855, Syn. Pl. gram.: 230; Hausskn., 1894, Mitteil. Thur. Bot. Ver., 6: 42; Mordvinkina 1936, Cult. Fl. USSR,: 432; Baum 1974, Canad. Jour. Bot., 52: 2243; Rodionova et al. 1994, Cult. Fl.,: 108–110. – *A. lagascae* Sennen, 1926, Pl. Esp.: 5980. – *A. strigosa* subsp. *hirtula* (Lagas.) Malz., 1930, Ovs. & Ov.: 247; Ladiz. et Zohary, 1971, Euphyt.,: 385–387. – *A. barbata* subsp. *hirtula* (Lagas.) Tab. Mor., 1939, Bot. Soc. Brot., ser. 2, 13: 622; Mansfeld., 1959, Kulturpflanze Beih., 2: 479; Romero Zarco 1996, Lagascal., 18, 2: 171–198.

Annual. Juvenile growth semi-erect to prostrate. Flowering stems erect, 70–150 cm high. Panicle equilateral. Spikelets awned small and containing 2–3 florets. Glumes nearly unequal, 10–20 mm long with 7–9 veins. Lemmas equal, small or medium sized, strongly pubescent. Lemma tips biaristulate with 1 denticulum. All florets disarticulate at maturity. Awn inserted at lower 1/3 of the lemma. Callus narrow elliptic, about 2 mm long.

A. hirtula with the highest degree of genetic polymorphism is observed in Spain, Portugal, France, Italy, and Greece. Besides, in the eastern Mediterranean, the species occurs in Algeria, Morocco, Tunisia, Israel, Turkey, Syria, and Jordan. Among its typical habitats are the areas with small-stony or limy soils and sand dunes (Vavilov 1965c; Rajhathy et al. 1966; Baum et al. 1972a; Baum 1977; Perez de la Vega et al. 1998).

Avena atlantica Baum et Fedak, 1985, Canad. Jour. Bot., 63: 1057–1060; Ladiz., 1989, Rep. Work. Gr. Avena,: 19–32; Rodionova et al. 1994, Cult. Fl.,: 117–120.

Annual. Juvenile growth prostrate. Flowering stems geniculate, about 95 cm high. Panicle equilateral. Spikelets awned small and containing 2–3 florets. Glumes nearly unequal with 9–10 veins. Lemma tips biaristulate. Only the lower floret disarticulates at maturity. Awn inserted at lower 1/3 of the lemma. Callus round-elliptical.

A. atlantica is a Moroccan endemic. Its population grows on the Atlantic and Mediterranean coasts. The species climbs up the northwestern slopes of the Atlas Mountains up to 1,000 m. The most favored by the species are very dry red-brown sandy and stony soils in the foothills (Baum and Fedak 1985a; Leggett et al. 1992).

Tetraploid Species

Avena barbata Pott ex Link autopsia spec. orig., 1796, Herb. Acad. Sc. Petrop.; idem in Link in Schrader, 1799, Jour. Bot., 2: 315; Ledeb., 1853, Fl. Ross., 4: 412; Coss. et Durieu, 1855, Expl. sc. Alger., 2: 112; Hausskn., 1894, Mitteil. Thur. Bot. Ver., 6: 40, 45, excl. var. *caspica* et var. *longiglumis* Hausskn.; Roshevitz 1934, Fl. USSR, II: 262; Mordvinkina 1936, Cult. Fl. USSR,: 422; Mansfeld., 1959, Kulturpflanze Beih., 2: 479; Baum 1974, Canad. Jour. Bot., 52: 2243; Tzvelev, 1976, Poaceae USSR,: 242; Rodionova et al. 1994, Cult. Fl.,: 91–97; Romero Zarco 1996, Lagascal., 18, 2: 171–198. – *A. hirsuta* Moench, 1802, Meth. Suppl.: 64; Roth, 1806, Catal. Bot., 3: 19; Marschall et Bieberstein, 1819, Fl. Taur.-cauc. suppl. III: 82; Baum 1974, Canad. Jour. Bot., 52: 2243. – *A. hispida* Hort. ex Steud., 1840, Nomencl. Bot., 2, 1: 172. – *A. japonica* Steud., 1855, Syn. Pl. gram.,: 231. – *A. strigosa* subsp. *barbata* (Pott) Thell., 1911, Veirt.

Natur. Ges., LVI: 330; idem. 1928, Rec. trav. bot. neer., XXVa: 434, excl. var. *solida* Hausskn.; Malz., 1930, Ovs. & Ov.; 268. – ***A. lusitanica*** Tab. Mor., 1939, Bot. Soc. Brot., ser. 2, 13: 624; Baum 1977, Monogr. Gen. Avena: 129. – ***A. matritensis*** Baum 1977, Monogr. Gen. Avena: 129. – *A. barbata* subsp. *castellana* Romer. Zarc., 1990, Lagascl., 16: 252.

Annual. Juvenile growth prostrate to erect. Flowering stems erect, 65–210 cm high. Panicle equilateral. Spikelets awned medium sized and containing 2–4 florets. Glumes nearly unequal with 9–10 veins. Lemma tips biaristulate. All florets disarticulate at maturity. Awn inserted at 1/2 of the lemma. Callus round.

A. barbata with the highest degree of genetic polymorphism is observed throughout the Mediterranean region and along the European Atlantic coast. In the east, it is spread through Asia Minor up to the Himalayas and also occurs on the Ethiopian Plateau at altitudes around 2,200–2,800 m. It grows on limy soils, on hilly terrain with loess-like soils, in depressions with alluvial deposits on red sandy, volcanic, and heavy basalt soils, which form on metamorphic rocks. It is also spread in Brazil, Japan, and Australia as an adventive species (Malzev 1930; Roshevitz 1934, 1937; Vavilov and Bukinich 1959; Ladizinsky 1971c, 1975; Baum et al. 1972a; Kliphuis and Wieffering 1972; Whalley and Burfitt 1972; Loon van 1974; Dillenburg 1984; ; Romero Zarco 1990; Sheidai et al. 2002; Loskutov 2007).

Avena vaviloviana (Malz.) Mordv., 1936, Cult. fl. USSR,: 422; Mansfeld., 1959, Kulturpflanze Beih., 2: 479; Baum 1974, Canad. Jour. Bot., 52: 2243; Rodionova et al. 1994, Cult. Fl.,: 90–91. – *A. strigosa* subsp. *vaviloviana* Malz., 1930, Ovs. & Ov., 278. – *A. barbata* subsp. *vaviloviana* (Malz.) Nevski 1934, Work Asie. univ., ser. 8в, 17: 4; Ladiz. et Zohary, 1971, Euphyt.,: 385–387.

Annual. Juvenile growth prostrate to erect. Flowering stems erect, 80–110 cm high. Panicle unilateral. Spikelets awned medium sized, and containing 2–3 florets. Glumes equal, longer than florets, 20–25 mm long with 8 veins. Lemma tips biaristulate 1 mm long with 2 denticula. All florets disarticulate at maturity. Awn inserted at 1/2 of the lemma. Callus short, oval, about 3–5 mm long.

A. vaviloviana is an endemic species in Ethiopia, Eritrea, and Yemen. In these countries, it occurs everywhere and is very common on the Ethiopian Plateau at altitudes between 2,200 and 2,800 m, mostly on cultivated fertile lands (Mordvinkina 1936; Vavilov 1965b; Ladizinsky 1975).

Avena agadiriana Baum et Fedak, 1985, Canad. Jour. Bot., 63: 1379–1385; Ladiz., 1989, Rep. Work. Gr. Avena,: 19–32; Rodionova et al. 1994, Cult. Fl.,: 120.

Annual. Juvenile growth prostrate. Flowering stems geniculate, about 60 cm high. Panicle unilateral. Spikelets small, and containing 2 florets. Glumes nearly unequal, 15–18 mm long with 8 veins. Lemma tips bidentate. Only the lower floret disarticulates at maturity. Awn inserted at lower 1/3 of the lemma. Callus rounded-elliptical.

A. agadiriana is a Moroccan endemic species. A number of its populations have been collected from the Atlantic and Mediterranean coasts. The species prefers red-brown sandy and stony soils (Baum and Fedak 1985b; Leggett et al. 1992).

Avena magna Murphy et Terrell, 1968, Science, 159: 103; Ladiz. et Zohary, 1971, Euphyt.,: 385–387; Baum 1974, Canad. Jour. Bot., 52: 2243; Rodionova et al. 1994, Cult. Fl.,: 98–100. – ***A. moroccana*** Gdgr., 1908, Bull. Soc. Bot. France, 55: 658; Baum 1977, Monogr. Gen. Avena: 129.

Annual. Juvenile growth prostrate. Flowering stems geniculate, 65–100 cm high. Panicle unilateral. Spikelets large and containing 3–4 florets. Glumes wide, long, membranous, nearly unequal with 8–10 veins. Lemma tips bidentate. Only the lower floret disarticulates at maturity. Awn inserted at 1/2 of the highly pubescent lemma. Callus strongly pubescent, rounded.

A. magna (syn. *A. moroccana* Gdgr.), an endemic species, have been collected on the Moroccan coast and in the mountains within the altitudinal range from 500 up to 1,000–1,300 m. Wherever it occurs, this species grows on fertile, loose, reddish-brown alluvial loamy soils (Rajhathy et al. 1966; Leggett et al. 1992).

Avena murphyi Ladizinsky, 1971, Israel Jour. Bot., 20: 24–27; Baum 1974, Canad. Jour. Bot., 52: 2243; Rodionova et al. 1994, Cult. Fl.,: 97–98; Romero Zarco 1996, Lagascal., 18, 2: 171–198.

Annual. Juvenile prostrate. Flowering stems geniculate, 70–80 cm high. Panicle equilateral. Spikelets large and containing 2–6 florets. Glumes equal with 8 veins. Lemmas wide, equal, mostly glabrous. Lemma tips bidentate. Only the lower floret disarticulates at

maturity. Awn inserted at lower 1/4 of the pubescent lemma. Callus oval.

A. murphyi originates from limited natural areas in southern Spain and northern Morocco, where it grows in a typically Mediterranean climate on thick alluvial soils (Ladizinsky 1971a; Leggett et al. 1992; Perez de la Vega et al. 1998).

Avena insularis Ladizinsky 1998, Gen. Res. Crop Evol., 45: 263–269; Hanelt, 2001, Mansf. Encycl. Agr. Hort. Crops, 5: 2507.

Annual. Juvenile growth prostrate. Flowering stems geniculate, about 60 cm high. Panicle unilateral. Spikelets V-shaped, large, and containing 3–5 florets. Glumes long, equal, with 9–10 veins. Lemmas medium sized, strongly pubescent. Lemma tips bisubulate or shortly biaristate. Only the lower floret disarticulates at maturity. Awn inserted at lower 1/3 to 1/2 of the pubescent lemma. Callus elliptical.

A. insularis has been found in southern Sicily and then in Tunisia at altitudes from 50 to 150 m on alluvial clay soils with sand-clay and conglomerate-stony subsoils varying in color from whitish to gray, black, brown, or red, with a pH of 7.8, with rainfall around 400 mm. Later on, the populations of this species were collected on the same type of soil in Tunisia (Ladizinsky 1998, Ladizinsky and Jellen 2003).

Avena macrostachya Balansa et Durieu, 1854, Bull. Soc. Bot. France 1: 318; Baum 1974, Canad. Jour. Bot, 52: 2243; Ladiz., 1989, Rep. Work. Gr. Avena,: 19–32. – *Helictotrichon macrostachyum* Holub 1958, Pflanzentaxonom,: 101–133.

Perennial, outcrossing autotetraploid plant. Juvenile growth semi-prostrate. Flowering stems erect, about 100 cm high. Panicle equilateral. Floret anthers large, yellow, with anthocyan. Spikelets small with the awns, and containing 6–8 florets. Glumes short, very unequal, lower glume one-half of upper one with 7 veins. Lemmas medium long, slightly pubescent. Lemma tips bisubulate. Awns inserted in lower 1/3 of the lemma. All florets disarticulate at maturity. Callus linear-elongated.

A. macrostachya is a narrow endemic species found at the very edge of snows (1,500 m) in the mountainous regions of Djurdjura and Aures (the Atlas Mountains) in northeastern Algeria. It grows predominantly on limy soils (Baum 1977; Baum and Rajhathy 1976; Baum et al. 1975; Guarino et al. 1991).

Hexaploid Species

Avena sterilis L., 1762, Sp. Pl., ed. 2:118; Ledeb., 1853, Fl. Ross., 4:412 incl. *A. fatua* β-*trichophylla* (C. Koch.) Griseb.; Coss. et Durieu, 1855, Expl. sc. Alger., 2: 109, excl. *A. sterilis* var. *minor*; Grillet et Magne, 1875, Fl. Franc. Ed. 3:532, excl. subsp. *ludoviciana* Durieu et Magne, subsp. *barbata* Gillet et Magne; Hausskn., 1894, Mitteil. Thur. Bot. Ver., 6:38, 44, excl. *A. sterilis* f. *abbreviata* Hausskn.; Thell., 1911, Veirt. Natur. Ges., LVI: 312–319, excl. *A. sterilis* subsp. *ludoviciana* (Durieu) Gillet et Magne; idem, 1928, Rec. trav. bot. neerlan., 25a: 429–433; Malz., 1930, Ovs. & Ov.: 359, quoad subsp. *macrocarpa* (Monch) Brig.; Roshevitz 1934, Fl. USSR, II: 269; Mordvinkina 1936, Cult. Fl. USSR,: 417; Baum 1974, Canad. Jour. Bot., 52: 2243; Tzvelev, 1976, Poaceae USSR,: 238, excl. subsp. *ludoviciana* (Durieu) Gillet et Magne; Rodionova et al. 1994, Cult. Fl.,: 86–90; Romero Zarco 1996, Lagascal., 18, 2: 171–198. – *A. macrocarpa* Moench, 1794, Meth. Pl.: 196. – ***A. atherantha*** Presl, 1820, Cyper. Gram. Sicul.,: 30; Baum 1977, Monogr. Gen. Avena: 129. – *A. maxima* Presl., 1826, Fi. Sic.,: 44; Baum 1974, Canad. Jour. Bot., 52: 2243. – *A. sterilis* subsp. *macrocarpa* Briq., 1910, Prodr. fl. Cors., 1: 105; Thell., 1911, Veirt. Natur. Ges., LVI: 312–319. – *A. sativa* subsp. *sterilis* (L.) Ladiz. et Zohary, 1971, Euphyt., 20: 385–387.

Annual. Juvenile growth erect to prostrate. Flowering stems erect, 30–145 cm high. Panicle equilateral. Spikelets V-shaped, large, and containing 3–5 florets. Glumes large equal. Lemma tips bidentate. Only the lower floret disarticulates at maturity. Awn inserted at lower 1/2 of the slightly moderate pubescent lemma. Callus rounded.

A. sterilis is widely spread throughout the Mediterranean, from the Atlantic Ocean to the Himalayas, namely in Spain, Portugal, Italy, Switzerland, southern France, northern Iran, Turkey, Ukraine, Transcaucasia, in all countries of northern Africa, and in Ethiopia. The highest degree of genetic polymorphism may be observed in the areas with rainfall from 50 to 800 mm, on almost all types of sandy and clay soils, on basalt rocks, and at altitudes above 600–700 m. Numerous form of *A. sterilis* can be found at present in Japan and South Korea (Roshevitz 1934; Holden 1966; Ladizinsky 1975; Yamaguchi 1976; Garcia-Baudin et al. 1978, 1981; Schuler 1978; Maillet 1980; Brezhnev and Korovina 1981; Costa

1988; Romero Zarco 1994; Kanan and Jaradat 1996; Loskutov 2007).

Avena ludoviciana Durieu, 1855, Act. Soc. Linn. Bordeaux, 20: 41; Roshevitz 1934, Fl. USSR, II: 269; Mordvinkina 1936, Cult. Fl. USSR,: 418; Rodionova et al. 1994, Cult. Fl.,: 78–82. – *A. persica* Steud., 1855, Syn. plan. gram., I: 230. – *A. sterilis* var. *minor* Coss. et Durieu, 1855, Expl. sc. Alger., 2: 109. – *A. sterilis* subsp. *ludoviciana* (Durieu) Gillet et Magne, 1875, Fl. Fr., Ed. 3: 532; Thell., 1911, Veirt. Natur. Ges., LVI: 313; Malz., 1930, Ovs. & Ov., 363; Tzvelev, 1976, Poaceae USSR,: 239; Romero Zarco 1996, Lagascal., 18, 2: 171–198. – *A. sterilis* f. *abbreviata* Hausskn., 1894, Mitteil. Thur. Bot. Ver., 6: 39, 44. – ***A. trichophylla*** C. Koch 1848, Linnaea, XXI: 393; Roshevitz 1934, Fl. USSR, II: 269; Baum 1974, Canad. Jour. Bot., 52: 2243. – *A. sterilis* subsp. *trichophylla* (C. Koch) Malz., 1930, Ovs. & Ov.,: 363; Tzvelev, 1976, Poaceae USSR,: 238.

Annual. Juvenile growth erect to prostrate. Flowering stems erect, 40–150 cm high. Panicle equilateral. Spikelets large and containing two or rarely 3 florets. Glumes equal, 25–30 mm long. Lemma tips bidentate. Only the lower floret disarticulates at maturity. Awn inserted at lower 1/3 of the pubescent lemma. Callus rounded.

A. ludoviciana is found throughout Europe, in eastern Mediterranean, Ukraine, southern Russia, Azerbaijan, Central Asia, Iran, Asia Minor, southwestern Asia, Afghanistan, as well as along the entire coast of northern Africa and Mediterranean Sea. It grows on almost all types of sandy and clay soils and demonstrates wide genetic diversity. The species has been brought over to northern and southern Australia and to New Zealand (Roshevitz 1934; Romero Zarco 1994, 1951; Vavilov and Bukinich 1959; Holden 1969; Guillemenet 1971; Kropac and Lhotska 1971; Whalley and Burfitt 1972; Garcia-Baudin et al. 1978; Schuler 1978; Costa 1988; Loskutov 2007).

Avena fatua L. 1753, Sp. Pl.,: 80; idem, 1762, Ed. 2: 113; Lebed., 1853, Fl. Ross., 4: 412, excl. *A. fatua*., β-*trichophylla* (C. Koch) Griseb. et syn. *A. byzantina* C. Koch; Coss. et Durieu, 1855, Expl. sc. Alger., 2: 109; Hausskn., 1885, Mitt. geogr. Ges., 3: 237–239; idem, 1894, Mitteil. Thur. Bot. Ver., 6: 37, 45, exl. subsp. *sativa* Hausskn.; Thell., 1911, Veirt. Natur. Ges., LVI: 319; Malz., 1930, Ovs. & Ov.,: 287; Roshevitz 1934, Fl. USSR, II: 267; Mordvinkina 1936, Cult. Fl. USSR,: 402–403; Baum 1974, Canad. Jour. Bot., 52: 2243; Tzvelev, 1976, Poaceae USSR,: 239; Rodionova et al. 1994, Cult. Fl.,: 72–78; Romero Zarco 1996, Lagascal., 18, 2: 171–198. – ***A. hybrida*** Peterm., 1841, Fl. Bienitz: 13; Koch, 1844, Syn. Fl. Germ. Helv. Ed. 2.II: 917; Steudel, 1855, Syn. Pl. gram.: 230; Baum 1977, Monogr. Gen. Avena: 129. – *A. sativa* subsp. *fatua* (L.) Thell., 1911, Veirt. Natur. Ges., LVI: 319; Ladiz. et Zohary, 1971, Euphyt.,: 385–387. – *A. septentrionalis* (Malz.) Roshevitz, 1934, Fl. USSR, II: 265; Baum 1974, Canad. Jour. Bot., 52: 2243. – *A. meridionalis* (Malz) Roshevitz, 1934, Fl. USSR, II: 266. – *A. cultiformis* (Malz.) Roshevitz, 1934, Fl. USSR, II: 268.

Annual. Juvenile growth erect to prostrate. Flowering stems erect, 40–150 cm high. Panicle equilateral. Spikelets large and containing 2–3 florets. Glumes equal, 20–25 mm long. Lemma can be hairy or glabrous. Lemma tips bidentate. All florets disarticulate at maturity. Awn inserted at 1/2 of the lemma. Callus rounded.

A. fatua grows on different soils under different climatic conditions ranging from the Tropics right up to the Polar Circle; it climbs high into the mountains up to the upper limit of crop cultivation (till 3,000 m) and demonstrates the highest degree of genetic polymorphism. Wild oat has penetrated almost all agricultural areas around the globe – from the Atlantic Ocean across Eurasia into Mongolia, Japan, and South Korea; spread throughout southern and northern Africa and got into North and South Americas, Australia, and New Zealand (Malzev 1930; Roshevitz 1934, 1937; Vavilov and Bukinich 1959; Guillemenet 1971; Baum et al. 1972a; Kuhn 1972; Whalley and Burfitt 1972; Nilsson et al. 1973; Yamaguchi 1976; Rines et al. 1980; Costa 1988).

Avena occidentalis Durieu, 1865, Jard. Pl. Bordeaux, 2: 24; Hubbard, 1937, Fl. Trop. Afr., 10: 122; Baum 1971, Canad. Jour. Bot., 49: 1055–1057; Rodionova et al. 1994, Cult. Fl.: 82.

Annual. Juvenile growth semi-erect to prostrate. Flowering stems geniculate, 45–100 cm high. Panicle equilateral. Spikelets large and containing 3–4 florets. Glumes equal, 16–20 mm long. Lemma can be hairy or glabrous. Lemma tips bisubulate or shortly biaristate. All florets disarticulate at maturity. Awn inserted at 1/2 of the lemma. Callus rounded.

A. occidentalis occurs mostly on the Canary Islands (Spain), but according to Baum (1977) can be also found on the continent in Portugal, Egypt,

Ethiopia, as well as on the Azores and on Madeira Island, though initially it had been described in course of processing herbarium material from Algeria. The species tends to grow on alluvial soils in valleys and on stony slopes (Durieu de Maisonneuve 1845; Baum 1971).

Therefore, wild *Avena* species occupy vast areas in the basins of the Mediterranean, Black, and Caspian seas and demonstrate a wide range of genetic diversity and good adaptation to different ecogeographical and soil conditions of the regions.

A detailed morphological description and study of features of this or that oat species helps to define precisely its systematic position within a complicated system of the entire genus showing different ploidy levels and the huge morphological diversity determined by the vast natural habitat occupied by wild, weedy, and cultivated species.

3.1.1.3 Karyotype and Cytology of *Avena* Species

Quite often, karyological analyses of cultivated plants and their wild relatives confirm their taxonomic status at the interspecific levels, thus making genomic characterization of the material in question possible. Karyological and cytological studies of *Avena* L. species have a long history (Avdulov 1931; Huskins 1927; Nikolaeva 1922; Rajhathy and Thomas 1974) (Table 3.2).

Diploid Species with C-Genomes

The widest karyotype diversity is found within diploid oat species. All of them split into three groups: those containing predominantly subterminal

Table 3.2 The karyotype structure of *Avena* species

Species	Genome	Type of chromosomes[a]						Reference
		SM^{t1}	SM^{t2}	ST^{t2}	M	SM	ST	
A. bruhnsiana	Cv	–	–	1	–	1	5	Emme (1932)
A. ventricosa	Cv	–	–	1	–	–	6	Rajhathy (1961)
A. clauda	Cp	1	–	1	–	–	5	Nikolaeva (1922)
A. pilosa	Cp	1	–	1	–	–	5	Nikolaeva (1922)
A. prostrata	Ap	1	–	1	3	1	1	Ladizinsky (1971b)
A. damascena	Ad	1	–	1	4	1	–	Rajhathy and Baum (1972)
A. longiglumis	Al	1	–	1	4	1	–	Malzev (1930)
A. canariensis	Ac	1	–	1	4	1	–	Baum et al. (1973)
A. strigosa	As	1	–	1	2	2	1	Kihara (1919)
A. hirtula	As	1	–	1	2	2	1	Huskins (1926)
A. wiestii	As	1	–	1	2	2	1	Huskins (1926)
A. atlantica	As	1	–	1	2	2	1	Baum and Fedak (1985a)
A. barbata	AB	1	–	1	4	6	2	Kihara (1919)
A. vaviloviana	AB	1	–	1	4	6	2	Emme (1932)
A. abyssinica	AB	1	–	1	4	6	2	Aase and Powers (1926)
A. agadiriana	AB?	–	–	2	2	7	3	Baum and Fedak (1985b)
A. magna	AC	1	1	1	4	2	5	Murphy et al. (1968)
A. murphyi	AC	1	–	1	4	6	2	Ladizinsky (1971a)
A. insularis	AC?	1	–	1	4	7	1	Ladizinsky (1998)
A. macrostachya	CC?	–	2	–	10	–	2	Baum and Rajhathy (1976)
A. fatua	ACD	1	–	2	4	7	7	Kihara (1919)
A. sativa	ACD	1	–	2	4	7	7	Kihara (1919)
A. byzantina	ACD	1	–	2	4	7	7	Kihara (1919)
A. sterilis	ACD	1	–	2	4	7	7	Kihara (1919)
A. ludoviciana	ACD	1	–	2	4	7	7	Huskins (1926)
A. occidentalis	ACD	1	–	2	4	7	7	Baum (1971)

[a]*M* median chromosomes; *SM* submedian, *ST* subterminal, SM^{t1}, SM^{t2}, ST^{t2} satellite chromosomes with different type of satellites (*t1* large satellite, *t2* small satellite)

chromosomes (*A. ventricosa, A. bruhnsiana*, *A. clauda*, and *A. pilosa*), the species with one pair of such chromosomes (*A. prostrata, A. wiestii, A. hirtula, A. atlantica*, and *A. strigosa*), and those with only median and submedian chromosomes (*A. damascena, A. longiglumis*, and *A. canariensis*).

A. ventricosa and *A. bruhnsiana* have been determined as diploid species with the somatic chromosome number equal to 14 (Emme 1932). Numerous studies have shown that *A. ventricosa* has the Av type of nuclear genome being similar in some features with *A. pilosa* (Rajhathy 1961, 1963). Later on, modification of the *A. ventricosa* genome relative to the *clauda–pilosa* complex has been confirmed (Thomas 1970) and underlined by assigning the Cv symbol to the *A. ventricosa* genome (Rajhathy 1966, 1971b). A study of an *A. bruhnsiana* population from the VIR collection revealed that all forms of this species collected in Azerbaijan possess five subterminal chromosome pairs (Loskutov 2007) as has been observed in an earlier investigation (Rajhathy 1971b). On the basis of similarity of submedian chromosomes, the species *A. ventricosa* and *A. bruhnsiana* have been identified as the very primitive ones in the *Avena* section (Rajhathy and Thomas 1974).

A study of another pair of closely related diploid species, *A. clauda* and *A. pilosa*, has found them to have similar karyotypes (Nikolaeva 1922; Emme 1932). An analysis of karyotypes of the species has shown that the presence of two pairs of satellite chromosomes and five of medium subterminal chromosome pairs in the karyotype may be regarded as their main character. On the basis of karyological analysis, the genome formula of *A. pilosa* and *A. clauda* was changed from previously suggested Ap (Rajhathy 1966) to Cp and the species were defined as primitive ones in the *Avena* section (Rajhathy and Thomas 1974).

The differences between these genomes confirm the rule deduced for the diploid species karyotypes (Thomas 1992), the essence of which is that the differences in the morphology of chromosomes in diploids are mainly in the position of the centromere and secondary constrictions. At the same time, all the above-mentioned species with the asymmetric karyotype are not primitive in the true sense of the word; they are only archaic, i.e., undergoing evolution at a slower bradytelic type (Stebbins 1971).

The diploid Cp- and Cv-genome species were studied using C-banding and fluorescence in situ hybridization (FISH). Species with the C-genome differed considerably from the species of the A-genome group in the karyotype structure, heterochromatin type and distribution, and position of 5S and 45S rDNA loci. These facts confirmed that the C-genome had diverged from the ancestral genome before the radiation of the various A-genome group. Presumably, further evolution of the A- and C-genome species occurred separately (Shelukhina et al. 2008b).

Diploid Species with A-Genomes

The number of chromosomes in somatic cells of *A. longiglumis* is 14 (Malzev 1930) that was later confirmed by other researchers (Holden 1966). A study of different populations of this species revealed that the early flowering short-stem forms had two pairs of satellites, while the late flowering tall forms had just one pair (Morikawa 1992). Some researchers believe the genome of this species to be similar to that of *A. strigosa* (Steer et al. 1970), while others think it is different (Griffiths et al. 1959). The genome had been designated as Al (Rajhathy and Morrison 1959), and later, the difference of the genome of this species from other variants of A-genome has been confirmed by a variety of other methods (Rajhathy 1961; Thomas and Jones 1965; Murray et al. 1970).

Chromosome number and karyotype structure were determined for *A. prostrata* (Ladizinsky 1971b), and its genome was designated as Ap (Rajhathy and Baum 1972).

The number of chromosomes of *A. damascena* is 14 (Rajhathy and Baum 1972). Its karyotype (designated as Ad) resembles that of *A. longiglumis* very much with the only difference in the ratio (1:1) between the large satellite and the chromosome arm to which it is attached (Rajhathy and Thomas 1974). On the other hand, later evidence indicated that this species occupied a unique position in the A-genome diploid species based on the pattern of in situ hybridization with rDNA probes (Shelukhina 2008).

A. canariensis was shown to be a diploid species (Baum et al. 1973). The karyotype of this species (Ac) is similar to the Al (*A. longiglumis*) and Ad (*A. damascena*) karyotypes with the only difference being one satellite slightly larger. This species was supposed to be the A-genome donor for hexaploid oats (Baum et al.

1973) and for the AC-genome tetraploid species (Leggett 1980). Different populations of this species had different numbers of satellited chromosomes. The early flowering population from Lanzarote Island (Spain) was found to have just one chromosome pair with a satellite, while the late flowering populations had two pairs of SAT chromosomes (Morikawa and Leggett 1990). The diploid oat species containing the Al- and Ac-genomes were studied by C-banding and in situ hybridization. Their karyotypes displayed similar C-banding patterns but differed in size and morphology of several chromosomes, presumably because of structural rearrangements that occurred during the divergence of A-genomes from a common progenitor. A considerable similarity of *A. canariensis* and *A. longiglumis* to *Avena* diploid species carrying the As-genome variant was demonstrated (Shelukhina et al. 2008a).

The chromosomes number of cultivated *A. strigosa* is $2n = 14$ (Kihara 1919). The karyotype of this species was characteristic of the whole diploid sandy oats group that included *A. hirtula* and *A. wiestii* with the As-genome (Huskins 1926; Emme 1932; Shepeleva 1939; Rajhathy 1961), though initially the former species had been grouped with the tetraploids (Huskins 1926, 1927). A study of the *A. wiestii* karyotype and its comparison with the A-genome of hexaploid species had initially confirmed their similarity (Holden 1966; Thomas and Thomas 1970), and its partial structural modification has been proved by a number of authors (Sadanaga et al. 1968). A cytogenetic investigation of the As-genome species has displayed significant closeness of *A. strigosa* and *A. weistii* and a somewhat isolated position of *A. hirtula* in this group. The results of this investigation contradict the findings of other authors that the rearrangement of chromosome 7 is species-specific to *A. strigosa* because all three studied species (as well as *A. hispanica*) are similar morphologically as well as in terms of the chromosome C-banding pattern (Badaeva et al. 2005).

Later-discovered species *A. atlantica*, with the number of chromosomes in somatic cells $2n = 14$, had a similar karyotype as the As-genome (Baum and Fedak 1985a), though its plants differed very much morphologically from other species in this group. *A. strigosa*-specific carried a chromosomal translocation relative to the diploid species *A. atlantica* and *A. hirtula* (Loarce et al. 2002).

Tetraploid Species with CC-Genomes

A. macrostachya, the most primitive perennial outcrossing species, has been identified as an autotetraploid ($2n = 28$). It has a symmetrical karyotype mainly with median chromosomes, which differ little in size and represent a gradually decreasing series in terms of length. The presence of a large number of chromosomes, which are similar in terms of size and structure, favor its autotetraploid nature. Cytologically, the nature of this species has been confirmed by behavior of chromosomes in meiosis. On the average, 56.6% of chromosomes formed quadrivalents, which is characteristic of the majority of autotetraploids in the Poaceae family (Baum and Rajhathy 1976). A symmetrical karyotype is not characteristic of the C-genome diploid species (Pohler and Hoppe 1991), but at the same time, this character brings *A. macrostachya* closer to the A-genome oat species (Loskutov 2001b). The presence of large C-heterochromatic blocks in the chromosomes' pericentromeric regions indicates similarity between this and the C-genome species and it is supposed that this species can have the hitherto undescribed CmCm-genome (Rodionov et al. 2005). At the same time, the karyotype symmetry suggests its primitive nature, being confirmed by outcrossing, perennial type of development, and a complex of morphological and cytological characteristics. According to Levitsky's (1976) results in 1931 obtained and those of Stebbins (1971) perfectly symmetrical karyotype is an indicative of species primitiveness.

Tetraploid Species with AB-Genomes

Morphologically, karyotypes of tetraploid species are subdivided into two groups that have two distinctively different genomes. In the first place, the studies of tetraploid species refer to the chromosome number analysis in the wild species *A. barbata* (Kihara 1919; Emme 1932; Shepeleva 1939). Morphologically, chromosomes of the tetraploid species differ somewhat from those of the diploid species belonging to the *strigosa* group, and the AsAb formula has been used to designate their karyotype (Holden 1966). On the other hand, the genome of this group of species has been designated as AB (Ladizinsky 1973b) as it was

determined earlier that the A-genome of the tetraploid species was similar to the As-genome of the diploid ones (Ladizinsky and Zohary 1968). A similarity of the main karyotypic features with other tetraploid species with AB-genomes has been confirmed by many researchers (Rajhathy and Morrison 1959; Thomas and Thomas 1970). This group includes two Ethiopian endemics: *A. vaviloviana*, a wild species (Emme 1932), and cultivated *A. abyssinica* (Aase and Powers 1926; Emme 1932). All these species have one and the same karyotype and AB-genome composition.

The recently discovered species *A. agadiriana* has been classified as tetraploid (Baum and Fedak 1985b). Its karyotype does not resemble those of any other tetraploids (Baum and Fedak 1985b), still its genome is presumed to be AB (Leggett 1988), though this species has an absolutely different morphological structure of lodicules and of the lemma tip. A study of different populations of this species has shown the early flowering forms to have three pairs of satellited chromosomes, while the late flowering ones had only two (Morikawa 1991, 1992).

The tetraploids *A. abyssinica*, *A. vaviloviana*, *A. barbata*, and *A. agadiriana* have been studied using C-banding and in situ hybridization, which confirmed affiliation of all the four species with the AB-genome group and demonstrated significant isolation of *A. agadiriana*. A supposition has been made that *A. abyssinica*, *A. vaviloviana*, and *A. barbata* had originated from a common tetraploid progenitor: however, the species diverged due to structural chromosome rearrangements and changes in the polymorphism system. Phylogenetic closeness has been demonstrated of the A- and B-genomes of these species to the A-genome diploids. The most probable progenitor of the A-genome for *A. abyssinica*, *A. vaviloviana*, and *A. barbata* is an As-genome species. The second diploid progenitor of these three species has not been identified, but most likely, it does not belong to the As-genome group. Diploid progenitors of *A. agadiriana* also have not been established; however, it is supposed that *A. damascen*a could be one of them (Shelukhina et al. 2009). FISH and Southern hybridization were used to investigate the chromosomal distribution and genomic organization of tetraploids. The study indicates that the Ethiopian endemic species *A. abyssinica* and *A. vaviloviana* have diverged from *A. barbata*. Differences between *A. barbata* and *A. vaviloviana* genomes were also revealed by both FISH and Southern hybridization using pAs120a rDNA probes. Whereas two B-genome chromosome pairs were found to be involved in intergenomic translocations in *A. vaviloviana*, FISH detected no intergenomic rearrangements in *A. barbata* (Irigoyen et al. 2001).

Tetraploid Species with AC-Genomes

A. magna (Murphy et al. 1968) and *A. murphyi* (Ladizinsky 1971a, d) represent the second group of tetraploid species. It has been established that the karyotype of *A. magna* differs from those of other tetraploid species and does not contain the B-genome (Murphy et al. 1968). Later, this genome was designated as AC instead of the previously determined AD-genome (Murray et al. 1970). A cytogenetic analysis of *A. magna* and *A. barbata* hybrids has found these species to have little relation to each other (Thomas 1988). Further study showed that the karyotype of *A. murphyi* has a somewhat different structure (Rajhathy and Thomas 1974), though this species also have the AC-genome (Rajhathy and Thomas 1974).

Another tetraploid species, *A. insularis,* is morphologically very similar to the above-mentioned group. The species was not found to contain the A-genome (Ladizinsky 1999): presumably, this species contains either AC- or CD-genome. Crosses between *A. insularis* populations collected on Sicily and in Tunisia gave partially sterile hybrids, confirming their genetic remoteness (Ladizinsky and Jellen 2003). Evaluation of *A. insularis* C-banding karyotype showed that the Sicilian wild tetraploid is a close relative of *A. magna* and *A. murphyi* and is more plausible than either of these two species as the immediate tetraploid progenitor of *A. sterilis* and the other hexaploid oats (Jellen and Ladizinsky 2000).

A comparative cytogenetic investigation of three tetraploid species *A. magna*, *A. murphyi*, and *A. insularis* has shown these species to be similar in terms of C-banding patterns of a number of chromosomes. According to the data obtained, *A. insularis* is phylogenetically closer to *A. magna*, while *A. murphyi* is somewhat isolated from two other species. It may be supposed that all of the three studied species have a common tetraploid progenitor and their divergence is associated with different species-specific chromosome rearrangements. Apparently, the evolution of

A. murphyi proceeded independently from other species (Shelukhina et al. 2007).

Hexaploid Species

Hexaploid species have similar karyotypes and in general have the same genome composition. Investigations of these species started by determining chromosome numbers for *A. fatua*, *A. sterilis* (Kihara 1919; Emme 1932), and *A. ludoviciana* (Huskins 1926). Eighteen chromosome pairs of hexaploid *A. sativa* and *A. fatua* were morphologically similar, while three pairs showed very different morphology (Shepeleva 1939). Later on, it was determined that such karyotype structure is typical for hexaploids. *A. fatua, A. sterilis, A. byzantina*, and *A. sativa* have been found to possess the same ACD-genome composition (Rajhathy and Morrison 1959; Rajhathy 1966). Initially, the genome was designated as ABC (Nishiyama 1929), but then, the genome formula was changed to ACD (Rajhathy and Morrison 1959; Rajhathy 1963). It has been later supposed that A-genome was the Ac-genome of the diploid *A. canariensis* (Thomas and Leggett 1974), while the Cv-genome of the diploid *A. bruhnsiana* was the source of the C-genome (Rajhathy 1966). However, similarity of the As-genome to the A-genome of hexaploid species has not been confirmed (Ladizinsky 1969). A cytogenetic study of populations of wild Mediterranean species concluded that *A. pilosa* (Cp) and the *A. strigosa* (As) group are the basic variants of the C- and A-genomes, respectively, while other species (*A. ventricosa* (Cv) and *A. longiglumis* (Al)) have structurally altered derivative karyotypes (Rajhathy 1963).

On the contrary, a study of the AB-genome of the tetraploid *A. barbata* did not identify it as a progenitor of the hexaploid species (Thomas et al. 1975).

Thus, the modern karyological and cytological investigations make it possible to identify chromosomes in a karyotype and thus provide the possibility of making a judgment about intraspecific and genomic differences at the chromosomal level. Therefore, the karyotypic peculiarities characterizing individual species facilitate a better understanding of the position of each species in the system of genus *Avena* and help to elucidate taxonomic problems.

3.1.1.4 Agricultural Status of Wild Oat Species

Some *Avena* species are truly wild and grow in natural habitats on different types of soils, and some of them are weeds. They comprise noxious weeds in agricultural fields such as *A. fatua* and *A. sterilis*, including the well-known *A. ludoviciana* and *A. barbata*, and truly wild plants as native components of their local flora as *A. clauda, A. pilosa, A, ventricosa, A. prostrate, A. damascena, A. hirtula*, *A. wiestii*, *A. canariensis*, *A. atlantica, A. murphyi, A. agadiriana, A. insularis,* etc. (see Sect. 3.1.1.2).

All oat species prefer in general a temperate hot, semi-arid and dry climate; in the east, they prefer temperate semi-arid or temperate arid climate and do not grow in hot and dry climates. For diploid and tetraploid species, a combination of favorable climate types in the African continent occurs in some regions of Morocco, Algeria, and coastal regions of Tunisia in the north, and Ethiopia in the east. In the European continent, this combination is offered by the southern Europe, especially Spain, Mediterranean islands, as well as coastal and mountainous territories of the Black and Caspian sea basins. In the Asian continent, favorable climates may be found in Israel, Jordan, Syria, Turkey, Azerbaijan, northern parts of Iraq, Iran, and Afghanistan, and Central Asian states, and it is here that some of the diverse species of oat are found. Hexaploid species *A. sterilis* and *A. ludoviciana* grow in the same territories and occupy wider areas to the south and north of the natural habitat. *A. fatua* has a wider natural habitat, which encompasses all European countries up to the northern ones as well as territories of Russia, Mongolia, and China. This species has never occurred below the 25th parallel in the Asian continent. In the African continent, this species occupies the entire northern part and reaches as far as Ethiopia and northern Kenya in the east. *A. barbata*, *A. ludoviciana*, *A. sterilis*, and *A. fatua* occur as adventitious species in most regions of the American continent, on the southern tip of Africa, in New Zealand, and Australia (see Sect. 3.1.1.2).

The the economic importance of the wild diploid species such as *A. clauda* and *A. pilosa* occurring in arid semi-deserts in the Caucasus and *A. canariensis* growing in the Canaries (Spain) is not great as they have limited value as forage for cattle (Grossheim 1967; Morikawa and Leggett 1990); however, the

nutritive value of another tetraploid species, *A. barbata,* is of special importance for breeding. The hybrids produced in Japan exceed standard cultivars by 15–40% in terms of total green mass yield. In the US, the latter species is regarded as a promising forage (Marshall and Jain 1968, 1970; Coffman et al. 1970). According to a number of explorers, the hexaploid species *A. fatua* and *A. ludoviciana* represent a fairly good pasture forage in the Caucasus and southern Russia and are promising for green fodder crop breeding and use for fodder purposes (Roshevitz 1934; Grossheim 1967; Musaev et al. 1976). A thorough study of local populations of *A. sterilis* applying different methods was carried out in California with the aim of involving them in fodder crop breeding (Singh et al. 1973). In India, *A. strigosa, A. magna, A. sterilis*, and *A. fatua* are widely used for breeding fodder cultivars (Choube et al. 1985).

Thus, on the one hand, wild oat species are noxious weeds of agricultural crops, while on the other, they can be used as natural forages for agricultural purposes.

3.2 Conservation Initiatives

3.2.1 Evaluation of Genetic Erosion at Space and Time Scale

Population growth, urbanization, developmental pressures on the land resources, deforestation, changes in land use patterns, and natural disasters are contributing to abundant habitat fragmentation and destruction of the crops and their wild relatives. Social disruptions or wars also pose a constant threat of genetic wipeout of such promising diversity. Overexploitation and also the introduction of invasive alien species are the other factors contributing to the loss of genetic resources. More recently, global warming and a high degree of pollution have also been recognized as further causes for the loss of biodiversity (Hammer and Teklu 2008).

Genetic diversity among domestic lines or cultivars is always more restricted than that of the parents or the general plant population(s) from which the cultivars originated. As improvements are made through further recombination and selection among progenies, genetic diversity narrows even more. When the oat crop was developed from the original wild species, early agriculturists selected successive individual plants over a long time on the basis of favorable appearance, seed or green forage production or both under cultivation, and personal preferences of those who harvested and saved seed for replanting. Each primitive cultivar had less genetic diversity than the source population or the species (Wesenberg et al. 1992).

Loss of such diversity is critical because it could be a source of pest resistance or adaptive characters not recognized at the time. Loss of genetic diversity associated with oat domestication and other species, however, dwarfs the loss of genetic diversity that modern plant breeders have been responsible for in the process of cultivar development. Thus, a continuing effort is required to conserve most wild *Avena* populations, old landraces, and populations; otherwise they will be lost forever.

Two types of genetic erosion can be distinguished in wild populations: the extinction of populations and the drastic change of genetic structure of populations. The first type means the total loss of genetic resources, which results from complete destruction of habitats, and all genotypes and/or alleles being lost, while the second one originates from isolated local populations due to the deterioration of habitats. In another case, there is significant risk of the ultimate loss of diversity because smaller populations are vulnerable to demographics and the decline in fitness associated with genetic drift and inbreeding. Genetic drift is the random change in allele frequency that occurs because gametes transmitted from one generation to the next carry only a sample of the alleles present in the parental generation (Hammer and Teklu 2008).

The threatened wild oat species and those with a shrinking natural habitat include *A. clauda, A. pilosa, A. ventricosa, A. bruhnsiana, A. damascena, A. prostrata, A. canariensis, A. atlantica, A. agadiriana, A. magna, A. murphyi*, *A. insularis*, and *A. occidentalis*. All these species represent components of natural habitats in some countries on the Mediterranean coast of Africa, southern coast of Spain, Italy, Greece, and a part of Mediterranean and Atlantic islands. Most natural habitats are disjunctive due to the island location of habitats; therefore, the species are narrow endemic in the outlined area. At present, natural habitats of these species are shrinking because of the

anthropogenic pressure. Apparently, the above-mentioned species once had wide natural habitats. For instance, Malzev (1930) noted in his work that in the early twentieth century, *A. ventricosa* used to be widely spread in Algeria and in Cyprus, while at present, the natural habitat of this species is very narrow, with single plants occurring in these regions. According to researchers who recently have been collecting *A. bruhnsiana*, in the Apsheron Peninsula (Azerbaijan), this species is disappearing at high rate, most likely because of activities of oil development and construction companies in this territory (Loskutov 2007). The endemic diploid species *A. canariensis* occurs only within a limited area of the Canaries (Spain); another tetraploid species (*A. agadiriana*), isolated from that diploid and resembling it morphologically, is distributed in a very narrow range near Agadir in Morocco. According to Spanish and Moroccan researchers, genetic diversity of the tetraploid species *A. murphyi* has sharply decreased in comparison with the results of previous collecting, and in some places, it could not be found at all (Perez de la Vega et al. 1998; Saidi and Ladizinsky 2005). The recently described new tetraploid species *A. insularis*, which has been discovered in both Italy (Sicily) and Tunisia, also has a very narrow natural habitat. Of special importance is the most primitive oat species – the perennial outcrossing tetraploid *A. macrostahya*. This narrow endemic species has been found at the very edge of snows (1,500 m) in the mountainous regions of Djurdjura and Aures (the Atlas Mountains) in northeastern Algeria only. Apparently, all the above-listed species are losing a part of their habitats, and some genotypes and/or alleles are being lost; other species may experience genetic erosion as a result of genetic drift and inbreeding caused by the shrinkage or split of natural habitats, or the decrease in plant number in a certain territory.

The problem of genetic erosion through inappropriate maintenance of ex situ collections is widely recognized. Genetic erosion can occur at many stages in the preparation, subsampling, exchange, storage, and regeneration of seed samples (Sackville Hamilton and Chorlton 1997). These authors also highlighted loss of diversity through genetic shifts and convergent selection during regeneration as a potentially severe and often underacknowledged problem. However, the different institutes are suffering from financial problems, lack of staff, and shortage of farms.

The threat of loss of genetic integrity from regeneration of wild oat species is very high. The reason is that practically all *Avena* species originate from the Mediterranean and adjacent regions, while the ex situ collections are maintained predominantly in countries that are quite distant from the regions of collection and natural habitats. As a result, regeneration of these species in the regions where the genebanks conserving these accessions are located may lead to genetic shifts and convergent selection during regeneration. Lengthening of regeneration backlogs due to inadequate funding, lack of technical staff, and its low proficiency causes partial loss of germination by seed material, which, in turn, leads to the loss of genetic integrity of the non-regenerated accessions. The long-term storage strongly reduces the metabolism and therefore highly limits viability and seed vigor. In this case, poor germination potential and vigor in the field also are indicators of genetic erosion.

Another important problem complicating regeneration of accessions and experienced by genebanks around the world in their work with wild oat species is the absence of distinct descriptors based on clearly visible and easily distinguishable morphological characters, which could be used for the identification of botanical diversity using morphological characters of the plant in the field and morphological characters of the kernels in the laboratory. On the whole, these problems may lead to genetic erosion of individual accessions even if they are under careful conservation and safety-duplication.

3.2.2 *Attempts of* In Situ *and* Ex Situ *Conservation*

The conservation of plant diversity is of critical importance because of the direct benefits to humanity that can arise from its exploitation in improved agricultural and horticultural crops, because of the potential for development of new medicinal and other products and because of the pivotal role played by plants in the functioning of all natural ecosystems (Hammer and Teklu 2008).

In formulating strategies for the conservation of any crop, it is essential to know areas of distribution and identify regions where its collecting for conservation activities could usefully be initiated. This will

be due to a combination of high levels of genetic diversity at the site(s), interest of the user community in the specific genetic diversity found at or believed to be found at the site, lack of previous conservation activities, and imminent threat of genetic erosion (Maxted et al. 1997).

There are two primary complementary conservation strategies, ex situ and in situ, each of which includes a range of different techniques that can be implemented to achieve the aim of the strategy. The products of conservation activities are primarily conserved germplasm, live and dried plants, cultures, and conservation data. The conservation materials are either maintained in their original environment or deposited in a range of ex situ storage facilities. To ensure safety, conservation products should be maintained at more than one location (Hammer and Teklu 2008).

The great genetic diversity to be found in the traditional agriculture stocks in the centers of genetic diversity, where the wild or weedy relatives of crop species can be found, were called gene centers or centers of origin according to Vavilov (1926) and centers of diversity according to Harlan (1975).

As agriculture progressed with the beginning of scientific plant breeding, modern varieties were widely distributed replacing landraces from cultivation. This increased the need to formally store plants and seeds in ex situ collections. Landraces were then gathered together, which resulted in fairly large collections, above all in the US and in Russia. The great Russian biologist N. I. Vavilov accumulated rich collections of diversity in the Institute of Plant Industry (now VIR) by systematically collecting material (Loskutov 1999).

Wild species are much more difficult to maintain and to regenerate than the cultivated forms. Their safeguard is a major concern in the oat community. As the convention of biological diversity (CBD) and the International Treaty (IT) reinforce the national sovereignty on biological diversity, centers of diversity will need primary consideration in developing a global conservation strategy, also with respect to complementary actions of ex situ and in situ conservation.

Such a complementary approach will not only increase the security of the conserved material, but it will also allow evolutionary processes to continue, use the safest conservation strategies, and facilitate a closer link between the more scientific orientated and static ex situ approach with the much more practically orientated and dynamic in situ conservation. In addition, it is expected that this complementary approach will also result in an improvement of the quality of conservation and of the knowledge of the existence of genetic material as well as in more transparency. These factors increase the possibility to integrate the various conservation strategies and to make use of the material collaboration, also through exchange, independently from where the material is located (ECPGR 2008).

Among the various ex situ conservation methods, seed storage is the most convenient for long-term conservation of plant genetic resources. Traditionally, many crops are conserved as seed in gene banks. This involves desiccation of seeds to low moisture contents and storage at low temperature. The ex situ conservation of large numbers of cultivated plants depends on the longevity of the seeds. Most species belong to the orthodox seed type with a logarithmical progression of shelf-life as humidity and storage temperature are reduced (Hammer and Teklu 2008).

The recently established Global Crop Diversity Trust has begun contributing to the development of regional and global crop-specific strategies aimed at more effective arrangements for ex situ conservation (*www.croptrust.org*). The Global Strategy for the ex situ conservation for oats was discussed at the meeting of genebank curators and stakeholders of oat in St. Petersburg in 2007.

Ex situ conservation of wild oat species has provided fairly good results. Some of these species, especially hexaploid ones, can retain their germinating ability for 10–15 years at room temperature. Under controlled conditions, at $+4°C$, $-10°C$, or $-18°C$ and standard humidity, seeds of these species keep viability well and therefore retain genetic integrity for a long period. Unfortunately, until now, no research has been undertaken by genebanks to elucidate the influence of duration of different types of ex situ storage facilities on the oat species genetic integrity. Judging from the data on genetic integrity of oat landraces conserved at VIR in ex situ storage facilities for the last 80 years, a positive conclusion about genetic stability of the studied material can be made (Zelenskaya et al. 2004).

In situ conservation is defined as the conservation of ecosystems and natural habitats, and the maintenance and recovery of viable populations of species in their natural surroundings, and, in the case of

domesticated or cultivated species, in the surroundings where they have developed their distinctive properties.

The first decision concerning the establishment of a nature reserve for the in situ conservation of cereal species in Ethiopia was taken at the First International Wheat Congress in Rome in April 1927 following the N.I. Vavilov's report entitled "Les centres mondiaux des genes du ble," in which he formulated preliminary results of his collecting mission to Ethiopia (Loskutov 1999). Over 50 years had passed before this method of genetic diversity preservation was adopted by the international community.

In situ conservation activities should be carried out for saving wild oat species, some of which are quite rapidly disappearing from Earth. Unfortunately, according to the Spanish researchers, only vegetation of the Canaries can at present be regarded as relatively protected from destruction, thanks to the ban on human activities expansion in effect on the islands. Among the species growing there are *A. canariensis* and *A. occidentalis*. At present, an EU project for the in situ conservation of the species of special importance for cultivated oat breeding (*A. magna, A. murphyi* and *A. insularis*) is being developed for implementation in Morocco, Spain, Italy, and Tunisia.

3.2.3 Germplasm Banks: National and International

About 220,000 oat accessions in ex situ collections have been estimated in the state of the world's plant genetic resources report. Large collections are held by the USDA (20,000 accessions), the PGRC, Canada (30,000 accessions), and within the framework of the ECP/GR (34,146 accessions), namely by the Vavilov Institute of Plant Industry (VIR, Russia) (about 12,000 accessions), which has a collection of about 10,000 accessions of four cultivated and 2,000 accessions of 21 wild species (Loskutov 2001b). For those 90,000 accessions, documentation is easily accessible on the internet. Further, large collections have been mentioned by FAO in Kenya (13,000 accessions), in Israel (7,500 accessions), the latter announcing an especially rich collection of *A. sterilis* (5,000 accessions), and in Australia (Germeier 2008).

In FAO/WIEWS (*http://apps3.fao.org/wiews/germplasm.htm*), 29 collections are listed that maintain accessions of wild *Avena* species (Table 3.3) of which 13 hold more than 20 respective accessions (Brazil, Canada, China, Germany, Israel, Morocco, Norway, Poland, Russia, Spain, Sweden, UK, USA).

Some genebanks accumulate specific and geographic diversity of wild oat species not only by means of natural collection but also through seed exchange with and ordering samples from other genebanks (Tables 3.4 and 3.5). This way, they managed to accumulate quite a wide diversity of these species. These genebanks are located in USA, Canada, Russia, Germany, Great Britain, and Poland. Other genebanks, such as those in Australia, Israel, Turkey, Brazil, and some other countries, have the mandate to collect and conserve national biodiversity including wild species that occur in these territories. Therefore, they possess small collections of the species in question. It should be noted that hexaploid species, *A. sterilis* (*A. ludoviciana*) and *A. fatua*, in the first place represent the

Table 3.3 Number of accessions of wild oat species in the main genebanks (Germeier 2008)

Institution	Country	Number of accesions
Agriculture and Agri-Food Canada, Plant Gene Resources of Canada, Saskatoon Research Center	Canada	14,935
USDA-ARS, National Small Grains Germplasm Research Facility	USA	10,908
N.I. Vavilov Research Institute of Plant Industry	Russia	2,001
Tel-Aviv University Institute Cereal Crop Development Lieberman Germplasm Bank	Israel	1,544
Agricultural Research Center, Australian Winter Cereals Collection	Australia	549
Aegean Agricultural Research Institute, Department of Plant Genetic Resources	Turkey	311
Institute for Plant Genetics and Crop Plant Research – Genebank	Germany	300
National Wheat Research Center	Brazil	254
National Plant Genetic Resources Center Plant Breeding and Acclimatization Institute	Poland	168
Agricultural Research Organization, Volcani Center, Israel Gene Bank for Agricultural Crops	Israel	117

Table 3.4 Representation of wild Avena species in ex situ collections in the world (Germeier 2008)

Species	Number of accessions
A. atlantica	18
A. brevis	87
A. canariensis	70
A. damascena	17
A. hirtula	75
A. hispanica	16
A. longiglumis	85
A. nuda	35
A. prostrata	2
A. strigosa	697
A. wiestii	76
A. bruhnsiana	1
A. clauda	111
A. pilosa (*syn. A. eriantha*)	156
A. ventricosa	8
A. macrostachya	13
A. abyssinica	615
A. barbata	2,526
A. lusitanica	30
A. vaviloviana	248
A. agadiriana	18
A. insularis	14
A. magna (*syn. A. moroccana*)	97
A. murphyi	12
A. diffusa	8
A. fatua	2,341
A. hybrida	24
A. ludoviciana	444
A. macrocarpa	2
A. occidentalis	71
A. sterilis	22,951

main part of wild oat accessions in the ex situ collections because they are of great importance as breeding material and are easy to conserve and propagate in the field (Table 3.5). Other species, especially the diploid ones, are rare in genebank collections as their natural ranges are narrower, their diversity is sufficiently smaller, and because most of them are difficult to distinguish from each other, and their conservation and propagation are very laborious. In this respect, the Canadian genebank is quite unique with its collection of oat species founded by B. Baum, who has been collecting oats worldwide in the 1970s–1980s (Baum et al. 1975). The American genebank maintains a small collection of diploid and tetraploid species; VIR (Russia) conserves a diverse collection of these species; small collections are also preserved in Germany and Poland. Besides, a small, very carefully composed unique collection of diploid and tetraploid species of oats is conserved in the Welsh Plant Breeding Station, University College of Wales (UK). The base of this collection is seed of *Avena* wild species obtained during Canada–Wales Expeditions (Rajhathy et al. 1964). Basically, this collection was assembled for research rather than practical purposes for studying cytogenetics and genomic interactions between wild oat species (Leggett 1992a).

Genebanks can employ standard methods of ex situ conservation, but organizationally they may differ. Two types of national genebanks exist, the centralized and decentralized ones. Genebanks of the first type unify management of all the plant genetic resource (PGR) activities within one single center (institution), while the stations, where collections are studied and regenerated, can be located elsewhere (Russia, Germany, Canada, etc.). The decentralized genebanks consist of a National PGR Council that coordinates activities of individual institutions and stations where different collections are conserved, studied, and regenerated (USA, France, Spain, etc.).

The institutions with a long-standing history of PGR activities belong to the group of centralized genebanks, while the recently established ones, especially those in the West-European countries, belong to the decentralized organizations.

United States' National Plant Germplasm System. Initially in 1898, at the Department of Agriculture of US was established the Section of Seed and Plant Introduction. Later on, the National Plant Germplasm System (NPGS) was organized. It constitutes a coordinated group of scientists from Federal, State, and private sectors of the US agricultural research community. Responsibilities include (1) the acquisition, maintenance, evaluation, enhancement, and distribution of a broad array of germplasm, (2) research on the preservation of genetic diversity and methods of preserving viability through improved storage procedures, and (3) monitoring of genetic vulnerability (Wesenberg et al. 1992).

The key elements of the NPGS pertinent to oat include:

1. USDA-ARS Plant Science Institute, Beltsville Agricultural Research Center, Beltsville, MD. This includes the Plant Introduction Office, the Germplasm Services Laboratory, the Germplasm Quality Enhancement Laboratory, and the Systematic Botany and Nematology Laboratory. Included in

Table 3.5 Representation of wild *Avena* species in the main ex situ collections (Germeier 2008)

Genebank	*atlantica*	*canariensis*	*damascena*	*hirtula*	*longiglumis*	*wiestii*	*clauda*	*pilosa*	*ventricosa*	*macrostachya*	*barbata*	*vavilioviana*	*agadiriana*	*insularis*	*magna*	*murphyi*	*fatua*	*hybrida*	*ludoviciana*	*sterilis*
Canada	15	45	3	51	45	46	91	132	5	1	1,685	135	14	3	34	2	579	23		11,461
USA					7	4		4			611	43			2	1	1,322	1		8,246
Russia	2	17	5	13	12	15	13	12	3	1	90	46	4	2	16	4	219		434	833
Australia										1							7			536
Germany		7			5	5					40				34	2	96	1		65
Poland	1		9	6	1					10	3			8	1	1	20			37
Israel				7	10	5	6	6			10									1,500
Turkey											32						71		4	204
Brazil					5	1		2			50	22			3		1			52

the Germplasm Services Laboratory is the Germplasm Resources Information Network (GRIN) Database Management Unit.
2. USDA-ARS National Seed Storage Laboratory (NSSL), Fort Collins, CO. The NSSL, established in 1958, is the only long-term storage facility in the US for crop germplasm normally maintained through seed.
3. USDA-ARS National Small Grains Germplasm Research Facility, Aberdeen, ID. The NSGC is housed in the USDA-ARS National Small Grains Germplasm Research Facility that was completed in 1988 at the University of Idaho Aberdeen Research and Extension Center, Aberdeen, ID (Wesenberg et al. 1992). This is main storage for all cereal crops and for cultivated and wild oat too.

Nearly one-half of the NSGC oat germplasm consists of wild *Avena* species, primarily *A. sterilis* and *A. fatua*. Since the latter two are hexaploids and can be crossed readily with *A. sativa,* they are currently the most useful of the related species. Adequate diversity is probably represented in the *A. sterilis* accessions obtained from Israel, but not from other regions of the world. There is inadequate diversity represented by the accessions available in the NSGC for most other wild species, including some diploids and tetraploids (Wesenberg et al. 1992). GRIN provides National Genetic Resources Program (NGRP) personnel and germplasm users continuous access to databases for the maintenance of passport, characterization, evaluation, inventory, and distribution data important for the effective management and utilization of national germplasm collections (*http://www.ars-grin.gov/*).

Plant Gene Resources of Canada. Agriculture and Agri-Food Canada appointed the first Plant Gene Resources officer and established Plant Gene Resources of Canada (PGRC) in 1970. Until early 1998, it was located at the Central Experimental Farm in Ottawa, but moved to a modern facility in Saskatoon. Canada's Plant Germplasm System is a network of centers and people dedicated to preserving the genetic diversity of crop plants, their wild relatives, and unique plants in the Canadian biodiversity. The system plays a significant part of Agriculture and Agri-Food Canada's commitment to the Canadian Biodiveristy Strategy in response to the Convention on Biological Diversity. The largest wild oat collection in the world is located there.

A multinodal system was established in 1992 to respond to the recommendations from study committees on the enhancement of germplasm conservation in Canada. It was initially funded through the Green Plan. This initiative links rejuvenation, evaluation, and documentation to research and plant breeding programs for specific crop plants. Seed storage facilities at PGRC consist of long-term, medium-term and cryopreservation units. For long-term storage, a large walk-in vault is available in which seed is preserved in laminated envelopes at −20°C. For medium-term storage, a large walk-in vault stores seed in paper envelopes at 4°C and 20% relative humidity. Seeds are evaluated for viability, dried to optimum moisture content of 6–8% and transferred to either medium- or long-term storage. Cryopreservation (a type of freezing) in or over liquid nitrogen at −196°C is the most highly developed of these techniques. Depending on the species, dry seeds can last from a few years to probably centuries (*http://pgrc3.agr.gc.ca/index_e.html*).

Vavilov Institute of Plant Industry – VIR (Russia) originated and developed from the Bureau of Applied Botany organized in 1894; in 1930, it was named the All-Union Research Institute of Plant Industry (VIR – Russian abbreviation), and since 1967, it has borne the name of N.I. Vavilov. The main directions in the work of the Institute were determined by N.I. Vavilov to be collecting, conserving, studying, and utilizing plant genetic resources. Besides, the Institute is involved in designing methods for studying collection materials and developing various long-term conservation techniques. The Vavilov Institute of Plant Industry is governed by the administration (located in St. Petersburg) that supervises and coordinates activities of the entire institute and experiment stations, including nine departments of crop genetic resources, the Department of Agrobotany, and In Situ Conservation with a unique herbarium collection, ten departments and laboratories dealing with methodology of PGR studies, a genebank consisting of several storage facilities (ensuring PGR conservation at +4°C, −10°C, −18°C and cryoconservation), and the Department of Biotechnology that includes an in vitro conservation unit. The network of the Institute also includes 11 experimental stations for PGR studies and regeneration, and the

National Seed Store at Kuban Experiment Station (+4°C, −10°C, −18°C).

On the VIR website, more information about Institute's activity and passport database of VIR collection is available (*http://vir.nw.ru/data/dbf.htm*). VIR collection of cultivated and wild oat is maintained in the Department of Genetic Resources of oat, barley, and rye. All information about taxonomy, characterization, evaluation, and breeding value of cultivated and wild species collection is available on their *Avena* webpage (*http://vir.nw.ru/avena/*).

Coordination activities of national genebanks are fulfilled by international organizations. The International Board for Plant Genetic Resources (IBPGR) was established in 1974 to create and coordinate a worldwide network of germplasm resource conservation centers. The IBPGR receives funds from the World Bank, the Food and Agriculture Organization of the United Nations (FAO), the United Nations Development Program, and individual donor nations. Like other international centers for agricultural research, the IBPGR is under the jurisdiction of the Consultative Group on International Agricultural Research (CGIAR), established in 1971. The Board's Secretariat, responsible for collecting and documenting information and administering the Board's financial program, is provided by the FAO in Rome. The IBPGR has been effective in assisting various international, regional, and national research centers in acquiring, multiplying, storing, documenting, evaluating, and distributing germplasm. The IBPGR or its designees have established a set of descriptors for many crop species, organized and sponsored collection expeditions, and designated specific locations as repositories for the world's base collections for seed of principal food crops. In 1991, IBPGR was renamed as International Plant Genetic Recourses Institute (IPGRI). IPGRI is an autonomous international scientific organization, supported by the CGIAR. Mandate of the Institute is to advance the conservation and use of plant genetic resources for the benefit of present and future generations. In 2006, IPGRI and the International Network for the Improvement of Banana and Plantain (INIBAP) became a single organization under new name Bioversity International (*http://www.bioversityinternational.org*).

Collection centers participate in national, regional, and global networks. National networks in many cases organize the national plans and responsibilities on genetic resources and often have to coordinate a multitude of institutions working in the sector. Important regional networks are the ECP/GR on the European level and related activities as EURISCO and the Central Crop Database. Information networking in the Nordic countries is affiliated with SESTO, a genebank management tool developed by the Nordic Gene Bank. In South America, some networking has been mentioned around the software product DBGermo. Global networks cover breeding interests, such as the Quaker nursery and a Uniform Oat Winter Hardiness Nursery, the FAO World Information and Early Warning System (WIEWS), and several international information projects such as the Global Biodiversity Information Facility (GBIF) and the Pedigree of Oatlines (POOL) database. Though GRIN is a national product of the USDA, it is of global relevance and is also used by Canada, and thus considered as an international network. A large part of networking focuses on information and documentation issues (Germeier 2008).

The Working Group on *Avena* was set up in 1984 with the task of coordinating the work on oat genetic resources in Europe (IBPGR 1984). The Group unites oat collection curators from all European genebanks. The Working Group coordinates activities aimed at collecting, documenting, studying, conserving, and utilizing oat genetic resources. A large part of networking focuses on information and documentation issues through *Avena* Database (*http://eadb.bafz.de/bgrc/eadb/avena.htm*). Periodically, the Group convenes its meetings to discuss the most important questions related to genebank activities (IBPGR 1986, 1989; Frison et al. 1993; Maggioni et al. 1998). At present, the *Avena* Working Group operates within the ECPGR Cereals Network that coordinates the work on genetic resources of all cereal crops in the European territory as well as that performed by the institutions holding significant cereal collections in some Asian and African countries (Lipman et al. 2005; Maggioni et al. 2009) (*http://www.ecpgr.cgiar.org/Workgroups/avena/avena.htm*).

At present, genebanks and international organizations involved in activities targeted at oat genetic resources conservation successfully perform and coordinate this work aimed at rational and effective utilization of oat species in breeding programs.

3.2.4 Modes of Preservation and Maintenance: Seeds/Propagule/Cryopreservation

The limited amount of genetic variation for vegetatively propagated species has led to efforts to develop *in vitro* conservation methods. It guarantees freedom from pest infestation and diseases. However, it is extremely laborious and cost intensive and can therefore only be used for special material as a long-term storage possibility (Hammer and Teklu 2008).

Cryoconservation is accomplished with liquid nitrogen at −196°C. It is also suitable for seeds and leads to a dramatic prolongation of germination rates. It allows for an extremely long storage of many species. The problem with cryoconservation is its high cost, especially for technical equipment. A constant supply of liquid nitrogen also has to be available at all times (Hammer and Teklu 2008).

In regard to seeds of wild oat species, some experimental work concerning their cryoconservation was carried out at VIR (Russia) in the 1970s–1980s but was terminated later, as the procedure for seeds of this type turned out to be unjustifiably expensive in comparison with conservation under different storage regimes ensuring good germinating ability of oat seeds for a long period.

3.3 Role in Elucidation of Origin and Evolution of Allied Crop Plants

Combined field studies performed in the late 1950s and early 1960s in the regions of the origin and maximum diversity of oat species *Avena* (Baum et al. 1972a, b; Rajhathy et al. 1964, 1966) drew attention of the researchers to the karyology and cytogenetics of cultivated oat species and their wild relatives. This added to understanding the mechanisms responsible for reproductive isolation of the species. Since the 1950s, the investigation of wild and cultivated oat species has involved interspecific crosses conducted for various purposes (Rajhathy and Thomas 1974). The theoretical objective of these studies was to establish the diploid ancestor of cultivated hexaploid oat, which, in turn, would add to understanding the cytogenetics of this species and clarify its genomic formula and pathway of evolution.

One of the first studies concerning the genomic formula of cultivated oat was performed by Nishiyama (1929), who crossed *A. sativa* and *A. strigosa*. In later experiments, crosses of *A. strigosa* with the diploid species *A. hirtula* and *A. wiestii* produced fertile offsprings, and their chromosomes were completely homeologous. This suggested that these species belong to one group having the same genome, thereby confirming Malzev's (1930) suggestion on close relatedness of these forms, which he assigned to one species *A. strigosa*. It was found that these species are identical in chromosome structure because their hybrids regularly formed seven bivalents (Rajhathy 1966).

The diploid species *A. atlantica* was crossed with *A. strigosa* and other species of this group and gave fertile progeny. On this ground, some authors regarded *A. atlantica* as a wild analog of *A. strigosa* instead of *A. hirtula* (Rajhathy and Morrison 1959).

Other diploid species having chromosome variants of the A-genome [*A. canariensis* (Ac), *A. damascena* (Ad), *A. prostrata* (Ap), and *A. longiglumis* (Al)] differ by the results of hybridization: *A. prostrata* is quite compatible with all the listed species, and *A. longiglumis* is readily crossable with *A. strigosa*, whereas hybrids between *A. canariensis* and *A. damascen*a are sterile (Rajhathy and Morrison 1959; Rajhathy 1961; Rajhathy and Baum 1972; Baum et al. 1973; Ladizinsky 1973a; Leggett 1984, 1987). On the other hand, *A. strigosa*, *A. hirtula*, *A. wiestii*, and *A. atlantica* have similar karyotype and are interfertile. *A. lusitanica*, *A. hispanica*, and *A. matritensis* have been demonstrated to be homologous to *A. strigosa* according to results of DNA in situ hybridization. *A. longiglumis* and *A. prostrata* are more related to each other than to *A. strigosa*, from which they differ by at least five chromosome rearrangements. *A. damascena* and *A. canariensis* are separate from *A. strigosa* with at least three translocations; a larger one is in *A. canariensis* and assume a common progenitor for *A. damascena*, *A. canariensis*, and *A. prostrata* (Jellen and Leggett 2006).

Crosses of the diploid species *A. pilosa* with cultivated oat revealed partial chromosome homeology for one of these species genomes. A high or

complete homeology was revealed in crosses between the A-genome diploid species and hexaploid species. However, these species are incompatible in crosses with *A. pilosa*. It was thus concluded that *A. pilosa* has a variant of the C-genome (Rajhathy 1966). Species with the C-genome are readily crossed with each other and their hybrids form seven bivalents. At the same time, no interspecific hybrids were obtained between the species with the genomic formulas A and C. The species *A. ventricosa* was generally used in such studies as the donor of the C-genome (Rajhathy and Thomas 1967). The natural habitat of this species (Algeria and Cyprus) is completely isolated from those of species with the A-genome, which confirms their incompatibility. The scarcity of bivalents in the meiosis of the *A. strigosa* × *A. pilosa* hybrid carrying the A- and C-genomes confirmed the significant difference between these genomes (Nishiyama and Yabuno 1975).

The results of cytogenetic studies of C-genome species are consistent with the suggestion by Malzev (1930), who pointed to a significant difference in the ecology and morphology of these taxa and recognized two groups of diploid species, *A. pilosa, A. clauda* and *A. ventricosa, A. bruhnsiana*. The species of the former group gave fertile offspring in crosses with each other (Rajhathy and Thomas 1967; Nishiyama and Yabuno 1975) and demonstrated reproductive isolation in crosses with the species of the latter group.

Interspecific crosses between diploid species and species with other ploidy levels demonstrated that the A-genome could have been originated from *A. strigosa* and *A. longiglumis* (Rajhathy 1971a) or *A. canariensis* (Baum et al. 1973). Only partial homeology was revealed between the chromosomes of A-genome diploids and those of cultivated oat (Rajhathy and Thomas 1974). The morphological traits of *A. canariensis* suggested that it could have been the progenitor of tetraploid and hexaploid species (Baum et al. 1973), but cytological studies did not reveal complete homeology between their chromosomes (Leggett 1980). Afterwards the A-genome developed independently from the C-genome, which brought about lots of A-genome variants (Al, Ap, Ad, Ac, As), and finally produced a cultivated diploid species (*A. strigosa*) with the As-genome (Loskutov 2008).

On the basis of ample factual evidence, it was suggested that *A. ventricosa* could have been the diploid donor of the C-genome for tetraploid and hexaploid species (Rajhathy 1966). All tetraploid species can be classified into four groups based on their karyotypes, morphology, and the pattern of chromosome conjugation in meiosis of interspecific hybrids. Group 1 includes *A. barbata*, *A. vaviloviana*, and *A. abyssinica*. They are genetically uniform and possess the AB-genome. Their relationships were later confirmed by the fact that the tetraploid species of this group are autotetraploids, which originated from the diploid species *A. hirtula* and *A. wiestii* (Holden 1966; Ladizinsky and Zohary 1968; Sadasivaiah and Rajhathy 1968). The AB-genome was suggested to have resulted from the divergence of the original diploid A-genome (Rajhathy and Thomas 1974). In recent studies, it is designated as AA′ (Fabijanski et al. 1990). These species are readily crossable with all species of the genus *Avena*, except for diploids with the C-genome (Leggett and Markland 1995). Both tetraploid species from this group *A. vaviloviana* and *A. abyssinica*, having found in Ethiopia the most favorable climate and soil conditions for distribution into the south of the Mediterranean center, were unable to move further because of more severe arid climate in the countries adjacent to Ethiopia. It should be mentioned that diploid and hexaploid cultivated species incorporate naked forms, while tetraploids do not contain them. The most probable reason for that, in our opinion, is that the species of this group were unable to disperse far from their center of origin and had no recessive mutations (Loskutov 2008).

The species with A- and AB (AA′)-genomes and a biaristulate lemma tip in most cases each floret disarticulated. Some of them have cultivated analog with the same ploidy level (*A. wiestii*, *A. hirtula* – *A. strigosa*; *A. vaviloviana* – *A. abyssinica*) and wider areas of distribution (*A. wiestii*, *A. hirtula, A. barbata,* etc.) being rather active weeds. Obviously, this group apparently had no part in the development of hexaploid oats (Loskutov 2008).

Group 2 includes *A. magna* and *A. murphyi*. Crossing of diploid species possessing the As-genome with *A. magna* gave partially sterile hybrids (Leggett 1987). Cytological examination of the F_1 hybrids from *A. barbata* × *A. magna* showed that these species are not related. Crosses *A. sativa* × *A. magna* also yielded sterile F_1 hybrids (Thomas 1988). The great morphological similarity between *A. magna* and wild hexaploids and the meiotic pattern of pentaploid hybrids obtained with the use of *A. sativa* suggested that

A. magna could have been involved in the evolution of hexaploid species as an AC tetraploid progenitor (Ladizinsky and Zohary 1971).

Reciprocal crosses of *A. magna* with cultivated oat and other hexaploid species demonstrated its important role in the evolution of hexaploid species (Ladizinsky 1988). The results of interspecific crosses brought some scientists to the conclusion that the tetraploids bearing the AC-genomes originated from diploid species bearing its components, presumably *A. canariensis* (A) and *A. ventricosa* (C). According to an alternative opinion, the A-genomes of *A. magna* and *A. strigosa* were identical, and the C-genomes of *A. magna* and *A. pilosa* were related. However, later, this hypothesis was discarded (Leggett and Markland 1995; Leggett 1998).

Group 3 of tetraploids includes *A. macrostachya*, which is a perennial outcrossing autotetraploid. According to Malzev's (1930) definition for the genus *Avena*, the morphological features of this species allow it to be assigned to the most primitive oat-grasses. Crosses of this species with diploids possessing various derivatives of the A-genome (*A. damascena*, *A. prostrata*, *A. atlantica*, and *A. canariensis*) demonstrate negligible chromosome homeology (Leggett 1985, 1992b). According to other studies, the most viable F_1 hybrids were obtained between *A. pilosa* and *A. macrostachya*. They often form trivalents in the metaphase. Hybrids between *A. atlantica* and *A. prostrata* formed seven bivalents each (Pohler and Hoppe 1991). The mitosis study of crosses *A. barbata* × *A. macrostachya* suggested autotetraploid origin of the latter and the close relatedness of the species, although the hybrids were sterile even after chromosome duplication by colchicine (Hoppe and Pohler 1989).

The apparent differences in the morphology of *A. macrostachya* and a low percentage of chromosome conjugation in the meiosis of hybrids of *A. macrostachya* with *A. sativa* and *A. murphyi* indicate that *A. macrostachya* was not involved in the evolution of tetraploid or hexaploid species (Leggett 1985). Further studies demonstrated that the *A. macrostachya* genome is closer to the C (*A. pilosa*) genome than to the A (*A. strigosa*) one (Leggett 1990).

A. macrostachya is considered as more related to the C-genome than the A-genome diploids and the situation may be complicated by the operation of a pairing control gene in the species (Jellen and Leggett 2006). It is the only autotetraploid species within the genus *Avena* (Rodionov et al. 2005), while all other tetraploid species are a result of hybridization of different diploid progenitors (Badaeva et al. 2005).

Meanwhile, according to Rodionov et al. (2005), the division of the phylogenetic oat lines carrying A and C-genomes was accompanied by accumulation of differences in dispersed repeat sequences and accumulation of transitions and transversions specific for each branch. Later, the C-genome line segregated into phylogenetic branches of *A. macrostachya* from the progenitor of the other species with the C-genome, and after that, the progenitors of *A. macrostachya* doubled their chromosome number and generated large blocks of C-heterochromatin that resulted in characteristic C-banding pattern of *A. macrostachya* chromosomes.

Group 4, including *A. agadiriana*, cannot be considered as independent since this species is little studied. *A. agadiriana* was discovered by finding tetraploid forms in collections of the diploid species *A. canariensis* rather than discovering it in nature. Later, it was found that these species have different natural habitats: the diploid *A. canariensis* is endemic to Canary Islands (Spain) and the tetraploid *A. agadiriana* occurs only in Morocco (Baum and Fedak 1985a, b). Nevertheless, it is believed that the latter is more closely related to *A. barbata* than any other tetraploid species and has the AB-genome composition (Leggett 1988). Moreover, *A. agadiriana* is similar to *A. magna*, *A. murphyi*, and other hexaploids in the structure of lodicules, forms of lemma tips, etc. The participation of *A. agadiriana* in the evolution of hexaploids is indicated by the above evidence and by the good crossability between hexaploids and this species (Thomas 1988; Alicchio et al. 1995).

Interspecific crosses were also used for determining the genomic constitution of the most important group of hexaploids. Crosses of diploid species having the As-genome (*A. strigosa* group) and tetraploid species having the AB-genome with *A. sativa* demonstrated that the hexaploid species has the A-genome but lacks the B-genome. In later studies, the A- and C-genomes were found to be similar to the corresponding genomes of diploids and tetraploids, whereas the origin of the D-genome is still unknown (Rajhathy and Thomas 1974). The following evolutionary pathway was proposed: *A. canariensis* > *A. magna* > *A. sterilis* (Baum et al. 1973). Later, the A-genomes of *A. magna* and *A. sterilis* proved to be closer to each other than the

A-genomes of *A. abyssinica* and *A. sativa*. It was suggested that *A. magna* was the donor of two genomes (AC) of the hexaploid *Avena* species (Thomas 1988). Later, it was found that the genomes A- and D- are similar to each other but different from C (Leggett and Markland 1995). The A-genome of the diploid progenitor appears to have been the ancestor of the A- and D-genomes of hexaploid species (Linares et al. 1996). Recent studies have demonstrated that the D-genome is likely to be a variant of the "A"-genome, like the B-genome, but differs from the latter (Leggett 1997). On the other hand, Katsiotis et al. (2000) reported on repetitive DNA elements common to *Arrhenatherum* and the *Avena* A-genome that were either absent, less abundant, or polymorphic in the C-genome of *Avena*.

A new tetraploid species, *A. insularis*, has been recently reported in 1998. It was suggested to be one of the progenitors of the hexaploid species and is tentatively assumed to have the CD- or CA-genome. This species gave fertile hybrids with *A. strigosa* only. With closely related *A. magna* and *A. murphyi*, the hybrids were sterile and partially fertile hybrids are obtained with hexaploid species (Ladizinsky 1999).

Species with C- and AC-genomes (*A. ventricosa*, *A. canariensis*, *A. magna*, *A. murphyi*, *A. insularis*) are considered as transitional progenitor forms in the evolution of hexaploid oats (Fig. 3.1). Some of these species are strictly endemic or have a very limited area of distribution as wild representatives of natural undisturbed habitats. There are no other (direct) cultivated analogs of these species. Together with the cultivated forms, these species are classified into a section *Avenae* (Loskutov 2008).

In *Avena*, areas of domestication seem not to coincide with the primary areas of distribution or the centers of origin of the weed progenitors (Vavilov 1965a, 1992). Malzev (1930) located the origin of the hexaploid oat in Southwest Asia region. Baum (1972) considered *A. septentrionalis* (= *A. hybrida*) as the closest progenitor to the cultivated oat. It is distributed in Mongolia to the Ural links the European center with the Chinese centers of diversity of cultivated oats (Holden 1979; Thomas 1995).

The species *A. sterilis* is a more likely hexaploid progenitor of cultivated oat than *A. fatua* (Zhou et al. 1999; Jellen and Beard 2000). It is suggested that large-seeded *A. sterilis*, disarticulated by separate spikelets, underwent mutations in the type of floret dispersal, which led to the development of the cultivated species *A. byzantina*, and on the other hand, of the wild species *A. occidentalis* shattering by separate florets and occurring presently only on the Canary Isles (Spain). It is highly probable that, owing to the changes in the disarticulation type, *A. occidentalis* had previously occupied vast areas; besides, its dominating type of development is winter or semi-winter, and we consider it as primary, compared to the spring type. In the process of eastward distribution, *A. sterilis* became differentiated into more adaptive small-seeded forms of *A. ludoviciana*, which underwent mutations in the Minor Asiatic center that changed their florets' disarticulation type. It led, in turn, to the appearance of weedy forms of *A. sativa*. As for *A. occidentalis*, when moving eastwards, it acquired earlier-ripening, typically spring forms, which combined into a separate species, *A. fatua* (may be included *A. hybrida*). This species, disarticulating by separate florets, became a harmful weed and infested vast areas in the north and east of Europe and Asia. Weak sensitivity to vernalization and strong reaction to the length of day was reported to indicate true spring nature of *A. fatua*, which enabled it to occupy by weeding the most extensive agricultural territories on Earth. True spring nature of this species proves that it was secondary in origin as compared with *A. sterilis* and *A. ludoviciana* (Loskutov 2007).

The ample evidence on interspecific crosses and other researches suggest that the evolution of the genus *Avena* involved two strikingly different genomes: A- and C-. Other genomes were their more or less distant derivatives. According to the generally adopted assumption that the diploid species *A. canariensis* and *A. ventricosa* are the progenitors of the A- and C-genomes, respectively, the evolution of the resulting allopolyploids should have involved structural chromosome rearrangements, making them partially homeologous.

3.3.1 List of Related Crop Plants

The cultivated hexaploid common oat ranks fifth among cereals in world production. Oats are cool-season crop, and while they are grown to some extent on every continent, their production is of far greater importance in the cool climate of the northern hemisphere.

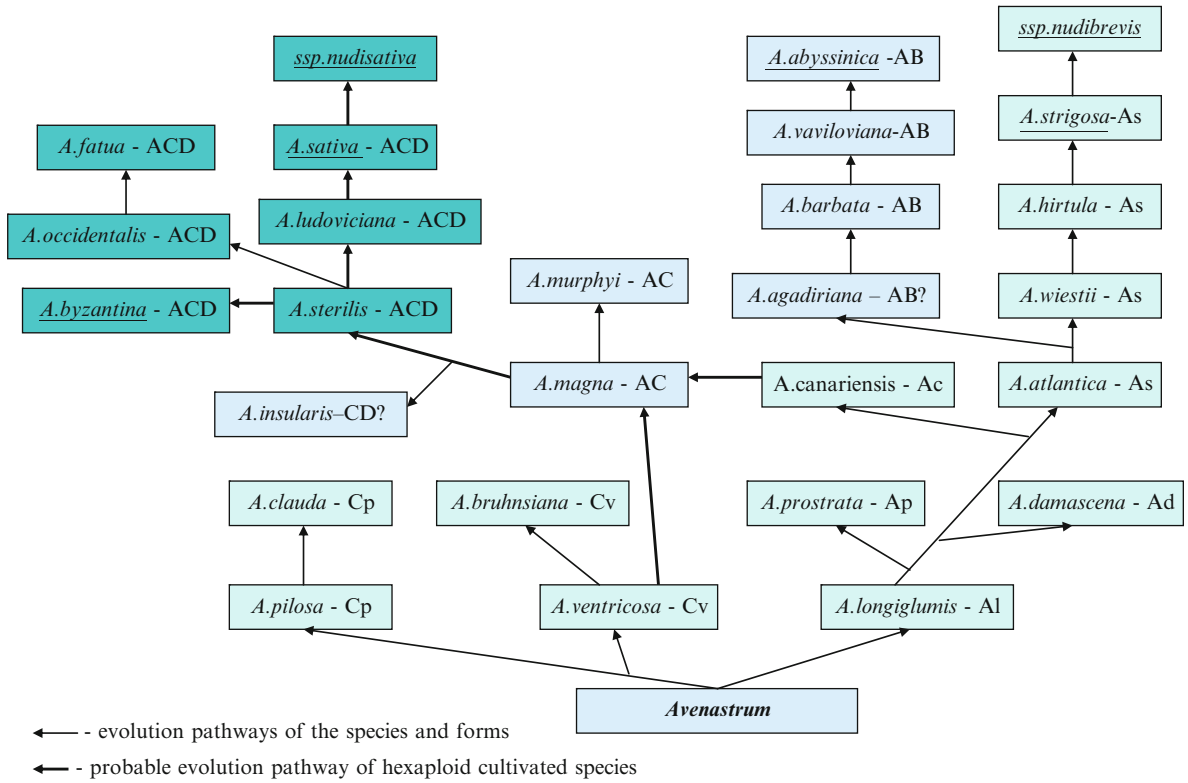
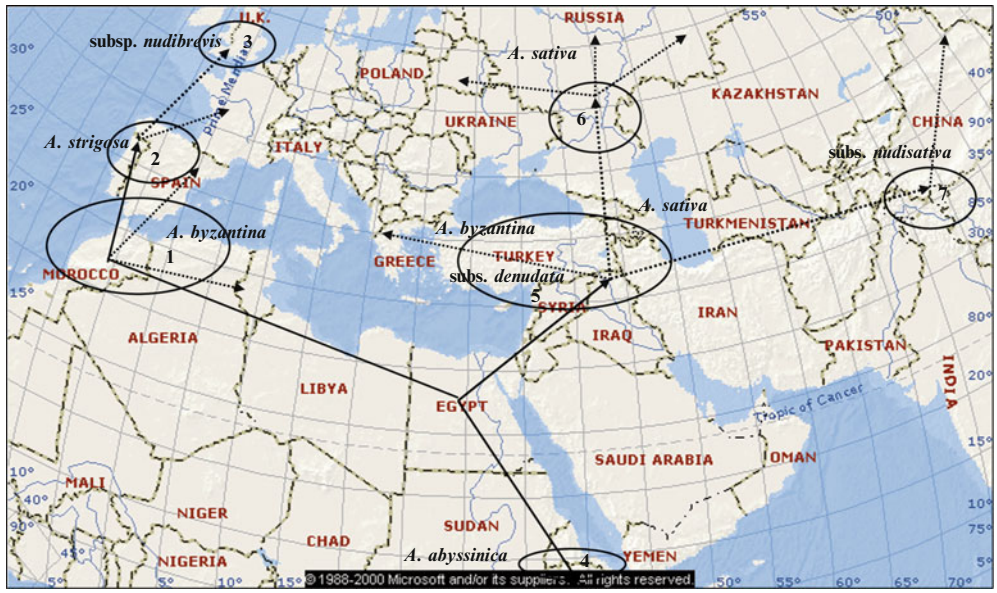


Fig. 3.1 Phylogenetic relationships of Avena species (Loskutov 2008). *Thin arrow*: evolution pathways of the species and forms, *bold arrow*: probable evolution pathway of hexaploid cultivated species

Avena is a polyploid series from diploid through tetraploid to hexaploid. There are cultivated forms at each ploidy level: the common oat (*A. sativa*, $2n = 6x = 42$), the red oat (*A. byzantina*, $2n = 6x = 42$), the Ethiopian oat (*A. abyssinica*, $2n = 4x = 28$), and the sandy oat (*A. strigosa*, $2n = 2x = 14$). The whole diversity of cultivated oats was proven by N. I. Vavilov (1926) to have a weedy field origin. As its species moved northwards, oat replaced basic crops by weeding them and became an independent crop for itself. This process may be clearly traced in Spain on the cultivated diploid species *A. strigosa*, in Ethiopia on *A. abyssinica*, in Turkey and Iran on *A. byzantina*, and in Iran and Russia on *A. sativa* (Fig. 3.2). The main difference between wild species and cultivated ones is disarticulation of the florets. All cultivated species have non-shattering panicle, mostly glabrous lemma and soft awn if it existed. Very high distribution of cultivated, especially hexaploid, species through the whole world is a reason for their high diversity (Loskutov 2008).

The hexaploid cultivated species *A. sativa* occurs in all agricultural regions of the world with moderate climate; it is cultivated in all European countries including Russia, in the north of the Asian continent, above the 30th parallel, in the North American countries, in the northern and southern parts of South America, at the southern tip of Africa, in New Zealand, and Australia. This species is represented by both hulled (*A. sativa* subsp. *sativa* L.) and naked forms (*A. sativa* subsp. *nudisativa* (Husnot.) Rod. et Sold.) (Rodionova et al. 1994), which, to all appearance, have originated in China (Loskutov 2008) (Fig. 3.2). The species *A. byzantina* has a more limited natural habitat and is characterized by a higher degree of drought tolerance. It is cultivated in countries of southern Europe, in northern Africa, in Southwest Asia above the 30th parallel, and in some South American countries. Under cultivation, this species is represented by hulled form only [*A. byzantina* (C. Koch) Thell.], while the existence of a naked form [*A. byzantina* subsp. *denudate* (Hausskn.) Rod. et Sold.] has been



1. Mediterranean center – Morocco, Algeria, Spain
2. Spain and Portugal – center of diversity of *A. strigosa*
3. Great Britain – center of diversity of *A. strigosa* subsp. *nudibrevis*
4. Abyssinian center – Ethiopia, center of diversity of *A. abyssinica*
5. South-West Asian center – Turkey, Iran, Iraq, Syria
6. Tatarstan, Bashkortostan – diversity of *A. sativa* convar. *volgensis*
7. China, Mongolia – center of diversity of *A. sativa* subsp.*nudisativa*

→ - pathways of distribution of cultivated species and forms.

Fig. 3.2 Evolution pathways of cultivated Avena species (Loskutov 2008)

identified by Malzev (1930) and mentioned by Rodionova et al. (1994). Recently, it has also been newly described and confirmed of Turkish origin (Loskutov 2007) (Fig. 3.2). It should be noted that the majority of modern oat cultivars widely spread in southern Europe, Asia, Africa, America, and Australia have originated as hybrids of *A. sativa* and *A. byzantina* with an intermediate manifestation of many morphological traits.

A. abyssinica is a tetraploid species typical of Ethiopia, which had progressed northwards to more humid regions, crowded out other cereal crops (e.g., barley), and had become a cultivated plant. In the south of the country, it is an ordinary segetal weed in spelt and barley fields (Vavilov 1965b, 1992). Most likely, the species has not developed naked forms because of the narrowness of its natural range. The diploid *A. strigosa* has been quite widely cultivated for forage and hay before Second World War in many countries of northern and central Europe as well as in the European Russia. At present, this species is a ruderal weed in southern Europe and in some places is cultivated for non-commercial use (Fig. 3.2). At the same time, such commercial cultivars of this species as Saia, Saia 2, Saia 4, Saia 6, etc. have been bred in Brazil through selection from local populations and are cultivated quite widely in South America. In the nature and majority of collections, this species is represented by hulled forms (*A. strigosa* subsp. *strigosa* Thell. and *A. strigosa* subsp. *brevis* Husn.), which, most likely, have originated from Spain, but sometimes unique naked forms originating from the mountainous regions of Great Britain may be found, which had been described by Linneaus (1762) as *A. nuda* L. In the beginning of the twentieth century, the name *A. nuda* L. started to be used for the naked forms of diploid cultivated oats, while the diploid forms were given the name *A. nudibrevis* Vav. In the 1970s, B. Baum (1977) restored the initial name *A. nuda* L. to the diploid naked forms, but the hexaploid naked forms were not determined in this taxonomy. On the other hand, it has been proved long ago that the diploid naked forms are easily crossed with the hulled forms of *A. strigosa* Schreb., and this pair has much in common concerning the majority of morphological traits.

According to Rodionova et al. (1994), these naked forms belong to the sandy oat subspecies *A. strigosa* subsp. *nudibrevis* (Vav.) Kobyl. et Rod.

Thus, the genus *Avena* includes four cultivated species, which had been quite widely used in agriculture of different countries of the Old World, while at present only cultivated hexaploid oat species keep leading positions in the world in terms of the occupied areas.

3.3.2 Application of Morpho-taxonomy

Classification based primarily on morphological traits is the fundamental basis for botanical research. There is no agreement among researchers regarding the systematics of the species in *Avena* L. The history of taxonomy of this genus started about 300 years ago (Linneaus 1762, 1753). Among the numerous publications of the nineteenth century dedicated to the systematics of *Avena* L., the most significant taxonomic surveys were by Marshall Bieberstein (1819), Grisebach (1844), Koch (1848), and Cosson and Durie de Maisonneuve (1855). Natural or phylogenetic classifications that outlined groups of related species and derived cultivated species from wild ones were developed later (Jessen 1863; Haussknecht 1885). Similar views on the polyphyletic origin of oats were shared by other researchers (Trabut 1909, 1914; Thellung 1911, 1919, 1928; Zade 1918).

The most detailed classification of this very important genus of Mediterranean origin was developed by Malzev (1930) and remains the most cited *Avena* monograph based on the main morphological characters – rachilla and lemma tip shape, lemma pubescence, and the disarticulation of florets. Many assumptions concerning the position of a number of species were made by him on the basis of analysis of a complex of morphological and biological characteristics. Malzev produced the most comprehensive phylogenetic system of the type section of this genus based on lemma tip characteristics by dividing it into two subsections and by analyzing all wild species known at that time. According to Malzev (1930), *Avena* includes 7 species represented by 22 subspecies and 184 recognizable groups within subspecies (varieties and forms). The work of Malzev drew heavily on that of Thellung (1928). Malzev made a thorough analysis of *Avena* based on the major classification principles dominating at that time, where subspecies was an important taxonomic unit (Table 3.6).

Further development of a natural classification for the genus resulted from explorations of Vavilov (1926, 1927, 1992), Nevski (1934), and Mordvinkina (1936). For the detailed structured systems of the genus, the authors used morphological characteristics and plant immunological data for species and subspecific taxa, genetic and cytological data, and new information on the natural habitats for each species. The system of Mordvinkina (1936) supplemented by genetic, karyological, and morphological data with the addition of newly described species served as a foundation for the development of classification of *Avena* L. suggested by Rodionova et al. (1994).

In the second part of the twentieth century, several classifications of *Avena* L. were developed, for example, by Sampson (1954), Stanton (1955), Mansfeld (1958), Coffman (1961), and Romero Zarco (1996). Two tendencies are obvious in the modern classifications of *Avena* (1) a decrease in number of species due to lumping based on karyological observations only, and (2) an increase in the number of species and subdivisions of species into smaller ones on the basis of morphological differences. Extreme examples of these trends are such classifications where the number of *Avena* species is either only 7 (Ladizinsky and Zohary 1971) and 14 (Ladizinsky 1988) or 34 (Baum 1977).

In the first case, one can see unjustified lumping of species based only on karyological data disregarding their natural habitats. Ladizinsky (1989) combined the whole diversity of oat forms in a number of composite (14), so-called biological species (*A. ventricosa* Bal ex Coss., *A. clauda* Dur., *A. longiglumis* Dur., *A. prostrata* Ladiz., *A. damascena* Rajhat. et Baum, *A. strigosa* Schreb., *A. atlantica* Baum et Fedak., *A. canariensis* Baum, Raj. et Samp., *A. macrostachya* Bal. ex Coss. et Dur., *A. barbata* Pott. ex Link, *A. agadiriana* Baum et Fedak, *A. magna* Murphy et Terrell., *A. murphyi* Ladiz., *A. sativa* L.). The remaining species are defined by him as taxonomic species and are incorporated in the former ones without being divided into cultivated and wild forms.

In the second case, the rank of species is attributed to hybrid species, forms, and mutants, which evolve quite frequently. In his researches, Baum (1974) employed a formal approach based on the analysis of

Table 3.6 Taxonomic system of section *Euavena* Griseb. of genus *Avena* L. (Malzev 1930)

Subsection	Seria	Species	Subspecies
Aristulatae Malz.	*Inaequaliglumis* Malz.	*A. clauda* Dur.	
		A. pilosa M. B.	
	Stipitae Malz.	*A. longiglumis* Dur.	
		A. ventricosa Balan.	*ventricosa* (Balan) Malz.
			bruhnsiana (Grun.) Malz.
	Eubarbatae Malz.	*A. strigosa* Schreb.	*strigosa* (Schreb.) Thell.
			hirtula (Lagas.) Malz.
			barbata (Pott) Thell.
			wiestii (Steud.) Thell.
			vaviloviana Malz.
			abyssinica (Hochst.) Thell.
Denticulatae Malz.		*A. fatua* L.	*septentrionalis* Malz.
			nodipilosa Malz.
			meridionalis Malz.
			macrantha (Hack.) Malz.
			fatua (L.) Thell.
			sativa (L.) Thell.
			cultiformis Malz.
			praegravis (Kraus.) Malz.
		A. sterilis L.	*ludoviciana* (Dur.) Gill. et Magn.
			pseudo-sativa Thell.
			trichophylla (C. K. et Hausskn.) Malz.
			nodipubescens Malz.
			macrocarpa (Monch.) Briq.
			byzantina (C. K.) Thell.

variability of 27 characters by taximetric methods. Among the most significant characters, he used the shape (type) of lodicules and epiblast as well as the ploidy level of the species. Both tendencies may prevent practical application of such taxonomic systems. Baum has analyzed a very wide variety of oat materials including more than 10,000 herbarium sheets, 5,000 accessions of field evaluation, and 5,000 collected accessions of wild species. On the basis of this exploration and evaluation, the genus *Avena* L. was divided into 7 sections and 34 species (Baum 1977; Table 3.7).

Summarized taxonomy of Malzev (1930), Mordvinkina (1936), and Rodionova et al. (1994) and based on the recent literature concerning the oats and on the evaluation of the 26 species, VIR world collection of the genus *Avena* L. under field conditions and different laboratory methods were confirmed. The genus is divided up into two sections of subgenus *Avena*: *Aristulatae* (Malz.) Losk. and *Avenae* Losk. Perennial outcrossing autotetraploid *A. macrostachya* belongs to subgenus *Avenastrum* (C. Koch) Losk. On the basis of a detailed morphology (presence of bidentate or bisubulate lemma tip), distribution, and ecology (most of them true wild, not weedy species), we concluded that diploids and tetraploids of *Avenae* section were involved in the evolution of hexaploid wild and cultivated oats (Loskutov 2007; Tables 3.8 and 3.9).

A consideration of different taxonomic systems based predominantly on morphological traits shows that these systems can substantially differ from each other depending on the keystone character.

3.3.3 Application of Biochemical and Molecular Markers

Morphological studies do not offer a complete understanding of the evolutionary and systematic position of some oat species and forms. There is no universal molecular approach for many of the problems faced by taxonomists and genebank managers, and many

Table 3.7 Taxonomic system of species of genus *Avena* L. (Baum 1977)

Section	Species
Avenatrichon (Holub) Baum	*A. macrostachya* Bal. ex Coss. et Dur.
Ventricosa Baum	*A. clauda* Dur.
	A. eriantha Dur.
	A. ventricosa Bal. ex Coss.
	A. clauda × *eriantha* F_1 hybrid
Agraria Baum	*A. brevis* Roth
	A. hispanica Ard.
	A. nuda L.
	A. strigosa Schreb.
Tenuicarpa Baum	*A. barbata* Pott ex Link
	A. canariensis Baum, Rajh. et Samp.
	A. damascena Rajh. eBaum
	A. hirtula Lag.
	A. longiglumis Dur.
	A. lusitanica Baum
	A. matritensis Baum
	A. wiestii Steud.
	A. lusitanica × *longiglumis* F_1 hybrid
Ethiopica Baum	*A. abyssinica* Hochst.
	A. vaviloviana (Malz.) Mordv.
	A. abyssinica × *vaviloviana* F_1 hybrid
Pachycarpa Baum	*A. maroccana* Gdgr.
	A. murphyi Ladiz.
Avena Baum	*A. atherantha* Presl.
	A. fatua L.
	A. hybrida Peterm.
	A. occidentalis Dur.
	A. sativa L.
	A. sterilis L.
	A. trichophylla C. Koch

techniques complement each other. However, some techniques are clearly more appropriate than others for some specific applications. In an ideal situation, the most appropriate marker(s) can be chosen irrespective of time or funding constraints, but in other cases, the choice of marker(s) will depend on constraints of equipment or funds (Spooner et al. 2005). The use of protein markers helps to sufficiently accelerate the works on specifying genomic composition of diploid and allopolyploid species, establishing phylogenetic relations between the species and clarifying some aspects of intraspecific diversity. The application of molecular markers, proteins and DNA, has offered possibilities for solving a series of theoretical problems related to plant phylogeny and taxonomy.

The use of protein markers permits successful identification of genetic resources and registration when solving different problems related to classification of cultivated plants and their wild relatives. A study of protein markers of the cultivated hexaploid oat *A. sativa*, cultivated diploid species *A. strigosa*, and wild *A. ventricosa* has shown that out of ten bands identified in electrophoretic banding patterns of *A. sativa*, seven were found in *A. strigosa* and six in *A. ventricosa*. At the same time, two components identified in the latter species were missing in *A. strigosa*, while another found in *A. sativa* has not been discovered in any diploid species. Presumably, this component can characterize the D-genome of hexaploid species (Thomas and Jones 1968). Electrophoretic banding patterns have demonstrated that the Cp-genome of diploid species probably represents the modified Cv-genome, that the C- and A-genome species have very different avenin patterns, that tetraploid species have demonstrated sufficient diversity of this trait, and that all the studied hexaploids had the most monomorphic patterns (Murray et al. 1970). Later, it was determined that diploid species with the A-genome types had different patterns, while those with the C-genome types were similar to each other. All the AB-genome tetraploid species were found to be monomorphic. Both hexaploid species and individual hexaploid accessions were mostly uniform, in comparison with other studied accessions. At the same time, all hexaploids species and only one tetraploid species *A. magna* (AC-genome) were found to have identical bands, indicating their systematic affinity (Lookhart and Pomeranz 1985). Comparative uniformity of electrophoretic banding patterns of the AB-genome tetraploid species proves their autotetraploid nature. In addition to affinity of the AC-genome species, closeness of the As-genome diploids to hexaploid oat species has also been proved (Ladizinsky and Johnson 1972). A study of two subspecies, *A. sterilis* subsp. *ludoviciana* and *A. sterilis* subsp. *macrocarpa* from Spain, has shown them to significantly differ from one another in terms of intensity of electrophoretic banding patterns (Cadahia and Garcia-Baudin 1978).

Out of the total of 34 bands identified in electrophoretic banding patterns, wild diploid species of *Avena* were found to contain 15 bands, tetraploid 16 and hexaploid 17, on the average. The presence of only 11–13 bands are characteristic of the C-genome (except *A. bruhnsiana*), as well as Ap- and Ad-genome

Table 3.8 Key for the identification of *Avena* species (Loskutov 2007)

1.	Perennial plants	*A. macrostachya* Balan.
	Annual plants	2
2.	Lemma tips biaristulate	3
	Lemma tips bidentate or bisubulate	14
3.	Glumes very unequal, lower glume one-half of upper one	4
	Glumes equal or nearly so	5
4.	Disarticulate each floret at maturity	*A. clauda* Dur.
	Disarticulation occurs at the lower floret only	*A. pilosa* M.B.
5.	Disarticulate each floret at maturity or panicle non-shattering	6
	Florets disarticulating at maturity, plant with juvenile growth prostrate	7
6.	Glumes 40 mm long, callus very long, awl shaped, 10 mm	*A. longiglumis* Dur.
	Glumes 10–20 mm long, callus round or absent	8
7.	Low floret disartuculates only, callus elliptic, awn inserted at 1/3 of lemma	*A. atlantica* Baum
	Disarticulate each floret at maturity	9
8.	Disarticulate each floret at maturity	10
	Panical non-shattering	13
9.	Spikelets very small 12–15 mm	*A. prostrata* Lad.
	Spikelets 20 mm long	*A. damascena* Raj. et Baum
10.	Lemma tips biaristulate, glumes with 9–10 veins	*A. barbata* Pott.
	Lemma tips biaristulate with 1–2 denticula or without, glumes with 7–9 veins	11
11.	Lemma tips biaristulate with 1 denticulum, lemma tips longer than glumes; first floret scar narrow elliptic	*A. hirtula* Lag.
	Lemma tips biaristulate with 2 denticula, lemma and glumes equal or nearly so; first floret scar oval	12
12.	Lemma tips biaristulate 3–6 mm long	*A. wiestii* Steud.
	Lemma tips biaristulate 1 mm long	*A. vaviloviana* Mordv.
13.	Lemma tips biaristulate with 1 denticulum, lemma and glumes nearly equal, panicle equilateral or unilateral	*A. strigosa* Schreb.
	Lemma tips biaristulate with 2 denticula, lemma tips shorter than glumes, panicle unilateral	*A. abyssinica* Hochst.
14.	Florets disarticulating at maturity	15
	Panical non-shattering	25
15.	Disarticulate each floret at maturity	16
	Disarticulation occurs at the lower floret only	17
16.	Spikelets with 2–3 florets, glumes 20–25 mm long	*A. fatua* L.
	Spikelets with 3–4 florets, glumes 15–20 mm long	*A. occidentalis* Dur.
17.	Callus very long, awl shaped	18
	Callus elliptic, oval or round shaped	19
18.	Callus 5 mm long, glumes 27–30 mm long	*A. ventricosa* Balan.
	Callus 10 mm long, glumes about 40 mm long	*A. bruhnsiana* Grun.
19.	Spikelets small size, glumes 15–20 mm long	20
	Spikelets large size, glumes 25–30 mm long	21
20.	Spikelets small size with 2–3 florets, glumes 18–20 mm long	*A. canariensis* Baum et Fed.
	Spikelets small size with 2 florets, glumes 15–18 mm long	*A. agadiriana* Baum et Fedak.
21.	Spikelets large size with 2 rarely 3 florets, glumes 25–30 mm long	*A. ludoviciana* Dur.
	Spikelets large size with 3–5 florets	22
22.	Callus round shaped	23
	Callus elliptic or oval shaped	24
23.	Spikelets with 3–4 florets, lemma highly pubescent	*A. magna* Murphy et Terr.
	Spikelets V-shaped with 3–5 florets, lemma slightly-moderate pubescent	*A. sterilis* L.
24.	Awn inserted at about one-quarter of lemma, callus oval	*A. murphyi* Lad.
	Awn inserted at lower one-third to one-half of lemma, callus elliptic	*A. insularis* Lad.
25.	Fracture surface at the base of the primary floret is straighting	*A. sativa* L.
	Fracture surface at the base of the primary floret is slanting	*A. byzantina* C. Koch.

Table 3.9 Speciation in the genus *Avena* L. (Loskutov 2007)

Section	Species			2*n*	Genome
	Wild		Cultivated		
	Floret disarticulation	Spikelet disarticulation			
Aristulatae (Malz.) Losk.	*A. clauda* Dur.	*A. pilosa* M. B.		14	Cp-
	A. prostrata Ladiz.				Ap-
	A. damascena Raj. et Baum				Ad-
	A. longiglumis Dur.				Al-
	A. wiestii Steud.	*A. atlantica* Baum			As-
	A. hirtula Lagas.		*A. strigosa* Schreb.		
	A. barbata Pott.			28	AB-
	A. vaviloviana Mordv.		*A. abyssinica* Hochst.		
Avenae Losk.		*A. ventricosa* Bal.		14	Cv-
		A. bruhnsiana Grun.			
		A. canariensis Baum			Ac-
		A. agadiriana Baum et Fed.		28	AB-?
		A. magna Mur. et Terr.			AC-
		A. murphyi Ladiz.			
		A. insularis Ladiz.			AC-?
	A. fatua L.	*A. sterilis* L.	*A. byzantina* Koch	42	ACD-
	A. occidentalis Dur.	*A. ludoviciana* Dur.	*A. sativa* L.		

species, the same number of bands being typical of the monomorphic tetraploid endemic oat *A. vaviloviana* (AB). The largest number of bands (over 20) is characteristic of the As-genome species and the tetraploid oat *A. barbata* (AB) only. All hexaploids (ACD) contained 18–19 bands. The number of electrophoretic banding patterns may be connected with the degree of distribution and adaptiveness of the given wild species in nature. The species with the minimum number of bands have a very limited natural habitat, while the species with the largest number of bands and types of electrophoretic banding patterns have quite a wide distribution, such as, for instance, the diploids *A. hirtula* and *A. wiestii*, the tetraploid *A. barbata*, hexaploid species *A. ludoviciana* and *A. sterilis*, and of course *A. fatua* that weeds crop fields in the cereal belt around the globe. In general, the data analysis has shown that the level of polymorphism in terms of types of electrophoretic banding patterns was the highest in hexaploid species and the lowest in tetraploid and diploid species (Loskutov 2007).

Among, other markers, a sufficient polymorphism was discovered by an analysis of isoenzyme systems. Definite isoenzyme loci were characteristic of different populations of *A. barbata* depending on the air temperature, soil type and place of growth (Allard 1997; Perez de la Vega 1997; Guma et al. 2006).

Although Spanish and Californian *A. barbata* gene pools are closely similar in allelic composition and allelic frequencies, large differences are in multilocus genetic structure. Isoenzyme analysis involving 15 loci revealed 33 alleles and 38 genotypes common in Spanish and Californian populations, 20 alleles and 45 genotypes were found only in Spain, and 2 alleles and 3 genotypes only in California. These results describe an increasing variability and less fixed genotypes when comparing populations from California vs. Spain or Spain vs. Israel and southwestern Asia, the former gene pools being more similar to each other than to the eastern Mediterranean ones, which suggests founder effects, genetic drift, and selection during the westward distribution. *A. barbata* was introduced to California by ship from southern Spain (Garcia et al. 1989).

Many researchers have obtained results concerning wide intraspecific isoenzyme diversity within wild species (Craig et al. 1974; Hoffman 1996). The largest differences in the isoenzyme composition of oat proteins were found to exist at the interspecific level, and the differences were stronger in species with different genomes (Craig et al. 1972; Sanchez de la Hoz and Forminaya 1989). Significant differences by these traits were discovered for *A. barbata* and *A. fatua* among the populations initially growing in the eastern

hemisphere and carried to the western one (Marshall and Allard 1970; Kahler et al. 1980). The widest diversity of the isoenzyme composition in the studied oat accessions has been found within forms of the diploid species *A. canariensis* from Fuerteventura Island (the Canaries), if compared with populations from Lanzarote Island in the same archipelago (Spain).

It should be noted that the isoenzyme composition data correlated very well with the data on plant morphological traits. The differences in isoenzyme composition among populations of the same species were stronger than between individual species and between populations found on different continents (Morikawa and Leggett 1990; Morikawa 1991, 1992). A similar result was obtained from a study of the tetraploid species *A. agadiriana* (Morikawa and Leggett 2005). A study of the diversity of mitochondrial DNA enzymes has shown that forms of *A. fatua* and *A. sterilis* were similar or identical to those of oat cultivars (Rines et al. 1983, 1988). A study of isoenzyme composition in a representative set of *A. sterilis* accessions of different geographic origin has recorded a large diversity both for separate groups of populations and within each group. The largest diversity has been registered within *A. sterilis* populations collected in different regions of Turkey (Phillips et al. 1993). Further studies of this trait in bred oat cultivars from USA and Canada and in wild species have shown wild populations to have a wider diversity compared to cultivars (Murphy and Phillips 1993). A study of isoenzyme composition and morphological traits of *A. sterilis* populations of different geographic origin has determined that the average coefficients of affinity between the accessions studied using various methods were very small. Therefore, the data on isoenzyme composition cannot be completely used for checking morphological purity of collection accessions and their genetic integrity after regeneration (Beer et al. 1993).

The DNA marker methods, the development of which has been recently going quite actively, are promising for investigating polymorphism in oats. Molecular marking of genetic systems in an organism is based on the use of biological specificity of nucleic acids. Restriction fragment length polymorphism (RFLP) markers applied for mapping genome and its loci using electrophoretic patterns has become widespread among the genetic marker techniques employing this feature. Moreover, randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), highly variable microsatellites markers, polymorphism of ribosomal DNA, etc. are commonly applied in botanical, genetical, and breeding research.

The RFLP, RAPD, and other marker techniques are used for studying polymorphism in diverse materials for different purposes, for instance, for genetic mapping and determining genetic affinity between oat species at the DNA level (O'Donoughue et al. 1992; Van Deynze et al. 1995), for identifying alleles of the genes controlling characters of importance for breeding (Howarth et al. 2000), or for clarifying intraspecific variation (Hayasaki et al. 2001).

The application of DNA markers gives valid and reliable results only when it is complemented by a complex study of a thoroughly selected specific diversity (Heum et al. 1994; Jellen et al. 1994; Katsiotis et al. 1996, 1997; Abbo et al. 2001).

The study of a representative set of wild *A. sterilis* populations collected on both Asian and African continents was carried out by RFLP markers. The highest genetic polymorphism was observed for the accessions collected in Iran and the lowest one for Ethiopian accessions. Cluster analysis performed using the test results has clearly split all populations into two groups according to the geographic principle, that is, from the eastern (Iran and Iraq) and western (all other countries) regions. Besides, the second group was subdivided into two subgroups, one including populations of Southwest Asian origin (from Israel, Lebanon and Syria) and the other one of African origin (from Algeria, Morocco, and Ethiopia) (Goffreda et al. 1992).

The questions of phylogeny and systematic position of species are actively disputed at present time. In this relation, the search for new approaches to solving the problems of genome affinity and systematic position of *Avena* species in genus is quite relevant. DNA markers are promising tools to be used when carrying out investigations in this direction, and RAPD analysis is one of the possible research methods.

For instance, a study based on RAPD analysis has demonstrated a possibility of distinguishing between representatives of 20 oat species according to their genomic composition, ploidy level, and intraspecific differentiation. Subsequent research has confirmed the findings (Drossou et al. 2004). Diploid species were found to display a wider range of polymorphism in

comparison with the polyploid oat species. It has been established that all the As-genome species differed from those with the A-genome types (*A. atlantica, A. longiglumis* и *A. canariensis*). Regardless of all distinctions between these species, the data may suggest their indirect evolutionary closeness, and this confirms the conclusions made by other researchers on the basis of molecular markers application (Morikawa 1992).

It should be noted that all representatives of diploid species with the C-genome types had a low level of similarity with the A-genome species, as well as within their group between the Cv- and Cp-genomes, thereby confirming the remoteness of both individual genomes (A- and C-) and even different genome types (Cv- and Cp-) from each other. The differences between *A. pilosa* and *A. clauda*, with the same structure of the Cp-genome, were also significant, thus confirming their remoteness and correctness for classifying them as two separate species. Substantial differences were characteristic of two groups of tetraploid species with the AB (*A. barbata* and *A. vaviloviana*) and AC-genomes (*A. magna* and *A. murphyi*). The degree of difference between all the species in these groups was significant and confirmed correctness of systematic individualization of each of them (Loskutov 2007).

Another molecular marker technique, AFLP, was applied to screen 163 accessions of 25 *Avena* species with diverse geographic origins. For each accession, 413 AFLP polymorphic bands detected by five AFLP primer pairs were scored. All the species were clustered together according to their ploidy levels. The C-genome diploids appeared to be the most distinct, followed by the Ac-genome diploid *A. canariensis*. The Ac-genome seemed to be the oldest in all the A-genomes, followed by the As-, Al-, and Ad-genomes. The AC-genome tetraploids were more related to the ACD-genome hexaploids than the AB-genome tetraploids. Analysis of AFLP similarity suggested that the AC-genome tetraploid *A. maroccana* was likely derived from the Cp-genome diploid *A. eriantha* and the As-genome diploid *A. wiestii* and might be the progenitor of the ACD-genome hexaploids. These AFLP patterns are significant for our understanding of the evolutionary pathways of *Avena* species and genomes, for establishing reference sets of exotic oat germplasm and for exploring new exotic sources of genes for oat improvement (Fu and Williams 2008).

The technique of microsatellites markers has many desirable marker properties. According to Li et al. (2000), using microsatellite polymorphisms, dendrograms were constructed showing not so completely clear phylogenetic relationships among *Avena* species.

On the other hand, according to Fu et al. (2007), the study attempted to characterize a structured sample of 369 accessions representing 26 countries and two specific groups with *Puccinia coronata avenae* (Pc) and *Puccinia graminis avenae* (Pg) resistance genes using microsatellite (SSR) markers. Analyses of the SSR data showed the effectiveness of the stratified sampling applied in capturing countrywise SSR variation. Accessions from Greece, Liberia, and Italy were genetically most diverse, while accessions from Egypt, Georgia, Ethiopia, Gibraltar, and Kenya were most distinct. Accessions with Pc and Pg genes had similar levels of SSR variation, did not appear to cluster together, and were not associated with the other representative accessions. They conclude that these SSR patterns are significant for understanding the progenitor species of cultivated oat, managing *A. sterilis* germplasm, and exploring new sources of genes for oat improvement.

Consensus chloroplast simple sequence repeat (ccSSR) makers were used to assess the genetic variation and genetic relationships of *Avena* species. The analysis of genetic similarity showed that diploid species with the A haplome were more diverse than other species, and that the species with the As haplome were more divergent than other diploid species with the A haplome. Among the species with the C haplome, *A. clauda* was more diverse than *A. eriantha* and *A. ventricosa*. As for the maternal donors of polyploid species based on this maternally inherited marker, *A. strigosa* served as the maternal donor of some polyploid species such as *A. sativa*, *A. sterilis*, and *A. occidentalis* from Morocco. *A. fatua* is genetically distinct from other hexaploid species, and *A. damascena* might be the A-genome donor of *A. fatua*. *A. lusitanica* served as the maternal parents during the polyploid formation of the AACC tetraploids and some AACCDD hexaploids (Li et al. 2009).

Later, the technique of ITS1 and ITS2 sequences was found to be effective for taxonomic and evolutionary pathway research.

To examine the genomic constitution of *A. macrostachya*, the individual genes from three oat species with AsAs karyotype (*A. wiestii, A. hirtula*, and *A. atlantica*) and those from *A. longiglumis* (AlAl), *A. canariensis* (AcAc), *A. ventricosa* (CvCv),

A. pilosa, and *A. clauda* (CpCp) were sequenced. All species of the genus *Avena* examined represented a monophyletic group, within which two branches, i.e., species with A- and C-genomes, were distinguished. *A. macrostachya*, albeit belonging to the phylogenetic branch of the C-genome species, has preserved an isobrachyal karyotype, probably typical of the common *Avena* ancestor. It was suggested to classify the *A. macrostachya* genome as a specific form of C-genome (Rodionov et al. 2005).

C-genome clones were sequenced and the analysis revealed close proximity to *A. ventricosa* ITS1-5.8S-ITS2 sequences, providing strong evidence of the latter's active role in the evolution of tetraploid and hexaploid oats. In addition, cloning and sequencing of the chloroplast trnL intron among the most representative *Avena* species verified the maternal origin of A-genome for the AACC interspecific hybrid formation, which was the genetic bridge for the establishment of cultivated hexaploid oats (Nikoloudakis and Katsiotis 2008).

Major genic divergence between the A- and C-genomes was revealed, while distinction among the A- and B/D-genomes was not possible. High affinity among the AB-genome tetraploids and the As-genome diploid *A. lusitanica* was found, while the AC-genome tetraploids and ACD hexaploids were highly affiliated with the Al-genome diploid *A. longiglumis* (Nikoloudakis et al. 2008).

The species and their genome relationships among 13 diploid (A- and C-genomes), six tetraploid (AB- and AC-genomes), and five hexaploid (ACD-genome) to infer evolutionary pathways in Avena were investigated by using the plastid matK gene and the trnL-F region and the nuclear ribosomal internal transcribed spacers (ITS). The evaluation is presented that the B- and D-genomes of *Avena* could be regarded as variants of the A-genome. *Avena wiestii* (AsAs) likely was the maternal parent of most AACCDD species, AACC tetraploids, and *A. agadiriana* (AABB). *Avena hirtula* (AsAs) was the maternal parent of the other three AABB tetraploids, and *A. damascena* (Ad Ad) is the maternal parent of *A. fatua*. A high degree of homogenization in the ITS sequences was found, except in *A. fatua*, which had two types in separate clades, one with A-genome and one with C-genome carrying species. The C-genome was always differentiated from the undifferentiated A-, B-, and D-genomes groups in each gene tree as well as in the tree obtained from the three genes combined (Peng et al. 2009).

The analysis of data has demonstrated that the different research methods involving molecular markers should not be regarded as universal tools in oat studies; however, their correct application combined with the use of other approaches can yield the desired results. The undoubtful advantage provided by such markers is in the possibility to study plant genetic diversity right at the level of DNA, the carrier of hereditary information. That is why these markers have recently got a wide application for genetic mapping, analyzing plant polymorphism, phylogeny, and taxonomy.

3.4 Role in Classical and Molecular Genetic Studies

The most promising method of reducing genetic erosion within the cultivated species is in using wild species along with cultivated ones in breeding activities. Practical significance of the breeding work is in successful transfer of valuable traits from wild species to cultivated forms. In terms of crossability, all *Avena* species are grouped into three gene pools based on the gene pool classification system proposed by Harlan and de Wet (1971). The primary gene pool includes all cultivated and hexaploids wild species, which directly cross with cultivated oat easily. The secondary gene pool contains some tetraploid species (*A. magna, A. murphyi*, *A. insularis,* etc.), which cross with cultivated oat directly, though they produce sterile progeny. Hybrids of *A. sativa* with these species are partially, and increasing with backcrossing, female fertile, and natural recombination occurs best with *A. insularis*. The secondary gene pool contains several desirable traits and needs to be better explored in respect to collection as well as evaluation. The tertiary gene pool includes diploid and the remaining tetraploid species requiring application of in vitro techniques for hybridization (Jellen and Leggett 2006). Nevertheless, it is considered a rich reservoir of diversity for oat breeding.

In their turn, the crosses between cultivated and wild oats can be subdivided into two groups (1) the ones that proceed easily and gave either fertile (with

all hexaploid species) or partially sterile (with AC-genome species *A. magna* and *A. murphyi*) progeny, and (2) the crosses that proceed with difficulty and gave a progeny that is either sterile to a considerable degree (e.g., with *A. barbata*) or completely sterile, as is the case with *A. prostrata* and *A. vaviloviana* (Leggett 1996).

The difficulty of transferring alleles from diploid and tetraploid species into hexaploid forms had been noticed by many authors (Leggett 1996; Mitrofanov and Mitrofanova 1972), and its essence is in crossing the crossability barrier. This problem can be solved by using mutants, genetic transformation, or by applying the method of backcrosses. Chromosome duplication induced by colchicine treatment is often used to overcome sterility of the hybrids between diploid species and the hexaploid cultivated oats. Irradiation with thermal neutrons made it possible to cross the tetraploid species *A. abyssinica* with *A. sativa* (Sharma and Forsberg 1977). The possibility of transferring entire chromosomes or their parts from diploid species to the cultivated hexaploid oat genome using genetic carriers has been confirmed by successful hybridization of many species. The most promising among the method for overcoming the inability of chromosomes to conjugate employs the CW-57 accession of diploid *A. longiglumis*, which facilitated the homeologous chromosome conjugation at the transfer of traits from tetraploid *A. barbata* to hexaploid *A. sativa* (Thomas 1988). The reason is that the diploid species contains the "wild" diploidization suppressor allele and the gene responsible for the genome recombination (Rajhathy 1966). A good example of using such a vector is the creation of the hexaploids Amagalon line by crossing *A. sativa* with *A. magna* via *A. longiglumis*. The same procedure may be used for crossing other species, too. Fertility of the pentaploid sterile hybrids, which can be obtained through simple crosses between tetraploid *A. magna*, *A. murphyi*, and hexaploid *A. sativa*, can later be completely restored through backcrossing these hybrids with the cultivated oat. This scheme of using tetraploid species in breeding oat for grain quality and large grain size was developed in Sweden (Hagberg 1983). The procedure of natural backcrossing by growing pentaploid F_1 hybrids on the plots of cultivated oat can also be applied when *A. barbata* and *A. macrostachya* are used in crossings.

For the first time, wild hexaploid oat species were experimentally used in breeding for an increased grain yield in 1936. The species *A. fatua* had been recommended for the purpose. It was proved that the main traits of cultivated oat dominated in the progeny from interspecific hybrids (Emme 1938; Vavilov 1962).

A study of interspecific hybrids did not confirm the opinion that the hybrid resulting from a cross with hexaploid *A. sterilis* is difficult to get rid of a set of such "wild" traits as the presence of callus increased huskness of grains, wild type of awnedness, etc. (Popovic 1960). The use of the species in cultivated oat breeding has been found to significantly increase biomass and vigor in hybrid plants. These traits were discovered to be controlled by additive alleles.

For the first time, wild oat species were used for practical breeding purposes in the 20s of the twentieth century. However, the earliest commercial varieties created with their use appeared only in the 1960s. In the former USSR, *A. fatua* had been used by breeders in their work for development of winter type of oat varieties (Loskutov 2007). In Great Britain, diploid and tetraploid wild oat species have been successfully used as sources of resistance to the most important oat disease, which is mildew. The trait of resistance had been transferred from *A. barbata* into a series of oat cultivars, namely Maris Tabard, Maris Oberon, Margam, and Maldwyn (Jones et al. 1984). *A. fatua* had been used for creating the winter oat Mostyn that carries genes for mildew resistance (Hayes 1970). Beginning in the 1960s, oat breeding in the US has widely employed the use of wild hexaploids species (Frey 1991, 1994), particularly for disease resistance. The species *A. fatua* and *A. sterilis* have served as major contributors in creating many oat cultivars, which have occupied or now occupy considerable areas in the US, Canada, Brazil, and Australia.

Therefore, the availability of well-developed breeding methods and numerous successful practical results confirm the possibility of effective transfer of many agronomically important traits into cultivated oat by means of interspecific hybridization for raising hereditary potential and broadening genetic basis of the entire genus.

Genetically, oat is insufficiently studied in comparison with other cereal crops. The trait-oriented genetic collections composed in different laboratories

in Europe and America are small (Dielz 1928; Litzenberger 1949; Fleischmann et al. 1971b; Simons et al. 1978; Marshall and Shaner 1992; Nielsen 1993), though the first genetic research on the inheritance of morphological traits in oats, e.g., color and pubescence of glumes (Surface 1916), has been carried out since the beginning of the twentieth century. Significant attention in genetic studies have been paid to morphological traits of generative organs, which were studied most thoroughly in relation to the problems with systematics and phylogeny of the genus *Avena*. Later on, the genes governing resistance and susceptibility to diseases and pests have been identified and genetic control of polymorphic protein systems and generative organs development has been established, thus making these data very useful for breeding purposes. In 1978, a list of genes was published (Simons et al. 1978) and extended in 1992 (Marshall and Shaner 1992). Over 300 genes controlling different traits have been identified in oats, and only single alleles responsible for morphological traits were localized in concrete chromosomes.

Later on, composition of the genetic collection kept growing continuously, mainly following new publications, primarily in "Crop Science" and "Oat Newsletter." In 1997, VIR published a Catalog with descriptions of accessions in the genetic oat collection (Loskutov 2007), which included cultivars, cultivated lines, and wild accessions with one or more identified genes controlling different morphological, agrobiological, biochemical, and other traits. A big part of the collection is represented by accessions with the most important genes of resistance to mildew, crown and stem rusts, and smut species. At present, the genetic collection of oats at VIR includes over 600 accessions belonging to both cultivated (*A. sativa, A. byzantina, A. strigosa, A. abyssinica*) and wild (*A. sterilis, A. barbata*, *A. magna*) species that contain over 200 identified genes controlling different morphological, physiological, biochemical, and other traits.

The use of donors with the identified genes that ensure clear manifestation of a trait makes it possible to predict the results of investigations with sufficient precision, it being quite important when carrying out breeding for quality, plant height, and other traits. The role of donors is especially important in broadening the genetic basis of cultivated oats concerning resistance to smut and rust species as well as other diseases. The use of the genetic collection increases efficiency of the breeding work, facilitates selection of parental pairs for crosses, and accelerates creation of cultivars with required parameters.

All information about the accessions with identified genes from the oat genetic collection is available in the European *Avena* Database (EADB) established on the initiative of ECPGR and *Avena* Working Group at FAL (now part of the Julius Kühn-Institute, JKI, Germany) (*http://eadb.bafz.de/CCDB_PHP/eadb/*).

3.5 Role in Crop Improvement Through Traditional and Advanced Tools

3.5.1 Potential of Wild Oat Germplasm for Oat Improvement

The main objectives of agricultural crop breeding are to increase their productivity and improve grain quality characters. Raising plant productivity requires breeding cultivars with high productivity and quality potential as well as resistance to biotic and abiotic stresses. Resistance of wild oat species to unfavorable environmental factors, pathogenic organisms, their wide adaptability to different soil and climatic conditions, and a number of traits determining high productivity and quality are of special interest in the context of oat breeding.

3.5.1.1 Vegetative Period

Oat displays great interspecific diversity in terms of duration of vegetative period. In oat breeding, duration of vegetative period is a very important character, which is directly related to grain yield, its quality, and seed sowing properties.

Wild forms may contain populations with very different duration of the vegetative period (Trofimovskaya et al. 1976). In terms of duration of vegetative period, Malzev (1930) subdivides all oat species into spring, intermediate, and winter forms. The spring forms produce fertile stems within one summer; the intermediate forms produce turf similar to the winter ones and generate shoots during the first year of vegetation, while the winter forms do not produce fertile stems during this period. The late or winter phenotype is

more characteristic of the diploid and tetraploid species. For *A. sterilis*, all three vegetative period phenotypes – from the winter to spring ones – are characteristic, while for *A. ludoviciana*, the winter one is more typical, and for *A. fatua*, the spring, early type of development is more characteristic. The northern forms of this species have been found to have a shortened vegetative period, while it is longer for the southern ones.

The wild species are traditionally believed to have an extended vegetative period and its individual stages, but the performed investigations have yielded a wide range of early forms, the use of which in breeding for earliness may be quite promising. Some accessions of such species as *A. canariensis*, *A. abyssinica*, *A. fatua*, and *A. sterilis* have a shorter vegetative period, if compared to the cultivated forms, and can be both potentially and practically used in breeding programs (Trofimovskaya et al. 1976; Mal 1987; Frey 1991). A study of the BC_2 breeding lines involving *A. fatua* has found them to burst out 3 days earlier than the parental forms (Stevens and Brinkman 1986). Another hexaploids species, *A. sterilis*, is believed by many authors to be an inexhaustible source of alleles determining a wide range of seed maturation dates (Welsh 1945; Hayes 1970; Hagberg 1983). Notable is that special earliness was characteristic of accessions of the species collected in Ethiopia (Rezai 1978).

A big set of accessions of wild species has been analyzed from the point of view of the duration of vegetative period, and a considerable degree of variation in duration of separate stages of development was revealed by forms of *A. clauda, A. pilosa, A. longiglumis, A. wiestii, A. hirtula, A. barbata, A. agadiriana, A. ludoviciana*, and *A. sterilis*. Thus, early spring forms as well as late intermediate and semi-winter forms of oat have been found among these species (Loskutov 2007).

The study has shown that the shortest individual stages of development and the entire vegetative period among diploid and tetraploid species were characteristic of *A. prostrata, A. canariensis, A. atlantica, A. vaviloviana*, and *A. magna*, while the longest – of *A. bruhnsiana, A. ventricosa*, and *A. agadiriana*. It should be noted that the difference between the lowest and highest average values amounted to over 20 days. Among hexaploid species, *A. fatua* showed the shortest individual stages of development and the entire vegetative period, while *A. sterilis, A. ludoviciana*, and *A. occidentalis* had the longest ones. The average value difference between these species was only 10 days (Loskutov 1998, 2007).

Therefore, the analysis of correlations between duration of different phases and the entire vegetative period shows a higher probability of discovering more early or spring forms in the northwest of the Mediterranean center of origin of cultivated plants, that is, in southwestern Europe and northwestern Africa and its archipelago, while intermediate spring and semi-winter forms are more likely to be found in the west of the Asian continent. Besides, the localities where the endemic Ethiopian species *A. vaviloviana* had been collected suppose the origin of the most early forms presumably from the regions located southeast of Addis Ababa and from the southern coastal regions of Ethiopia (Loskutov 2007).

To sum it up, investigations of the cultivated forms of oat have been accompanied by the analysis of data from studies of wild oat species with different ploidy levels, which has shown the presence of early spring forms possessing either individual shortened stages of plant development or a shorter vegetative period. These forms can be used in breeding early cultivars.

3.5.1.2 Response to Photoperiod and Vernalization

The most important factors determining a plant's vegetative period duration, especially before heading, are the day-length and temperature regime. Not only winter crops need vernalization at the early stage of development but also almost all oat species require it to a small degree. The influence of day-length and low temperatures on the initial stages of oat species development had been noted by many researchers (Qualset and Peterson 1978; Thomas and Naqvi 1991; Rodionova et al. 1994). A study of *A. byzantina* has singled out a unique, photoperiod-insensitive Turkish oat landrace, which was later on quite widely used worldwide in breeding oat for photoperiodic sensitivity (Sampson and Burrows 1972). Besides, weak response to photoperiod has been described for some *A. abyssinica* accessions (Razumov 1961; Arias and Frey 1973; Loskutov 2001a).

Some forms of wild species have been shown to be insensitive to photoperiod and vernalization. The reason is that the center origin and diversity of wild

oat species are located in the Mediterranean region, countries on the Black and Caspian seas, and in Central Asian countries. The widest diversity is found between 30 and 40° N (Vavilov 1965a, 1992; Baum 1977). Initial stages of development of many wild oat species fall on cold months; besides, they grow at high altitudes (up to 2,000 m), hence are peculiarities of their response to the environmental factors including photoperiod and temperature (Paterson et al. 1976; Darmency and Aujas 1986).

Screening of a large set of wild species has identified truly winter genotypes within *A. clauda, A. barbata, A. ludoviciana*, and *A. sterilis,* and truly spring forms within *A. wiestii, A. canariensis*, and *A. magna*. *A. vaviloviana* and *A. fatua* may be regarded as truly spring species because of their neutral or weak response to vernalization. Besides, forms of *A. fatua* have displayed a strong response to day-length (Loskutov 2007). The typically spring type of development of these species indicates their secondary origin compared to those characterized by winter and semi-winter forms, it being confirmed in literature (Jellen and Beard 2000). Apparently, the availability of spring forms and a strong day-length sensitivity allow the forms of *A. fatua* to spread to the northernmost territories and climb up high into the mountains to the altitudinal limits of high mountain agriculture (Loskutov 2007).

Forms of *A. hirtula*, *A. vaviloviana*, and *A. occidentalis* displayed weak response to photoperiod, while forms of *A. clauda, A. murphyi*, and *A. sterilis* showed a very strong response to day-length variation (Loskutov 2001a). A perennial autotetraploid species *A. macrostachya* displayed a strong photoperiodic response under vernalization, while forms of *A. magna* were strong day-length sensitive (Sampson and Burrows 1972; Loskutov 2007).

Response to vernalization has been shown to depend on geographic origin of particular accessions, while response to photoperiod variation has been found to be predominantly species-dependent. There exists no direct relation between geographic origin of a species and photoperiodic response. At the same time, several accessions with a very weak or neutral photoperiodic sensitivity originated from the regions either adjacent to, or located south of 40° N, that is, from the Canaries (Spain), Corsica (France), Crete (Greece), Azerbaijan, Turkey, Morocco, Tunisia, Lebanon, and Ethiopia (Loskutov 2001a, 2007).

3.5.1.3 Plant Height and Lodging Resistance

Variation in plant height is quite high among species of the *Avena* genus. This helps to select and create new initial material combining optimal plant height with other agronomically important traits. Wild oat species with different ploidy levels have been studied with the aim of broadening the genetic basis of such traits as semi-dwarfness and lodging resistance. The use of a fairly short-stem diploid species *A. pilosa* in stepwise hybridization has been found to reduce plant height in cultivated oat (Hoppe and Hoppe 1991). According to many authors, hexaploid species *A. fatua* and *A. sterilis* are the sources of new alleles of genes determining wide variation in plant height (Welsh 1945; Hayes 1970; Frey 1991).

A study has shown that among diploid and tetraploid species, the lowest plant height was characteristic of endemic species from Spain (*A. prostrata*), the Canaries (*A. canariensis*), and from Morocco (*A. agadiriana*). Some semi-dwarfness forms of other species have been found in Azerbaijan (*A. pilosa*), Cyprus (*A. ventricosa*), Iran (*A. clauda*), Syria (*A. pilosa*), Morocco (*A. damascena, A. magna*), and Algeria (*A. hirtula*). Plants of *A. fatua* and *A. ludoviciana* with an average height of up to 65 cm have been found in Turkey, Iran, Iraq, Israel, Morocco, Ethiopia, and Kenya. The overwhelming majority of semi-dwarf accessions belong to *A. sterilis*. Itsgrows to an average height of up to 50 cm and it originated from Turkey, Iran, Iraq, Syria, Israel, Morocco, Tunisia, and Lebanon (Loskutov 2007).

The problem of plant height is closely related to oat lodging; it occupies a special place in oat breeding and attracts significant attention because of the plant habit peculiarities. Due to morphological characters, (juvenile growth prostrate to semi-prostrate, flowering thin stems geniculate and high "windage" of the panicle), the majority of wild species are sensitive to lodging. However, some forms of the hexaploid *A. occidentalis* have been found to have an erect juvenile growth and thicker stem walls, the structure of which resembled that of *A. magna*. These characters may be used for improving lodging resistance in cultivated oat (Baum 1971). A study of a diploid species *A. wiestii* has shown that its forms possess a wide range of adaptive responses of the root system related to lodging resistance (Holden 1969). At the same time, a study of root volume and growth rate in *A. sterilis* has identified an

accession from Sicily with quantitative characters (volume of the root system, root dry weight, panicles, and straw dry weight) 2–3 times higher than those in other forms of wild and cultivated oats (Carrigan and Frey 1980). Among the diversity of wild hexaploid species of plant height and lodging resistance, the most interesting semi-dwarf and lodging resistant forms have been found among *A. fatua, A. ludoviciana*, and *A. sterilis* (Loskutov 2007).

To sum it up, selection of new sources of shorter plant height and lodging resistance among both cultivated and wild species allows the breeders to enjoy a higher degree of flexibility in their work and thus reduce the risk of genetic erosion of genotypes at the intraspecific level.

3.5.1.4 Agronomical Important Characters

The main trend in breeding is the raising of a cultivar's grain productivity. In the first place, this character and quality of oat cultivars depend on the panicle size, number of grains in the panicle, size, and presence/absence of husk on the caryopsis. A study of the elements of productivity in wild oat species has shown that the highest number of spikelets in the panicle and the highest density of the latter were characteristic of some forms of *A. prostrata, A. wiestii, A. vaviloviana, A. abyssinica, A. fatua*, and *A. sterilis* (Trofimovskaya et al. 1976; Thomas and Griffiths 1985; Mal 1987; Kanan and Jaradat 1996). High values of the same characters were demonstrated by accessions of *A. hirtula* and *A. wiestii* collected in Italy (Sicily and Sardinia), Azerbaijan, Iran, Israel, and Egypt, of *A. barbata* from Azerbaijan, Israel, and Lebanon, of *A. vaviloviana* collected in Ethiopia, and of *A. fatua* collected in Georgia, Kazakhstan, Bulgaria, and China (Loskutov 2007). Some accessions of *A. sterilis* from Central Asia and the Middle East were also noted for these characters (Rezai and Frey 1988, 1990).

To a greater degree, a lower huskness correlates positively with grain size and high grain test weight and negatively with the degree of lodging and high susceptibility to rust diseases. Sources possessing these characters may be found among wild species, too. When crossed with cultivated oat, a diploid species *A. pilosa* with small grain and high percentage of huskness has reliably demonstrated an increase in the values of the characters (Hoppe and Hoppe 1991). The related tetraploid species *A. magna, A. murphyi*, and *A. insularis* have been noted for large grain size (1,000-grain weight reached 35 g) (Martens et al. 1980; Ladizinsky 1988, 1998; Loskutov 2007).

A. sterilis is a rich source of alleles of genes governing variation of grain size in a wide range (Welsh 1945; Hayes 1970). The largest grain size and the highest 1,000-grain weight have been recorded for the accessions collected in southwestern Europe and northern Africa (Rezai and Frey 1989b, 1990). At the same time, some African forms of *A. sterilis* had impressive spikelets, which were 2.5-times larger than those of other species (Vavilov 1965a, 1992). Hybrid lines obtained by crossing *A. fatua* with cultivated oat had larger grain than the parental forms (Stevens and Brinkman 1986).

The largest grain size has been found among the forms of *A. fatua* from Russia, Tajikistan, Poland, and Mongolia, of *A. ludoviciana* from Azerbaijan, Georgia, Turkey, and Iran, and of *A. sterilis* from Turkey, Iran, Iraq, Israel, Tunisia, and Morocco. The correlation analysis has shown the most large-grained forms of hexaploid species to be associated with southwestern Europe and northwestern Africa (Loskutov 1998). It should be noted that the most interesting forms with the weakest huskness and the largest 1,000-grain weight were the forms of *A. fatua* from Russia, Poland, and Mongolia, of *A. ludoviciana* from Azerbaijan and Iran, as well as of *A. sterilis* from Turkey and Tunisia (Loskutov 2007).

Another character related to yielding ability is the productive tillering. A higher degree of productive tillering has been found to be characteristic of some forms of wild diploids *A. prostrata, A. damascena, A. wiestii*, and *A. hirtula* (Mal 1987; Kanan and Jaradat 1996) and tetraploids *A. murphyi* and *A. magna* (Ladizinsky 1988). When transferring this character from tetraploid species to a hexaploid cultivated species, two backcrosses are sufficient for obtaining stable hexaploid genotypes (Zadoo et al. 1988).

The best forms of *A. fatua* are regarded by many authors as the best partners in breeding for higher yields and grain quality (Yamaguchi 1977). This species is promising for creating cultivars with different duration of seed dormancy, which permits to increase grain yields by 13–24% (Burrows 1970). The species transfers a higher spring sprouting ability and shattering resistance to cultivated oats (Frey 1985, 1991).

A consideration of yielding ability and other characteristics of interspecific hybrids involving *A. sterilis* shows that alleles of genes from wild species can increase vegetative vigor, grain yield, and straw weight in hybrid plants (Cox and Frey 1984a, b; Frey et al. 1984; Takeda and Frey 1985, 1987; Gupta et al. 1986a, b, 1987). To transfer these characters by means of interspecific crosses, the method of backcrosses is applied quite efficiently (Takeda et al. 1985; Frey 1988; Holland et al. 1996). Cytoplasm of a wild species had a significant influence on grain yield in certain combinations (Beavis and Frey 1987), total straw weight, plant height, and the vegetative vigor. The findings indicated the potential of *A. sterilis* for creating cultivars with a more stable productivity and less dependence on the environmental conditions (Robertson and Frey 1984).

Thus, the study has found that wild species possess agronomically important characters, which may be used in improving the existing cultivars through breeding.

3.5.1.5 Resistance to Diseases and Pests

Oat diseases and pests are still the main factors reducing yields and grain quality. The most efficient method of protecting plants from diseases and pests is the breeding of resistant cultivars. Their development requires different donors and sources of resistance. The main task of breeding for disease resistance is to restore in cultivated oats the lost genetic diversity as regards resistance to diseases and pests, initially possessed by wild progenitors. Therefore, a complex phytopathological study of all the specific diversity of *Avena* genus promotes identification of new sources and donors of resistance for their use for broadening genetic basis of the created oat cultivars (Clifford 1995).

Resistance to Diseases

Crown and stem rusts. Crown rust, caused by the fungus *P. coronata* Cda. f. sp. *avenae* Fraser et Led., and stem rust, caused by the fungus *P. graminis* Pers. f. sp. *avenae* Eriks. & Henn., are spread everywhere and affect the majority of oat crops. Numerous investigations of wild oat species show that their majority possesses a high degree of resistance to rusts (Table 3.10). For example, these are the diploid species *A. clauda, A. pilosa, A. longiglumis, A. damascena, A. prostrata, A. canariensis, A. wiestii*, and *A. hirtula* and tetraploids *A. barbata, A. vaviloviana, A. abyssinica*, and *A. magna* possessing complex resistance to rust species (Sebesta et al. 1987; Harder et al. 1992; Saidi 1998).

According to Vavilov's research (1935), the diploid species *A. clauda* and *A. pilosa* possessed resistance to crown rust (Vavilov 1951, 1964a). It had been established that the extreme variants of resistance to crown and stem rusts and to loose smut and powdery mildew are displayed by the diploid oat species (Vavilov 1951, 1964b, c). Consequent experiments with artificial infection of *A. barbata* have shown the existence of two very different groups, that is, a group strongly susceptible to all parasites and very resistant one. Forms of *A. barbata* from Persia, as well as forms of *A. vaviloviana* from Ethiopia, were strongly affected by rust species, while forms of *A. sterilis* happened to be susceptible to stem rust and medium resistant to crown rust (Vavilov 1965a, 1992). The performed analysis made it possible to suppose that high degree of resistance is displayed by representatives of the groups, which had formed under the conditions favorable for the development of infection, and that resistant forms should be sought in the areas where the parasite originated. It was further confirmed in his later works (Vavilov 1957).

According to Mordvinkina (1969a), the Pyrenean and Moroccan groups of *A. hirtula* demonstrated complex resistance to fungal diseases, while the South Palestinian group and the forms from Corsica were susceptible to them. Medium resistance to crown rust was characteristic of the Mediterranean forms of *A. barbata*, though South-Asian forms got affected by this disease. In general, resistance of these species was confirmed in consequent studies (Simons et al. 1959; Leggett 1992a). The highest degree of resistance to crown and stem rusts was demonstrated by accessions of *A. barbata* collected in Israel (Dinoor and Wahl 1963). Forms of this species collected in northern Australia were more resistant to stem rust than southern population (Burdon et al. 1983, 1992). Some forms of *A. barbata* possessed resistance only at the juvenile stage of development and were losing it during the reproductive stage (Karow et al. 1987). It had been established that crown rust resistance could

Table 3.10 Sources of diseases resistance in oat species

Species	Genome	Sources of resistance to					
		powdery mildew	Crown rust	Stem rust	BYDV	Smut ssp.	Septoria leaf blight
A. bruhnsiana	Cv-	+	+				
A. ventricosa	Cv-	+	+	+			
A. clauda	Cp-	+	+	+	+		
A. pilosa	Cp-	+	+	+			
A. prostrata	Ap-	+	+				
A. damascena	Ad-	+	+	+	+		+
A. longiglumis	Al-	+	+	+	+		+
A. canariensis	Ac-		+	+	+		+
A. wiestii	As-	+	+			+	+
A. hirtula	As-	+	+	+	+		+
A. atlantica	As-	+					+
A. strigiosa	As-	+	+	+	+	+	+
A. barbata	AB-	+	+	+	+	+	+
A. vaviloviana	AB-	+	+		+	+	+
A. abyssinica	AB-	+	+	+		+	
A. agadiriana	AB-?	+					
A. magna	AC-		+	+	+		
A. murphyi	AC-	+	+		+		+
A. insularis	AC-?		+	+			
A. macrostachya	CC-?	+	+	+	+		+
A. fatua	ACD-	+	+	+	+	+	+
A. occidentalis	ACD-	+	+	+	+		
A. ludoviciana	ACD-	+	+	+	+		+
A. sterilis	ACD-	+	+	+	+	+	+

be transferred to hexaploid species by means of amphiploid synthesis (Williams and Verma 1956). Reciprocal crosses have yielded a hexaploid line that contained an allele of the *A. barbata* gene governing resistance to stem rust *Pg16* and had a grain yield nearly that of cultivated oat (Martens et al. 1983; Brown 1985; Ohm and Shaner 1992).

When considering diversity of resistance to crown rust, it was found that resistance to this pathogen was displayed by forms of diploid species *A. bruhnsiana, A. ventricosa, A. longiglumis, A. hirtula,* of tetraploid species *A. magna, A. insularis, A. murphyi,* and *A. macrostachya,* while resistance to stem rust was found to be possessed by such diploids as *A. pilosa, A. longiglumis,* and *A. hirtula* and by a tetraploid *A. macrostachya* (Loskutov 2007).

A. abyssinica may be of much importance for synthetic breeding in the case of stepwise interspecific hybridization involving 14−, 28−, and 42-chromosome oats aimed at transferring the well-expressed resistance to fungal diseases from the group of diploid oat species to cultivated oats (Vavilov 1965a, 1992).

The species *A. magna* and *A. murphyi* have been found to possess a higher resistance to crown rust (Sebesta et al. 1987; Ladizinsky 1988; Leggett 1992a). High resistance to the most aggressive races of crown rust was displayed by sprouts and adult plants of *A. magna* (Murphy et al. 1968). This species and *A. longiglumis* as the carrier were used to produce the Amagalon (***A. mag-****na* + ***A. lon-****giglumis*) line, in which allele of the gene has been identified with molecular markers (Rooney et al. 1994; Wilson and McMullen 1996b).

All investigations have found *A. macrostachya*, a perennial tetraploid species, to be characterized by resistance to both crown and stem rusts and that can be successfully crossed to cultivated oat (Leggett 1992b; Loskutov 2007).

A study of the forms of *A. fatua* has found individual populations to have a higher degree of crown and stem rust resistance (Suneson 1948; Burdon et al. 1983; Simons and Briggle 1984; Johnson and Rothman 1986; Burdon and Muller 1987; Sebesta and Kuhn 1990). The accessions of *A. fatua* from Central Asia

displayed high resistance to 200 races of rust. The American populations of this species collected in the North Central states of the country happened to be susceptible to the stem rust isolates tested (Rines et al. 1980). On the whole, this species is characterized as having some resistance to stem and crown rusts (Frey 1991). According to many authors, the hexaploid species *A. sterilis* represents a rich source of alleles of genes controlling resistance to different races of crown and stem rusts (Welsh 1945; Suneson 1948; Hayes 1970; Harder et al. 1980, 1984; Frey 1983, 1991, 1994; Harder and McKenzie 1984; Wahl and Segal 1986; Leggett 1992a). It has been proved that in crosses involving the forms of *A. sterilis,* resistance of the progeny is influenced not only by the nuclear genetic material (Wilson and McMullen 1997a, b) but also by cytoplasm (Simons 1985; Simons et al. 1985). The inclusion of this species in the list of identifiers of physiological races of rust causal agents made it possible to identify almost 500 races of stem rust and 800 races of crown rust (Fleischmann and McKenzie 1968; Fleischman and Baker 1971; Fleischmann et al. 1971a, b). A study of several forms of this species has found correlations between crown rust resistance, protein content, potential grain yield, and powdery mildew resistance (Simons 1965, 1979; Popovic 1980). Alleles of rust resistance genes are frequently linked with the alleles controlling morphological traits of the grains, particularly color of lemma or its pubescence (Kiehn et al. 1976; Wong et al. 1983). Individual accessions of *A. sterilis* from southwestern Europe and countries of Asia and Africa are noted for a high resistance to rust diseases (McKenzie and Fleischmann 1964; McKenzie et al. 1970; Martens and McKenzie 1973; Kim 1974; Brodny et al. 1976; Rezai 1978; Simons 1985; Wahl and Segal 1986; Sebesta et al. 1987; Simons et al. 1987; Harder et al. 1990). The forms of *A. sterilis* from Turkey, Iran, and Iraq are resistant to crown rust and, apparently, possess an allele of the *Pc54* gene or alleles of stem rust resistance genes (Martens et al. 1980, 1981).

A. ludoviciana, related to *A. sterilis*, is also characterized by resistance to crown rust (Welsh 1945; Griffiths et al. 1959; Lupton and Thompson 1961; Clamot 1969; Hayes 1970; Sebesta et al. 1987) and to stem rust (Leggett 1992a). The accessions collected in northern Australia were more resistant to stem rust than southern forms of this species (Burdon et al. 1983; Oates et al. 1983).

The accessions of *A. sterilis, A. ludoviciana*, and *A. occidentalis* with the highest resistance to crown rust originated from Spain, Italy, France, Turkey, Israel, Iran, Lebanon, Algeria, Tunis and USA. Complex resistance to the main obligate fungal parasites (crown and stem rusts) were characteristic of the forms of tetraploid species *A. magna, A. insularis*, and *A. macrostachya* and hexaploid species *A. occidentalis, A. ludoviciana*, and *A. sterilis* (Loskutov 1998, 2007).

An analysis of the obtained data has shown that the forms of wild species with a higher resistance to crown rust were concentrated in the northwestern regions of the African continent, while resistant forms of *A. barbata* were concentrated in south of this species' natural range where strong epiphytotics of crown rust are most frequent. Besides, it has been discovered that crown rust weakens the diseased plants and it leads to their medium infection by stem rust (Loskutov 2007).

Powdery mildew is caused by the fungus *Erysiphe graminis* D. C. f. sp. *avenae* Em. March., is recorded everywhere and reveals itself on leaves, in leaf sheaths, and on plant stems (Table 3.10).

According to Vavilov, accessions of the wild diploid species *A. clauda* and tetraploid *A. barbata* were characterized by a higher resistance to powdery mildew (Vavilov 1965a, 1992). Later on, Mordvinkina (1969b) determined geographic boundaries of these groups of *A. barbata*: powdery mildew resistance was characteristic of the Mediterranean forms, while South-Asian ones were susceptible to the pathogen. Further investigations undertaken by many authors have confirmed the supposition that these species possess resistance to powdery mildew (Aung et al. 1977; Aung and Thomas 1978; Thomas and Aung 1978; Harder et al. 1992; Leggett 1992a). According to many researchers, resistance to powdery mildew is exhibited by many wild species, namely diploid species *A. pilosa, A. ventricosa, A. longiglumis, A. prostrata, A. damascena, A. hirtula,* and *A. atlantica*; tetraploids *A. barbata, A. vaviloviana, A. abyssinica, A. agadiriana, A. magna,* and *A. murphyi*, and hexaploids *A. fatua, A. occidentalis, A. sterilis* and *A. ludoviciana* (Welsh 1945; Jones and Griffiths 1952; Lupton and Thompson 1961; Clamot 1969; Hayes 1970; Ladizinsky 1988, 1992; Thomas 1988; Frey 1991, 1994; Harder et al. 1992; Leggett 1992a; Zwatz et al. 1994; Herrmann and Roderick 1996).

The highest resistance was registered for all Azerbaijanian accessions of *A. bruhnsiana, A. clauda*, and some forms of *A. pilosa*, *A. wiestii*, and *A. barbata* (Loskutov 2007).

A series of works on the transfer of alleles of the powdery mildew resistance genes from diploid species to cultivated oats have been undertaken at the Welsh Plant Breeding Station, University College of Wales (UK). The accession Cc 3678 (*A. hirtula*) was used to transfer powdery mildew resistance to hexaploid species via a genetic bridge of artificial amphiploids involving *A. longiglumis* (CW 57) (Thomas 1968). The accession Cc 4852 *A. ventricosa*, bearing an allele of the gene for resistance to powdery mildew races 2, 3, and 5, was included in the breeding program and crossing program (Thomas and Thomas 1970). The F_4 line with a chromosome replacement from *A. prostrata* bearing a gene of powdery mildew resistance was obtained through production of an amphidiploid (*A. longiglumis* (CW 57) × *A. sativa*) × (*A. sativa* with chromosome *A. prostrata*) (Thomas and Griffiths 1985). A cross of *A. macrostachya* and *A. prostrata* has yielded a hybrid that was morphologically similar to *A. macrostachya* but was resistant to powdery mildew (Hoppe and Pohler 1988; Hoppe et al. 1990). Hexaploid species were not characterized by high resistance to the pathogen. At the same time, it should be noted that some resistant forms have been found among *A. sterilis*, *A. ludoviciana*, and *A. occidentalis* (Sebesta et al. 2000).

Helminthosporium leaf blotch is caused by *Drechslera avenae* (Eidam.) Ito et Kuribay. (syn. *Helminthosporium avenae* Eidam; *Pyrenophora avenae* Ito et Kuribay.) and has been recorded everywhere (Sebesta et al. 2001).

Resistance to this disease was noted in forms of diploid species *A. bruhnsiana* and *A. wiestii* from Azerbaijan, *A. clauda* from Turkey, *A. longiglumis* from Morocco and Algeria, and *A. hirtula* from Spain, Italy (Sardinia), and Tunisia. Among wild tetraploid species, high resistance was exhibited by *A. macrostachya* from Algeria. Resistance to Helminthosporium leaf blotch displayed by the AC-genome species *A. magna* and *A. murphyi* was above the average. Disease appearance was minimal also in other species, such as *A. barbata* from Turkmenistan, Portugal, Italy, Turkey, Israel, and Tunisia, *A. magna* from Morocco, and *A. murphyi* from Spain and Morocco. Among wild hexaploid species, there can be noted resistant forms of *A. fatua* from Russia, Ukraine, Azerbaijan, Turkey, and Iran, of *A. ludoviciana* from Azerbaijan, Georgia, and Iran, and of *A. sterilis* from Russia, Iran, Israel, Morocco, and USA. It should be noted that all accessions of *A. occidentalis* from Spain (the Canaries) had juvenile resistance and that their majority was resistant to the disease. An analysis of the gathered data has shown that the forms of wild species most resistant to Helminthosporium leaf blotch had been collected in the northern parts of natural ranges of wild oat species, which is near 40° N (Loskutov 2007).

Septoria leaf blight is caused by the fungus *Septoria avenae* Frank. (syn. *Leptosphaeria avenaria* Weber.). It occurs everywhere and reveals itself from the tillering stage (Table 3.10).

Among wild species, *A. wiestii* and *A. sterilis* apparently possess high resistance to Septoria leaf blight under both natural and artificial inoculation (Clark and Zillinsky 1960; Thomas 1988; Harder et al. 1992; Zwatz et al. 1994; Sebesta et al. 1999).

Resistance to the disease was also characteristic of accessions of such diploid species as *A. atlantica* from Morocco, *A. canariensis* from Spain (the Canaries), *A. damascena* from Morocco, and *A. hirtula* from Italy (Sicily, Sardinia). Among wild tetraploid species, perennial *A. macrostachya* from Algeria has been noted for resistance to the disease in question. Moderate susceptibility has been recorded for *A. vaviloviana* and *A. murphyi*, and resistance has been displayed by forms of *A. barbata* from Azerbaijan, Portugal, and Israel. A sufficient number of forms with resistance to Septoria leaf blight have been found among hexaploid species. Many forms of *A. fatua* have been found to be resistant; they have geographically diverse origins: Russia, Ukraine, Georgia, Azerbaijan, Armenia, Kazakhstan, Tajikistan, Poland, Bulgaria, Czechoslovakia, Iraq, Turkey, Mongolia, and Argentina. The forms of *A. ludoviciana* from Czechia, Morocco, and USA have been found to be resistant to Septoria leaf blight. Among *A. sterilis* accessions, resistant forms originated from Turkey, Iran, Syria, Israel, Algeria, Morocco, Tunisia, Lebanon, and Ethiopia (Loskutov 2007).

Myrothecium necrotic blight is caused by the fungus *Myrothecium verrucaria* Ditmar. ex Fran. and manifests itself in the beginning of vegetation, most often at the tillering stage or during the leaf-tube formation. Resistance to this pathogen has been displayed by accessions of diploid species from different

regions of Azerbaijan, such as *A. bruhnsiana, A. pilosa*, and *A. wiestii*, by forms of *A. hirtula* from Italy, Spain, Israel, and Tunisia, as well as by forms of tetraploid *A. murphyi* from Spain and Morocco, *A. agadiriana* from Morocco, *A. barbata* from Iran and Israel, and *A. macrostachya* from Algeria. The majority of hexaploid forms of *A. fatua* possessing resistance to *Myrothecium* necrotic blight originate from Russia, Armenia, Azerbaijan, Mongolia, and Ethiopia; those of *A. ludoviciana* have Russian, Ukrainian, Azerbaijanian, Georgian, Turkish, and Algerian origin and those of *A. sterilis* originate from Russia, Turkey, Iran, Syria, Israel, Algeria, Morocco, Tunisia, and US (Loskutov 2007).

Smuts (caused by the fungi *Ustilago avenae* Jens. and *Ustilago kolleri* Wille.) occur everywhere and do much damage to oat crops. A study of complex resistance in oat species has shown that *A. wiestii* is not resistant to loose smut (Table 3.10). Experiments on artificial inoculation of *A. strigosa* and *A. barbata* with smut have identified two very contrasting groups within these species: that is, a group with strong susceptibility to all parasites, and another group with high resistance. The strongly susceptible forms of *A. barbata* happened to be typical of Persia. Among them, in some accessions, smut infection was localized to anthers only, while in most forms, it affected all organs of the inflorescence (Vavilov 1965a, 1992). According to Mordvinkina (1969a), moderate resistance to smut was characteristic of the Mediterranean forms of *A. barbata*, while the South-Asian forms were affected to a greater degree. According to Vavilov, the cultivated species *A. abyssinica,* its wild analog *A. vaviloviana*, and *A. sterilis* were quite susceptible to loose smut under conditions of Russia (Vavilov 1964c, 1965a, 1992).

Further studies on resistance to loose and covered smut have shown that almost all previously studied species had forms that were resistant to this disease and could be used as donor sources in breeding for this character. The species *A. strigosa, A. wiestii, A. barbata, A. vaviloviana, A. fatua*, and *A. sterilis* (Forsberg and Shands 1989; Frey 1991; Harder et al. 1992; Leggett 1992a; Rodionova et al. 1994) as well as the majority of *A. abyssinica* accessions (Nielsen 1978, 1993) are regarded as resistant. A study of a large set of oat accessions has established that the majority of most resistant forms of *A. sterilis* originated from Ethiopia, Israel, Lebanon, Syria, and all North-African countries, but some of them were found in Iran and Iraq (Nielsen 1978).

Fusarium is caused by fungi of the *Fusarium* genus. They are distributed everywhere and affect oats in the second half of the vegetation period. It is noted in numerous works that *A. sterilis* and *A. ludoviciana* represent a diverse source of resistance to *Fusarium* agents (Welsh 1945; Hayes 1970; Frey 1991; Gavrilova et al. 2008).

Halo blight is caused by *Pseudomonas coronofaciens* Starr. and affects oat plants after the stage of tillering and leaf-tube formation. *A. vaviloviana* is resistant to halo blight and is of importance in breeding for increased productivity (Trofimovskaya et al. 1976).

Barley yellow dwarf virus (BYDV) has recently become the most harmful disease of oat. It is caused by *Hordeum virus nanescens* Rademacer et Schwarz. When searching for new sources of resistance, a wide range of species has been studied and a number of BYDV-tolerant forms identified (Table 3.10). All these forms belong to the species *A. longiglumis, A. strigosa, A. barbata, A. magna, A. murphyi, A. macrostachya, A. fatua*, *A. occidentalis*, and *A. sterilis* (Frey 1983, 1991, 1994; Comeau 1988; Forsberg and Shands 1989; Harder et al. 1992; Leggett 1992a; Saidi 1998).

A study of a representative set of accessions has found moderate tolerance in variants of the A-genome diploid species *A. canariensis* (Ac) and *A. wiestii* (As), while the most susceptible happened to be variants of the C-genome oat species *A. bruhnsiana* (Cv), *A. ventricosa* (Cv), *A. clauda* (Cp), and *A. pilosa* (Cp). Individual accessions had moderate tolerance, e.g., *A. canariensis* from Spain (the Canaries), *A. clauda* from Greece (Crete Island), *A. damascena* from Morocco, and *A. hirtula* from Algeria. Among the wild tetraploids, the overwhelming number of species displayed moderate tolerance to BYDV. The highest tolerance was exhibited by the forms of *A. barbata* from Azerbaijan and Israel, of *A. vaviloviana* from Ethiopia, of *A. magna* from Morocco, and of *A. macrostachya* from Algeria (Soldatov et al. 1990).

The natural American populations of *A. fatua* collected predominantly in the Red River Valley in the northeastern states of the US were found to have a higher BYDV tolerance (Rines et al. 1980). A. Comeau (1984) noted high tolerance in *A. occidentalis* and stressed the necessity of further collecting

populations of this species, which are insufficiently represented in genebanks around the world, though these forms cross with cultivated oat easily. According to some authors, *A. sterilis* represents a source of alleles of genes governing resistance to different viral diseases, and therefore it should be used for breeding purposes to a greater degree (Welsh 1945; Hayes 1970). The majority of the most tolerant forms of this species were found in Greece, Algeria, Tunisia, Morocco, Lebanon, Ethiopia, Kenya, western Iran, and in the Mediterranean Turkey. Some tolerant accessions originated from Iraq, Libya, Israel, eastern Iran, and Anatolia (Turkey) (Comeau 1984; Landry et al. 1984).

Accessions belonging to the group of hexaploid species were moderately tolerant to BYDV, depending on the species. The highest percentage (31%) of tolerant accessions was found within *A. occidentalis*, an endemic from the Canaries (Spain). The highest tolerance was characteristic of the *A. fatua* forms from Russia, Ukraine, Georgia, Tajikistan, Poland, and Mongolia, the forms of *A. ludoviciana* from Azerbaijan, Bulgaria, Afghanistan, Israel, Morocco, and of the *A. sterilis* forms from Turkey, Japan, Israel, and Morocco (Loskutov 2007).

A comparison of the data on BYDV tolerance with those on the abundance of aphid population has identified the accessions with true resistance to BYDV, which belonged to the diploid species *A. clauda, A. pilosa, A. canariensis, A. hirtula*, and the tetraploid species *A. barbata*. Such forms have not been found within hexaploid species, as all of them got weakly populated with aphids. An analysis of the obtained data has shown that the forms of wild species with a higher BYDV tolerance had been collected from the regions located in the western parts of the natural ranges of wild oat species (Loskutov 2007).

Resistance to Pests

Pests cause serious damage, for instance, crop thinning, reduction of the productive tillers per plant, total or partial seed set failure, and lower grain and sowing material quality.

Cereal cyst nematode and stem nematode (*Heterodera avenae* Woll. and *Ditylenchus dispaci* Filip., respectively) affect plants at different stages of their development and occur in many regions of oat cultivation. Cereal cyst nematode resistance has been found in the forms of the following wild species: *A. canariensis, A. wiestii. A. strigosa, A. barbata, A. vaviloviana, A. abyssinica, A. magna*, and *A. murphyi* (Harder et al. 1992; Leggett 1992a). In Australia, the forms possessing resistance to stem nematode have been selected from local populations of *A. fatua* (Scurrah et al. 1992). Other hexaploid species *A. fatua, A. sterilis*, and *A. ludoviciana* displayed resistance to different nematode species (Lupton and Thompson 1961; Hayes 1970; Cotten and Hayes 1972; Hagberg and Mattsson 1986; Jain and Hasan 1988; Frey 1991; Leggett 1992a).

Fruit fly (*Oscinella frit* L.) is an internal stem feeder causing substantial damage to oat crops. Among wild species, *A. pilosa* and *A. fatua* were tolerant to fruit fly (Rodionova et al. 1994).

It should be noted that some accessions of diploid species possessed moderate tolerance to the pest. These forms originated mainly from the African continent and from Azerbaijan, e.g., *A. canariensis* from Spain (the Canaries), *A. hirtula* from Tunisia, *A. longiglumis* from Morocco, and *A. wiestii* from Azerbaijan and Algeria. The level of resistance was, in general, higher among tetraploid species than among the diploids. High resistance to fruit fly has been recorded for the forms of *A. macrostachya* from Algeria, *A. barbata* from Azerbaijan, Italy, Greece, France, Iran, and Turkey, and *A. vaviloviana* from Ethiopia. Among the hexaploids, resistant forms have been found within *A. fatua* from Russia, Ukraine, Georgia, Azerbaijan, Tajikistan and Poland, *A. ludoviciana* from Azerbaijan, and *A. sterilis* from Georgia, France, Italy, Greece, and Morocco. An analysis of the obtained data has shown that the forms with a higher degree of resistance originated from the regions located in the western parts of the natural ranges of wild oat species (Loskutov 2007).

The bird-cherry oat aphid (*Rhopalosiphum padi* L.) does significant damage to oat crops by itself and as the BYDV transmitter. A study of a broad range of species has identified aphid-resistant forms of such diploid species as *A. clauda* from Greece (Crete Island) and Algeria, *A. pilosa* from Azerbaijan, *A. ventricosa* from Algeria, *A. longiglumis* from Israel and Morocco, *A. prostrata* from Spain, and *A. wiestii* from Israel and Iran (Loskutov 2007).

Resistance to different aphid species has been found in the tetraploid species *A. barbata* (Weibull

1986; Weibull and Hanson 1986). According to the literature, the perennial outcrossing tetraploid species *A. macrostachya* is characterized by very high resistance to the aphid (Leggett 1992a). Probably, resistance of this species to aphids may be explained by larger number of cuticle cells in leaves and an increased content of the glutamic acid and decrease in the aspartic acid in the cell sap (Weibull 1988b); it is the reason why larvae develop poorly and excrete less fluid (Weibull 1988a).

Two related AC-genome species from Morocco, *A. magna* and *A. murphyi,* may be regarded as resistant. The AB-genome species *A. agadiriana, A. vaviloviana*, and *A. barbata* were moderately resistant, though the last species was not quite uniform in this respect as the former two. Degrees of resistance within *A. barbata* strictly depended on the geographic origin: high resistance was displayed by accessions from Russia, Portugal, Spain (the Canaries), Italy, Turkey, Morocco, and Tunisia. All hexaploid species were resistant to the pest: resistant forms within *A. fatua* were found to originate from Russia, Kazakhstan, France, Albania, Greece, Germany, Slovakia, Poland, Turkey, Iran, Canada, Mexico, and Argentina; all resistant accessions of *A. occidentalis* originated from the Canaries (Spain); those of *A. ludoviciana* from Tunisia, Ethiopia, and the US; and resistant forms of *A. sterilis* originated from Spain, Greece, Turkey, Iraq, Iran, Syria, Israel, Algeria, Morocco, Lebanon, Tunisia, Ethiopia, Kenya, and the US (Loskutov 2007).

Cereal leaf beetle (*Oulema melanopus* L.) causes damage to a plant at different stages of its development and is regarded as one of the most harmful insects affecting cereals. It has been established that leaf pubescence is a trait characterizing resistance to this pest. A study of resistance in American populations of *A. fatua* has identified some forms from Wyoming and Idaho with a medium resistance to the phytophage (Rines et al. 1980). Resistance of some forms of *A. sterilis* to the insect is connected with a higher leaf pubescence and with the differences in composition of the cell sap in the sprouts (Steidl et al. 1979).

It follows from the above that one of the most important means of creating resistant cultivated genotypes is the interspecific hybridization, which may lead to the introgression of genes into oat cultivars. Such crosses involving wild diploid and tetraploid sources of resistance are possible with the use of genetic carriers, while hexaploid species can directly transfer a trait.

3.5.1.6 Resistance to Abiotic Factors

Oat possesses quite a range of physiological traits, which help this crop grow in diverse conditions. The main regions of origin and diversity of the whole genus are predominantly located in the arid zones with insufficient moisture and different degrees of soil salinity and acidity and sometimes with low temperatures in the high mountain regions; therefore, drought and cold resistance, aluminum and salt tolerance, and related traits are characteristic of many oat species.

The search for new sources of edaphic resistance among wild relatives of cultivated cereals has acquired special importance, since in most cases, many characters initially possessed by wild ancestors have been lost by cultivated species in the course of evolution. Wild species *A. wiestii, A. ludoviciana*, and *A. sterilis* have been found to contain highly xeromorphic forms with hardiness to such unfavorable environmental factors as drought, heat, sharp temperature fluctuations, and certain salt tolerance (Holden 1969; Udovenko 1977; Hagberg 1983).

Investigations have shown that the C-genome species (diploid and tetraploid) have a low level of resistance to excessive aluminum and hydrogen ions in the nutrient medium, while the A-genome species with different ploidy levels more often displayed high tolerance to aluminum, and the correlation analysis has proved it. The largest group of tolerant forms belonged to the hexaploid species *A. sterilis, A. fatua*, *A. ludoviciana*, and *A. occidentalis*. All wild oat species have been found to be sources of salt-tolerance regardless of their ploidy level. The most tolerant were the forms of *A. fatua* from Georgia and Kazakhstan and of *A. ludoviciana* from Azerbaijan (Loskutov 2007).

Another set of characters is related to the edaphic stress-related oat adaptation to low and negative temperatures. Such abiotic characters as cold-resistance and winter-hardiness are very important for the wintering forms of cultivated oats. These properties are most characteristic of the majority of wild species

because of their predominantly winter- or semi-winter type of development. A tetraploid perennial outcrossing species *A. macrostachya* is characterized among other oat species by an increased winter hardiness, which may be transferred to cultivated species (Leggett 1992a; Loskutov 2007). Almost all forms of hexaploid weedy species are cold-tolerant and winter-hardy. A study of *A. fatua* and *A. ludoviciana* in the field and laboratory conditions has confirmed them to be cold-resistant (Pier 1964; Frey 1991; Leggett 1992a). Hybrids involving this species were cold resistant at the sprouting stage (Aujas and Darmency 1983, 1984). Cold resistance has been recorded for accessions of *A. sterilis* from Greece, Israel, and Turkey (Ephrat 1962; Hetzler and Dambroth 1990). An analysis of F_1 hybrids has found cold resistance to be controlled by recessive genes with additive effect; transgression has been observed in F_2; therefore, *A. sterilis* may serve as a source of alleles of the cold resistance genes (Rajhathy et al. 1966). According to Malzev (1930), another species *A. ludoviciana* truly belongs to the winter plants, especially its forms from Ukraine (the Crimea peninsula). This species has been included in the program for development of winter oat cultivars in Great Britain (Thomas and Thomas 1970).

To sum it up, the range of acid and salt tolerance displayed by wild oat species regardless of their ploidy level is quite wide. All the selected accessions can serve as sources of resistance to the considered abiotic factors, hexaploid forms being able to directly transfer these characters when crossed with cultivated oats.

3.5.1.7 Grain Quality Traits

Along with agronomic characters, cultivated oat has good grain quality and green matter traits. At the same time, it is believed that the percentage of quality components in the oat caryopsis can be augmented to a very high level through breeding.

Protein. Percentage of protein in oat and its yield unit quite often exceed these traits in other cereals, and the amino acid composition in oat is balanced better indicating good nutrient properties of the crop (Table 3.11).

In addition to cultivated species, wild diploids *A. pilosa, A. clauda, A. ventricosa, A. longiglumis, A. canariensis*, *A. damascena*, *A. hirtula, A. atlantica*, and *A. strigosa* are noted for a high (at a 20% level) protein content in grain (Hoppe and Hoppe 1991; Harder et al. 1992; Leggett 1992a; Miller et al. 1993; Welch et al. 2000; Loskutov 2007). Among the accessions with the highest indices (above 20%), there should be a mention of the forms of *A. atlantica* (Morocco), *A. longiglumis* (Morocco), and *A. wiestii* (Azerbaijan) (Loskutov 2007). Among tetraploid species, a higher content of protein in grain (above 20%) has been demonstrated by the species *A. barbata* and *A. agadiriana* (Trofimovskaya et al. 1976; Miller et al. 1993; Welch et al. 2000; Loskutov 2007). In some accessions, the amount of protein with an increased lysine content may reach 30%. Such forms have been found within *A. magna* and *A. murphyi* (Ladizinsky and Fainstein 1977; Ladizinsky 1988; Butler-Stoney and Valentine 1991; Harder et al. 1992; Leggett 1992a; Welch et al. 2000; Loskutov 2007). The best indices concerning this trait have been demonstrated by the accessions of *A. barbata* from Azerbaijan (over 21%) and Portugal (22.9%). The maximum value of protein content in grain, i.e., 25.2%, was displayed by the forms of *A. magna* and *A. murphyi* from Morocco (Miller et al. 1993; Loskutov 2007). When transferring these characters to cultivated oats, two backcrosses are sufficient for obtaining stable hexaploid genotypes (Zadoo et al. 1988). In Sweden, an oat breeding program with an elaborate system of crosses and the use of backcrosses have been developed for involving the species *A. magna* and *A. murphyi* (Hagberg and Mattsson 1986). When crossing these species with cultivated oats, it has been determined that the genes governing a higher protein accumulation were probably localized in the homeologous chromosomes.

The species *A. fatua* is considered by many authors as a good partner in breeding for higher yield and grain quality (Thompson 1966; Trofimovskaya et al. 1976; Frey 1991; Leggett 1992a). A detailed study of American populations of this species collected predominantly in the North-Central states of the country has identified some forms with an increased content of protein and some amino acids (though the average concentration of protein was lower than in *A. sterilis*). Individual forms of *A. fatua* (with protein content above 26%) can serve as donors of these characters (Rines et al. 1980). The highest average values of protein content were demonstrated by *A. fatua* accessions from Ukraine, Georgia, Azerbaijan, Tajikistan, Poland, and Greece (Loskutov 2007). The hybrid

Table 3.11 Sources of quality traits in oat species

Species	Genome	Sources of quality				
		Protein	Oil	β-Glucan	Starch	Avenanthramindes
A. bruhnsiana	Cv-					
A. ventricosa	Cv-	+				
A. clauda	Cp-	+	+			
A. pilosa	Cp-	+	+			
A. prostrata	Ap-					
A. damascena	Ad-	+	+	+		
A. longiglumis	Al-	+	+	+		
A. canariensis	Ac-	+	+			
A. wiestii	As-	+	+			
A. hirtula	As-	+	+	+		
A. atlantica	As-	+	+	+		
A. strigiosa	As-	+	+	+	+	
A. barbata	AB-	+	+	+		
A. vaviloviana	AB-		+			
A. abyssinica	AB-		+		+	
A. agadiriana	AB-?		+	+		
A. magna	AC-	+	+	+		
A. murphyi	AC-	+	+	+		
A. insularis	AC-?					
A. macrostachya	CC-?					
A. fatua	ACD-	+	+	+	+	
A. occidentalis	ACD-	+	+	+		
A. ludoviciana	ACD-	+	+	+	+	
A. sterilis	ACD-	+	+	+	+	
A. sativa	ACD-	+	+	+	+	+

plants produced by crossing *A. fatua* with cultivated oat had a higher percentage of protein (Reich and Brinkman 1984). The dark-glume forms with grain shattering (the wild type) had a lower protein percentage in the caryopsis and a lower percentage of caryopses in comparison with the light-colored non-shattering forms (the cultivated type) (Luby and Stuthman 1983).

According to many authors, the species *A. sterilis* possesses and transfers to its progeny the character of an increased (above 25%) accumulation in grain of protein with a well-balanced amino acid composition (Zillinsky and Murphy 1967; Briggle et al. 1975; Trofimovskaya et al. 1976; Frey 1991, 1994; Leggett 1992a; Welch et al. 2000; Loskutov 2007). A study of several forms of this species has shown the existence of a strong correlation between protein content, resistance to crown rust, and other traits (Spilde et al. 1974; Popovic 1980). It has been proved that in crosses involving forms of *A. sterilis*, the content of protein in the grain of the progeny is influenced not only by the presence of nuclear genetic material (Browning and Frey 1972) but also by cytoplasm (Rezai and Frey 1989a). A study of a large set of *A. sterilis* forms has shown that the an increased percentage of protein in the caryopsis and straw was characteristic of the accessions from Libya and Iraq (Eagles et al. 1978). The centers of the highest diversity for this trait were found in the Mediterranean Region, Central Asia, Middle East, and Israel (Rezai 1978). The highest average values have been displayed by the forms of *A. sterilis* from Iran, Israel, Algeria, Morocco, Tunisia, and Lebanon. A large group of accessions from Israel has been found to stably accumulate as much as 23.1–28.0% protein in grain during the years of the study (Loskutov 2007).

Besides, some forms of the hexaploid species *A. ludoviciana* and *A. occidentalis* have also been characterized by a higher content of protein in the caryopsis (Trofimovskaya et al. 1976; Miller et al. 1993). The highest average values of protein content in grain (20–23%) have been recorded for the accessions of *A. ludoviciana* from Azerbaijan, Turkey, Iraq, Iran,

Israel, and Algeria. An analysis of protein content in the grain of *A. occidentalis* from the Canaries (Spain) has shown that this species may be promising in breeding for grain quality (Loskutov 2007).

In hexaploid wild oat species, the protein content in grain may be as high as 27–28% (Campbell and Frey 1972) and even reach 35% according to some reports (Frey 1975). A comparison of wild and cultivated hexaploid species has shown that the percentage of protein in the caryopsis cross-sections of wild species was higher, while the content of protein per caryopsis was lower due to the small size of the caryopsis in wild species (Youngs and Peterson 1973).

The most promising species from the point of view of the availability of high-protein forms may be *A. murphyi* and *A. occidentalis*. Among the forms with high content of protein in the caryopsis, those belonging to hexaploid species *A. fatua, A. ludoviciana*, and *A. sterilis* are promising for breeding purposes. An analysis of the long-term data for a correlation between the protein content and the geographic locality of collecting a particular form has shown that the accessions of hexaploid species with the highest content of protein in grain originated predominantly from the Northwest of the African continent (Loskutov 2007).

Interspecific hybridization proved to be more efficient in breeding for a higher protein content in grain (Alexander 1975; Axtell 1981; Clamot 1984; McFerson and Frey 1990). When crossing cultivated and wild hexaploid species, protein content and yielding ability were found to be inherited independently (Cox and Frey 1985; Kuenzel and Frey 1985). In both cultivated and wild species, high protein content was inherited through recessive genes with additive effect (Sraon et al. 1975). According to many published data, a large number of forms of wild oat species have been analyzed for this trait (Peterson and Brinegar 1986). This has resulted in the identification of accessions, which are currently used in oat breeding for developing highly productive lines and cultivars with an increased content of protein with a well-balanced amino acid composition in grain (Lyrene and Shands 1975).

High protein content in grain is important not only for food but also for forage purposes. At present, the forms of naked oat are used as forage for cattle, especially in horse breeding. To increase protein concentration in grain of these oat species, wild species are involved in the breeding process (Valentine 1987).

A study of the nitrogen index (the ratio of nitrogen content in grain to the total nitrogen in the biomass) in *A. sativa* and *A. sterilis* has established that this index was on the average higher in the cultivated species, while in the interspecific hybrids, it exceeded that of both parents (Fawcett and Frey 1982).

Besides, some forms of wild species had a high protein content in straw and green matter. Such accessions have been found among the tetraploid *A. abyssinica* and *A. magna* and hexaploid *A. ludoviciana* and *A. sterilis* (Mal 1987). The accessions of *A. sterilis* collected in southwestern Europe had a higher protein percentage in straw (Rezai 1978). The absence of a connection between protein content in grain and in straw evidences for a possible combination in one genotype of genes controlling high protein content (Campbell and Frey 1974; Frey et al. 1975b).

Amino acid composition. Amino acid composition of proteins in cultivated oat is well balanced. Amino acid composition of the high-protein tetraploid species *A. magna* and *murphyi* is similar to that of cultivated oat (Rodionova et al. 1994). The content of individual essential amino acids was found to be distributed in the studied wild species as follows: the content of valine was the highest in *A. barbata* and the lowest in *A. fatua*, that of methionine was the highest in *A. barbata*, and that of isoleucine was the highest in *A. barbata* and *A. sterilis*. The content of leucine was the highest in *A. fatua* and the lowest in *A. barbata,* while the content of tyrosine was higher in *A. barbata*. All the studied species had the highest values of phenylalanine accumulation in comparison with cultivated oats; the content of glutamic acid in cultivated oat was lower than in *A. fatua* and higher than in *A. barbata*, and that of proline was higher in *A. ludoviciana* and lower in *A. barbata*. In terms of nutrient qualities, protein of the tetraploid species *A. barbata* should be noted, and in terms of lysine and other essential amino acids content in protein, hexaploid species demonstrated values that were at the level of cultivated oats (Loskutov 2007).

The share of albumins and globulins in hexaploid species *A. sterilis*, *A. ludoviciana*, and *A. fatua* was 1.5–2 times lower than in cultivated species, while, on the contrary, the percentage of the alkali-soluble proteins was higher in the three former species. It means that the total protein nutrient value in wild species was lower than that of cultivated species (Trofimovskaya et al. 1976). According to many authors, *A. sterilis* has

and transmits the trait of the well-balanced amino acid composition of protein in grain to its progeny (Briggle et al. 1975; Frey 1991; Leggett 1992a).

The correlation analysis has found regularities in accumulation of protein and individual amino acids in oat grain. It should be noted that the content of all essential amino acids (except for methionine) correlates closely and positively with the content of protein (0.78–0.94) and lysine (0.62–0.80). The existence of a correlation between the content of protein and lysine in grain protein has been determined in 60.1% of the cases, thus making it possible to carry out preliminary selection of the high-lysine forms on the basis of total protein in grain (Loskutov 2007).

Fats. In addition to protein, oat grain is rich in other chemical compounds and fats in particular (Table 3.11). A search for genes capable of improving this trait in cultivated oat while maintaining the stability of yield level has not achieved the desired results so far. Besides, it has been established that the genes controlling this trait in cultivated and wild species are not allelic (Thro and Frey 1985; Frey 1991). Interspecific hybridization proved to be more efficient in breeding for this trait. In some diploid and tetraploid species, the content of oil in the caryopsis reaches 12–13% (Welch and Leggett 1997). The majority of studied forms of such species as *A. clauda*, *A. pilosa, A. canariensis*, *A. longiglumis, A. damascene*, *A. wiestii*, *A. hirtula, A. atlantica, A. agadiriana, A. barbata*, and *A. vaviloviana* had the caryopsis oil content above 7% (Welch et al. 2000; Loskutov 2007; Leonova et al. 2008).

A high content of oil in grain has also been recorded for the tetraploid species *A. magna* and *A. murphyi* (Ladizinsky 1988; Welch et al. 2000; Leonova et al. 2008). The highest average values for this trait in *A. barbata* has been demonstrated by its accessions from Azerbaijan, Spain (the Canaries), and from Italy. The highest average values of oil content in grain have been registered for the forms of *A. magna* and *A. murphyi* from Morocco (Loskutov 2007).

In *A. ludoviciana* and *A. fatua*, the content of oil in grain reached 7–9% (Trofimovskaya et al. 1976; Leonova et al. 2008). The highest average values for this trait were displayed by accessions of *A. fatua* from Ukraine, Kazakhstan, and Tajikistan. The highest average values for oil content in grain (9.7–11.1%) were discovered in accessions of *A. ludoviciana* from Azerbaijan, Georgia, and Armenia. The percentage of oil (above 7.8%) in grain of a form of *A. occidentalis* from the Canaries (Spain) indicated that this species may be promising in breeding for grain quality (Welch et al. 2000; Loskutov 2007). On the average, a hexaploid *A. sterilis* has been found to have a high fat content (upto 10%) in grain (Thro 1982; Frey 1991, 1994; Leggett 1992a; Welch et al. 2000; Leonova et al. 2008). The largest number of samples with such characteristics has been collected in Israel (Rezai 1978). A group of accessions with oil content in grain in the range of 8.3–10.7% has been found to include forms of *A. sterilis* from Iraq, Israel, Japan, and Algeria (Loskutov 2007).

The percentage of oil in hybrid plants produced by crosses with said species increases (Frey 1991). The dark-glume, shattering forms have been noted to have low percentage of oil in grain and low percentage of grain in comparison with the light-colored, non-shattering forms (Luby and Stuthman 1983). This trait is controlled polygenically and shows incomplete dominance. The additive nature of gene action, high heritability, and a close and positive link between the general combining ability (GCA) effects and oil content in the caryopsis have been noted (Elliott et al. 1985). The hybrids involving cultivated oat may be transgressive (Frey et al. 1975a). The main method of transferring the trait of high oil content from wild to cultivated species is recurrent breeding. Since the alleles for high oil content from *A. sativa* and *A. sterilis* are complementary (Thro and Frey 1985), a program to improve the oil content by using recurrent selection was carried out that resulted in increases in groat oil concentration of upto 18% in relatively short time (Branson and Frey 1989a, b; Frey and Holland 1999; Schipper and Frey 1991).

Fatty acid composition of oil. The oil contained in grain of wild hexaploids practically does not differ from that of cultivated species: oleic and linolenic acids are the main ones in its composition, the content of the latter being sometimes lower or higher than that of oleic acid. The total polyunsaturated acids fit the amplitudinal range of cultivated species. Therefore, the oil content differentiates the wild from cultivated species more than the fatty acids ratio in the oil (Trofimovskaya et al. 1976). The highest content of oleic acid is characteristic of the diploid species *A. damascena*, *A. longiglumis, A. canariensis, A. atlantica, A. hirtula,* and *A. wiestii*, of the tetraploid ones: *A. barbata, A. vaviloviana, A. murphyi*, and

A. magna, and of the hexaploid species *A. occidentalis*, *A. fatua, A. sterilis*, and *A. ludoviciana* (Welch et al. 2000; Loskutov 2007; Leonova et al. 2008). At the same time, biological activity of such oil is determined by the linoleic to oleic acid ratio, which should equal 1.0. Such a value was displayed by the diploids *A. ventricosa, A. clauda*, and *A. pilosa* and by the tetraploid *A. vaviloviana* (Loskutov 2007).

A correlation analysis has shown that an increase in the content of saturated (palmitic and stearic acids) and monounsaturated (oleic) fatty acids in oil of wild oat species will be accompanied by the decrease in the content of polyunsaturated fatty acids, which oxidize easily during grain storage (Loskutov 2007).

Crosses of the forms with high and low content of linoleic acid have shown that inheritance of linoleic and oleic acids was oligogenic (Karow 1984). Other authors believe that inheritance of palmitic, oleic, and linoleic acids are controlled polygenically with additive effect and that inheritance is partially dominant (Thro 1982).

Starch. An important aspect determining grain digestibility is the presence of starch in it (Table 3.11). A content of amylose comparable with that in cultivated oats has also been recorded in the wild species *A. sterilis, A. ludoviciana*, and *A. fatua*. These data evidence closeness of starch composition in cultivated and wild oat species (Rodionova et al. 1994).

β-glucans. The (1–3) (1–4)-β-D-glucan, or the non-starch water-soluble polysaccharide β-glucan, is regarded as a physiologically important dietary component of grain (Table 3.11). The data on this trait in wild oat species are very small as compared to the cultivated ones. Among diploid species, an increased content of β-glucans has been noted for *A. strigosa* and *A. hirtula* (4.6–5.5%), *A. damascene, A. longiglumis*, and *A. atlantica*. In such tetraploids as *A. agadiriana, A. barbata, A. magna*, and *A. murphyi*, this value was around 3.2% on an average (Miller et al. 1993; Leggett 1996; Howarth et al. 2000; Welch et al. 2000). A study of a limited set of accessions of wild species including *A. sterilis* has shown that all hexaploid species (*A. fatua, A. occidentalis, A. byzantina*) had a higher content of β-glucans (upto 6%) in grain (Frey 1991; Welch et al. 1991; Cho and White 1993; Miller et al. 1993). Groat β-glucan concentration in wild species showed very wide variation (2.2–11.3%) as there were substantial interspecific and intraspecific differences (Welch et al. 2000).

Vitamins. Oat contains different vitamins, including the fat-soluble vitamin E (tocopherol and tocotrienol), which possesses increased antioxidant properties. The data on this trait in wild oat species are extremely poor. A study of the influence of different mutagens on oats has found that the high content of tocopherols in *A. ventricosa* plants can be explained by a lesser sensitivity of this species to physical and chemical mutagenic damage in comparison with the affined species *A. bruhnsiana*, which has a low content of tocopherols and a more narrow distribution range in comparison with the former species (Alekperov 1982; Alekperov and Sinitsina 1982).

A study of nutritional properties of oats from India has identified a number of species with a decreased content of lignin and silica compounds in green matter thus suggesting their suitability for consumption by animals. Among these species are the diploids *A. strigosa*and *A. ventricosa*, tetraploids *A. barbata, A. vaviloviana*, and *A. abyssinica*, and hexaploids *A. sterilis* and *A. ludoviciana* (Mal 1987).

Thus, it has been established that the wild species *A. magna*, *A. sterilis*, and *A. ludoviciana* are the most promising and important ones in terms of grain quality and the transfer of this trait to cultivated oats. Besides, there also exist other sources of various traits related to grain and green matter quality, which may be successfully used for improving these characters in cultivated oats.

3.5.2 Use of Wild Oats in Crop Improvement: Challenges, Approaches, and Successes

The tremendous potential of wild *Avena* species as donors of genes for a wide spectrum of biotic and abiotic traits is evident from the preceding deliberations of numerous species sources harboring resistance for various oat pathogens and wide ranges of variation in other quantitative traits. The transfer, capture, and sustainable use of these traits in oat cultivar development and releases can prove challenging for from genetic techniques to breeding line selection, especially with transfer from lower ploidy species. This section describes genetic and breeding approaches employed with different *Avena* species as trait sources, breeding line development

including examples of cultivars released with transferred improved traits, some difficulties and limitations encountered, and how use of molecular markers can facilitate transfer and selection.

The ease of transfer and the utility of the variation identified in wild and lower ploidy *Avena* species accessions depend on the nature of variation, whether qualitative or quantitative, and the genetic relationship between the alien donor and the recipient cultivated species. Qualitative variation, such as disease resistance when controlled by major genes, is often simply inherited and readily scorable independent of genetic background and growth environment. Quantitative variation, such as is observed for many agronomic and seed quality traits, in contrast, is often controlled by multiple genes and its expression is strongly influenced by genetic background and the growth environment. Oat cultivars with a portion of wild oat germplasm in their parentage and with enhanced quantitative traits have been released where the enhanced trait is attributed to the wild oat germplasm component. This attribution is often based on an enhanced level of that component in the wild germplasm donor. The extent of trait contribution, however, and even the amount of wild species germplasm genome present after rounds of crossing and selection cannot currently be documented. The use of gene- and genome-specific DNA markers will not only aid tremendously in the identification and transfer of quantitative trait loci (QTL) for these traits from wild species sources but also enable documentation of their presence and contribution.

As discussed in Sect. 3.4, the species of *Avena* have been assigned into three gene pools – primary, secondary, and tertiary – by Leggett and Thomas (1995) based on the ease of transfer (introgression) of genes from alien species into cultivated hexaploid oat according to the concept of gene pools for cultivated species proposed by Harlan and de Wet (1971). In this system, the primary gene pool consists of all the hexaploid species, the secondary pool of the tetraploid AACC species *A. magna*, *A. murphyi*, and *A. insularis*, and the tertiary pool the diploid species and the remaining tetraploid species. In *Avena*, the transfer of traits has been mostly from within the primary gene pool. Even there, documented contributions to cultivar development have been restricted primarily to qualitatively inherited major genes for disease resistance where trait detection is more readily accomplished.

3.5.2.1 The Primary Gene Pool

All the hexaploid oat taxa, including the most common wild oats *A. sterilis* and *A. fatua*, were grouped into a single biological species with cultivated oats by Ladizinsky and Zohary (1971) because of their high interfertility in crosses. Thus, the hexaploids constitute the primary gene pool for transfer of desired traits to cultivated hexaploid oat. Because of the high interfertility, the introgression of traits from the wild hexaploid can be accomplished fairly readily by conventional crossing and backcrossing procedures. However, greater frequencies of meiotic abnormalities, such as univalents and micronuclei, were observed in wild/cultivated species hybrids of *A. sterilis*/*A. sativa* by McMullen et al. (1982) and in *A. fatua*/*A. sativa* by Luby et al. (1985) than in intraspecific hybrids. These higher frequencies indicate that in spite of high interfertility, there are likely reductions in recombination associated with meiotic irregularities due to chromosomal heteromorphology between the wild and cultivated hexaploid oats. These irregularities could hinder breaking of linkages between useful and deleterious genes during wild to cultivated oat introgression.

A. sterilis, the progenitor species of cultivated oat, has been found to be quite a rich source of diverse traits, particularly disease resistance, for use in oat breeding programs (Frey 1985). In the spring, in oat regions of the Midwest US and the eastern prairie regions of Canada, and also in the winter oat regions of the southern US, where crown rust (*P. coronata* f. sp. *avenae*) periodically can devastate oat production, most oat breeding programs have become reliant on the use of crown rust resistance (*Pc*) genes from *A. sterilis* in their cultivar releases. More than 30 of these *Pc* genes from *A. sterilis* have been identified and backcrossed into susceptible oat lines to form sets of differentials that are used to monitor changes in rust virulence patterns (Chong et al. 2000; Carson 2008). However, most of the *Pc* genes used to-date have been major genes providing rust race-specific resistance. Thus, when deployed singly, though initially highly effective in protecting the cultivar, they can rapidly lose their effectiveness due to high selection pressure causing shifts in the rust population's virulence pattern. Various schemes have been developed over the years to try to extend the effective use of these genes. In the Canadian oat breeding programs, an *A. sterilis*

gene, *Pc39,* added singly in the cultivar Fidler (McKenzie et al. 1981) provided crown rust resistance that was improved compared to that of other cultivars when the cultivar was released. Later, combining *Pc38* and *Pc39* in the release "Dumont" (McKenzie et al. 1984) conferred resistance to all known crown rust races at the time. This *Pc38–Pc39* combination together with possible other non-identified *A. sterilis* genes was used in many other Canadian releases and in North Dakota releases including "Steele," "Valley," and "Newdak" (McMullen and Patterson 1992). The *Pc38–Pc39* combination provided effective resistance until it was finally overcome around 1990 (Chong and Seaman 1991). The addition of *Pc68* to *Pc38–Pc39* in "AC Assiniboia" (Brown et al. 2001), "AC Medallion" (Duguid et al. 2001), and several subsequent releases provided protection for several additional years, but this combination too lost effectiveness with race shifts to virulence on *Pc68* (Chong et al. 2008). This breakdown necessitated a search for additional sources of resistance including new *A. sterilis* lines for use in combined or stacked combinations. Winter oat breeding programs in the southern US made use of other *A. sterilis*-derived *Pc* genes for crown rust resistance including *Pc58* in "TAM-0-301" (McDaniel 1974a) and *Pc59* in "TAM-0-312" (McDaniel 1974b), two releases from Texas A&M University, and *Pc60* and *Pc61* in releases by the Coker's Pedigree Seed Company in South Carolina (Leonard and Martinelli 2005). *Pc60* and *Pc61* subsequently also were used in spring oat cultivars "Don" (Brown and Kolb 1989a) and "Hazel" (Brown and Kolb 1989b), respectively, released from Illinois.

An alternative method of deploying crown rust resistance genes, including numerous ones from *A. sterilis*, to extend the effective life of individual resistance genes was the "multiline" approach utilized by K.J. Frey, J.A. Browning, and M. D. Simons at the Iowa State University (Browning and Frey 1969). In this approach, different crown rust resistance genes were introgressed into a common oat cultivar or line to form a rust gene composite cultivar of otherwise agronomically similar lines. The deployment of this multiline should then put less selective pressure on the rust population to develop resistance to any particular resistance gene. In the multiline cultivar "Webster," all nine resistance genes used were from *A. sterilis* (Frey et al. 1988). While this approach appeared to be effective in delaying or preventing a build-up of virulence on specific genes, it proved too slow and cumbersome to make adequate continued progress on all the other traits involved in a cultivar improvement program and thus has not been widely utilized.

In addition to the named *Pc* genes, many other instances have been reported on transfer of crown rust resistance genes from *A. sterilis* into oat germplasms and cultivars. Many of these provide adult plant partial resistance and are quantitative and multigenic, and hence more difficult to characterize, but may be less race-specific and hence more durable in effectiveness (Simons 1985). For example, Hoffman et al. (2006) have shown that the *Pc58* resistance of "TAM-0-301" entails at least three genetically separable components including one showing possible non-race-specific partial resistance.

Crown rust resistance from *A. sterilis* has been used in oat improvement in other countries besides the US and Canada, either from direct crosses with *A. sterilis* or indirectly using *A. sterilis*-containing germplasm from the US or Canada. Of particular note in this regard is the likely presence in various South American cultivars with *Pc58*, *Pc59*, *Pc60*, and *P61* introduced through the Quaker International Oat Nursery, which has been grown for many years in several South American countries (Leonard and Martinelli 2005).

Two genes, *Pg13* and *Pg15*, conferring resistance to the other major rust of oat, stem rust (*P. graminis* f. sp. *avenae*), are included in the set of differentials currently used to characterize stem rust virulence in the US and Canada (Fetch and Jin 2007). *Pc13* combined with *Pg2* and *Pg9*, when used in developing "Dumont" (McKenzie et al. 1984) and several subsequent cultivar releases from the Winnipeg group and in "Steele" (McMullen and Patterson 1992) and subsequent releases from North Dakota, provided effective resistance to the prevalent stem rust races in those regions for several years. However, a rapid rise in prevalence of races with virulence on *Pg13* in those regions in the late 1990s necessitated a search for new resistance gene sources (McCallum et al. 2000). Screening of almost 7,000 *A. sterilis* accessions failed to identify any source with strong resistance to the current prevalent stem rust race in that region, but resistance was found in accessions of lower ploidy oat species (Gold Steinberg et al. 2005).

The use of *A. sterilis* as a source of resistance to oat powdery mildew (*Blumeria graminis* (DC.) Speer

f. sp. *avenae* Em. Marchall) in Britain and continental western Europe was included in a review by Roderick et al. (2000). Powdery mildew is described as the most important foliar pathogen of oats in Britain and in the cooler, humid regions of western Europe. Resistance from *A. sterilis* (var. *ludoviciana*) Cc4346 was introduced in the cv. Mostyn in Britain; however, the effectiveness of this race-specific gene was overcome within a few years by a shift in pathogen virulence, although it remained effective for a few additional years in continental Europe. Similarly, the release of cv. Maris Tabard with the resistance gene from an *A. sativa* × *A. sterilis* (var. *ludiviciana*) hybrid resulted in a pathogen virulence shift within a few years. An apparently more durable form of powdery mildew resistance expressed as a partial or adult plant resistance was discovered in the line PC54, which had been developed with a crown rust-resistant gene, *Pc54,* differential by backcrosses of *A. sterilis* CAV1832 into *A. sativa* cv. Pendak (Sebesta et al. 1993). This source became the main for powdery mildew resistance deployed in British and European oat breeding programs (Roderick et al. 2000).

Resistance transferred from *A. sterilis* into cultivated oats has also been documented for cereal cyst-nematode *Heterodera avenae* Woll. (Cook 1974). Nullisomic analysis indicated that the dominant resistance gene in the oat cultivar "Panema" derived from *A. sterilis* line I.376 appeared to be at the same locus as the one in cv. "Nelson" derived from *A. sativa* CI 3444 (Chew et al. 1981). Nematode resistance from *A. sterilis* was also used in developing cultivars at Svalof, AB, Sweden (Mattsson 1988).

In addition to being a rich source of genes for disease resistance, *A. sterilis* accessions have been characterized, which have other agronomic and seed quality traits of value in cultivated oat improvement. Most of these traits, however, are quantitative in nature, controlled by multiple genes and with expression influenced by the genetic background and the environment. Thus, the transfer of these traits into a desirable cultivated oat type is much more challenging than that for qualitative major genes, which often can be introgressed simply by backcrosses to a recurrent parent cultivar with minimal impact on other traits of the recipient. In his review on oat improvement, J. B. Holland (1997) pointed out that transfer of a polygenic quantitative trait from a wild oat species usually requires larger amounts of wild germplasm to be maintained in the resulting breeding lines than transfer of a single gene trait. Thus, the transfer of quantitative traits fits more into the concept developed by Simmonds (1993) of "incorporation" of exotic germplasm versus simple "introgression" of a single trait.

Another problem for incorporation of quantitative traits from wild germplasm besides potential linkage drag of deleterious genes is the same as for incorporation of such traits from any germplasm source – a resulting undesirable correlated change in other traits in the resulting lines. For example, groat (karyopsis) protein as high as 35% was identified in some *A. sterilis* accessions (Campbell and Frey 1972; Ohm and Patterson 1973; Frey 1983) but proved difficult to capture in high grain yielding breeding lines due to the well-known negative association between these characters in cereals (Simmonds 1995). This complication arises due to competition between physiological processes for a limited pool of nitrogen and carbon metabolites. Also, apparent high levels of grain components such as protein, oil, β-glucan, or other nutrients are often expressed on a percentage basis such that thin-kernels with low starch content appear deceivingly favorable. In spite of these problems, germplasm and breeding lines with enhanced levels of seed composition traits have been produced from *A. sterilis–A. sativa* hybridizations (Frey 1992).

In his review, Holland (1997) pointed out that recurrent selection has proven to be a technique particularly well-suited for incorporating genes from *A. sterilis* into adapted gene pools due to the opportunities to break linkages to undesirable traits through successive rounds of recombination and selection. He then described two examples from the extensive work of Frey and his students at the Iowa State University on the use of recurrent selection using *A. sterilis* germplasm to generate high protein and high oil oat breeding lines.

Several progressive studies were involved in the incorporation of high groat protein from *A. sterilis* including the demonstration that some high-protein genes from *A. sterilis* were different from and complementary to those in *A. sativa* (Cox and Frey 1985), the identification of several mating lines involving both *A. sterilis* and *A. sativa* germplasm in which there was little or no negative correlation between grain protein percentage and groat yield (Kuenzel and Frey 1985),

and the selection of progeny from those matings and their use as the source parents for cycles of recurrent selection for high grain protein yield (grain yield × protein percentage) (McFerson and Frey 1991; Moser and Frey 1994). A similar progressive set of experiments were conducted to use *A. sterilis* germplasm in the development of high groat oil concentration oat breeding lines with first the demonstration that genes for high groat oil content from *A. sterilis* are complementary to those from *A. sativa* (Thro and Frey 1985), then crossing of high oil *A. sterilis* accessions to high oil *A. sativa* lines followed by crosses to agronomically superior *A. sativa* cultivars with cycles of recurrent selection for high oil content with independent culling for agronomic adaptation and cultivated plant type (Branson and Frey 1989a, b). These efforts resulted in lines after cycle 6 with oil content over 16% (Schipper and Frey 1991).

The presence of yield enhancing genes from *A. sterilis* were reported by Frey and Browning (1971) based on increased yields of some of the lines derived from crosses made to introgress crown rust resistance genes from *A. sterilis* into *A. sativa*. Enhanced yield was also found in backcross F_2 generations of several other *A. sterilis* × *A. sativa* crosses by Lawrence and Frey (1975). Further studies of such materials indicated that the enhanced yield was likely attributable to enhanced growth rate (Takeda and Frey 1976), increased biomass (Cox and Frey 1984a, b), and greater leaf area duration (Brinkman and Frey 1977; Bloethe-Helsel and Frey 1978).

Several cultivars with improved traits and containing large amounts of *A. sterilis* germplasm have been released including "Sheldon" (Frey 1992) and Ozark (Bacon 1991). In these lines, high yield potential and, in Ozark, winter hardiness have been attributed, at least in part, to contributions from the *A. sterilis* component. Similarly, although no specific genes for barley yellow dwarf virus (BYDV) tolerance (inherited as a polygenic trait) were identified, the finding of BYDV tolerance or resistance in almost half of 1,718 *A. sterilis* accessions tested led Comeau (1982) to postulate that many of the recent cultivars in USA and Canada derive some BYDV resistance factors from *A. sterilis*.

Enhanced yield was also observed in early backcross generation lines, which contained *A. sterilis* cytoplasm when the lines were compared to derived lines from reciprocal crosses involving the same *A. sterilis*–*A. sativa* parents but with *A. sativa* cytoplasm (Robertson and Frey 1984). This observation led them to propose use of *A. sterilis* cytoplasm for oat cultivar improvement. However, failure to find evidence of a consistent nuclear–cytoplasm heterotic effect across numerous *A. sterilis*–*A. sativa* matings (Beavis and Frey 1987) and in later backcross generations of *A. sterilis*–*A. sativa* matings (Rines and Halstead 1988) indicated that the positive effect of the *A. sterilis* cytoplasm was specific depending on the accession combination and the *A. sterilis* nuclear genes retained in each line.

The other common wild hexaploid oat, *A. fatua* L., has been used to a much lesser extent than *A. sterilis* as a germplasm source for oat improvement. This reduced use is probably because of its lack of identified major genes for disease resistance and overall lack of diversity of traits as found in *A. sterilis*. *A. fatua* germplasm was a major component of releases of the cultivars "Rapida" (Suneson 1967a), "Sierra" (Suneson 1967b), "Montezuma" (Suneson 1969), and "Mesa" (Thompson 1967). The use of locally collected *A. fatua* as parents provided desired adaptation characteristics for the arid regions of the Southwest US including extreme earliness in Rapida and Montezuma. Stevens and Brinkman (1986) were less successful in the use of *A. fatua* accessions to improve yield performance in crosses to Midwest US cultivars. One backcross selection did outyield its recurrent parent but lacked straw strength and crown rust resistance as required for cultivar release.

Several dwarfing genes were identified in short statured *A. fatua* plants collected in Japan and the surrounding areas of eastern Asia when these plants were crossed to cv. Kanota by Morikawa 1989 and Morikawa et al. (2007). However, when one of these genes was used in further crosses by Milach et al. (1998), the resulting dwarf phenotype was found to be too extreme to be directly used in cultivar development. Further efforts to identify appropriate modifier genes or the use of other of the identified dwarfing genes might prove useful in developing reduced height oat cultivars. An uncharacterized gene (or genes) for dormancy in *A. fatua* accessions was used by Burrows (1986) to develop experimental lines (termed "dormants") of cultivated oat that could be planted in the fall, lie dormant over winter, and then germinate in early spring to take advantage of a longer growing season in the northern regions

where oats do not successfully overwinter. Adequate synchrony of germination combined with good cultivated oat phenotype proved difficult to attain for use in cultivar release.

3.5.2.2 The Secondary Gene Pool

Leggett and Thomas (1995) defined the secondary gene pool as the AACC tetraploid species *A. magna* (*maroccana*) and *A. murphyi* based on observations that, although in hybridizations with *A. sativa* the F_1 plants are highly self-sterile, F_1 female fertility enables crossing to hexaploid to produce low seed set, and pairing between chromosomes of the tetraploid and hexaploid species seems sufficient to allow recombination to occur. *A. insularis*, a more recently discovered AACC tetraploid (Ladizinsky 1998) should also be included in this secondary gene pool. The capability to transfer genes from the AACC tetraploid to hexaploid cultivated oat was indicated by the recovery of derivatives of backcrosses of the pentaploid F_1 having 42 chromosomes but with traits characteristic of the tetraploid parent (Ladizinsky and Fainstein 1977; Thomas et al. 1980a).

The successful introgression of crown rust resistance from an *A. magna* accession into a hexaploid cultivated oat germplasm was accomplished by Rothman (1984) through the use of a synthetic hexaploid. The synthetic hexaploid was derived from a colchicine treated F_1 hybrid between diploid *A. longiglumis* accession CW57 and *A. magna* accession CI 8330. Although the initial C_1 (progeny of colchicine-treated F_1) plant set few seed, a C_6 segregants with cultivated-type spikelet components and stable fertility was recovered, perhaps through accidental outcrossing. This germplasm containing the resistance gene *Pc-91* (Rooney et al. 1994) was used in developing the cultivar HiFi (McMullen 2005), which, due to the presence of *Pc91* together with some unidentified *Pc* genes, has retained its effective resistance for several years (Chong et al. 2008).

Features of *A. magna* that provoked the initial interest in transfer of genes to cultivated oats was the large groat size and high groat protein content (over 25%) in certain accessions (Ladizinsky and Fainstein 1977; Thomas et al. 1980a). In spite of extended crossing, backcrossing, and selection efforts, successful incorporation of these polygenically inherited traits into high-yielding cultivars has not proven possible (Valentine personal communication, Aberystwyth Univ., UK). As an alternative to trying to transfer these complex polygenic traits into hexaploid cultivated oat, Ladizinsky and Fainstein (1977a) proposed transfer of more simply inherited domestication traits from *A. sativa* into *A. magna* and *A. murphyi* to produce a domesticated tetraploid oats. Ladizinsky (1995) reported transfer of the characteristics of non-shedding spikelets, glabrous and yellow lemma, and reduced awn formation into tetraploids with each of the characteristics segregating in an apparent single gene manner. He further pointed out that such transfers into otherwise *A. magna* and *A. murphyi* genetic backgrounds might make domesticated derivatives more successful than common oat in the warm habitats of Morocco and Spain where these wild tetraploid species grow naturally.

3.5.2.3 The Tertiary Gene Pool

The tertiary gene pool as defined by Leggett and Thomas (1995) consists of all the diploid *Avena* species and the tetraploids *A. barbata*, *A. vaviloviana*, *A. abysinnica*, and *A. macrostachya*. Characteristic of this more difficult to exploit group is that members do not readily hybridize with *A. sativa* to produce F_1 plants, F_1 plants when produced are highly sterile both in self-fertilization and in backcrosses to the F_1, and the introduced desired traits are usually difficult to introgress into stable cultivated breeding lines free of accompanying deleterious genes.

Use of the lower ploidy parent as female in crosses usually enhances the frequency and quality of seed set (Rajhathy and Thomas 1974). Even then, embryo rescue is often required in many diploid × hexaploid crosses to recover F_1 plants. Some level of fertility in these highly sterile interspecific F_1 plants, presumably resulting from a lack of chromosome homology and subsequent lack of chromosome pairing, is often attained by treating the plants with colchicine to double the chromosome number, thus producing plants with the full sets of chromosomes from both parents. Backcrossing of these chromosome-doubled lines to *A. sativa* as recurrent parent with selection for the introduced desired trait often produces gradually increasing fertility to lines similar to the recurrent parent with the trait introgressed or with the trait

present on an added or substituted chromosome or chromosome pair. Examples of successful trait introgression using synthetic octaploids from embryo-rescued colchicine doubled F_1 plants from diploid × hexaploid crosses include introgression of powdery mildew resistance from *A. pilosa* by Hoppe and Kummer (1991), now in the recently released cultivar "Champion" (Herrmann, BAFZ, Gross Lusewitz, Germany, personal communication, 2009), and crown rust resistance *Pc94* by Aung et al. (1996), now in the cultivar "Leggett" (Chong et al. 2004).

Several other "bridging" schemes have been used to transfer genes, usually for rust resistance, from diploids to *A. sativa*. These involve incorporating the resistance into a tetraploid or synthetic hexaploid that will produce a viable F_1 in crosses to *A. sativa* and include production of a colchicine-doubled autotetraploid of the diploid (Zillinsky and Derick 1960), introgression of the rust gene from the diploid into a tetraploid species (Zillinsky et al. 1959), and construction of a synthetic hexaploid by crosses of the diploid to a tetraploid followed by colchicine doubling to construct a synthetic hexaploid. These latter $2x + 4x$ synthetic hybrids have been made both with AABB (AAA′A′) tetraploids such as *A. abysinnica* (Brown and Shands 1954; Forsberg and Shands 1969a, b) and AACC tetraploids *A. magna* (Rothman 1984) and *A. murphyi* (Rines et al. 2007). The $2x \times 4x$ synthetic Amagalon from *A. magna* × *A. longiglumis* also permitted access to the crown rust resistance gene *Pc91* (Rothman 1984) from the tetraploid *A. magna*, now in cv. HiFi (McMullen 2005).

The next difficulty frequently encountered in gene transfer from a diploid or tetraploid oat species into common cultivated oat is to integrate that gene into breeding lines stable for transfer of the added gene through generations and free of deleterious linkage effects. Dyck and Zillinsky (1963) reported two crown rust resistance genes originally from *A. strigosa* CD3820 present in different selections from crosses of a derived tetraploid to *A. sativa*. One of these, *Pc23*, appeared to be completely incorporated into the normal *sativa* lines and was transmitted in a stable fashion. The other, *Pc15*, was inherited in an unstable fashion. They suggested that, based on cytological observations of varying frequencies of pairing between *A. strigosa* and *A. sativa* chromosomes in *A. strigosa* × *A. sativa* hybrids, there were segments of diploid chromosomes (e.g., the one carrying *Pc15*) not fully compatible with the *sativa* chromosomes and thus responsible for the irregular meioses. Comparative analyses between molecular marker maps of A-genome diploids and *A. sativa* revealed larger syntenic regions to *A. sativa* linkage groups with some of the diploid linkage groups than with others (O'Donoughue et al. 1995; Portyanko et al. 2001). Also, Jellen et al. (1995) found that certain chromosomal linkage groups tended to remain more syntenic across cereals than others. Thus, the possibility or ease with which a foreign gene (e.g., from *A. strigosa*) is incorporated cleanly into a chromosome of the recipient species likely depends on the location of the gene in the donor genome and the retained homoeology of that donor chromosome or chromosome segment to a chromosome of the recipient species. For example, Rines et al. (2007) obtained normal male and female transmission of a crown rust resistance gene (*Pc94*) in 42-chromosome derivatives in transfers from *A. strigosa* CI6954SP into an "Ogle" oat background. Loss in further backcrosses of a molecular marker SCAR94-2 (developed by Chong et al. 2004) linked about 5 cM from *Pc94* in other backcross lines indicated that meiotic recombination was occurring in the region. Field tests of backcross 6 lines revealed no evidence of linkage drag of any detrimental traits (Rines unpublished). In contrast, several efforts were made over years to incorporate into stable lines the *Pc15* resistance from *A. strigosa* CD3820 by selfing or backcrossing of *strigosa*-derived materials. Paired cytological and resistance transmission studies revealed that the resistance was always associated with a retained alien addition, substitution, or telosomic chromosome and transmitted unstably, usually only through the female gamete. Irradiation of an alien monosomic substitution allowed recovery by Sharma and Forsberg (1977) of a stably transmitting line, presumably from translocations from the alien substitution chromosome to a non-homoeologous chromosome. The performance of three cultivars "Centennial," "Horicon," and "Dane" with resistance derived in this manner indicates that derivatives can be produced by this approach and further selection with no accompanying significant deleterious effects (Forsberg 1990).

Difficulties have also been encountered in transferring desired genes cleanly from tetraploid species of this secondary gene pool, again presumably due to chromosomal heteromorphology between the donor species and *A. sativa*. Attempts to transfer mildew

resistance from tetraploid *A. barbata* (Cc4897) to cv. Manod through backcrossing and selection resulted only in lines with the resistance carried on an alien addition chromosome (Thomas et al. 1975). Irradiation of the addition line with a CO^{60} source enabled recovery of a resistant translocation line with normal transmission of the resistance in the "Manod" background, but the translocation showed irregular transmission in certain other cultivar backgrounds. This translocation was shown to involve exchange between non-homoeologous chromosomes (Aung and Thomas 1978). Transfer of stem rust resistance from *A. barbata* by irradiation of alien chromosome additions was similarly accomplished by Brown (1985) producing relatively stably transmitting translocation lines, but with accompanying yield reductions, presumably due to the presence of deleterious genes on the translocated alien segment. More stably transmitting mildew-resistant breeding lines were produced by Thomas et al. (1980b) who employed through crosses the action of a "suppressor" of non-homoeologous chromosome pairing present in *A. longiglumis* CW57. The use of CW57 to induce homoeologous pairing of desirable alien variation into cultivated oat and thus enhanced introgression is discussed by Thomas and Al-Ansari (1988). That the diploid oat component of the "Amagalon" synthetic hexaploid in the earlier described transfer of crown rust resistance from *A. magna* by Rothman (1984) was *A. longiglumis* CW57 may account for his success in the recovery of a stable *sativa*-like resistant derivative. The CW57 line was also used to facilitate transfer of a dominant gene conferring mildew resistance from diploid oat *A. prostata* (Griffiths 1984; Morikawa 1995). Successful introgression of powdery mildew resistance from the perennial tetraploid oat *A. machrostachya*, however, was accomplished simply by various crosses and selection (Yu and Herrmann 2006).

Of interest relative to the degree of homology of an introduced alien chromosome segment relative to chromosomes of the recipient is not only its initial pairing and recombination with one of the recipient cultivated oat chromosomes but also the disruptive effect the introgressed segment may have on meiotic pairing once it is introgressed. Wilson and McMullen (1997a, b) reported that the introgressed *A. sterilis* crown rust resistance gene *Pc38* was located on one chromosome in the cultivar "Steele" but on a different chromosome in cultivar "Dumont" such that in crosses of "Steele" × "Dumont" the two sites could segregate producing progeny with 0 to 4 copies of *Pc38*. The alternative site in "Dumont" apparently was generated as a result of a reciprocal interchange presumably involving homoeologous *sativa* chromosomes. Similarly, the location of another introgressed alien chromosome segment, *Pc94* from *A. strigosa* CI6954SP, has been found at independently segregating sites in various backcross derived lines, again indicating possible occurrence of a translocation between homoeologous *sativa* chromosomes caused by abnormal meiotic pairing involving the introgressed alien chromosome segment (H. Rines unpublished).

Another obstacle that can arise in interspecific trait transfer and which can occur across all levels of ploidy transfer is a suppression or inhibition of expression of the introduced trait. This suppression may be either a gene-specific or a general species-specific suppression and has been observed in many crops including wheat (Singh et al. 1996). A crown rust resistance gene *Pc38*, or some factor closely linked to it, was reported to suppress the resistance of another *A. sterilis*-introduced gene *Pc62* (Wilson and McMullen 1997a, b) and also a resistance gene *Pc94* introduced from *A. strigosa* (Chong and Aung 1996). Rines et al. (2007) reported a suppressor of *Pc94* being co-introduced from *A. strigosa* CI6954SP that segregated from it in backcross derivatives. This suppressor also inhibited *Pc62* but differed from the *Pc38*-associated suppressor in other *Pc-gene* specific suppression (Rines et al. 2008).

Apparent general species-specific suppression was reported by Rines et al. (2007) where excellent crown rust resistance in *A. murphyi* P12 was suppressed in crosses and backcross derivatives of *A. murphyi* P12 × *A. sativa* "Ogle." A similar lack of expression of the *A. murphyi* resistances in crosses to five other susceptible cultivars indicated a general suppression by *A. sativa* (Rines et al. 2008). A lack of resistance expression was also found in hybridizations to *A. sativa* of stem-rust-resistant *A. strigosa* PI258731 (Rines unpublished) and BYDV-resistant *A. strigosa* (Ladizinsky 2000).

3.5.2.4 Use of Molecular Markers

Although genes for many traits, particularly disease resistance, have successfully been introgressed from wild species into cultivated oat by backcrossing with selection for the trait at each generation, the process

could now become much more efficient (efficacious) if one were able to use molecular markers to facilitate the transfer. Markers could be used to both identify the gene of interest, being either tightly associated with the gene or a part of it, and to select for maximum recovery of the recipient cultivated oat parental genome. Linked molecular markers have been found for several introduced genes (as summarized in Rines et al. 2006), but the identification has usually occurred only post-introgression for use in further transfer to other breeding lines. Whereas tightly linked or gene-specific markers are valued for selection for the introduced trait, also of value are markers identified in the surrounding region of the donor genome segment. Post-introgression selection against such markers can allow recovery of recombinants with smaller alien segments lacking possible deleterious genes linked to the gene of interest. For example, powdery mildew resistance and stem rust resistance have been introduced into stably transmitting breeding lines, but negative agronomic effects were associated with them (Brown 1985; Valentine et al. 1994), presumably due to linked deleterious genes from *A. barbata*. The effective transfer of the many possible quantitative multigenic traits identified in wild *Avena* species will require marker use to identify and track QTLs governing these traits. Also, selection for maximal recovery of the desired cultivar background in any transfer effort will require a large number of well-distributed markers. Thus, the capability to capture and utilize all the potential in wild oat species for cultivated oat improvement depends on the development of cheap, high-throughput markers allowing ready whole genome scans and well characterized molecular linkage maps at all ploidy levels. Many of the trait-associated markers identified to date in oat have been difficult or expensive to assay and with limited polymorphisms in possible recipients.

3.6 *Avena* as Weeds and Invasive Species

Some oat species are weeds infesting cereal crops (see Sect. 3.1.1.2). Among the diploid species, this is *A. longiglumis*, represented by populations of tall, vigorous plants (up to 2 m tall) weeding crops on fertile, well moistened sandy soils, and *A. atlantica*. Both species weed cereal crops in Morocco (Leggett et al. 1992). Some other diploid species occur as ruderal weeds only along the roads or around human dwellings. On the Ethiopian plateau, the tetraploid *A. vaviloviana* is a common weed in wheat and barley fields, reaching up to 2,200–2,800 m (Ladizinsky 1975). A large population of *A. magna* (upto 2 m tall) has been found in Morocco on alluvial soils as a weed of cereal crops (Leggett et al. 1992). Aggressive nature of this species has been later confirmed by other researchers (Saidi and Ladizinsky 2005).

Among all tetraploid species, *A. barbata* is the most noxious weed that grows in all Mediterranean countries. It invades fields of all agricultural crops, and in the east, its natural range stretches through Asia Minor, Iraq, and Iran to the Himalayas. It should be noted that according to the opinion of Malzev (1930), in the early twentieth century, this species was entirely wild and did not weed agricultural fields.

The hexaploid species *A. sterilis, A. ludoviciana*, and *A. fatua* are noxious weeds, especially *A. fatua*, against which herbicides are being developed. These invasive species infest vast territories in the basins of the Mediterranean, Black and Caspian seas, and extends through the Southwest Asia to the easternmost point of the Asian continent, occupying all agricultural regions.

It should be noted that in the American continent, south of Africa, Japan, Australia, and countries of Oceania, *A. barbata, A. sterilis, A. ludoviciana*, and *A. fatua* are noxious adventitious weeds (Malzev 1930; Whalley and Burfitt 1972; Yamaguchi 1976; Rines et al. 1980; Dillenburg 1984).

Gene flow via pollen dispersal may occur within the oat crop-wild-weedy complex, which consists of domesticated oat and its two fully interfertile hexaploid wild relatives *A. sterilis* and *A. fatua*. There is evidence of hybridization between the crop and the wild species under natural conditions. Although the levels of outcrossing are usually low, the potential for gene flow and introgression into wild oats is substantial because of their widespread occurrence.

Thus, the cultivation of transgenic cultivars of oats and other cereals in future may lead to the transformation of these noxious weeds into super-weeds due to gene flow from transgenic crops.

3.7 Recommendations for Future Actions

The international community faces the task of raising funds and designing programs of safeguarding other oat wild species: *A. clauda* (in Greece, Bulgaria, Turkey, Algeria and Morocco), *A. pilosa* (Greece, Turkey, Algeria and Morocco), *A. ventricosa* (on Cyprus and in Algeria), *A. bruhnsiana* (in Azerbaijan), *A. damascene* (Syria and Morocco), *A. prostrata* (Spain and Morocco), *A. atlantica* (Morocco), *A. agadiriana* (Morocco) and *A. macrostahya* (in Algeria). Being a unique component of the Earth's biodiversity, each of these species contains genetic information that can be used in practical oat breeding (see Sect. 3.2.1).

3.8 Conclusions

There is considerable interest in extending the range of cultivated oat through the incorporation of genes from the wild oat species. A number of considered wild species are reflected in a wide range of botanic, ecological, and genetic diversity. The results of presented researches of wild *Avena* species made it possible to display intraspecific diversity on all the characters involved. Numerous researches in this direction and practical results of oat breeding have evidenced that utilization of wild species is the most promising trend of oat breeding, capable of broadening genetic base, and reducing genetic erosion of this crop.

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Chapter 4
Dasypyrum

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4.1 Introduction

Members of the grass family (Poaceae) have diverged from a common ancestor 50–70 million years ago (Mya) (Bolot et al. 2009). While earlier forms of the current taxa in the Ehrhaetoideae (i.e., rice), Pooideae (*Avena, Brachypodium, Hordeum, Triticum,* etc.), and Panicoideae (i.e., *Setaria, Pennisetum, Sorghum, Zea*, etc.) subfamilies branched out from the phylogenetic tree about 46 Mya, the larger divergence within them started 20 Mya, and by 13 Mya all the ancient forms of the extant genera were already differentiated (Bolot et al. 2009). Those ancestral forms dispersed around the primitive areas of what nowadays constitute the surrounding territory of the south side of the Mediterranean basin and gave rise to the *Dasypyrum* taxa within the Pooideae subfamily.

The genus name *Dasypyrum* Cosson et Durieu (Ordo CCVII Gramineae) was validated by Durand (1888, p. 504), in substitution for the genus 8273 285 *Haynaldia* Schur (Durand 1888, p. 479), to avoid confusion with other *Haynaldia* genera. Since then, according to Humphries (1978), three species were recognized: *D. villosum* (*Dv*), *D. hordeaceum*, and *D. sinaicum*. The last taxon was considered an annual species (Humphries 1978) occurring in eastern Mediterranean environments (Durand 1888) and was taken as a species also by Candargy (1901) (Löve 1984). However, Frederiksen (1991a) in her taxonomical revision of *Dasypyrum* (Poaceae) indicated that *Dasypyrum sinaicum* (Steudel) Candargy being based on *Triticum sinaicum* Steudel, whose lectotype belonged to *Eremopyrum bonaepartis* (Sprengel) Nevski did not belong to *Dasypyrum*. Therefore, she recognized only two species in the genus: the annual *D. villosum* (L.) Candargy [syn. *Haynaldia villosa* (L.) Schur] and the perennial *D. breviaristatum* (syn. *D. hordeaceum*). We use the abbreviation *Dv* throughout to designate *D. villosum* and *Db* for *D. breviaristatum*. The following review on the botanical, ecological, genetical, cytogenetical, and breeding aspects of the members of the *Dasypyrum* genus contain updates to the comprehensive review made by Gradzielewska (2006a, b).

4.2 Basic Botany and Phyletic Relationships of the Species

4.2.1 *Geographic Distribution and* Dv*-Dominated Phytoassociations*

The distribution of *Dv* is mostly Mediterranean, extending from southwestern Europe: Corsica and Hérault, Vaucluse, Bouches-du-Rhône, Alpes maritimes in south of France, Baleares in Spain (Rouy 1913), then to the core distributional center in southeastern Europe*:* Italy (including Sicily and Sardinia), Slovenia, Croatia, Bosnia-Erzegovina, Serbia, Albania, Macedonia, Greece (including Crete) (Fig. 4.1). Over the centuries, it has also been found in Middle Europe*:* Austria, Hungary, Switzerland; East Europe*:* Romania, Bulgaria, Moldova; Ukraine (Krym); western Asia: Turkey; Caucasus*:* Armenia, Azerbaijan, Georgia, and

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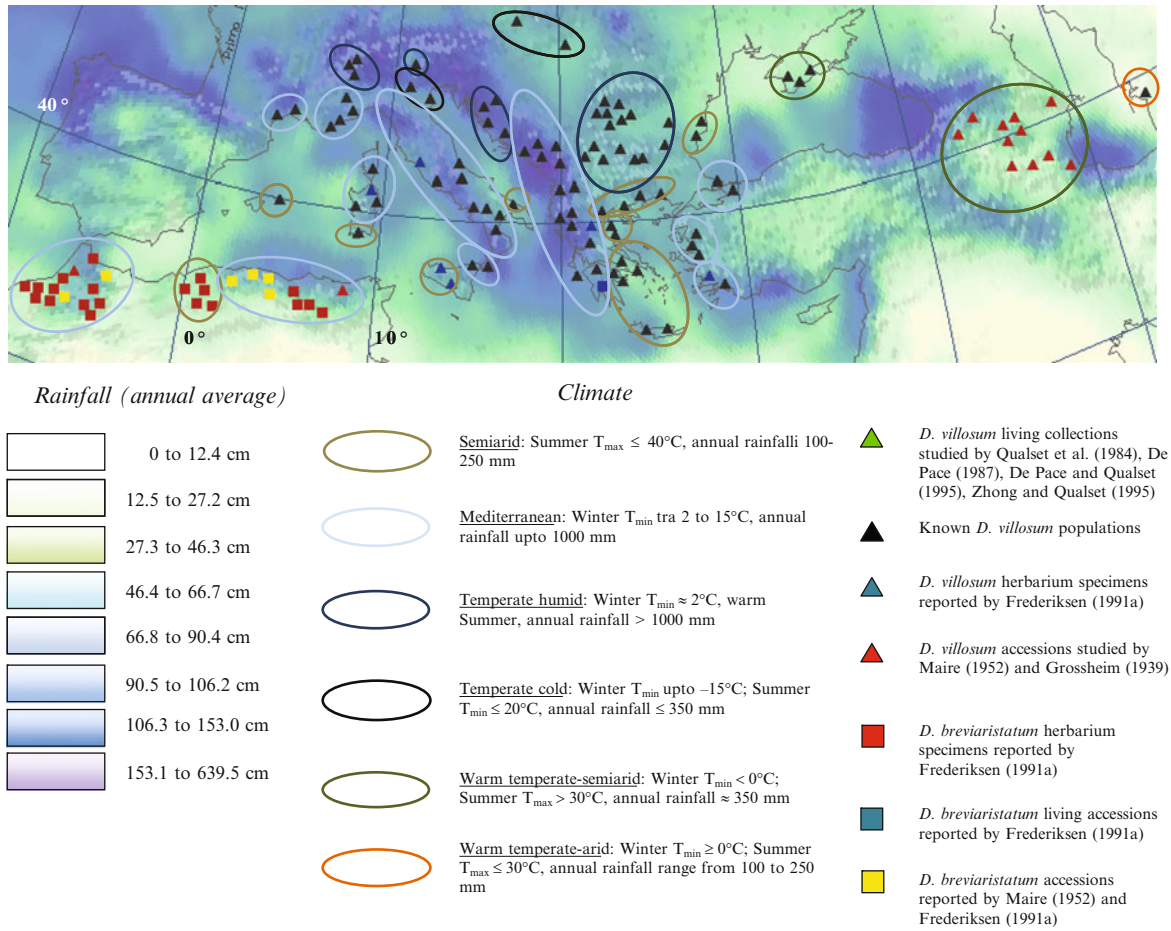

Fig. 4.1 Geographical distribution of *D. villosum* and *D. breviaristatum* accessions studied or reported by several authors (*see list on the left side*). Territorial physical map and average annual rainfall adapted from *Microsoft Encarta*. Climatic regions defined as in Köppen (1900)

possibly Kabardino-Balkaria, Karachay-Cherkessia, and Krasnodar within the Russian Federation; Middle Asia: Turkmenistan (West part). *Dv* is common in Sicily and Sardinia islands but rare in Corsica, where it has been found in Bonifacio and vallon de Canalli (Briquet 1910). The species appears to be rather uncommon in northern Italy (Pignatti 1982), as well as in northern and West Europe. It has been cited in the floras of France, Austria, Belgium, Germany, Luxemburg, the Netherlands, Switzerland, Bulgaria, Romania, and South Russia, but it is not reported in the floras of Spain (Frederiksen 1991a), Cyprus, Ethiopia, Egypt, Iran, Iraq, Israel, Lebanon, Libya, Malta, South Africa, and Syria. It was found in Bône and Oran in Algeria in the nineteenth century by Ernest Saint-Charles Cosson. However, Maire (1952) doubted that *Dv* was endemic in the region and suspected that the plants sampled in the area were introductions. Later expeditions did not find *Dv* at Oran (Quezel and Santa 1962).

The above reports suggest a distribution limit for *Dv* towards the Near-East regions of the Caucasus (Grossheim 1939) and Transcaucasus and eastern Turkey (it has recently been found in the Ephesus area south of Izmir; observation of C.O. Qualset), and the East-North limit in Ukraine, Hungary (sporadic since the nineteenth century), and Austria. A recent excursion along the main roadsides from Budapest to Dunakeszi, Szeged, Győr, Gyöngyös, and Gödöllő, did not reveal any presence of *Dv*, although it was reported occurring around those areas in the Magyar flora of

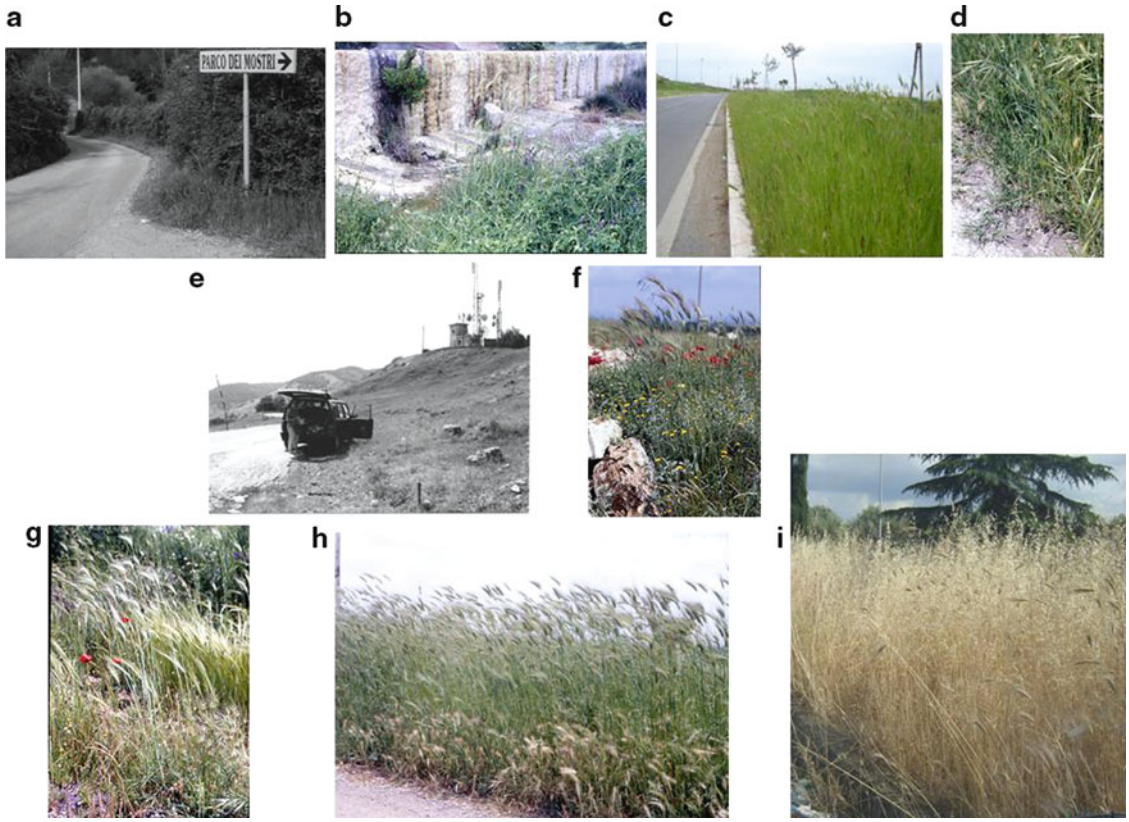

Fig. 4.2 Habitats where *Dv* ecotypes thrive: (**a**) trails along parks such as "Monster park" near Bomarzo (Viterbo, Italy) where the original I 84-16 population was found (see Sect. 4.2.2); (**b**) sulfur-water ditch (i.e., Bulicame sulfur spring near Viterbo, Italy); (**c**) roadside (i.e., Trigoria near Rome, Italy); (**d**) soil paved with asphalt; (**e**) harsh-soil at high altitude (i.e., Mt. Armizzone 1,300 m asl, Basilicata Region, Italy); (**f**) quarry dumps; rocky-soil in semi-arid areas (i.e., Puglia Region, Italy) in association with (**g**) *Aegilops ovata*, (**h**) *Hordeum murinum*, and (**i**) *Avena* spp.

Rezső (1973). Accessions have been collected in Bulgaria (Angelov 2003a, b). Occasional specimens found in North European countries and North Africa are introductions and not part of the endemic flora.

Dv is a vigorous plant on disturbed and moisture-stressed sites; smaller forms grow on compacted soils (Fig. 4.2). The most dense stands of *Dv* have been found in central Italy (Fanelli 1998), southern Italy (De Pace 1987), Adriatic coast of Croatia, Bosnia and Herzegovina, Montenegro (Qualset et al. 1984), and Greece (see Sect. 4.7.2). In Italy, it constitutes an important component of the wild grass plant communities on the Murgia (Apulia-Italy), Rome (where the earliest-heading plants in March are found in the fields between landing and take-off runways at the Fiumicino airport due to early and repeated mowing of the field grasses and regrowth ability of *Dv* plants), and Tuscia areas (Latium-Italy) (Fanelli 1998), and other summer-dry hills of southern Italy. *Dv* grassland in Latium is dominated by tall annual herbs (1–1.5 m) such as *Avena barbata* and *Phalaris brachystachys*. Perennials are also present, such as *Asphodelus ramosus* and *Carlina corymbosa* (Bianco et al. 2003). Two new association of fallows, dominated by *Dv* or less frequently by *Hordeum bulbosum* and corresponding to the "anthropogenous steppe" have been found near Rome: *Laguro-Dasypyretum villosi* on sublitoraneous calcareous sands of recent fossil dunes (up to 4–5 km from the Tirrenean Coast) (Figs. 4.3 and 4.4) and *Vulpio-Dasypyretum villosi* within non-litoraneous environments (over 5 km from the coast) at various lower altitude soils (less than 700 m above sea level, asl) but usually on sands or tuffs (Fanelli 1998). The first ecotypic community is dominated by *Dv* and *Lagurus ovatus*, associated to less represented species such as *Bromus rigidus*,

Fig. 4.3 High-density *Dv–Lagurus* phyto-association in a trail of the pine wood near sandy dunes along the Ionic-sea coast of the Puglia region near Termitosa–Castellaneta Marina railway stations in southern Italy

Anthemis mixta, *Centaurea sphaerocaephala*, *Euphorbia terracina,* and *Vicia pseudocracca*. The second phytoassociation is dominated by *Dv* and *Vulpia ligustica*, associated with less represented species such as *Knautia integrifolia*, *Hordeum bulbosum*, *Vicia bithynica*, and *Trifolium pallidum* (Fig. 4.5).

The two phytoassociations share a common ecotypic structure composed mainly by a stratum of 1–1.5 m tall annual species such as the grasses *Hordeum bulbosum*, *Avena* spp., *Dactylis glomerata*, *Bromus* spp., *Poa trivialis*, and a few dicots as *Verbascum sinuatum,* and a second stratum made by 0.3–0.4 m tall dicots in which such legume plants prevail, as *Trifolium* spp. and *Medicago hispida*, and a small proportion of other species such as *Sherardia arvensis* and *Plantago lanceolata*. Those plant communities have been found in similar habitats near the Ionio-coast areas in the Puglia region (Termitosa-Castellaneta Marina; Figs. 4.3 and 4.4), and are established within few years in recently colonized disturbed habitats, along the borders of new trails of litoral pine woods or at the edges of inland areas abandoned from cultivation. In experimental plots settled at University of Tuscia, Viterbo, by seeding *Dv* spikelets containing caryopses at a density of 400 spikelets m^{-2} in soil that remained unploughed for over 10 years, a spontaneous herbaceous flora stabilized during the last 6 years in which *Dv* was the prevalent species. In the meanwhile, the majority of the former-widespread dicot species (such as *Soncus tenerrimus*) remained in the control area near the *Dv* plots, but they were out-competed by *Dv* within the experimental plot area (Fig. 4.6). Once established, the *Dv* phytoassociations are very stable over time (20–50 years' observations reported by Fanelli et al. 2006).

Dv rarely has been found at altitude higher than 1,000 m above sea level (asl) in Italy, except in Calabria sites on Sila Mountain "Monte Armizzone," at 1,350 m asl (Massiccio del Pollino, Basilicata, Italy; Fig. 4.2e). In the past, *Dv* was common in wheat fields, but it now occurs in massive stands along roadsides and on the borders of crop fields, but rarely is it found in large stands within uncultivated fields. Mowing the grasses along the borders of local roads and highways (superstrada or expressway) occurs at the end of April to early May (Fig. 4.2). At that time, some early *Dv* populations are already at the grain-filling period; in addition, by the end of May, new regrowing tillers from the mowed *Dv* plants are already in anthesis. Both events provide ample opportunity for dispersal in the same habitat and selection pressure for early-heading genotypes.

Vegetative propagation has not been found in sampled ecotypes growing at lower altitude, but some ecotypes (*Dv*_T) sampled at altitude higher than 900 m asl displayed regrowth ability. In a transplanting experiment from the native habitat to the greenhouse, dormant axillary buds at the basal nodes of mature culms of those plants, during summer, sprouted epicotyles terminating with a rooted shoot (Fig. 4.7), which in turn may produce further epicotyles (Fig. 4.8) forming a stolon-like structure eventually persisting up to April–May next year, when heading takes place. Mesocotyls can be formed from the late growth of dormant buds on basal nodes (Fig. 4.9) or from middle (Fig. 4.10) or top nodes (Fig. 4.11) of old and dry culms.

This may suggest a tendency of *Dv* to vegetative propagation when it reaches the extreme range of its habitat at high altitude, where it expresses regrowth ability when humidity persists in the soil along steep tracks. Although *Dv* has been found in Italy and elsewhere at altitudes below 1,350 m asl, native stands of *Dv* at lower altitude have not been found in Morocco, and when there have been reports, those probably represented introductions. *Dv* occurs frequently as native stands at lower altitudes in Greece.

Db, contrary to Dv, is common in pastures and forests of the mountains in Algeria above 500 m asl (the Massif de l'Ouarsenis, Titteri mountains, Ksour

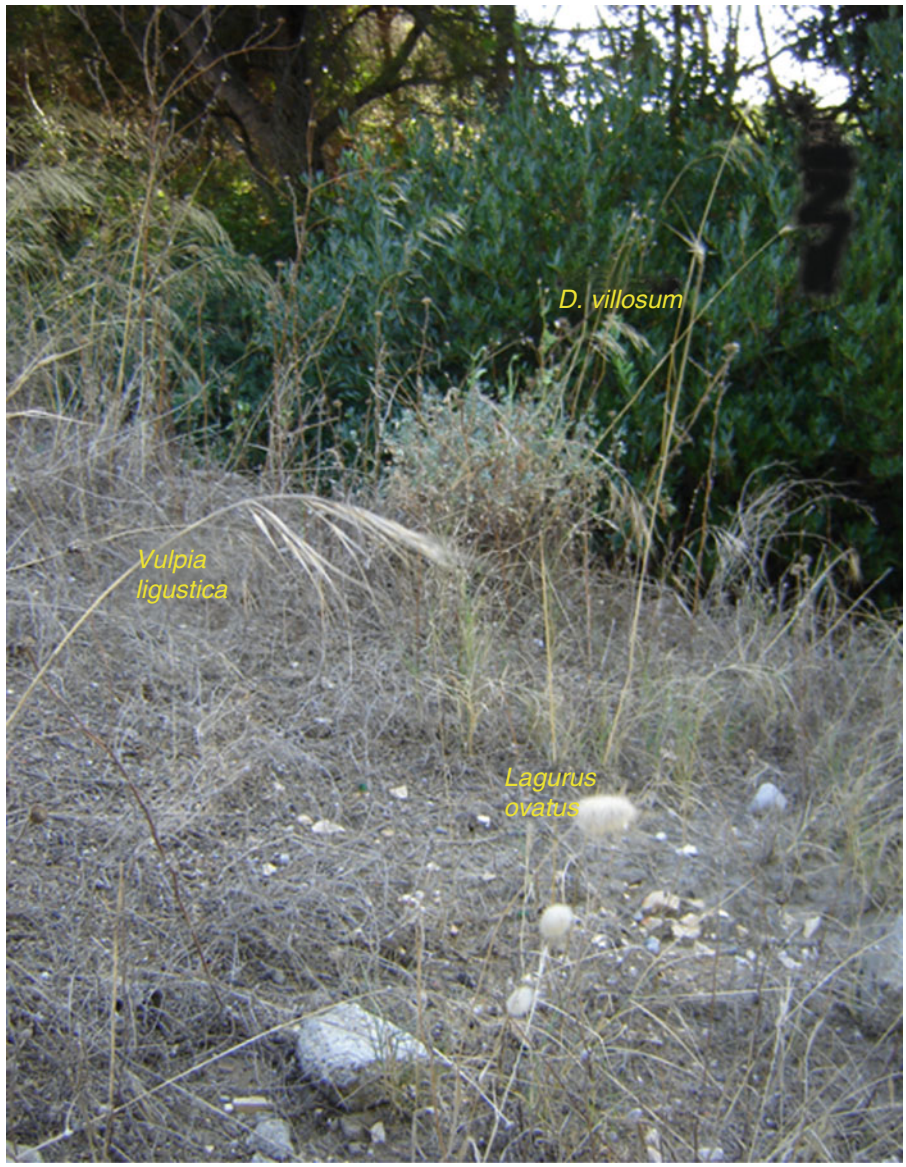

Fig. 4.4 Low density *Dv–Lagurus–Vulpia* phyto-association in a trail of the pine wood near sandy dunes along the Ionic sea coast of the Puglia region near Termitosa–Castellaneta Marina railway stations in southern Italy

mountains, Djebel Amour, Oulad Nail mountains, and Aures mountains) (Quezel and Santa 1962; Battandier 1888) and on the Great Atlas mountains in Morocco at 1,000–2,000 m elevation (Ohta et al. 2002; Fig. 4.12). This species probably is the only indigenous species of the genus in North Africa.

At elevations ranging from 1,100 m asl (Asni and Marrakech sites) to 2,250 m asl (Lake Tislit) in Morocco and at elevation above 1,080 m asl in Greece (Mt. Taygetos) west of Anogia near Sparti in Peloponnisos (Frederiksen 1991a; Fig. 4.1) isolated populations of the tetraploid form of *Db*, or *Db*(4x), were found, which expressed high capacity of both vegetative propagation through rhizomes and seed dispersal ability by disarticulating spikelets. In Greece, *Db*(4x) was found at the forest line between conifers and open grass slopes (Sakamoto 1991). In one out of 20 sites explored in Morocco, the perennial diploid form ($2n = 14$) of *Db, or Db*(2x), has been identified at 9% frequency; in all the other sites, the perennial

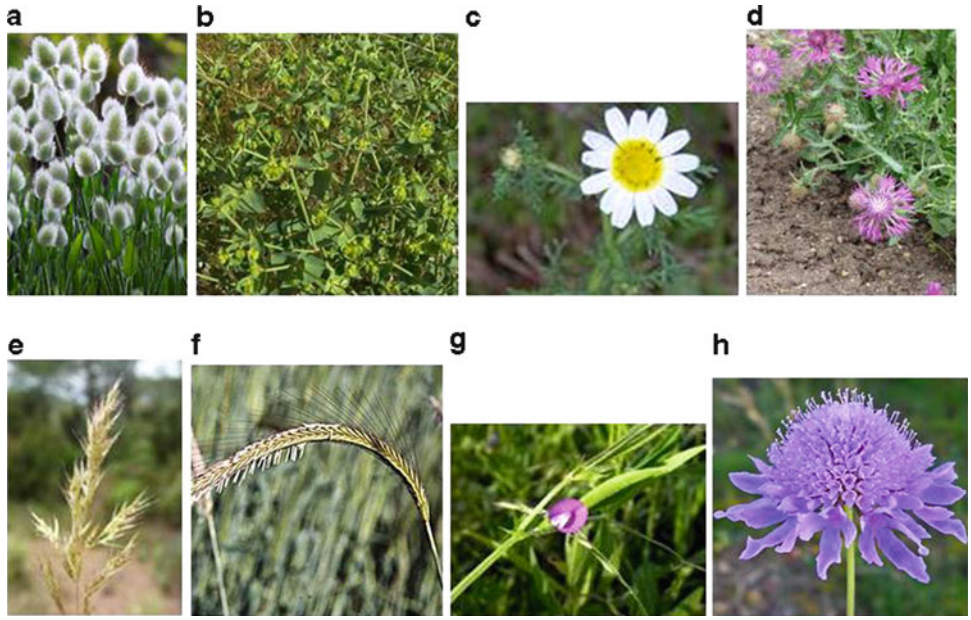

Fig. 4.5 Inflorescence of the species involved in the Laguro–Dasypyretum villosi plant association: (**a**) *Lagurus ovatus* (Poaceae), (**b**) *Euphorbia terracina* (Euphorbiaceae), (**c**) *Anthemis mixta* (Compositae), (**d**) *Centaurea sphaerocaephala* (Compositae), and the Vulpio-Dasypyretum villosi plant associations: (**e**) *Vulpia ligustica* (Poaceae), (**f**) *Hordeum bulbosum* (Poaceae), (**g**) *Vicia bithynica* (Leguminosae), (**h**) *Knautia integrifolia* (Dipsacaceae)

Fig. 4.6 Ecotipic differentiation in an experimental plot at the Experimental farm of University of Tuscia, Viterbo, Italy, where *Dv* reseeds every year since 6 years. The species *Soncus tenerrinus* is absent where *Dv* form dense stands, while prevail where *Dv* is absent although there is dense stand of other weeds

form displayed chromosome number ranging from 27 (7% of the plants) to 29 (9.9%), with the majority of the plants (81.7%) showing $2n = 28$ (Ohta et al. 2002). The habitats of the diploid *Db* in Morocco are disturbed oak park forest and calcareous bed rock (Ohta et al. 2002), and the diploid form was found in a

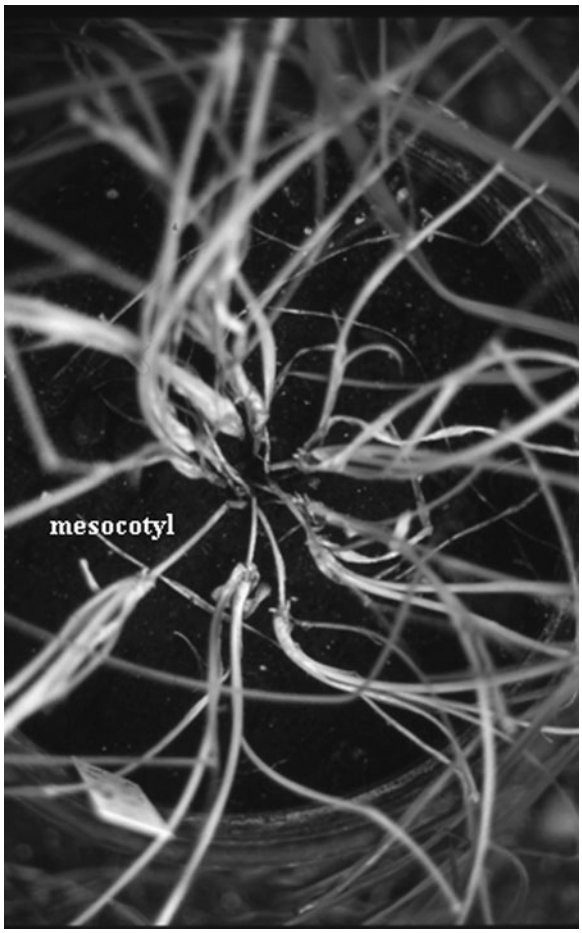

Fig. 4.7 Greenhouse grown plants from the DV-T ecotype displaying early proliferation from basal non-dormant buds each producing a new mesocotyl with crown roots and aerial tiller

cleared oak park forest. It grows slower than the tetraploid form (Sarkar 1957; Ohta et al. 2002) and it seems unlikely that diploids colonize the intensively disturbed habitats where tetraploids thrive. These ecological aspects of locally narrow distribution of the diploid species and larger geographic distribution of the tetraploid *Db* populations fit the trend observed for other diploid–tetraploid taxa comparisons in *Aegilops* and *Triticum*, for which the range of phenotypic variation and geographic distribution within any diploid species are limited and sometimes even very narrow compared to the tetraploid counterpart (Zohary 1965).

The above account indicates that *Dv* is a successful annual colonizer of opened-up man-made territories (roadsides, quarry-dumps, etc.) in ecologically specialized environments occurring in central (Latium) and southern (Puglia, Calabria) Italy, from the sea coast up to 1,000 m asl and rarely at 1,300 m asl, and in similar environments in Croatia, Serbia, Bulgaria, Greece, Turkey, and Ukraine; *Db* is a better colonizer of upland and near-forest habitats.

4.2.2 Geographical Locations of Genetic Diversity

Sampling and evaluation of populations from a wide range of geographic areas for monogenic and multigenic traits are extremely important from the point of view of conservation strategy, detection of intensity of species and population fitness, and assessment of gene resources for breeding purposes.

The first common garden study to gauge *Dv* population differentiation, involved ecotypes surveyed in southern Italy in 1981 and 1984 and scored for morphological traits (Fig. 4.13a; De Pace 1987). Out of the 22 variables measured on the ecotypes collected in 1981, three characters were the most discriminating of the total detected phenotypic variation: flag leaf sheath length, number of bristle tufts on the main keel-ridge, and flag leaf lamina width (Table 4.1). Two groups of ecotypes were clearly differentiated: the first group included the two populations from near the Adriatic Sea coast of Puglia (I-81-1 and I-81-2) and the second group comprised populations from near the Ionic Sea coast of Puglia (I-81-4, I-81-6, I-81-7, and I-81-8). The collection sites of these two population sets were 50 km distant (Fig. 4.13b). The greatest geographical distance between population sites within each of these two groups was 18 km.

The analysis of genetic variation at isozyme loci provided information on genetic differentiation among *Dv* ecotypes and allowed the assessment of their outcrossing rates. The observed isozyme genetic differentiation (Fig. 4.13c) was high within populations and relatively low between populations, as detected using the weighted average of F_{ST} (Wright 1943, 1951), which corresponds to $G_{ST} = l - (H_s/H_T)$ in Nei 1972s notation. Genetic differentiation among populations accounted for only up to 10% of the total differentiation, and the rest of variability was observable within populations. The genetic differentiation was greatest for population I-84-145, I-84-120, and I-84-85. These

Fig. 4.8 Mesocotyl formation from basal bud with terminal rooted tiller which in turn produce, from a basal bud, a further mesocotyl with terminal tiller

populations occur at the latitudinal extremes of the collection sites (Fig. 4.13d).

The pattern of diversity observed for isozymes was similar to the results obtained for the population genetic differentiation at the *Glu-V1* locus (Zhong and Qualset 1993), although the total differentiation among populations at this locus was lower. There was little divergence between *Dv* ecotypes collected in Italy as compared to those gathered from former Yugoslavia. The high molecular weight (HMW) glutenin subunits migrated into the same gel-region as *Glu-B1* subunits of wheat after sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Fourteen alleles were identified: one null, ten encoding single subunits, and three coding for two subunits (Zhong and Qualset 1993). Five to ten alleles (mean = 7) were found in 12 Italian and two Yugoslavian populations. Only two alleles were found in one population, and seven were found in ten or more populations.

The multilocus outcrossing rate (t_m) estimated from the allele frequencies at three isozyme loci indicated that for six of the populations examined, the t_m ranged from 0.82 to 0.99, while three other populations had much lower values (range 0.51 to 0.58). Consequently, the breeding system varied from almost complete allogamy to mixed selfing-random mating (De Pace and Qualset 1995).

Although there might have been a sampling effect in the estimation of the t_m values, there was adequate evidence to conclude that the breeding system in the analyzed *Dv* ecotypes is a predominance of outcrossing. Outcrossing is a source of heterozygosity and, as in many other outcrossers, when *Dv* plants are forced to self-pollinate, there is a considerable reduction of seed output and vigor of S_1 and S_2 plants. Some

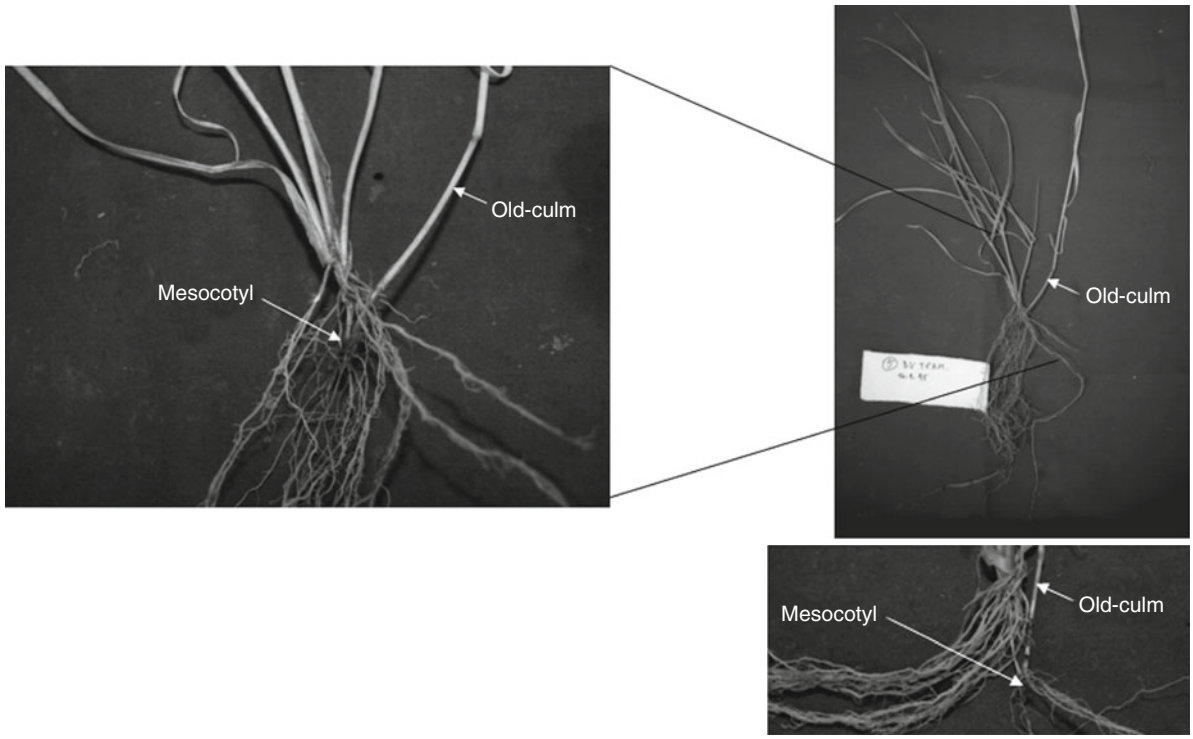

Fig. 4.9 Mesocotyl resulting from the late growth of basal dormant axillary bud of a dry culm

S_2 progenies produced up to 15% albino plants. The anther extrusion from the florets is almost synchronous on the same spike (Fig. 4.14) and pollination is wind-aided.

Outcrossing generally increases effective population size (N_e), reduces population subdivision, enforces pollen movement, and increases the probability of long distance gene flow (Loveless and Hamrick 1984). Several theoretical studies assuming neutral alleles have shown that only a small amount of long distance gene flow is needed to prevent population differentiation (Wright 1946; Slatkin and Maruyama 1975). Predominant outcrossing species usually have the following characteristics (1) low between-population genetic variation, (2) low phenotypic plasticity, (3) absence or rarity of coadaptive gene combinations, (4) large and continuous populations, (5) low colonizing ability, and (6) small response to selection in a new environment due to weak gene associations and small population turnover (De Pace 1987; De Pace and Qualset 1995).

The above genetic data revealed, as expected, low interpopulation genetic diversity. This result is made more relevant if we consider that it was obtained from populations representing a wide geographic range in Italy and growing conditions in human-influenced habitats (mainly roadsides) under strikingly different temperature and rainfall regimes. For example, lower rainfall (400 mm per year) and relatively high temperature during the growing period typify the sites occupied by populations I-84-85, I-84-50, and I-84-136 compared to the sites of populations I-84-120, I-84-16, and I-84-145. The site of population I-84-120 is at one of the highest altitude (about 1,000 m asl) where *Dv* has been found; all the other sites are at 5–300 m asl. Furthermore, it should be considered that the studied populations represent sampling from a diffused and often continuous *Dv* plant-stand, all over the studied area. Although the interpopulation genetic diversity was small, there is evidence of a positive relationship between spatial distance and genetic distance. As matter of fact, populations I-84-85 and I-84-145, which show the highest Nei's D_{ij} (0.417) and Hedrick's (Hedrick 1971) distance values (0.663), come from sites that are the most different in latitude.

Therefore, the outcrossing rate deduced from the genetic data alone and the geographical distance of the diverse population-sites suggest that for maximizing

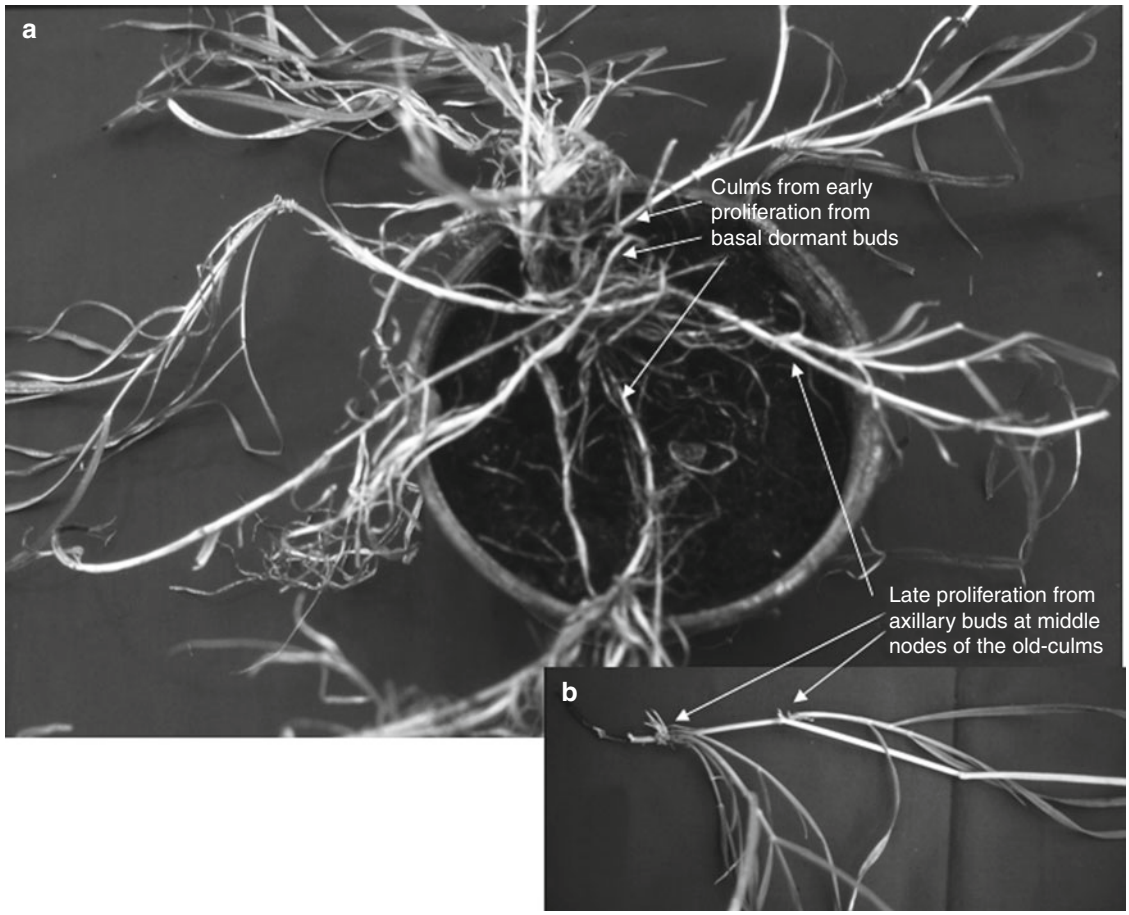

Fig. 4.10 Greenhouse grown DV-T ecotype collected at 1,000 m asl (**a**) Late proliferation from axillary buds at nodes of the old culms becoming stolon-like structures, and (**b**) Late mesocotyl proliferation from basal and middle dormant buds on old culms

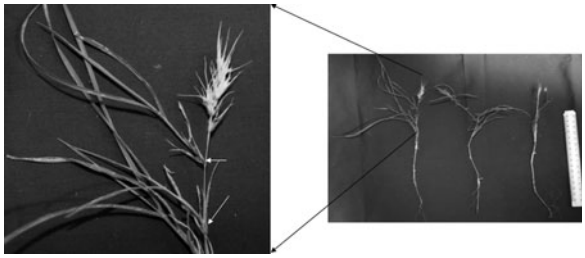

Fig. 4.11 Late proliferation from axillary buds at the upper nodes of an old dry culm

the sampling efficiency during the collection to capture 99% of the genetic variability, populations more than 100 km apart should be sampled, and 28–50 plants for each ecotype should be collected if each plant sets 50–100 caryopses under the assumption of an average outcrossing rate of 0.8 (Sapra et al. 2003).

The mentioned ecotypes were also studied for measuring phenotypic variability for plant height and number of culms per plant. Substantial interpopulation diversity was shown, with the populations I-84-145 and I-84-3 from Tuscany significantly taller than the remaining populations. Number of culms per plant was greater for populations I-84-50 and I-84-136 from Puglia (or Apulia). Among the populations with more than 70 plants, populations I-84-3 and I-84-50 were the most different for these two traits: population I-84-50 had short plants with high culm number, and the reverse was true for population I-84-3. In general, genetic differentiation has occurred in *Dv* throughout its geographic range for plant height and number of culms per plant. Population I-84-16, although evaluated on a small number of families, showed the shortest plants and the lowest number of culms per plant. Population I-84-145, from the most northern

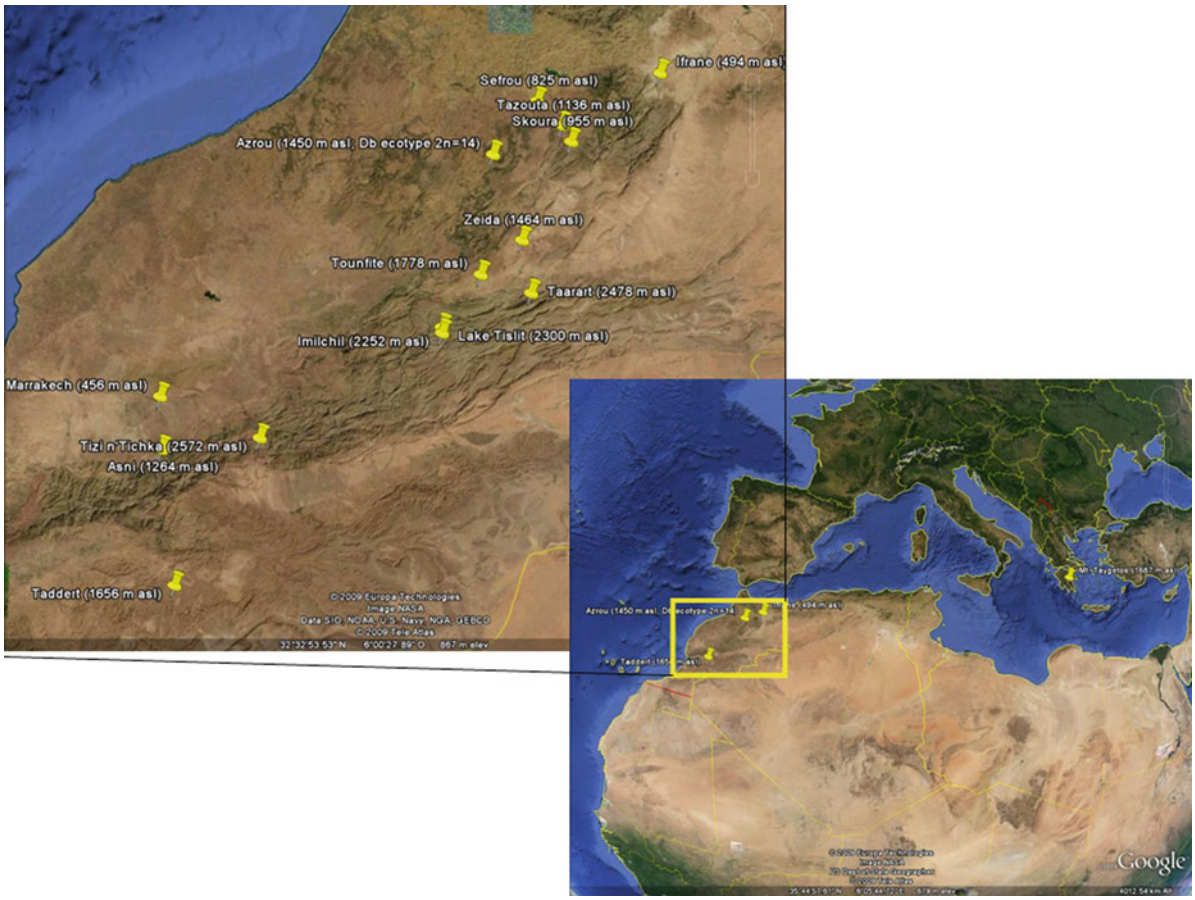

Fig. 4.12 Geographical distribution of *D. breviaristatum* according to collection passport data of Ohta et al. (2002)

collection site in Italy, showed the tallest plants and the same number of culms per plant as for population I-84-16. Population I-84-50, from the southern collection site in Italy, showed mean plant height not statistically different from that of population I-84-16 but significantly shorter than that of population I-84-145 and had the highest number of culms per plant. Although the phenotypic means differed among populations, the amount and distribution of variability within populations did not. In fact, the within-family variation, with the only exception being population I-84-16, was the largest source of variation. Comparing the isozyme and quantitative variation, it is evident that in both cases, there is evidence of interpopulation variability, but the contribution to this variability comes mainly from the populations obtained from the extreme latitude and altitude: the southern and high elevation population I-84-120 and the northern and low elevation population I-84-145 for isozymes; the southern population I-84-50 and the northern populations I-84-145 and I-84-16 for plant height and number of culms.

This trend of relationships between great geographic distance or gross habitat differences and large phenotypic interpopulation differences were also detected by Zhong and Qualset (1995) for 31 morphological characters on half-sib plants from *Dv* populations collected also in southern Italy and the former Yugoslavia (see Sect. 4.7.2). Six traits were measured on the spikes, three related to flowering and anthesis, in addition to flag leaf length and width and mature plant height. Uni- and multivariate analyses were conducted using data for all traits. Genetic variation was found for all traits. Most interesting was the partition of phenotypic variation: the percentages of variance due to countries, populations, families, and plants in families were 38, 28, 9, and 25, respectively, with a mean genetic coefficient of variation of 18%. These results contrast somewhat with the

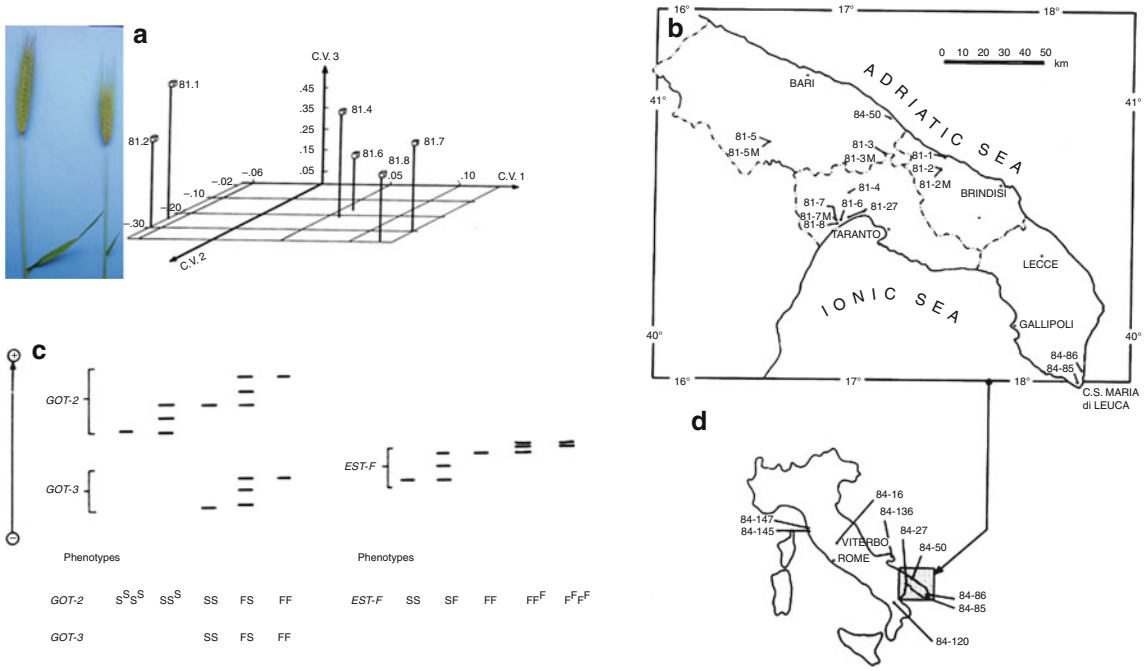

Fig. 4.13 Diversity for culm and leaf morphology (**a**) in *Dv* ecotypes collected in 1981 from Puglia (**b**), and GOT and esterase isozyme polymorphism (**c**) in *Dv* ecotypes collected in 1984 from central and southern Italy (**d**)

Table 4.1 Means with standard error and coefficients of variation (cv) representing principal factors of differentiation for three morphological traits in six *Dv* ecotypes collected in 1981

Population	Flag leaf sheath length (cm)		Flag lamina leaf width (cm)		Number of bristle tufts on glume keel	
	Mean	CV	Mean	CV	Mean	CV
Group 1						
81-4	15.7 ± 1.23	10.5	4.1 ± 0.06	39	3.8 ± 0.12	30.0
81-8	16.8 ± 2.30	10.0	3.6 ± 0.09	26	2.7 ± 0.07	60.2
81-7	16.8 ± 2.01	9.5	3.8 ± 0.11	24	3.5 ± 0.17	45.0
81-6	15.0 ± 2.80	12.8	3.9 ± 0.09	23	3.1 ± 0.05	43.0
Group 2						
81-1	14.2 ± 1.00	14.0	5.5 ± 0.07	36	4.8 ± 0.17	43.0
81-2	13.5 ± 1.05	11.2	4.4 ± 0.11	34	4.3 ± 0.15	32.0
Mean						
Group 1	16.1 ± 0.89	10.7	3.9 ± 0.05	28	3.3 ± 0.08	44.5
Group 2	13.9 ± 0.79	12.3	5.0 ± 0.04	35	4.5 ± 0.05	37.5
Mean difference (Group 1–Group 2)	2.2	−1.6	−1.1	−7	−1.2	7

analysis carried out for the same populations at protein loci for which a greater variation between countries and among populations was found.

The trend of latitudinal and altitudinal diversity observed among the Italian ecotypes is in concordance with the climatic conditions characterizing such areas. For example, relatively few, but intensive, rain storms occur on the Adriatic Sea coast area, and low and uniform precipitation occurs on the Ionic Sea coast. Different temperature and rainfall regimes also characterize the sampled Murgia areas of Puglia with arid conditions at the lowest latitude and semi-arid conditions at the highest latitude. The present-day populations represent the products of microevolution in such

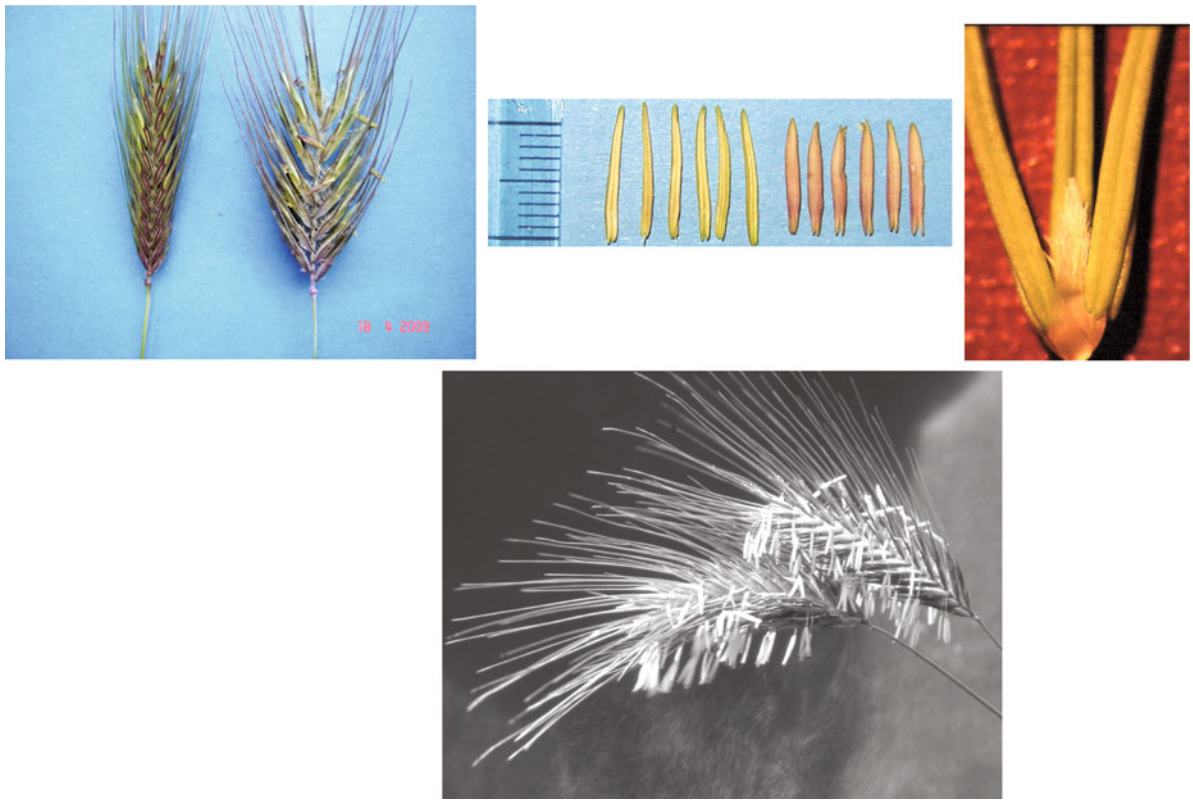

Fig. 4.14 *Dv* spike and anther morphology at anthesis

environments. The populations from the most dry and warm area (populations I-81-4, I-81-6, I-81-7, and I-81-8; Fig. 4.13b) showed a narrow long flag leaf compared to those from colder and wet areas (populations I-81-1 and I-81-2). Distance of the populations from the sea and the altitude might affect such patterns of variability. In fact, populations I-81-2, I-81-6, I-81-7, and I-81-8 from higher altitudes and inner zones of Puglia tended to have shorter flag leaves than the other populations of their respective group. Populations experiencing the coldest temperatures in winter (populations I-81-3M and I-81-5M) had smaller seeds and more rapid germination and longer coleoptile growth compared to populations (I-81-2M and I-81-7M) from relatively warmer climates in winter (De Pace 1987).

Some ecotypes of *Dv* require vernalization for flowering induction. Plants from three populations (I-84-16, I-84-85, and I-84-145) grown under long-day photoperiod and non-vernalized reached the heading stage, but that was not the case for population I-84-120. This result indicated that *Dv* populations differ in the vernalization requirement for induction of flowering.

The above information on morphological, isozyme, and seed storage proteins can be used to define the sampling strategies suitable to collect ecotypes that represent the pattern of variation using mainly the criteria of the distance between sampled sites (i.e., sample one population every 50 or 100 km, depending on the altitude of the site).

4.2.3 Spike and Plant Morphology

Dv (Fig. 4.15) and *Db* spikes have an articulated rachis, which at maturity shows wedge type disarticulation. Shattering is an essential seed dispersal mechanism in *Dasypyrum* species due to a wedge-type spikelet disarticulation gene on chromosome 3VS in *Dv* (Urbano et al. 1988). Spikes with different length

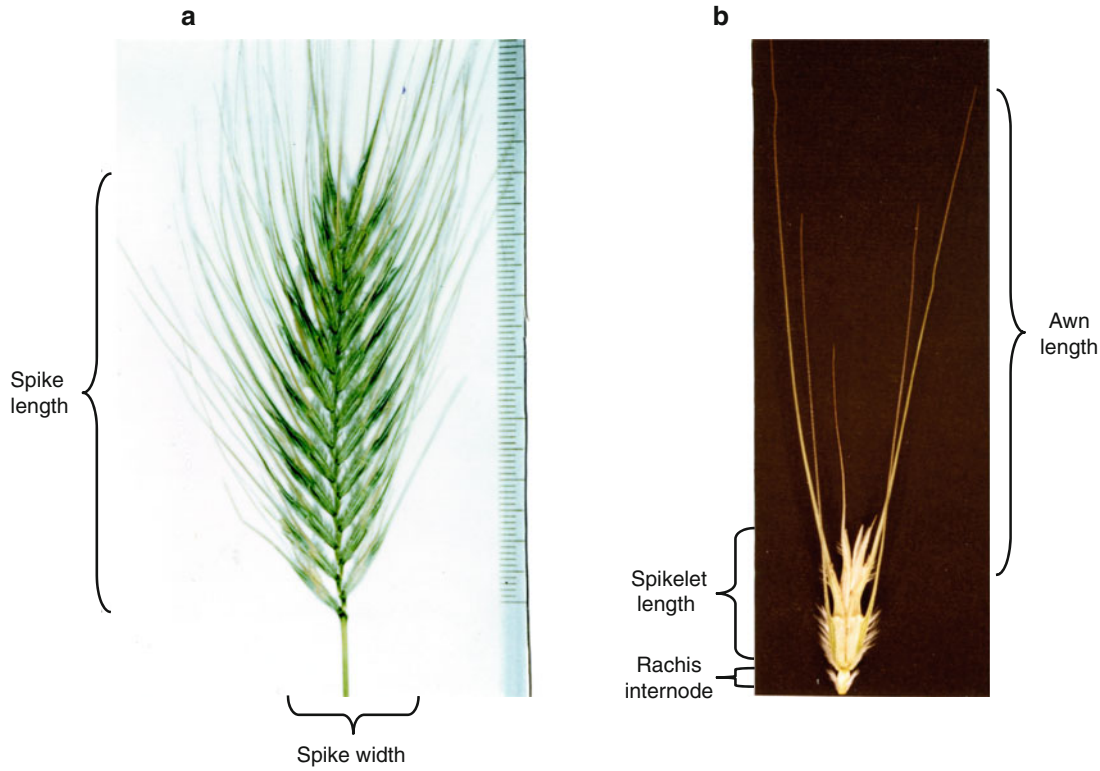

Fig. 4.15 *Dv* spike (**a**) and spikelet (**b**) morphology

within and between plants, glume color, and glume glaucosness have been found (Fig. 4.16). Waxy or anthocyanic bicarenate glumes (Fig. 4.17), long- and short-awned (*Dv* in Fig. 4.18) and very short-awned glumes (*Db* in Fig. 2 of Ohta et al. 2002), and pubescent leaves and auricles (Fig. 4.19) are displayed. A peculiar characteristic of *Dv* is the presence of tufts of bristles 1–4 mm long on the keel of the glume and apex of the lemma (Fig. 4.20). The bristles of the *Db* glumes are shorter on the main keel and are not grouped in tufts, rather they are sparse along the main glume keel (Fig. 3 in Ohta et al. 2002).

Dv produces a considerable amount of pollen (Fig. 4.14). Under natural conditions, pollen fertility varies from 90 to 100%. The duration of pollen formation in *Dv* is about 20 days, with variations depending on climatic conditions and the tillering ability and chronology of tiller heading within (Fig. 4.21) and between plants at the ecotype site (Stefani and Onnis 1983; Stefani et al. 1993). The pollen grain has a diameter of over 50 μm at anthesis (Stefani 1986), three nuclei, and is rich in starch. It has sculptured walls and onIy one germination pore, closed by an operculum presenting the same sculpturing as the walls (De Gara et al. 1993). Dark-red and yellow-colored kernels are present within the same spikelet of *Dv* (Fig. 4.22) and *Db*. The main feature of *Db* is the production of underground creeping rhizomes (Fig. 4.23). Sando (1935a) has given a thorough description of the *Dv* plant morphology, and *Db* morphology has been described by Maire (1952), Frederiksen (1991a), and Ohta et al. (2002). In all the F_1 plants obtained from crossing *Dv* with the diploid species *T. aegilopoides* and *S. cereale*, and with the tetraploid *T. timopheevi, T. dicoccoides, T. dicoccum, T. durum, T. turgidum,* and *T. polonicum* (Sando 1935a), the rachis fragility, the bicarenate, and canaliculate glume (presence of a deep channel or depression between the two prominent keels) traits of *Dv* are dominant over the tough rachis, unicarenate, and non-canaliculate glume conditions showed by the wheat parents.

Fig. 4.16 *Dv* intrapopulation variability for spike size, glume color, and glume glaucosness

4.2.4 Reproduction and Caryopsis Somatic Dimorphism

All the plants of every *Dv* and *Db* open-pollinated population examined showed dimorphism for kernel color within a spike (Fig. 4.24). The color may range from yellow-pale (named also "light color," "amber color," "light yellow," "yellow 246" by Onnis (1967a) according to Seguy (1936) color codes, or simply "yellow") to red up to almost black (named "brown," "dark," "red 112" by Onnis (1967a) according to Seguy (1936) color codes, or simply "dark-red"), which defined two classes of caryopses within the same spikelet: "yellow" and "dark-red" (Fig. 4.24). The inheritance of the seed color does not show any dominance effect, nor does it follow any Mendelian segregation, although in some *Dv* ecotypes the yellow to dark-red kernel ratio per spike is 2:1 (Meletti and Onnis 1961). Physiological differences are reported between the kernel color classes: dark-red seeds have longer seed dormancy and slightly higher energy and power of germination than the yellow ones and maintain germination ability until after 8 years of storage (Stefani et al. 1998; Table 4.2).

In other ecotypes, about 55% of the kernels of each spike were yellow-colored. Investigations carried out by De Pace (1987), De Pace et al. (1994b), and unpublished data by the authors, evidenced several aspects of the *Dv* caryopses somatic polymorphism that might be related to the population biology response to climatic changes. The dark-red and yellow-colored kernels showed a mean frequency of 42.7 and 57.3% in three-examined populations, and there was little evidence for interpopulation variation in these frequencies: the frequencies of yellow-colored kernels in populations I-84-27, I-84-86, and I-84-147 in Fig. 4.13d were 55%, 63%, and 54%, respectively.

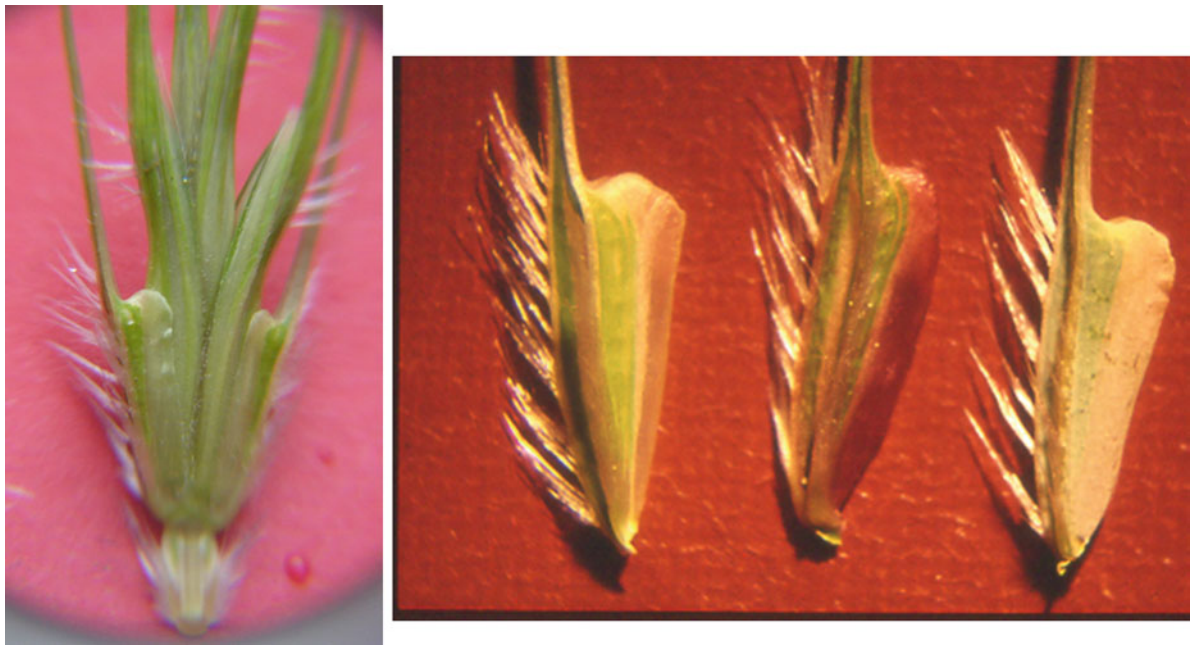

Fig. 4.17 *Dv* spikelet, glume morphology, and tufts of bristles along the main glume keel

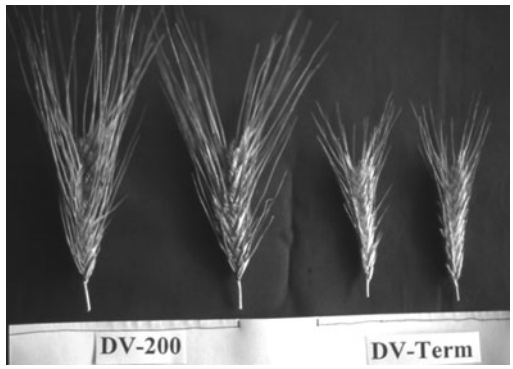

Fig. 4.18 *Dv* diversity for awn length and spike size for two ecotypes collected at low altitude (DV_200; Bomarzo, Viterbo, Italy; 380 m asl) and high altitude (DV_Term; Terminillo mountain, Rieti, Italy; 980 m asl)

Yellow kernels were more frequent on the second floret (Fig. 4.24), were heavier than dark-red kernels, and germinated faster (De Pace et al. 1994b). Coleoptile length is usually correlated with seed size: dark-red caryopses produced shorter coleoptiles than yellow-colored ones (Fig. 4.25, treatment (−). The differences among populations for coleoptile length were significant. In some ecotypes, red-coleoptile seedlings are produced (Fig. 4.26).

Under random mating, floret fertility ranged between 57 and 93% depending on the spike section in which the floret was located and was very similar in upper and lower florets. The proportion of yellow versus dark-red kernels was the same in plants derived either from yellow or dark-red kernels. No significant differences were detected in the comparison of the mean number of yellow kernels per spikelet of open-pollinated progenies from yellow kernels versus number of yellow kernels per spikelet of open-pollinated progenies from dark-red kernels. Selfing caused a drastic reduction in seed fertility and increased the proportion of yellow to dark-red-colored kernels. The dark-red kernels are lower in number in all cases, compared to the number of yellow kernels. Such discrepancy is higher for the selfed spikes than for the open-pollinated ones.

On the average, selfing caused a 90% reduction in spikelet seed set (De Pace 1987; De Pace et al. 1994b). Increased selfing may be a response to lower than optimal climatic conditions by producing a reduced number of seeds and an increased proportion of the better endowed (yellow) seeds for rapid germination and seedling growth. This might occur through modulation of the duration of microsporogenesis and palinogenesis under normal (Stefani et al. 1993) and drought and salt stresses (Stefani and Colonna 1994),

Fig. 4.19 Ligule and pubescent auricles at the junction of the flag leaf lamina and sheath of one *Dv* culm from the ecotype "Montespaccato" (Rome–Italy)

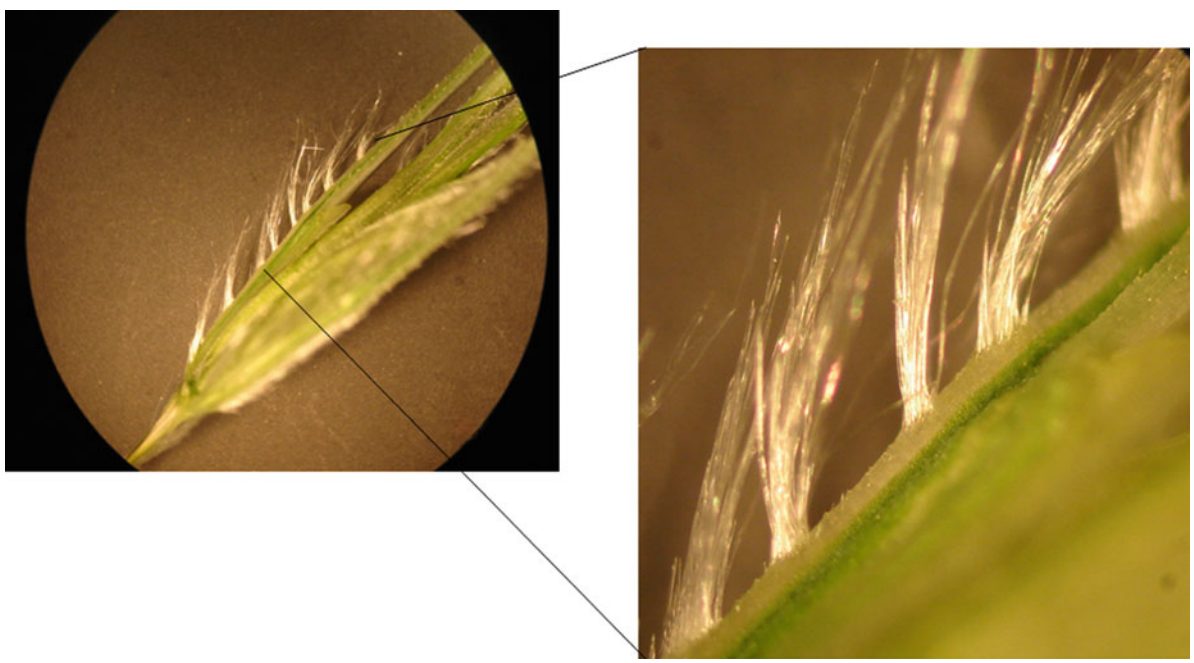

Fig. 4.20 Tuft of bristles on glume main keel

which affect the consequent rate of seed set (Stefani 1992). Therefore, the low proportion of dark-red caryopses in an ecotype might be an indicator of ecological conditions that limited outcrossing and favored inbreeding; on the other hand, equal proportion of the two caryopses morphs indicate they were produced under prevailing outcrossing. The interaction of the differential germination ability of the dimorphic caryopses with the breeding system of the plant may burst multifaceted ecological adaptations of *Dv* populations to the varying environment (Stefani and Onnis 1984, 1987).

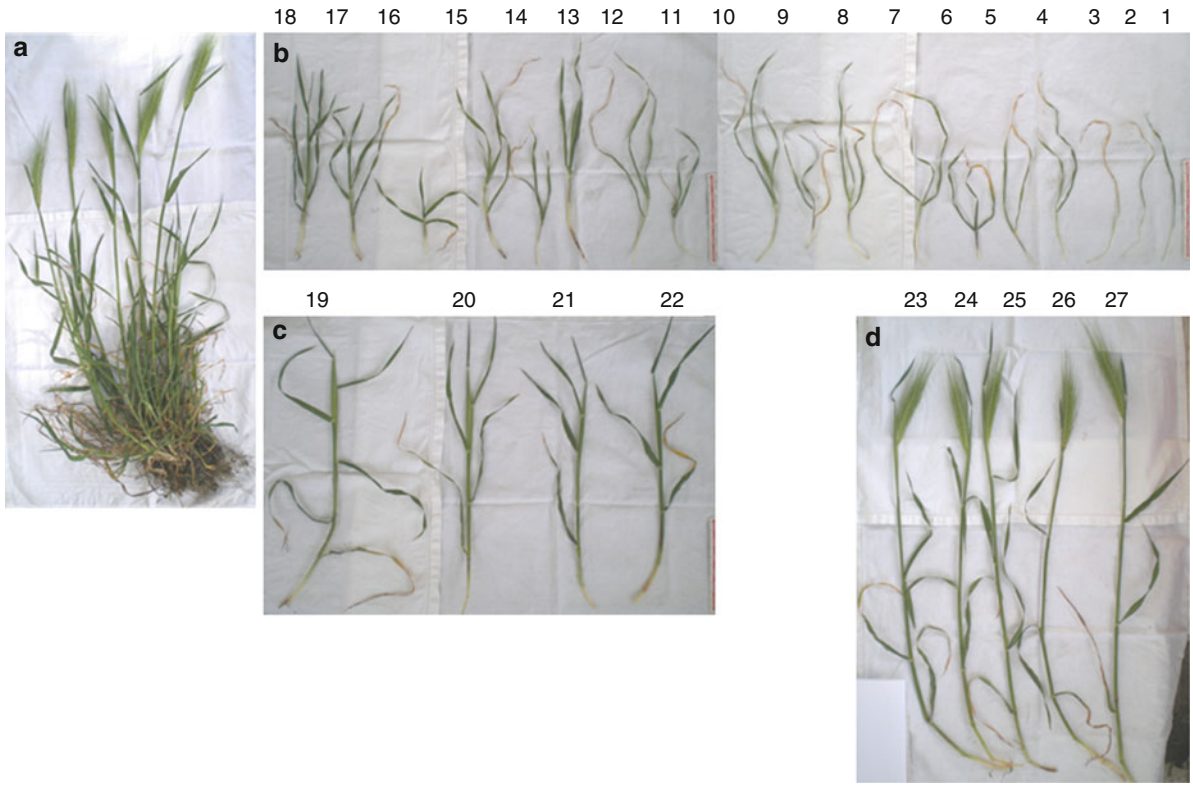

Fig. 4.21 The tillers from the same *Dv* plant (**a**) sampled within an ecotype at a site located in the area of "Montespaccato" near Rome; the tillers have been detached at the heading stage and ordered according to their size and age within plant, from the youngest (1st tiller in **b**) to the oldest (tiller 27th in **d**). Size has been calibrated by a 10 cm-ruler in **b** and **c**

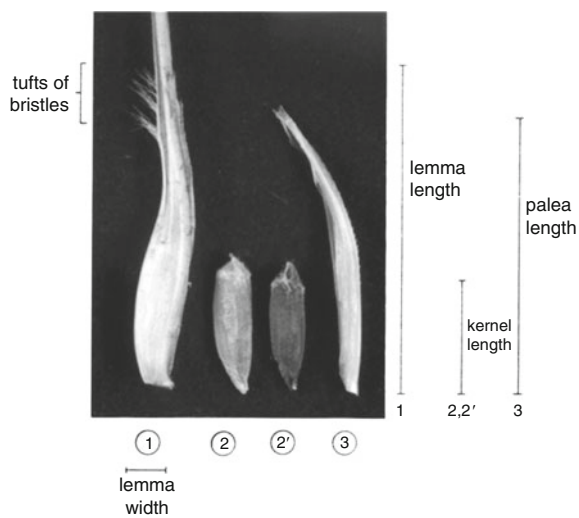

Fig. 4.22 Floret structures (1) lemma, (2) yellow-kernel, (2′) dark-red kernel, (3) palea

Dv plants enter the reproductive stage between March and June, depending on climatic conditions in the Italian sites. Under field conditions of Pisa area, meiosis in May has a duration of about 35 h (Stefani 1992), and in July it lasts 22 h. These differences are related to temperature differences between May and July. In controlled conditions, duration of meiosis was as follows: at 5°C, it lasted 136 h, at 10°C, 88 h; at 20°C, 24 h; at 28°C, 21 h; and at 35°C, 17 h. Meiotic abnormalities were observed after 48 h incubation either at Iow or at high temperatures(Stefani 1992; Stefani and Colonna 1994). At 5°C and 10°C, the presence of dyads (0–10%) instead of tetrads was observed at telophase II together with telophasic and microspore nuclei that were uncontracted. At 28°C and 35°C, high condensation of the chromatin of telophasic and microspore nuclei was clearly detectable. Abnormalities such as asynapsis, break-up of the nucleolus, or failure of condensation affecting some chromosomes were not observed. Populations from the driest habitats showed less chromatin abnormality and a higher photosynthetic activity than those from optimum soil water conditions (Angelini et al. 1994). Ecotypes from high

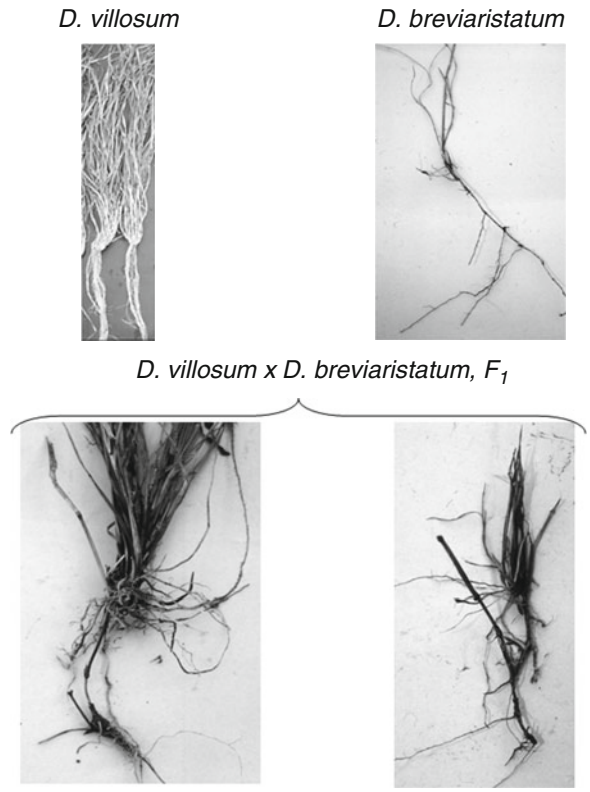

Fig. 4.23 Roots in *Dv* and rhizomes in *Db* and in the *Dv* × *Db* F_1 progeny (photos made by the authors on materials kindly provided by A. Blanco, University of Bari, Italy)

altitude display a high proportion (>75%) of plants with pubescent glumes, a trait that is absent in populations from sites lower then 300 m asl (Kotsonis 1999). The ascertained resistance to thermal stresses in terms of viable and normal pollen formation and performance in photosynthetic activity suggest a complex genetic architecture within *Dv* populations for adaptation to fluctuating environmental conditions. It should be noted that the possibility of obtaining diploid spore formation at low temperatures (up to 10% diad at telophase II) could facilitate autopolyploid formation (see discussion on *Db*(4*x*) origin in Sect. 4.2.8.1).

The duration and regularity of *Dv* meiosis and microspore behavior have been examined in plants grown in pots under different water availability conditions (2.8–22.6 mm a week) and salt concentrations (62–250 mM of NaCl) (Stefani and Colonna 1994). Meiosis and initial microspore development were analyzed in anthers excised from each spikelet at successive times from the central spike area at the boot stage. It was ascertained that meiosis duration is about 4–6 h shorter in plants under stress than in control ones.

After pollination and embryo maturation, the dark-red kernels show a higher and longer lasting activity of ascorbate peroxidase, a key enzyme involved in removing the hydrogen peroxide produced by cell metabolism during aging processes and some types of stresses (De Gara et al. 1991; Table 4.2). This occurrence led those authors to postulate that some morphological anomalies observed in seedlings from yellow caryopses and the decreased seed germination capacity could be due to the decreased activity of that enzyme (De Gara et al. 1991). The differences in ascorbic acid metabolism are not correlated to adaptation of *Dv* populations to various environments; however, an ecotype from the dry and warm environment of Pachino (Sicily) had a lower activity of the two main oxidoreduction enzymes of the ascorbate system: ascorbic free radical reductase and ascorbic acid peroxidase (Paciolla et al. 1991). A peroxidase, rather than catalase, has been found to be the key enzyme

Fig. 4.24 Caryopses somatic polymorphism: (**a**) when both basal (1) and upper (2) florets set caryopses, then the lower floret sets a dark-red caryopses and the upper floret sets a yellow (*amber*) colored caryopses. Floret 3 and 4, when fertile, set always yellow colored caryopses although much smaller than those set on florets 1 and 2. There are only rare exceptions to this pattern of kernel somatic polymorphism distribution within spikelet. (**b**) Ten pairs of yellow and dark-red caryopses; each pair was taken from a single spikelet sampled from a spike of ten different *Dv* plants

to remove hydrogen peroxide produced in *Dv* pollen metabolism (De Gara et al. 1993). In addition, for the dark-red seeds, Innocenti and Bitonti (1980, 1983) observed an almost constant histone/DNA ratio in embryo root meristems over time, in contrast to an increasing ratio in the yellow ones, and shorter mitotic cycles. Later, Innocenti and Bitonti (1986) found differences for spontaneous mutations between plants derived from brown and black caryopses of *Secale cereale*. Cremonini et al. (1994) found 20–24% higher DNA concentration in early prophase nuclei of *Dv* seedlings from yellow caryopses. Frediani et al. (1994), studying the modulation of genome size by cytophotometry and in situ hybridization with a 396 bp *Dv* repeated sequence, found that during germination of *Dv* caryopses from seven geographically distant populations collected in Italy, the basic amount of nuclear DNA increased to a higher extent in seedlings from yellow caryopses than in those from the dark-red ones. In 2-day-old seedlings from yellow caryopses, the DNA content was 12% higher than in seedlings of the same age from the dark-red ones. DNA content also differed up to 13% between plants within a caryopses-color group and up to 40% between populations. It was also shown that during germination and further plant development, there were fluctuations in

Table 4.2 Differences detected by various authors for dimorphic *Dv* caryopses and their derived seedlings

Trait	Caryopsis color		Reference
	Yellow	Dark red	
Caryopsis size	Large	Small	Meletti and Onnis (1961); De Pace et al. (1994b)
Germination ability[a]	2	7	Stefani and Onnis (1984)
Dormancy period:			Stefani and Onnis (1984
(a) days from caryopses harvest to 50% germination ability	0–15	0–15	Stefani and Onnis (1984)
(b) days from caryopses harvest to 100% germination	0–15	0–30	Stefani and Onnis (1984)
Ascorbate content (AA) (millimoles/g fresh wt) in 4-day-old seedlings	2.74	2.68	Paciolla et al. (1991)
Enzyme activity in 4-day-old seedlings:			Paciolla et al. (1991)
Ascorbate free radical reductase (nanomoles AA formed/min/mg protein)	<242[b]	228[b]	Paciolla et al. (1991)
	169 ± 12[c]	203 ± 1[c]	Paciolla et al. (1991)
Catalase	<57[b]	59[b]	Paciolla et al. (1991)
	71 ± 4[c]	99 ± 4[c]	Paciolla et al. (1991)
Ascorbate peroxidase (nanomoles AA ox/min/mg protein) in seedlings from:			Paciolla et al. (1991)
1-year-old caryopses	343[b]	334[b]	Paciolla et al. (1991)
	270 ± 15[c]	155 ± 18[c]	Paciolla et al. (1991)
5-year-old caryopses	100	320	De Gara et al. (1991)
Seed longevity:			De Gara et al. (1991); Stefani et al. (1998)
Germination after 3 years	90%	90%	De Gara et al. (1991); Stefani et al. (1998)
Germination after 5 years	10%	80%	De Gara et al. (1991); Stefani et al. (1998)
Germination after 7 years	0%	28%	De Gara et al. (1991); Stefani et al. (1998)
Duration of mitotic cycle	12 h	9.5 h	Innocenti and Bitonti (1983)
Histone/DNA ratio in 2C nucleus of root meristematic cell during aging	Increase	Constant	Innocenti and Bitonti (1980)
Basic amount of nuclear DNA (in mean Feulgen absorption units) in 2-day-old seedlings	2,185	1,904	Frediani et al. (1994)
Copy number of subtelomeric 396-bp tandem repeats in:			Frediani et al. (1994)
Resting embryos	2.5×10^3	1.5×10^3	Frediani et al. (1994)
10-day old seedlings	15.5×10^3	6.0×10^3	Frediani et al. (1994)
50-day old seedlings	8.0×10^3	3.5×10^3	Frediani et al. (1994)

[a]Number of days for 90–100% germination in non-dormant caryopses at 20°C
[b]Average of measurements in populations from Campobasso, Pisa, and Villanovaforru (Italy)
[c]Value in the population from Pachino (Sicily, Italy)

genome size, and redundancy modulations of subtelomeric and other repeated DNA sequences were involved in these genomic changes.

Obermayer and Greilhuber (2005) investigated 29 *Dv* accessions from various countries, totalling 186 plants, for genome size using flow cytometry, and found no evidence for a consistent reduction in genome size in seedlings from dark-red caryopses compared to seedlings from yellow caryopses. A linear regression model through the origin relating genome size of seedlings from dark-red caryopses (Y_i) to the genome size of sib-seedlings from the yellow caryopses (X_i) of 17 *Dv* accessions provided a regression line $Y_i = 0.99X_i$. This indicated similar increments in genome sizes of seedlings from yellow and dark-red caryopses. However, the regression $Y_i = 1.61 + 0.68X_i$ fitted to the same set of data after correction for the mean, gave a smaller residual, and

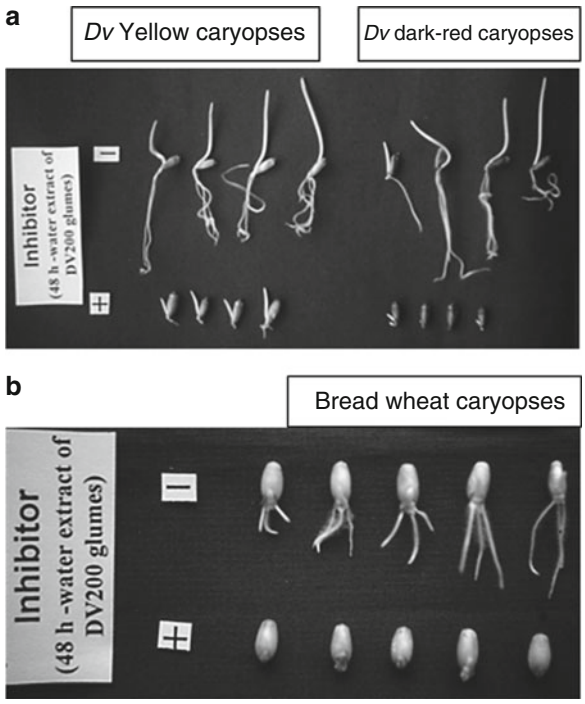

Fig. 4.25 Yellow- and dark-red caryopses from the *Dv*_200 ecotype (**a**) and bread wheat Chinese Spring (**b**), after 5-days in Petri-dishes containing two disk-layers of filter paper imbibed with a water-extract of *Dv* glume powder (48-h extraction) (*plus*) or distilled water (*minus*)

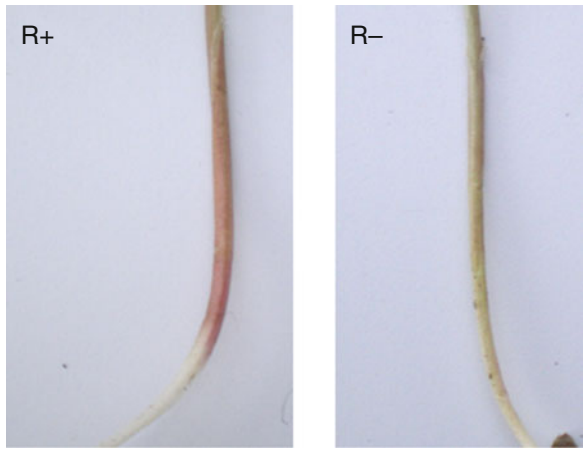

Fig. 4.26 *Dv* seedlings with (R+) and without (R−) red pigmentation in coleoptile

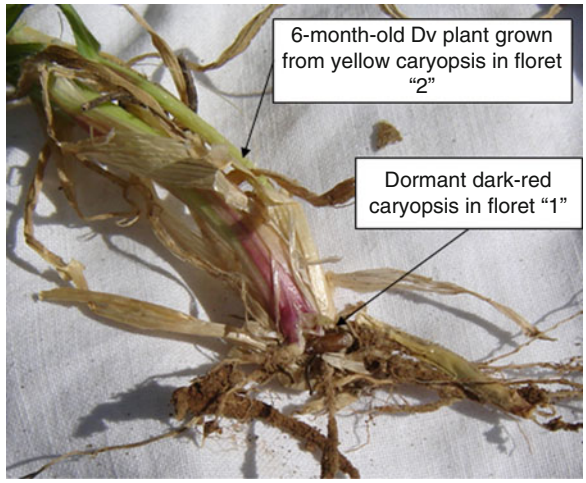

Fig. 4.27 Results from germination, under field conditions, of *Dv* dark-red and yellow caryopses developed in floret "1" and "2," respectively, of the same spikelet

the probability that this was due to chance was $P = 0.066$ (Steel and Torrie 1960, pp. 179–180). If such regression model ($b = 0.68$) was used to predict changes in genome size among accessions, then for an increase (of 0.01 pg) in the genome size of cells in seedlings from yellow kernels of a pair of accessions, the increase of the genome size of cells in sibling plantlets from dark-red kernels from the same pair of accessions should have been expected to be lower (i.e., 0.0068 pg), maintaining a trend of lower genome size of the seedlings from dark-red kernels compared to the trend of the genome size of the sib-seedlings from yellow kernels. Therefore, more experiments are required to identify a consistent pattern of genome size variation in *Dv* seedlings in relation to the caryopses color from which the seedlings originated.

A cline for the proportion of yellow and dark-red caryopses produced by plants across Italian sites at different latitudes and altitudes has not been observed (De Pace et al. 1994b), which suggests that the polymorphism might be a fixed feature of the species for the plastic phenotypic response to sudden changes in local environment (i.e., site temperature changes affecting meiosis and breeding system within *Dv* populations; see above in this paragraph). In other genera, such as *Halogeton* and *Atriplex,* seed color polymorphism is common and environmentally governed (Frankton and Bassett 1968). Seed size polymorphisms are found in Cruciferae and Poaceae (Zohary 1962, 1969). Harper (1977) indicated that somatic polymorphism is the optimum strategy adopted by the fugitive annuals of disturbed habitats, if the environments are very different.

Dv has a wedge disarticulating spike, but the lower spikelets are retained by the plant for 1–2 months, and

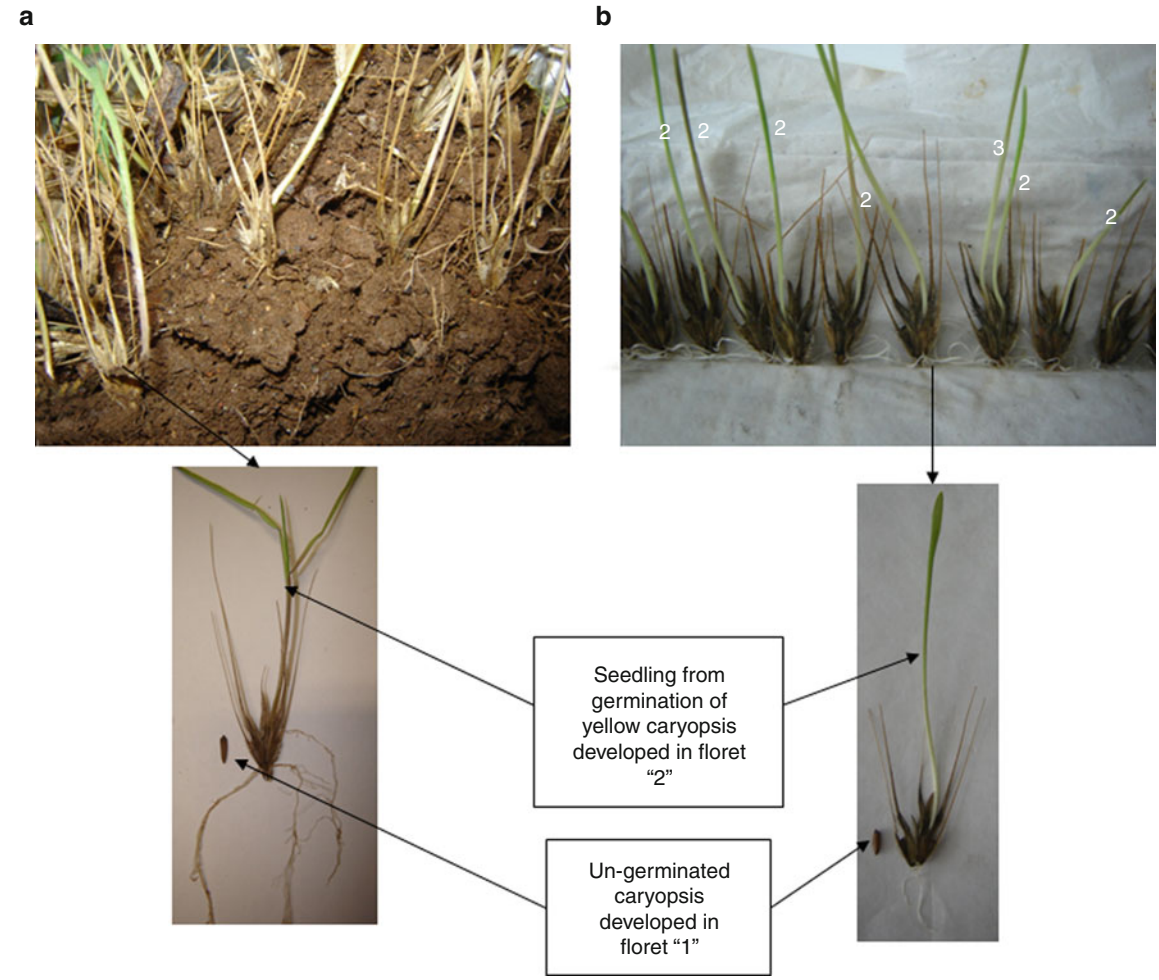

Fig. 4.28 Results from germination, under both field environmental conditions (**a**) and growth chamber controlled environment (**b**), of *Dv* dark-red and yellow caryopses developed in floret "1" and "2," respectively, of the same spikelet

they reach the soil only when the culm is forced down. The different time of caryopses (spikelet) release might cause a time-controlled contribution of spikelets with different germination capacity to the seed bank in the soil. However, the timing of caryopses release from *Dv* spikes does not seem to be related to the time of caryopses germination. The lower spikelets retained by the mother plant show the same distribution and the same germination ability of dark-red and yellow caryopses as the upper spikelets.

Caryopses germination rate seems to be related only to caryopses color and size and not to the position of the spikelet carrying the caryopses. There are indications that dark-red and yellow caryopses differ in dormancy only for few weeks after maturation. A germination test carried out on a dark-red and a yellow caryopses pool from one population (I-81-7a) few days after collection showed an average of 5 days to germination for yellow caryopses and of 11 days for dark-red caryopses. However, after storage for about 1 month, the difference in days to germination of yellow and dark-red caryopses did not exceed 1 day. Compounds within the glumes are mobilized by water imbibition and impede germination of dark-red caryopses but not of those yellow colored in spikelets either dispersed in the topsoil (Figs. 4.27 and 4.28a) or after water imbibition under controlled conditions (Fig. 4.28b). Extract prepared from glume powder immersed in distilled water exerts a strong inhibitory effect on *Dv* and wheat germination of naked caryopses (Fig. 4.25).

Table 4.3 *Dv* caryopsis polymorphism for quality traits in comparison to bread wheat kernels. *Dv* caryopses were from ecotype "200" (former I-84-16), collected near Bomarzo, Viterbo, Italy

Species	Kernel color	PC (% dry matter)	Hardness	Gluten content (% dm)	Gluten index	SSV (mL)	Specific SSV (SSV/PC)
Dv	Yellow	21.1	6	14.7	90	75	3.55
	Dark-red	19.5	8	15.5	85	70	3.59
Bread wheat cv. Bologna	Pale yellow	14.3	85	9.8	98	54	3.80

SSV SDS sedimentation volume; *PC* Protein content

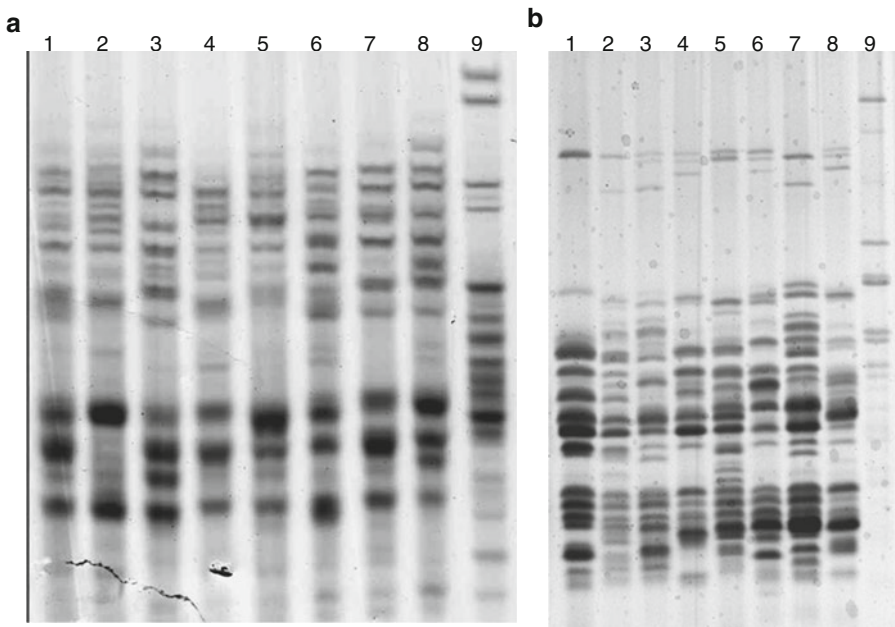

Fig. 4.29 A-PAGE separation of gliadins (**a**) and SDS-PAGE separation of glutenins (**b**) from the same set of single kernels of *Dv* "ecotype 200." *Lanes* 1–4: dark-red kernels; *lanes* 5–8: yellow kernels; *lane* 9: *T. aestivum* cv. Chinese Spring

Seed dormancy for annual plants such as *Dv* can be a crucial link between generations. The dormant phase in the life cycle of a plant evolved in response to the probability of suffering greater hardship by initiating growth. In many environments, the supply of water is uncertain and seeds that are triggered into germination by an early rain after the dry season may die if the next rain is delayed. Thus, there is a fitness advantage for polymorphism in seed dormancy (Stefani and Onnis 1984). This behavior, called "wild type" regulation of germination in wild cereals by Zohary (1969), endows strong fitness to the progeny of a spike, assuring the contribution to the gene pool by progeny from each spike. The difference in germination rate of dark-red and yellow-colored kernels is an example of Zohary's wild-type regulation of fitness. Natural selection may operate at very low intensities when wild-type regulation of germination exists in the *Dv* seed-bank, and this may contribute to the equilibrium gene frequencies in *Dv* populations. Such equilibrium will be reached at different gene frequencies only for populations reproductively isolated and facing distinct ecological conditions. *Dv* somatic polymorphisms for caryopses size and color and genetic polymorphisms for isozymes and morphological characters are both operative to maintain genetic diversity. There were no differences in vernalization requirement for heading between plants derived from yellow- and dark-red kernels.

Some grain quality traits measured on two subsamples of yellow and dark-red caryopses from the same population (ecotype DV-200, former I-84-16, collected near Bomarzo, Viterbo, Italy and maintained in field plots at the Experimental Farm of the University of Tuscia, Viterbo) are reported in Table 4.3. The yellow-kernel subsample had a higher protein content

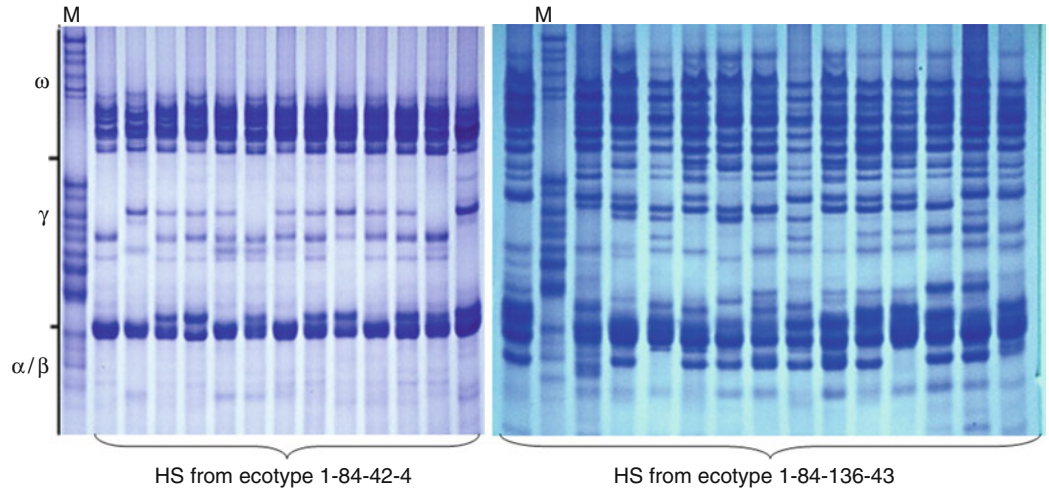

Fig. 4.30 Contrasting patterns for gliadin-like seed storage proteins in half-sib progenies (HS) of two *Dv* ecotypes: I-84-42-4 and I-84-136-43; M = *T. aestivum* cv Marquis used as reference

(21.1%) compared with the dark-kernel one (19.5%), paralleled with a higher sodium dodecyl sulfate sedimentation volume. On the contrary, the gluten content of the yellow-kernel subsample (14.7%) was lower than the dark-red kernels (15.5%); however, when the gluten index, which is a measure of the ratio of strong/to weak gluten, was considered, the yellow-kernel subsample confirmed its higher quality for leavened bread products (Table 4.3). Gliadin-like and glutenin-like seed storage polypeptides extracted from the yellow and dark-red kernel of the same ecotype (DV-200) did not show apparent differences in polypeptide concentration and representativeness (Fig. 4.29). However, ecotypes from different Italian regions show significant differences in concentration and frequencies of the α-type, β-type, and γ-type prolamin polypeptides (Fig. 4.30; De Pace et al. 1994a).

4.2.5 Cytology and Karyotype

The karyotype observed in an Italian population of *Dv* from Latium is shown in Fig. 4.31a. Following the nomenclature proposed by Levan et al. (1965), there are one *M* chromosome pair (arm ratio 1.0), three *m* pairs (arm ratio 1.0–1.7), and one satellited pair. This karyotype is basically similar to those reported by other authors (Gill 1981; Friebe et al. 1987; Gill and Appels 1988; Linde-Laursen and Frederiksen 1991), but discrepancies between published karyotypes of *Dv* occur, as well as differences between the karyotypes of plants originated from yellow or dark-red caryopses (Cremonini et al. 1994). Moreover, polymorphism within chromosome pairs in the length and arm ratio and the banding pattern after differential staining was often observed (Cremonini et al. 1994; Blanco et al. 1996). Intraspecific variation of the DNA content affects the length and arm ratio of different chromosome pairs (Caceres et al. 1998). The metaphase chromosome complement has been characterized also by the use of different banding techniques, including staining with fluorochromes (Fig. 4.31b), C-banding, and Ag-NOR (Blanco et al. 1996 and references therein), and by the chromosomal localization of a species-specific, 380 bp long satellite DNA sequence (De Pace et al. 1992). The results obtained allowed identification of each chromosome pair in the complement so that tagging of individual V chromosomes in different genomic backgrounds after interspecific or intergeneric hybridization can be made.

A karyotype of *Db* ($2n = 4x = 28$) is shown in Fig. 4.32. Aneuploid plants are not rare (Ohta et al. 2002), and diploid forms have been occasionally found in Moroccan populations (Sarkar 1957; Ohta et al. 2002). The illustrated karyotype is from a population from Mount Taygetos, Peloponnisos, Greece. There are six M pairs, six m pairs, and two satellited

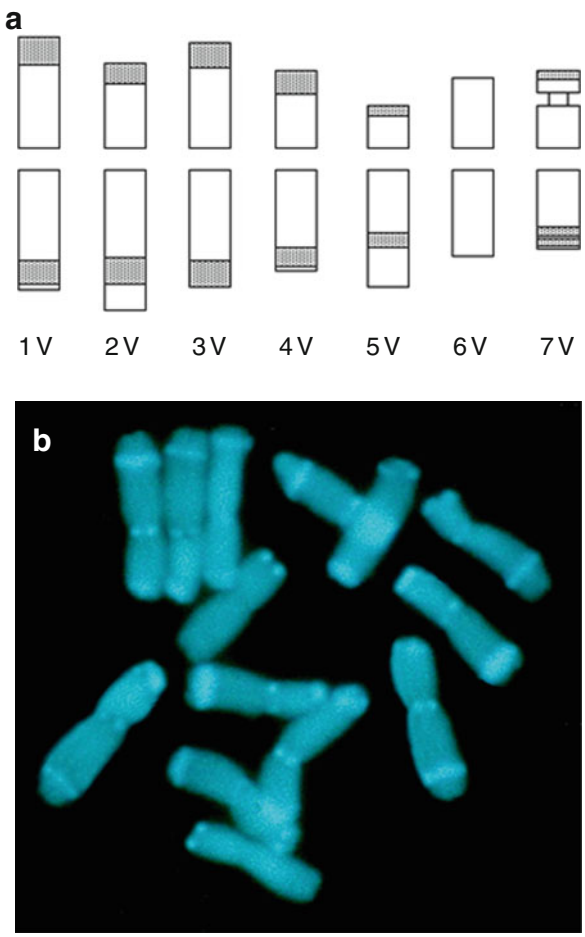

Fig. 4.31 (**a**) Ideogram of the haploid chromosome complement of *Dv* from a population sampled in Latium (Italy); chromosomes were arranged according to their length. (**b**) DAPI-stained metaphase plate of *Dv*. Bands of enhanced fluorescence can be seen at the centromere regions and at the ends of each chromosome (×2,000)

pairs. As for *Dv*, substantially different karyotypes of *Db*(4*x*) have been published. For example, different numbers of satellited chromosome pairs (up to 4) have been reported (Frederiksen 1991a). The origin and genomic constitution of *Db*(4*x*) is debated. An autotetraploid origin has been suggested by several authors (Sarkar 1957; Sakamoto 1986; von Bothmer and Claesson 1990). However, a direct derivation from *Dv* was denied on the basis of the results of molecular cytogenetic investigation (Galasso et al. 1997), RAPD analysis of the genomic DNAs (Yang et al. 2006), and studies of the meiosis in reciprocal crosses of the two species (Sakamoto 1986; Ohta and Morishita 2001). By contrast, the autotetraploid origin of *Db*(4*x*) from *Dv* has been suggested by Blanco et al. (1996) on the basis of the results of DNA fragment analyses and isozyme systems, as well as from those of FISH using structural genes as target sequences. Other authors have proposed an allopolyploid origin for *Db*(4*x*), most likely with *Dv* as the one parent (Frederiksen 1991a; Linde-Laursen and Frederiksen 1991). Further comments on these aspects are reported in Sect. 4.2.8.1.

4.2.6 Genome Size

Cytophotometric determinations of the nuclear DNA content carried out on the two accessions whose karyotypes are reported above showed the genome size of *Db*(4*x*) to be twice of that of *Dv* (Blanco et al. 1996). Using flow cytophotometry, the average estimated 1C-genome size of *Dv* was 5.065 pg (4,953.6 Mb; Obermayer and Greilhuber 2005). However, it is not simple to state the genome size of *Dv* as large intraspecific variation of genome size has been detected between populations and differences have been evidenced even between individual plants within the same population. Greilhuber (2005) found differences in genome size up to 1.07-fold among 17 *Dv* accessions from the IPK Gatersleben germplasm collection. Simultaneous DNA flow cytometric separation of two individuals from two different *Dv* accessions differing, on average, about 1.08-fold in genome size gave two clearly distinguishable peaks. That difference was more than just technical "noise."

Cytophotometric determinations have shown that the genome size varies significantly between plants grown from yellow caryopses (4C = 23.7 pg) and those grown from dark-red caryopses (19.1 pg) (Cremonini et al. 1994) (see also Sect. 4.4.2.4). Similar differences were also observed by Blanco et al. (1996), Frediani et al. (1994) and Colonna et al. (1991). These latter authors also showed that the DNA content varies to different degrees during the germination of yellow or dark-red caryopses. Clear-cut differences cannot be seen by comparing resting embryos contained in differently colored dry caryopses. During seed germination, the 1C DNA content increases in all seedlings but to a higher extent in those from yellow caryopses than in those from dark-red caryopses. Frediani et al. (1994)

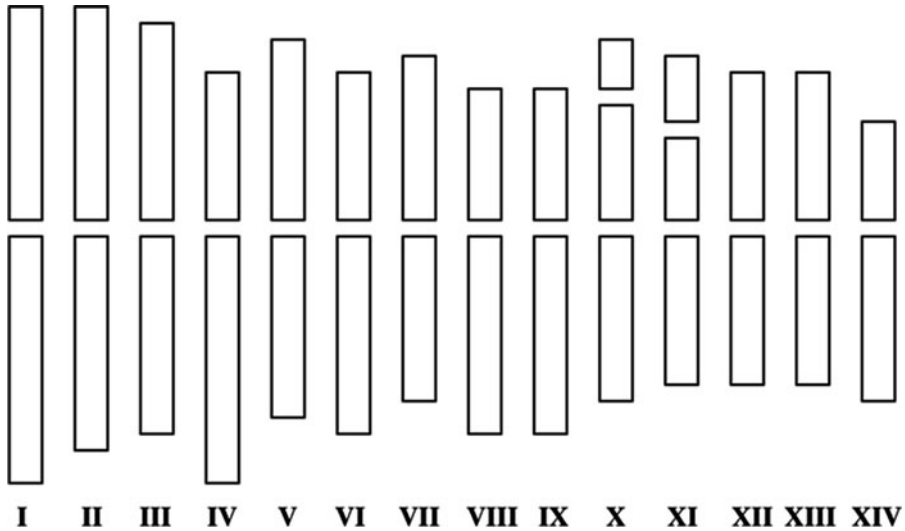

Fig. 4.32 Ideogram of the haploid chromosome complement of *Db* as observed in a population from Mount Taygetos, Peloponnesos, Greece. Chromosomes were arranged according to their length

also showed that these variations of genome size are correlated to differential amplification of the family of *Dv*-specific DNA sequences mentioned above.

These modulations do not exhaust the nuclear DNA plasticity found in *Dv*. Feulgen/DNA cytophotometric determinations showed that substantial quantitative alterations in the nuclear DNA occur between and within 15 natural populations in Italy differing for geographical and environmental parameters of their sites (Caceres et al. 1998). When considering the most divergent values, there was a 17.6% difference between the population mean genome size and a 66.2% difference between the genome sizes of individual plants within a population. A highly significant positive correlation was found between the genome size of *Dv* plants and their altitude of origin, and differences between individual plants were greater in populations from mountain sites, where the environment is expected to be particularly limiting and/or variable. Moreover, significant negative correlations were found between genome size and both the mean flag leaf length and flag leaf width. In contrast, the genome size of individual plants is positively correlated with the weight of the caryopses from which they originated and their flowering interval.

Caceres et al. (1998) also studied intraspecific variation of genome size during reproduction. The results obtained showed another aspect of the DNA plasticity in *Dv* that might aid in interpreting certain behaviors of its chromosomes after hybridization with wheat. A wide range of genome sizes was found in half-sib progeny of plants having a relatively large genome. In contrast, in the half-sib progeny of plants having a small genome, the genome sizes of individual plants were less divergent and similar to that of the mother plant. All siblings from crosses between plants with differing genome sizes had similar DNA contents, which were intermediate between those of the parental plants, even if closer to the DNA content of the parent plant having the smaller genome size, indicating that small genome sizes are somehow "dominant" in determining DNA contents in progeny. Size polymorphism within chromosome pairs was not observed in plants obtained from these crosses or in half-sibs, whose genome size differed from that of the mother plant, although a correlation between chromosome morphology and genome size was ascertained in *Dv* (see Fig. 4 in Caceres et al. 1998). This observation suggests that imbalances in DNA sequences redundancy are recognized and are overcome rapidly after fertilization, even if it is currently impossible to speculate on the way in which this is achieved.

Some hypotheses have been worked out to explain genome remodeling in Poaceae through events such as whole genome duplications from an ancestral five-chromosome genome, orthologous gene shuffling through gene loss, and translocations occurred between 53 and 94 million years ago (Bolot et al. 2009).

Most recent variation was attributable to the amount of genome duplications and repetitive DNA modulation, which comprises 70% of cereal genomes. Genome sizes range from 450 Mb for rice, 2,500 Mb for maize, 5,000 Mb for barley and *Dv*, and 16,000 Mb for hexaploid wheat. However, identification of the cause of the intraspecific variation in genome sizes remains a controversial topic in the study of plant genome size variation (Bennett and Leitch 2005). Shaked et al. (2001) and Ozkan et al. (2001) presented evidence that prompted appreciation about the extreme dynamic range, the rates of change that are possible, and the various mechanisms for promoting change or maintaining homeostasis within genomes at the chromosome level. They found that DNA sequence elimination is a major and immediate response to allopolyploidization in wheat that can affect up to 15% of polymorphic loci, in some cases within a single generation. The sequences analyzed are present in all diploid wheat species but occur in only one genome in polyploid wheat, either as a single homologous pair (chromosome-specific sequence) or in several pairs on more than one chromosome (genome-specific sequence). Their results show that sequence elimination was a widespread and immediate response to allopolyploidization among *Aegilops* and *Triticum* species and followed a reproducible pattern characterized by preferential elimination of sequences from one of the parental genomes. Although this mechanism affecting genome size variation cannot be extended to explain *Dv*-genome size variation, it nonetheless indicates that there are sequences within the Triticeae genomes that are prone to be eliminated under certain biological circumstances.

Bennetzen and Kellogg (1997) suggested that plants may have a directional increase towards a larger genome sizes via amplification of retrotransposons. The propagation of these RNA, or Class I transposable elements ("retroelements"), where a static parental element generates copies at new sites via an RNA intermediate, contrasts strongly with that of DNA, or Class II, transposable elements ("transposons") (Petersen and Seberg 2000).

In the case of the most sophisticated retroelement, retrotransposons, each new copy is potentially as active as its parent, leading to the possibility of exponential increases in copy number. In plants, particularly the cereals, massive increases in retrotransposon abundance do appear to have occurred during recent evolution so that these elements make up 50% or more of the genomes of species such as maize, wheat, and barley (Kumar and Bennetzen 1999). In the large genome of maize (2,500 Mb), some retrotransposons have copy numbers exceeding 2×10^4 per haploid genome (San Miguel and Bennetzen 1998), and all of the retrotransposons sequenced were shown to have inserted within the last six million years, leading to a doubling of the size of the maize genome. Other retrotransposon insertion events date back to the root of the Triticeae lineage. A *Db*(4*x*)-genome-specific RAPD product of 1,182 bp was cloned and designated as pDb12H. Sequence analysis revealed that pDb12H was strongly homologous to a long terminal repeat (LTR) *Sabrina* retrotransposon newly reported in *Hordeum* (Yang et al. 2006). In FISH experiment probed by the fluorescence labeled pDb12H, hybridization signal appeared in all *Db*(4*x*) chromosomes, while no signal was detected in wheat and *Dv* chromosomes (Yang et al. 2006), although *Sabrina*-like elements were detected in wheat A-, B-, an D-genomes (Gu et al. 2004).

Transposons are mobilized by a cut-and-paste mechanism. This mechanism not only allows movement into new sites but also, depending on the success of the transposition event and its timing relative to DNA replication, may increase or decrease the copy number of the element.

MITE is a class II element that has expanded the grass genome as the CACTA family transposons. A lineage of CACTA family has been identified in temperate grasses and cereals where it maintains an unexpected high copy number and strong conservation (Langdon et al. 2003). In a study to investigate whether RPB2 gene sequences can provide information on the origin of Y-genome and its relationship to other genomes, it was found that the longest MITE *stowaway* insertion (about 100 bp) in the 130–180 nucleotide region was found for the V-genome of *Dv* compared to insertions occurring in species with H-, Y-, W-, P-, and E^b-genomes (Sun et al. 2008).

Revolver is Class II-like transposable element dispersed as transposons in the Triticeae genomes. The name *Revolver* means a dynamic factor to construct genomes through evolution of the Triticeae. Revolver cDNA hybridized strongly to *Secale* species and *Dv* and moderately (3×10^3 to 28×10^3 copies) to *T. monococcum* (AA), *Ae. speltoides* (SS), *Ae. tauschii* (DD), and *T. dicoccum* (AABB). In contrast, *Revolver* was not detected in the common wheat genome. These

facts indicate that *Revolver* has existed since the diploid progenitor of the Triticeae lineage of the Poaceae (3,000–8,000 copies), and it has been amplified in rye and *Dv* (about 20,000 copies), but it has been lost from bread wheat after the allopolyploidy event (Tomita et al. 2008) probably through a mechanism similar to that advocated by Feldman et al. (1997).

The *Dv*, *Db* (2*x*), and *Db* (4*x*) species group represent an attractive study material to test hypotheses about chromosome evolution in Triticeae and to shed light on the dynamic range of orthologous gene shuffling through gene loss and translocations and on the effect of Class I and II transposon abundance on genome size.

Retrotransposons and other repeated elements are useful sequences for genome analysis and chromosome evolution in *Dasypyrum*. The LTR *Sabrina* retrotransposon (Yang et al. 2006) and two dispersed repetitive DNA sequences pDbKB45 (distributed in the subtelomeric regions) and pDbKB49 (less amplified in terminal region) from *Db* (Galasso et al. 1997) have been used for that purpose. Other repeated elements from *Dv* that share homology with the 350 bp-family repeated element of rye (Yuan and Tomita 2009) have been used for inferring evolutionary trends. The 350 bp-family repeated element mark specifically the telomeric region of all V-genome chromosomes except 7V of *Dv* [such as the 380 bp telomeric tandem repeat isolated by De Pace et al. (1992) and the cloned 292 bp species-specific repeated sequence pHvNAU62 isolated by Li et al. (1995)] or the telomeric and centromeric region of all V chromosomes (pDvTU383 isolated by Yuan and Tomita 2009).

4.2.7 Taxonomic Position

Dasypyrum (Cosson & Durieu) T. Durand and the other Triticeae species *Eremopyrum, Agropyron sensu stricto*, *Crithodium* Link (the diploid part of *Triticum* including *T. monococcum*), and *Australopyrum* (Tzvelev) Löve seem to represent the only diploid genera of the tribe with keeled glumes (Kellogg 1989). However, keeled glumes are expressed as two character states; 2-keeled in *Dasypyrurn* and *Crithodiurn,* and 1-keeled in *Agropyron, Eremopyrurn* (Frederiksen 1991b), and *Australopyrurn.*

According to Löve (1984), the genus name *Dasypyrum* derives from Greek *dasy* (bushy, shaggy) and *pyros* (wheat), the haplome is V.

Dv was named *Secale villosum* in 1753 by C. Linnaeus (Linnæi 1753, p. 84) (*http://botanicus.org/page/358103*). Frederiksen (1991a, b) explains that in Linnæi (1753, p. 84), two varieties of *S. villosum* were identified: The typical one and the β. Under the typical variety, she cites Tournefort (1700, *http://botanicus.orghttp://botanicus.org/page/502743*, p. 518, *http://www.botanicus.org/title/b11931589#*) where, under the genus VIII-Gramen, *Chien-dent,* there was the type described by Parkinson (1640, p. 1144) as *Gramen secalinum, maximum.*

The type β of Linnæi (1753, p. 84) is the same as that reported by Tournefort (1703, *Corollarium*, *http://botanicus.orghttp://botanicus.org/page/503460*, p. 39, *http://botanicus.org/page/503503*), as "*Gramen creticum spicatum secalinum*, glumis ciliatibus," and Buxbaum (1740), *Centuriae* V. (*http://bibdigital.rjb.csic.es/ing/Libro.php?Libro=1744*, p. 21, Table XLI, illustration in Làm. XXV). As Frederiksen (1991a) pointed out, the illustration in Table XLI of Buxbaum (1740) does not remind the *Dv* spike morphology, which make difficult the determination of the taxon, considering that the authentic specimen has been lost.

In 1798, Jean Baptiste Pierre Antoine de Monet de Lamarck used the name *Hordeum ciliatum* Lam. (Lamarck 1798, p. 604; *http://botanicus.org/page/739642*) for a plant from Italy he received by M. Vahl[1] (*http://www.lamarck.cnrs.fr/herbier.php?lang=en* Liasse no 90 GRAMINEAE (18); Fig. 4.33), which seemed to fit the description that Linnæi (1753) made for *Hordeum bulbosum* with some differences. Later (Lamarck 1816, p. 184 *http://botanicus.org/page/737230*), he proposed that *H. ciliatum* was the same as *S. villosum* L. rather than *H. bulbosum* L., which M. Desfontaines described under the name of *H. strictum*. Frederiksen (1991a), Humphries (1978), and others reported that *Dv* was also named *Triticum caudatum* by Persoon (1805, p. 110).[2] However, a species different from *Dv* embodied *Triticum caudatum* Persoon, because

[1] *http://www.lamarck.cnrs.fr/ice/ice_page_detail.php?lang=en&type=img&bdd=lamarck&table=corpus_lamarck&bookId=285&title=Liassen°90GRAMINEAE&pageOrder=212&typeofbookDes=Herbier&nump=204&nav=1&cfzoom=1.9&facsimile=off*

[2] *http://books.google.it/books?id=vOsCAAAAYAAJ&pg=PA113&lpg=PA113&dq=persoon+synopsis+plantarum&source=bl&ots=yotRaFANtB&sig=_NdhjCEvPP9Ni3RAbOTHaZ0O6ZI&hl=it&ei=8Qe4SqP3B46UnwOU8KjUDw&sa=X&oi=book_result&ct=result&resnum=1#v=onepage&q=&f=false*

Fig. 4.33 Herbarium specimen of *Dv*: Author: Lamarck Jean-Baptiste. Muséum National d'Histoire Naturelle de Paris/CRHST Herbier Lamark P00564587 Liasse no 90 Plate group 18

Persoon (1805, p. 108)[3] described *Dv* under *Secale villosum* with a clear reference to the description made by Linnæi in the 2nd (Linnæi 1762, p. 124, *http://botanicus.org/page/1159878*) and the 4th edition of Species Plantarun edited by C. L. Willdenow (1797, p. 471, *http://botanicus.org/page/602659*) and to Buxbaum (1740). In 1808, *Secale villosum* L. was transferred to the genus *Triticum* [*Triticum villosum* (L.) M. Bieb.] (Marschall von Bieberstein 1808, p. 85)[4] and as such was described also by Link (1821, p. 96, *http://botanicus.org/page/948474*) although later Link (1827, 1:31)[5] placed *Dv* under *Agropyrum villosum* (L.). Since then it has been considered as a distinct section of the genus *Triticum* sect. *Dasypyrum* (Cosson and Durieau 1855) or *Triticum* sect. *Pseudosecale*[6] (Grenier and Godron 1856, p. 599; Humphries 1978). Schur (1866, p. 807) was the first botanist to recognize that *Secale villosum* L. was distinct from other species of *Secale*, *Triticum*, and the other genera in tribe Hordeae on the basis of singly arranged spikelets and the long-awned, ciliate glumes and lemmas. Therefore, in his book, whose preface was dated April 1866,[7] but the publication date remains uncertain, possibly May or June 1866, he placed *Secale villosum* L. in a new genus, *Haynaldia* Schur[8] to honor Cardinal Stephan Franz Ludwig (Lajos) Haynald (1816–1891) (Fig. 4.34), a Hungarian by birth, not only as a great churchman but also for his interest in the science of botany (Bor 1970). However, on 2 Oct 1865,[9] at the Zoologisch-botanische Gesellschaft, held in Wien, the paper of Schulzer von Müggenburg et al. (1865), p. 37) was presented in which the genus name *Haynaldia* was used also to honor Cardinal L. Haynald,[10] and to describe a fungus growing on rotten cooked tomatoes, *Haynaldia umbrina*[11] (later the genus wad renamed *Helicostelium*). The paper was cited in Flora 49:224 and published as a book on 30 May, 1866 (Schulzer von Müggenburg et al. 1866). Bor (1970) maintained that the fungus genus *Haynaldia* Schulzer appeared earlier than *Haynaldia* Schur. Also, Kanitz (1876) and Pantocsek (1889,

[3] *http://books.google.it/books?id=vOsCAAAAYAAJ&pg=PA113&lpg=PA113&dq=persoon+synopsis+plantarum&source=bl&ots=yotRaFANtB&sig=_NdhjCEvPP9Ni3RAbOTHaZ0O6ZI&hl=it&ei=8Qe4SqP3B46UnwOU8KjUDw&sa=X&oi=book_result&ct=result&resnum=1#v=onepage&q=&f=false*

[4] *http://www.archive.org/stream/floratauricocau00biebgoog#page/n101/mode/1up*

[5] *http://books.google.it/books?id=a6QCAAAAYAAJ&pg=PA65&dq=Link+berolinensis&lr=&ei=76a4StnND6bUyQSgq7zyDg#v=onepage&q=&f=false*

[6] *http://www.archive.org/stream/floredefranceoud03gren#page/598/mode/2up*

[7] *http://www.archive.org/stream/enumeratioplanta00schu#page/n21/mode/2up*

[8] *http://www.archive.org/stream/enumeratioplanta00schu#page/806/mode/2up*

[9] *http://www.archive.org/stream/diebisherbekann00mggoog#page/n9/mode/1up*

[10] *http://www.archive.org/stream/diebisherbekannt00schu#page/n5/mode/2up*

[11] *http://www.archive.org/stream/diebisherbekann00mggoog#page/n42/mode/1up*

Fig. 4.34 A sculpture of Reverend Lajos Haynald (**a**) has been erected in the city of Kalocsa (Hungary) (**b**) where he established the botanical garden and an observatory (**c**), and was named Archibishop in 1867 and Cardinal in 1879, and where he died in 1891

p. 120)[12] honored Cardinal L. Haynald by naming after him a genus of the family Campanulaceae (later changed in Lobeliaceae) and a genus of fossilized diatom, respectively. Durand (1888) was the first author to validate *Dasypyrum*, to avoid confusion with other *Haynaldia* genera, as a distinct genus in substitution of the genus *Haynaldia* Schur. *Haynaldia* Schulzer and *Haynaldia* Kanitz have disappeared into synonymy. *Haynaldia* Pantocsek (1889) and *Haynaldella* J. Pantocsek (1892)[13] are still used in the literature.[14]

Three other authors, namely, von Borbás (1898), Candargy (1901), and Maire (1942) raised the section *Dasypyrum* to the generic rank. However, according to the rules of the International Code of Botanical Nomenclature (Stafleu et al. 1978), only the descriptions of Candargy (1901) for the genus *Dasypyrum* and its species are considered valid. Compared to the other genera of the subtribe Triticinae, namely *Secale*, *Agropyron, Aegilops*, and *Triticum*, the genus *Dasypyrum* has the distinctive trait of bicarenate (two-keeled) glumes with tufts of bristles along the keels. A botanical variety of *Dv,* indicated as *Dv* var. *glabratum* (von Borbás 1898, *nomen nudum*), with small spike and spikelet and glabrous palea has been reported as occurring in Budapest, Hungary, since 1898.

Maire (1952) classified *Dasypyrum* in the Gramineae (Poaceae) and recognized only two species: *D. hordeaceum*, with the awn on the lemma shorter than the awn on the glume, and *Dv*, with awn on the lemma longer than the awn on the glume. *D. hordeaceum* (Coss. et Dur.) Maire was also known before Maire (1952) as (1) *Triticum hordeaceum* (Cosson and Durieau 1855); (2) *Haynaldia hordeacea* (Coss. et Dur.) Hackel (Engler and Prantl 1887); and (3) *H. breviaristata* (Lindberg 1933). *D. hordeaceum* (Coss. et Dur.) Maire

[12] *http://www.archive.org/details/beitrgezurkenn21889pant*

[13] *http://www.archive.org/stream/beitrgezurkenn31892pant#page/n131/mode/2up*

[14] *http://research.calacademy.org/research/Diatoms/genproject/gp2.html http://www.algaebase.org/search/genus/detail/?genus_id=46840&-session=abv4:5D29D2F91689513 E8DOnL428CA0B*

corresponds to *D. hordeaceum* (Cosson & Durieu) Candargy, the latter being, according to the priority rules of the International Code of Botanical Nomenclature (Stafleu et al. 1978), the only valid name recognized for the species. Maire (1952) described the species as a rhizomatous plant, 0.4–1 m tall, spike 3–6 cm long; rachis fragile, spikelet 11–12 mm long (without the awn); glume bicarenate with long, isolated bristles. Three botanical varieties were recognized by Maire on the basis of the leaf pubescence: *D. hordeaceum* var. *genuinum* has glabrous leaf lamina and rare hairs along the leaf edges; *D. hordeaceum* var. *velutinum* has pubescent leaf lamina with dense and short hairs; *D. hordeaceum* var. *breviaristatum* has sparse pubescence on the leaf lamina and long hairs on both lamina surfaces and along the leaf edges. *D. hordeaceum* var. *genuinum* Maire and *Dv* var. *glabrata* Borbas are the only two *Dasypyrum* botanical entity not fully expressing the "*dasy* or shaggy" trait.

The revision of the *Dasypyrum* (tribe Triticeae) made by Frederiksen (1991a) definitely recognized two species: the annual *Dv* ($2n = 14$) and the perennial tetraploid *Db*(4*x*) ($2n = 4x = 28$). She demonstrated that the combination *D. hordeaceum* is based on a later homonym, and for that reason, a new combination, *Db,* was made. The diploid *Db*(2*x*) cytotype has been described by Ohta et al. (2002).

4.2.8 *Speciation in the* Dasypyrum *Genus and Evidence Used to Place the Genus in the Triticeae Phylogeny Tree*

The phyletic relationships within the *Dasypyrum* genus and among *Dasypyrum* species and other Triticeae species can be examined at the level of morphology, protein, chromosome, and chloroplast and nuclear DNA fragments and nucleotide sequences.

4.2.8.1 Speciation in the *Dasypyrum* Genus

Morphologically, the most conspicuous evolutionary divergence between diploid *Dv* and *Db*(2*x*)–*Db*(4*x*) is found in their vegetative propagation device: tendency to form stolon-like structures and absence of rhizomes in *Dv* (Figs. 4.7–4.11) and presence of rhizomes in *Db*(2*x*)–*Db*(4*x*) complex. These differences clearly reflect the major trends of adaptive radiation between *Dv* and the *Db*(2*x*)–*Db*(4*x*) complex during colonization of high altitude habitats and further adaptation and differentiation of the tetraploid *Db*(4*x*) cytotype to the environmentally disturbed habitats in the neighborhood of forests and pastures at high altitude. The F_1 plants from *Dv* × *Db* hybridizations produced rhizomes indicating that the perenniality trait was dominant (Blanco and Simeone 1995; Ohta and Morishita 2001).

Sarkar (1957), for the first time, analyzed the diploid *Db*(2*x*) and tetraploid *Db*(4*x*) forms from a collection made by G. L. Stebbins in Morocco in 1954. Both forms were perennial and at meiosis-I of *Db*(4*x*) plants, up to six quadrivalents were observed, suggesting an autotetraploid derivation of *Db*(4*x*) from the diploid *Db*(2*x*). Nakajima (1958), for the first time, compared the chromosomal and plant morphology of *Db*(2*x*) and *Dv*. *Dv,* compared to *Db*(2*x*), had longer culms (114 vs. 88 cm), longer awns (3.7 vs. 1 cm), and higher number of tillers (105 vs. 39). The frequency of pollen mother cells (PMC) with univalents was 7.6% in *Dv* and 2.2% in *Db*(2*x*). Ohta et al. (2002) observed that the bristles on the glume keel that terminate with awn in *Db*(2*x*) and *Db*(4*x*) are not grouped in tufts as in *Dv*. Nakajima (1958) was the first also to make direct and reciprocal hybridization between *Dv* and *Db* at the diploid level using the Moroccan ecotype found by G. L. Stebbins. He obtained six F_1 seeds (2.78% seed setting) from *Dv* (♀) × *Db*(2*x*) hybridization but none of them germinated (Table 4.5). However, when the similar hybridization was attempted by Ohta and Morishita (2001), 36.1% seed set was obtained, and 30% of those shriveled caryopses germinated. The reciprocal *Db*(2*x*) (♀) × *Dv* combination did not produce seeds at all when it was attempted by either Nakajima (1958) or Ohta and Morishita (2001). The plants from F_1 caryopses obtained when *Dv* was used as female parent allowed the assessment of the pairing ability of the V chromosomes of *Dv* and *Db* (2*x*) at meiosis I (Ohta and Morishita 2001). The mean pairing configuration was 11.12^{I} and 1.44^{II} per cell, and the mean arm pairing frequency was 0.107. Only 0.1% of the pollen grains were viable, resulting in nearly-sterile F_1 plants. These data suggest that strong evolutionary divergence involving copy number and nucleotide sequence rearrangement of the repeated sequences has driven the two genomes to lose their homology and entering the homoeology status.

A similar pattern of seed set and chromosome homoeology was observed after hybridization of *Db* (4*x*) and *Dv*. About 50% and 12% seed settings were obtained from *Dv* (♀) × *Db*(4*x*) hybridization carried out by Ohta and Morishita (2001) and Blanco et al. (1996), respectively, and over 80% of them germinated (Table 4.5). However, when the same authors attempted the reciprocal *Db*(4*x*) (♀) × *Dv* cross combination, they were not able to get any F_1 caryopsis. Parents and F_1 of the *Dv* × *Db*(4*x*) hybridization are presented in Fig. 4.23 where the perenniality trait is evident in *Db*(4*x*) and F_1 due to the formation of rhizomes.

Sakamoto (1986, 1991) was also successful in the interspecific hybridization between *Dv,* used as female parent, and *Db*(4*x*) as pollen parent. At meiosis I, the triploid F_1 hybrid displayed chromosome configurations that, in average, were formed by 6.5^{II} and 7.9^{I}. However, the *Db*(4*x*) parent itself formed up to seven quadrivalents, indicating that the bivalents observed in the *Dv* × *Db*(4*x*) F_1 were from autosyndesis of the *Db* chromosomes. A critical intergeneric hybrid between *Ae. squarrosa* L. and *Db*(4*x*) was also produced, which formed PMCs exhibiting chromosome configuration with a mean of 6.1^{II} and 8.8^{I}. The chromosome pairing was attributed to autosyndesis between the two genomes of *Db*(4*x*), giving more support to the autotetraploidy hypothesis of Sarkar (1957) for the speciation events that lead to *Db*(4*x*). That hypothesis received further sustainment from von Bothmer and Claesson (1990) and Ohta and Morishita (2001). The latter authors obtained F_1 plants from the *Dv* (♀) × *Db*(4*x*) hybridization whose *Dv* and *Db* chromosomes did not pair at meiosis-I, and pollen fertility was about 1%. To take into account these results and those obtained previously, the symbol V^v for the haploid *Dv*-genome and V^bV^b for the haploid *Db*(*4x*)-genome was suggested (Ohta and Morishita 2001; Yang et al. 2005).

Linde-Laursen and Frederiksen (1991) observed nucleolar dominance of V^b NOR region on the V^v NOR region and wide C-band karyotype differences between the two genomes. Galasso et al. (1997) using the fluorescein (FITC)-labeled DNA from *Dv* and rhodamine (TRITC)-labeled DNA from *Db* were able to label, simultaneously, *Dv* and *Db* chromosomes in meiotic metaphases of a *Dv* × *Db*(4*x*) F_1 and observed seven bivalents of *Db* and seven univalents of *Dv*. Yang et al. (2005, 2006) confirmed the divergence of the *Dv*- and *Db*-genomes based on GISH and RAPD markers. When the total *Db* genomic DNA was labeled to hybridize a somatic mitotic metaphase of CS-*Db*(4*x*), partial amphiploid (TDH-2; $AABBV^bV^b$) with 42 chromosomes, 14 chromosomes were strongly and uniformly labeled along the entire chromosome length, which belonged to *Db* (Fig. 1a in Yang et al. 2005). That was not the case when *Dv* was used as a probe, and many arms of the 14 *Db* chromosomes displayed large regions in their distal half with reduced strength of labeling. These differentiated GISH patterns not only reflected the large genomic divergence between the *Db* and *Dv* chromosomes, as was described by Galasso et al. (1997), but also helped to identify the *Db* chromosome pairs in wheat background with labeled *Dv*-DNA as a probe.

Because in the PMC of the *Dv* × *Db*(4*x*) F_1, the observed number of trivalents was lower than 0.14 (Table 4.5), while in the PMC of the *Db*(4*x*) × *Db* (2*x*) F_1 the number of trivalents was 3.74, and considering that *Db*(2*x*) was perennial as was *Db*(4*x*), then, most likely, the diploid species in which the genome duplication event occurred to give rise to the *Db*(4*x*)-genome, was *Db*(2*x*) rather than *Dv*. Therefore, at present, the hypothesis that *Db*(4*x*) was derived from *Db*(2*x*) by autoploidy is the only one to have concurrent supports (Ohta et al. 2002). Indirect evidence for the hypothesis came from experiments of Nakajima (1960c; see Sect. 4.2.9.2) where the diploid *Db* produced unreduced gametes. Other authors have proposed an allopolyploid origin for *Db*(4*x*), most likely with *Dv* as one of the parent (Frederiksen 1991a; Linde-Laursen and Frederiksen 1991).

The autotetraploid origin of *Db*(4*x*) from *Dv* suggested by Blanco et al. (1996) did not find consensus on the basis of the lack of pairing of the V^v and V^b chromosomes in hybrids containing the two V^v and V^b-genomes. Nonetheless, the homology between the *Dv* and *Db* species for the peculiar spike morphology, the shared unilocus molecular and biochemical markers (Blanco et al. 1996), as well as the few signals detected on *Db*(4*x*) chromosomes but not on other Triticeae species (except *Th. bessarabicum*) using the pHv62 *DV*-species-specific repeated sequence (Uslu et al. 1999), are indicative of *Dv-Db* common ancestry. The genetic distance between *Dv* and *Db* for 320 RAPD loci was lower than their distance from *Secale* species (Yang et al. 2006). Therefore, differentiation between *Dv* and *Db* might be due to adaptability to the diverse ecogeographic

Table 4.4 Chromosome and genome specific molecular markers for *Dv* and *Db*

V chromosome	Chromosome arm	RFLP	SSR primers based on:		PCR markers	Reference
D. villosum			*Wheat microsatellite* (*marked wheat chromosome*)	*Wheat and rice EST* (*Cao et al. 2009*)		
1V	S	PSR 596	wmc 49 (1BS)		–	Qi et al. (1998a, b, c, 1999) used RFLP; PSR (wheat cDNA) clones provided by MD Gale, John Innes Center, UK; BCD (barley cDNA) and CDO (aot cDNA) clones were provided by ME Sorrels, Cornell University. RAPD markers were developed by Liu et al. (2003); SSR markers were used by Zhang et al. (2006): gdm primers from Pestsova et al. (2000), gwn primers from Röder et al. (1998), and wmc primers were from Gupta et al. (2002). $Xgwm498_{110}$ $Xgwm498_{190}$ were specific for 1V (Liu et al. 2004).
	L	BCD 240	$Xgwm498_{110}$ $Xgwm498_{190}$	$CINAU32_{300}$		
2V	S	BCD 855	wmc 25 (2BS)			
	L	PSR 388	gwm 469 (6DS)	$CINAU33_{280}$		
		BCD 240	–	$CINAU34_{510}$		
				$CINAU35_{1100}$		
				$CINAU36_{380}$		
				$CINAU37_{400}$		
3V	S	PSR 926	gdm 36 (3DS)	$CINAU38_{250}$		
	L	BCD 589				
4V	S	PSR 584, 139		$CINAU39_{950}$		
	L	BCD 327, CDO 795	gdm 145 (4AL)	$CINAU40_{800}$		
5V	S	CDO 1335	wmc 233 (5DS)	$CINAU41_{745}$		
	L	BCD 1088		$CINAU42_{1051}$		
6V	S	PSR113; PSR312	wmc 256 (6AL)	–	Codominant marker NAU/xibau-15_{902} for *Pm21* locus	Cao et al. (2006)
		–	–		Xcinau15_{902}, homologous to NAU/xibau-15_{902}	Chen et al. (2008)
					$SCAR_{1400}$	Liu et al. (1999)
	L	PRS149; CDO497	gdm 107 (2DS)			Pestsova et al. (2000)
7V	S	BCD385	gwm 344 (6DS)	$CINAU44_{765}$		Röder et al. (1998)
	L	PSR311; WG466		$CINAU45_{495}$		
All	–	–	–	–	Xgwm301/415 (405bp)	Liu et al. (2006)
All					BV679208 (755 bp)	Li et al. 2006 unpublished
All					*Revolver* transp.: a class II transposon-like seq.	Tomita et al. (2008)
All except 7V					Telomeric repeated sequence p380	De Pace et al. (1992)
					pHvNAU62	Li et al. (1995)
					pDvTU383	Yuan and Tomita (2009)
D. breviaristatum						
All	–	–	–	–	pDb12H: homologous to LTR of *Sabrina* retroelement	Yang et al. (2006)

area occupied by the *Dv*-*Db* common ancestor rather than the lack of common ancestry. Therefore, the formation of the *Dv*-*Db*(2*x*)-*Db*(4*x*) species complex and their biological and taxonomical status may be explained by a sequel of events starting at the earlier stages of the Triticeae clade separation (13–15 Mya) through the reproductive isolation of the lower-altitude *Dv* ecotypes from the high-altitude *Db*(2*x*) prototype, followed by the *Db*(2*x*)→*Db*(4*x*) auopolyploidization event and incipient reproductive isolation of *Db*(4*x*) from *Db*(2*x*). Such divergence has not occurred for other V^v-V^b-genome syntenic and generich DNA segments, as suggested by the strong similarity between *Dv*- and *Db*-genomes for restriction fragment patterns of genomic DNA, the phenotypes for some isozyme systems, and the location of gliadin genes (Blanco et al. 1996). The *Db*(2*x*)→*Db*(4*x*) autopolyploidization event favored the extensive *Db* (4*x*) colonization of the high altitude habitat on the mountains from North African Atlas to the Mt. Taygetos in the Peleponnesus in Greece. Chromosomal divergence between the Moroccan and Greek *Db*(4*x*) ecotypes is not evident yet by the comparison of the within and between ecotype meiotic chromosome pairing pattern (Table 4.5).

4.2.8.2 The Phyletic Relationships of *Dasypyrum* to Other Triticeae Genera

Morphology

The pioneering study of Baum (1978, 1983) in making a phylogenetic analysis of the Triticeae based on morphology has been followed by Kellogg (1989), Frederiksen and Seberg (1992), and Seberg and Frederiksen (2001). In those analyses, *Dasypyrum* branched in a sister group of *Secale* within the same clade. *Dv* resulted morphologically similar to *Crithodium monococcum* (Seberg and Frederiksen 2001) or to *Triticum* in general (Baum 1978). In the cladistic analysis of Triticeae done by Kellogg (1989) and based on morphology, *Dasypyrum* was placed near *Agropyron* and *Crithodium* (*Triticum*), and Baum (1978, 1983), based on homology for morphology, considered *S. cereale* and *Dv* as evolutionarily more contiguous to *Triticum* and *Aegilops* than to the rest of the galaxy of species that fall within the tribe Triticeae.

Protein

Dv, Agropyron cristatum (L.) Gaertner (P-genome), and the E-genome species *Lophopyrum elongatum* (Host) A. Löve [synonymous names *Thinopyrum elongatum* (Host) D. R. Dewey, *Elytrigia elongata* (Host) Nevski, *Agropyron elongatum* (Host) Beauv.] were examined for isoenzyme variation of esterase, peroxidase, and acid phosphatase (Angelov 2003a). Four indices of protein phenotypic similarity had their lowest estimates in the comparison of *L. elongatum* with *Dv*. That was an indication that both species were most distantly related as judged by the enzymes surveyed. *Dv* and *A. cristatum* were also positioned remotely. The species *A. cristatum* and *L. elongatum* demonstrated greater affinity as estimated by all indices used. Isoenzyme variation of esterase and acid phosphatase in natural populations of *Elytrigia repens* (L.) Nevski and *Elymus caninus* (L.) L. proved to be phylogenetically distant from *Dv* but equally far-away from this species (Angelov 2003b).

Jaaska (1982) found that a common superoxide dismutase alloenzyme (SOD-A) with pI 4.55 was shared by all wheat, goatgrass, and rye species except *Dv*, which carries a rare alloenzyme with pI 4.45. The other genetically independent SOD-B isoenzymes had a form, B^U, present in *Dv* and wheat but missing in rye.

Shewry et al. (1987) studied the degree of polymorphism and structural diversity of prolamin storage proteins of grasses and established that *Dv* prolamins were more closely related to cultivated wheat than to rye.

Montebove and De Pace (1988) compared the electrophoretic mobility of polypeptides for six isozyme systems (ADH-1, GOT-2, GOT-3, EST-2, EST-3, and EST-4) and gliadin and glutenin seed storage proteins in 11 Triticeae species and found that 57% of the *Dv* polypeptides had the same electrophoretic mobility of those expressed by homoeoalleles in the A-genome, and 29% and 14% of the *Dv* polypeptides had the same electrophoretic mobility of those expressed by homoeoalleles in the B- and D-genomes, respectively.

Chloroplast DNA Sequences Affinities

The chloroplast genome (cpDNA) is uniparentally inherited and generally lacks heteroplasmy, is present in high copy number in the cell, and is fairly well

Table 4.5 Mean percentage of kernel set, kernel with well developed endosperm, and kernel germination after mating within and among *Dv*, *Db*(2x) and *Db*(4x) species, and mean number of chromosome configurations at meiosis I of the PMC and pollen fertility of the plants risen from those kernels

Species or mating combination	Reference	Kernel set (%)	Kernels with well dev.endosp. (%)	Germination rate (%)	I[f]	II	III	IV	Pollen fertility (%)
Dv	Ohta and Morishita (2001; Tables 1 and 2)[a]; De Pace (unpublished data)	> 60	100	100	0.04	6.98	–	–	95.0
Db(2*x*)[17]or *Db*(2*x*)[8]	Ohta and Morishita (2001; Table 2)[a]	–	–	–	0.04	6.98	–	–	80.7
Db(2x)[17] × Db(2x)[8]	Ohta and Morishita (2001; Tables 1 and 3)[a]	43.1	100	100	0.14	6.92	–	–	82.2
Dv × Db(2x)	Ohta and Morishita (2001, Tables 1 and 4)[a]	36.1	0	30	11.12	1.44			0.1
	Nakajima 1958	2.8	–	0	–	–	–	–	–
Db(2x) × Dv	Nakajima 1958	0.0	–	0	–	–	–	–	–
	Ohta and Morishita (2001, Tables 1 and 4)[a]	58.5	0	0	–	–	–	–	–
Dv × Db(4x)	Ohta and Morishita (2001, Tables 1 and 4)[b]	50.6	100	86	8.10	6.20	0.14	0.040	1.0
	Sakamoto (1991)[c]				7.90	6.50	0.03	–	–
	Blanco et al. (1996)	12.1	100	100	7.23	6.78	0.07	–	–
Db(4x) × Dv	Ohta and Morishita (2001, Table 4)[b]			0	–	–	–	–	–
	Blanco et al. (1996)	50.7	100	0	–	–	–	–	–
Db(2x) × Db(4x)	Ohta and Morishita (2001, Table 1)[b]	35.4	0	0	–	–	–	–	–
Db(4x) × Db(2x)	Ohta and Morishita (2001, Table 4)[d]	11.1	31	50	3.40	3.20	3.74	0.005	17.2
Db (4x)	Sarkar (1957)	–	–	–		11.70		1.100	
	Blanco et al. (1996)	–	–	–	0.82	10.16	0.19	1.560	
	Sakamoto (1991)	–	–	–	1.32	7.37	0.38	2.680	
	Ohta and Morishita (2001, Table 2)[d]	–	–	–	0.55^{M} 0.31^{G}	5.08^{M} 6.87^{G}	0.33^{M} 0.15^{G}	3.57^{M} 3.15^{G}	73.8^{M} 66.5^{G}
Db(4x) × Db(4x)	Ohta and Morishita (2001, Table 1)	40.0	93	96	–	–	–	–	–
$Db(4x)^{M} \times Db(4x)^{M}$	Ohta and Morishita (2001, Table 3)[d]	–	–	–	0.69	4.73	0.40	3.520	70.4
$Db(4x)^{M} \times Db(4x)^{G}$	Ohta and Morishita (2001, Table 3)[e]	–	–	–	0.98	6.06	0.49	3.300	72.9

[a]Average of three plants
[b]Weighted average of nine plants
[c]Average of 233 cells of the same plant
[d]Average of three plants
[e]M and G indicate Moroccan and Greek ecotypes, respectively
[f]Chromosome configuration: I Univalent; II Bivalent; III Trivalent; IV Quadrivalent

conserved both in gene arrangement and sequences. These features make the cpDNA the choice for phylogenetic studies based on subtle restriction site variation, base pair substitution, mutation, and variable intergenic sequences, such as insertion/deletions (indels) and inversions, and microsatellite loci (such those designated WCt1-24 by Ishii et al. 2001; Provan et al. 2004). The restriction fragment patterns of chloroplast and mitochondrial DNA of *Dv* and wheats was described by Shu et al. (1993). *Dv* chloroplast genome sequences have been identified and cloned (Table 4.6).

Table 4.6 List of *Dv* and *Db* nucleotide sequences searched across the NCBI databases

D. villosum	Code for the NCBI GeneBank Seq.
Mitochondrial sequences	
tRNA-Met gene, complete sequence; and 18S ribosomal RNA gene, partial sequence; mitochondria	DQ307257.1; DQ307266.1; DQ307266.1; DQ307267.1; DQ307268.1
Mitochondrial *rps2* gene for ribosomal protein S2, W-type sequence	AB158207
Dav3 ScF13 retrotransposon, partial sequence; mitochondrial	DQ075443; DQ075442; DQ075441
Chloroplast sequences	
Chloroplast ACCase gene for acetyl-CoA carboxylase	AJ966451
Chloroplast rpoA gene	Z77741
Ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbcL*) gene, complete cds; *rbcL*-psaI intergenic spacer, complete sequence; and photosystem I subunit VIII (*psaI*) gene, partial cds; chloroplast	AY836163
ndhJ gene, partial sequence	EU334376
rpo C1 gene	EU334375
rpo B gene	EU334374
Ribosomal protein S4 (*rps4*) gene, partial cds; chloroplast	EF442875
NADH dehydrogenase subunit F (*ndhF*) gene, partial cds; chloroplast	DQ247881
InfA gene, complete cds; chloroplast	DQ269010
NADH dehydrogenase subunit F (*ndhF*), chloroplast gene encoding chloroplast protein, partial cds	U71030
RNA polymerase alpha subunit (*rpoA*) gene, complete cds; chloroplast gene for chloroplast product	AY115926AY115925
trnT gene, partial sequence; *trnL* gene, complete sequence; and *trnF* gene, partial sequence; chloroplast genes for chloroplast products	AF519129; AF519128
Nuclear sequences involved in:	
Transcription	
Second largest subunit of RNA polymerase II (*rpb2* gene), partial sequence	EU187471
Translation	
Translation elongation factor G (*EF-G*) gene, partial cds	AY836195
Translation initiation factor gene, partial cds	DQ279900
ITS1, 5.8S rRNA gene and ITS2	AJ608150
IGS (partial), ETS and 18S rRNA gene (partial), isolate 501004 IPSR	AJ315031
5S DNA (Scole et al, 1988)	Z11435
Ribosomal RNA (rRNA) gene (Hsiao et al. 1995)	L36489
Gene specific function	
Chloroplast glutathione reductase (GR) mRNA, partial cds; nuclear gene for chloroplast product	EF555119
Acetyl-CoA carboxylase gene, partial cds; nuclear gene for plastid product	DQ456971

(*continued*)

Table 4.6 (continued)

D. villosum	Code for the NCBI GeneBank Seq.
Gibberellin 3-beta-hydroxylase gene, complete cds	EU142950
Gibberellin 20 oxidase gene, complete cds	GQ365640; GQ365636; GQ365621; GQ365622; GQ365624; GQ365625; GQ365626; GQ365634; GQ365627; GQ365628; GQ365633; GQ365639; GQ365629; GQ365630; GQ365631; GQ365632; GQ365637; GQ365635EU142949
Pto kinase interactor 1 (*Pti1*) mRNA, complete cds	FJ711059
kinase-START 1 (*WKS1*) gene, partial cds	FJ154106FJ154105
3-phosphoglycerate kinase (*Pgk1*) gene, partial cds	FJ711027
Serine/threonine protein kinase gene, complete cds (5101 bp sequence of Hv-S/TPK)	EU153366
Putative ethylene responsive element binding protein (EREBP) mRNA, complete cds	FJ711058
Leucine-rich repeat protein (LRR2) mRNA, partial cds	FJ711057
Phytochrome B (*phyB*) gene, partial cds	EF442837
M14S cyclophilin 5 mRNA, complete cds. Cyclophilins are cellular receptors for the immunosuppressive drug, cyclosporine A (CsA), with intrinsic peptidyl-prolyl cis-trans isomerase activity. CYPs are known to be involved in numerous aspects of signal transduction pathways and protein folding, interacting with many other signal transduction components in various eukaryotes	EU070909; EU070908**:** EU070905; EU070906; EU070907
Glutathione S-transferase (*gst*) mRNA, complete c	EU070904
Blue copper-binding protein (bcb) mRNA, complete cds	EU070903
GH phosphoenolpyruvate carboxylase (*pepC*) gene, exons 2 through 4 and partial cds	AY548403; AY553240
atpI-atpH intergenic region	AB207718; AB207472AB207349AB207595
Alcohol dehydrogenase 1 (*Adh1*) gene, partial cds	AY294174
TCP1 gene, partial cds; similar to teosinte branched 1	AF543436
Disease resistance	
A family of disease resistance gene analogs from *Dv* telosome 6VS	AY040671; AY040672
Carbohydrate metabolism	
Fructan 6-fructosyltransferase (6-SFT) gene, partial cds. Sucrose: fructan 6-fructosyltransferase (6-SFT), that catalyzes the formation and extension of b-2,6-linked fructans in barley (Hordeum vulgare L.) The 20 bp length polymorphism in the 6-SFT intron cosegregated with the ABG704, ABG312, and Plc markers and the Rpg1 locus on barley chromosome 1 (7H) in the "Steptoe" × "Morex" DH mapping population (Wei et al. 2000)	AF222943
Beta amylase gene, partial cds	AY821699; AY821698
Granule-bound starch synthase (GBSSI) gene, exons 9 through 14 and partial cds	AY556480
Granule-bound starch synthase (waxy) gene, partial cds	AF079274
Seed-storage protein synthesis	
Dvgli2 α-gliadin pseudogene, partial sequence	EU325713; EU325709; EU325712; EU325711; EU325697; EU325710; EU325708; EU325696;. EU325689; EU325690;EU325691; EU325688; EU325692; EU325695; EU325693;EU325694
VLG-2 low-molecular-weight glutenin subunit gene, partial cds	GQ169796; GQ169790
y-type HMW glutenin subunit pseudogene, partial sequence	AY608740
High molecular weight glutenin gene, complete cds	FJ804126
Gamma gliadin gene, partial cds	FJ804125
High molecular weight glutenin gene, complete cds	FJ804124
High-molecular-weight glutenin subunit gene, complete cds	FJ600492; FJ600491; FJ600490; FJ600489

(*continued*)

Table 4.6 (continued)

D. villosum	Code for the NCBI GeneBank Seq.
γ-type high-molecular-weight glutenin subunit gene, promoter region and 5′ UTR	EU074236
I-type low-molecular-weight glutenin subunit gene, partial cds	FJ600497; FJ600496
New members of the *Stowaway* family in an intron of the DMC1 gene (disrupted meiotic cDNA1) in species of the Triticeae.	AF277238
Revolver-cDNA mRNA for transposase, complete cds	AB304273
Molecular polymorphism and genome and chromosome typing	
pDv19, pDv40, pDv24, pDv27 identifying *Dasypyrum* choromatin in the wheat genome	AB074442; AB074441; AB074439; AB074440
STS marker for V-genome of Dv	BV679208
ISSR marker pDv848/388 genomic sequence	EF411201
Genome-specific STS marker for V-genome	BV679208
RAPD marker M2 genomic sequence	EF566895
Species-specific sequence	AF358924; AF358923
Tandem repeat sequence	AF472572;
Chromosome-specific RAPD marker genomic sequence	DQ234072
RAPD marker RM874	DQ869606; DQ869605; DQ869589DQ869590; DQ869591; DQ869588; DQ869592; DQ869593; DQ869594; DQ869595;DQ869596; DQ869597; DQ869598 DQ869585DQ869586;DQ869599; DQ869600; DQ869601; DQ869604; DQ869602;DQ869603; DQ869587
D. breviaristatum	
Chloroplast sequences	
InfA gene, complete cds; chloroplast	DQ269011
Mitochondrial sequences	
tRNA-Met gene, complete sequence; and 18S ribosomal RNA gene, partial sequence; mitochondrial	DQ307261
Nuclear sequences	
Translation	
Eukaryotic translation initiation factor eIF5A gene, partial cds; nuclear	DQ414518; DQ234390
Seed-storage protein synthesis	
Dbgli2 α-gliadin pseudogene, partial sequence (Li G R et al. 2009)	EU325719; EU325699; EU325700; EU325701; EU325702 EU325703; EU325704; EU325705; EU325706 EU186106; EU186105; EU186108; EU325707; EU325698; EU325714; EU325715; EU325716; EU325717 EU186102; EU186103; EU325718; EU186104
Glutenin subunit 1*Dby2* gene, complete cds	EF524116 EF524115
Disease resistance	
Y10-like protein (RGAYr10) gene, complete cds; similar to wheat stripe rust resistance protein	EU428764
Molecular polymorphism and genome and chromosome typing	
Satellite DNA (clone pDbKB49), Galasso et al. (1997) distributed along chromosomes, reduced in terminal regions	Z71270; Z71269
Satellite DNA (clone pDbKB45), Galasso et al. (1997) distributed along chromosomes, amplified in terminal regions	Z71269
Clone J10-2 genomic sequence New repetitive DNA sequence from *Db*-genome	EU313788
ISSR marker pDu848/364	EF371927
RAPD marker OPH12 genomic sequence; allows detection of *Db* chromatin in *Triticum*	DQ167398

Previous phylogenetic analyses based on molecular data suggested that *Secale* is the closest relative of the *Triticum–Aegilops* genera (Kellogg et al. 1996; Huang et al. 2002; Mason-Gamer et al. 2002), indicating the relatively close relationship between the three genera. Therefore, *Secale* species are used as in-group in phylogenetic analyses. When *Hordeum* and *Dasypyrum* are used with the *Secale, Triticum*, and *Aegilops* genera, they stand at the base of the tree topology as out-groups (Yamane and Kawahara 2005; Kawahara et al. 2008). This implies a much earlier divergence between *Dv* and the common ancestor of *Triticum–Aegilops*.

Two cpDNA data sets, one based on restriction site variation (Mason-Gamer and Kellogg 1996) and the other on sequences encoding the rpoA-subunit of RNA-polymerase (Petersen and Seberg 1997), placed *Lophopyrum* (*Thinopyrum*) and *Dasypyrum* together on cpDNA cladograms. Sequencing of the nuclear starch synthase gene has also revealed a close affinity between *Lophopyrum* and *Dasypyrum* (Mason-Gamer and Kellogg 2000).

Usually, molecular data sets from chloroplast genomes give consistent results, while comparisons between cpDNA and nuclear data give incongruent placement of taxa on cladograms. For instance, the sequence of the starch synthase gene supports the basal position of *Psathyrostachys*, followed by the clade containing *Hordeum* and *Critesion,* which is consistent with the tree from ITS (Hsiao et al. 1995) and 5S short-spacer (Kellogg et al. 1996) nuclear sequences and the cpDNA data (Mason-Gamer and Kellogg. 1996). However, while for the cpDNA tree two *Dasypyrum* accessions appear monophyletic, the starch synthase exon data reject this placement and *Dasypyrum* appears polyphyletic. Furthermore, the cpDNA tree contained a well supported clade including *Dasypyrum*, *Thinopyrum*, and *Pseudoroegneria*, which was not found in cladograms based on sequences for the starch synthase gene, although one accession grouped with *Thinopyrum* and the other with *Pseudoroegneria* (Mason-Gamer and Kellogg 2000). The *Dasypyrum–Pseudoroegneria* monophyly was also observed in cladograms obtained from: (a) RFLP similarity pattern based on 14 cloned fragments covering the entire cpDNA of *T. aestivum* (Kellogg1992), morphological data (Kellogg 1989), and 5S RNA (Appels and Baum 1991).

Nuclear DNA Sequence Affinities and Chromatin Organization

The nuclear DNA data (Hsiao et al. 1995; Kellogg and Appels 1995) are incongruent with the cpDNA data, as these studies suggest different affinities of *Lophopyrum* and *Dasypyrum* within Triticeae. Evidently, there are considerable discrepancies among different studies treating the relationships among *Agropyron, Dasypyrum*, and *Lophopyrum,* depending on the cellular genome where the molecular markers reside.

Partial sequences of disrupted meiotic cDNA1 (DMC1), where at least two excisions events of the *Stowaway* family of MITEs have occurred, was carried out for 30 species of the Triticeae (Petersen and Seberg 2000). Six *Stowaway* elements were found in five species of Triticeae; all were located in intron 14 of the *DMC1* gene. Species of the *Taeniatherum* and *Australopyrum* genera share the presence of short elements of 76, 59, 59, and 75 bp, respectively, inserted some 20 bases downstream of the 5′ end of intron 14 of the *DMC1* gene. Seven species (*Peridictyon sanctum, Thinopyrum bessarabicum, Crithodium monococcum, Lophopyrum elongatum, Crithopsis delileana, Comopyrum comosum*, and *Patropyrum tauschii*) share footprints (a C preceding the TA recognition site) left after excision of the short *Stowaway* element. This element and the footprint of its excision are absent in *Dv*. However, two other *Stowaway* elements were discovered in *Heteranthelium piliferum*, 100 bases downstream of the insertion site of the short element in *Australopyrum* and *Taeniatherum*. One element of 123 bp seems to be inserted into a 160 bp element. A 4 bp footprint sequence corresponding to the *Heteranthelium* element is present in *Dasypyrum* but absent in the seven species mentioned above. It should be interesting to see if this *Dv* specific footprint is also present in *Db*(2*x*) and *Db*(4*x*). *Heteranthelium piliferum* and *Dv* stand at one extreme of the phylogenetic relationships based on variation in the PCR sequences of 6-SFT (sucrose:fructan 6-fructosyltransferase). This enzyme catalyzes the formation and extension of β-2,6-linked fructans (levans) and is important to fructan synthesis in many cool-season Triticeae species where diploid species of *Secale*, *Triticum*, and *Aegilops* are at the other extreme (Wei et al. 2000). Molecular phylogeny of the RPB2 (the second largest subunit of RNA polymerase II) gene sequence reveals

multiple origin and geographic differentiation of the H-genome. The V-genome of *Dv* is sister to the St-genome and both diverged from the H-genome (Sun et al. 2008). This result is in line with the phylogenetic relationships of the monogenomic species of the Triticeae inferred from nuclear rDNA (internal transcribed spacer) sequences, where *Heteranthelium* and *Dasypyrum* were closely related to *Pseudoroegneria*. An intraspecific polymorphism was established for the *Dv* nuclear rDNA (Delre et al. 1988).

Linde-Laursen and Frederiksen (1991) observed that the chromosomes of *Dv* and *S. cereale* tend to have large bands of heterochromatin preferentially in the subtelomeric region, while the wheat chromosomes show smaller heterochromatin bands located in the middle or proximal portion of the arms. Vershinin and Heslop-Harrison (1998) found that the DNA of *Dv* and *S. cereale* is digested by micrococcal nuclease much faster than the wheat DNA. In DNA/DNA hybridization experiments in which the genomes of rye and *Dv* were hybridized with labeled nuclear DNA from wheat and rye, revealed greater homology between the V- and R- than between V-, A-, B-, and D-genomes (Lucas and Jahier 1988). FISH analysis in which the genomes of different species of the Triticeae were hybridized with species-specific molecular probes prepared from tandem repeated DNA sequences of *Dv* (pHv62) and *S. cereale* (pSc119.2), exhibited a greater homology between the R- and V-genomes than the homology among R- or V-genomes and those of *Triticum* and *Aegilops* (Uslu et al. 1999). Major telomeric hybridization signals of pHv62 were visualized on four pairs of chromosomes in *Th. bessarabicum* and also in the CS/*Th. bessarabicum* amphiploid. When pHv62 was similarly hybridized to chromosome preparations of *Db*(*4x*), it revealed only one major and two minor sites of hybridization. On this basis, Uslu et al. (1999) reasoned that although Löve (1984) placed *Db*(4*x*) taxonomically in the same genus as *Dv*, the above results suggest that it is less close to *Dv* than *Th. bessarabicum* and *S. cereale* to *Dv*. Moreover, the limited *Db*(4x) chromosome labeling achieved with pHv62 probe, suggested that the genomes of *Db*(4*x*) have little homology with that of *Dv*. Absence of FISH signals when *Dv* chromosomes were probed with the labeled *Db*(4*x*) repeated element pDb12H (Yang et al. 2006) and pDBKB45 (Galasso et al. 1997) were further demonstration of chromosomal divergence between *Dv* and *Db*(4*x*).

Analysis of phylogenetic relationships in the Triticeae tribe using RFLPs (Monte et al. 1993) placed *Dv* alone in its clade near the *Critesion* and *Hordeum* clades, at the opposite side of the *Triticum* clade position in the cladogram.

Chromosome Pairing

Earlier patterns of phylogenetic differentiation in the tribe Triticeae based on chromosome pairing have been reported by Sakamoto (1973, 1991). Gupta (1972), based on review of the studies on chromosome pairing in hybrids between members of the subtribe Triticinae, demonstrated that synteny and pairing exist for most of the chromosomes of the species within the subtribe. The only exception is *Dv*, which showed also poor crossability. Hybridization experiments performed by Lucas and Jahier (1988) involving *Dv* and other diploid Triticeae species, including the relatives of the putative donor species of the A- and B-genomes of wheat, indicated a low average number of chromosomes pairing between homoeologous arms when *Dv* was one of the parents, compared to good mean pairing, ranging from 3.14^{II} to 7^{II}, expressed in hybrids with the same species but having *Ae. squarrosa* as common parent (Table 4.7). *Dv* chromosomes showed an average number of homoeologous chromosome associations of 2.47, 1.70, and 1.24, respectively, with *T. boeoticum* (genome-A), *T. urartu* (genome-A^u), and *Ae. squarrosa* (genome-D). In those experiments, hybrids between *Dv* and *Ae. longissima* (genome-S) were not obtained, and it is not possible to determine the degree of similarity between the V-genome and the genome of the sibling species of *Ae. speltoides*, the putative donor of genome-B of wheat. However, in *Ae. longissima* × *T. boeoticum*, the pairing was 1.29. Therefore, *Dv* seems to be taxonomically as distant as *Ae. longissima* from the relatives of the species donor of the A-genome.

Low levels of homology between the chromosomes of *S. cereale* and *Dv* have been reported in hybridization experiments carried out over the decades since 1951, when Nakajima (1953) first studied meiosis in the *S. cereale* × *Dv* F_1 hybrid. He noted, on average, that for each PMC in meiosis, only one bivalent was formed. The average number of associations between chromosome arms for the hybrid *S. cereale* × *Ae.*

Table 4.7 Average number of bivalent configurations observed at MI of F_1 obtained from the hybridization of *Dv* to some Triticeae species related to the putative donor species of the A-, B-, and D-genome of wheat (From Lucas and Jahier 1988)

Species	*T. urartu*	*T. boeticum 165*	*T. boeticum 166*	*Ae. longissima*	*Ae. squarrosa*	*Ae. umbellulata*	*Ae. uniaristata*	*Ae. comosa*	*Ae. caudata*	*S. cereale*	*H. villosa*
T. urartu	–	–	–	–	6.92	–	–	–	–	–	1.7
T. boeticum 165	13.19	–	–	–	3.14	–	–	–	–	–	–
T. boeticum 166	13.43	–	–	1.29	4.32	4.12	2.35	–	2.88	–	2.47
Ae. squarrosa	7.23	–	–	7.38	–	7.01	3.53	5.78	5.82	0.62	1.24
Ae. umbellulata	4.58	4.56	4.12	2.38	6.51	–	3.64	3.26	–	1.33	1.31
Ae. uniaristata	–	–	2.89	3.13	–	–	–	5.37	–	0.69	0.71
Ae. comosa	5.27	–	2.63	–	5.54	4.08	–	–	4.49	–	–
Ae. caudata	–	–	2.43	–	–	5.96	–	–	–	–	1.18

squarrosa was one-half than that found for *Dv* × *Ae squarrosa* (0.62 against 1.24) (Lucas and Jahier 1988). Extremely low pairing ability was observed between V and R chromosomes in PMCs of hybrid plants generated by crossing two amphidiploids: *Ae. uniaristata* × *Dv* ($2n = 4x = 28$, U^nU^nVV) and *Ae. uniaristata* × *S. cereale* ($2n = 4x = 28$, U^nU^nRR) (Jahier et al. 1988). Similar findings were drawn from the cytological PMCs in meiosis of the hybrid triticale [AABBRR] × (*T. turgidum* ssp. *durum* × *Dv*) [AABBVV]. It thus appears that possibilities of pairing between the chromosomes of *Dv* and those of wheat or of the *Aegilops* are low but greater than that between rye chromosomes and those of *Dv*. However, Jahier et al. (1988) did not reject the working hypothesis that V and R chromosomes share homologous sequences. Rather, they attribute the V and R asynapsis to causes unrelated to non-homology, such as asynchronous meiotic rhythm between R- and V-genomes. Similar causes can explain lack of pairing between V^v and V^b reported in Sect. 4.2.8.1.

Nakajima (1960c) attempted several hybrid combinations between six tetraploid *Triticum* species and *Db* (4*x*) and was able to obtain adult F_1 plants only in the hybrid involving *T. turgidum*. The cytological observation at meiosis of the F_1 indicated no homology between the chromosomes of AB-genomes and those of V^b-genome. The combination *T. turgidum* var. *durum* × *Db*(4*x*) was produced also by Blanco and Simeone (1995), and an average of 11.50 chiasmata/cell and 7.86 bivalents, and 11.37 chiasmata and 7.68 bivalents, and 0.07 trivalents were observed in two hybrids with and without the *Ph* gene, respectively. The high pairing mostly involved autosyndesis among the V^bV^b-genomes.

The combination *T. aestivum* × *Db*(4*x*) was produced (von Bothmer and Claesson 1990) and seed set of hybrid F_1 was 2.9%. Two F_1 hybrid plants displayed a high level of pairing of up to 12 bivalents and average of 7.8 bivalents, with means of 10.42 and 13.34 chiasmata per cell, respectively. Pairing was attributed to V^bV^b or ABD autosyndesis. Other information on chromosome pairing in interspecific hybrids is reported in the following paragraphs and Table 4.8.

4.2.9 *Use of* Dv *and* Db *as Parents in Interspecific/Intergeneric Crosses Facilitating Gene Transfer to Crop Species and Preparation of Mapping Populations*

The production of the earliest interspecific and intergeneric hybrids was accomplished by the aid of the simplest techniques of emasculation and pollination that were in conventional use in wheat breeding programs. With these techniques, many important hybrids were and still are being made. A tabulation of hybrids involving *Dv* and *Db* and their relatives is reported in Table 4.8. In the list of this large range of hybrids, various levels of difficulty have been found in attaining the formation of hybrid embryos.

Several researchers have used *Dv* in interspecific hybridization with the aim of reporting the origin and unique morphological and chromosomal pairing characteristics of the hybrids. Those studies interested the evolutionists, biosystematists, and cytogeneticists, but they rarely went further in the backcrossing and selection programs for releasing cultivars. Von Bothmer

Table 4.8 Phenotype and chromosome configurations of F_1 and F_2 progenies after hybridization of *Dv* to species of other Triticeae genera

Triticeae parents[1]					F_1									
Triticeae species used as seed parent (rarely used as ♂)				*Dv* ecotype	Embryo formation		Chromosome configuration at meiosis					Number of F_1 plants and phenotype	Amphiploid obtained	Aneuploid derivative	Reference
Essential names and synonyms of the species	Genome symbol	Ploidy level (*n*)	Chrom number (2*n*)		Embryo rescue	Mature seed (% seed set)	I	II rod	II ring	III	IV				
Triticum monococcum	A-	Diploid	14		No	No									Oehler (1935)
Triticum monococcum	A-	Diploid	14		No	Yes	9.12	1.78	0.14	0.08					Sears (1941b)
Triticum monococcum	**A-**	Diploid	14		No	Yes									Piralov (1980)
Triticum monococcum	A-	Diploid	14		(No)	Yes (5.2)		0.76 (av.)				9			von Bothmer and Claesson (1990)
Triticum boeoticum	A-	Diploid	14		No	Yes		2.47							Lucas and Jahier (1988)
Triticum urartu	A-	Diploid	14					1.70							Lucas and Jahier (1988)
Triticum urartu	A-	Diploid	14		No	No									von Bothmer and Claesson (1990)
Triticum monococcum ssp aegilopoides (Link) Thel.	A-	Diploid	14		–	Yes		Up to 5							Sando (1935b)
Triticum monococcum ssp aegilopoides (Link) Thel.	A-	Diploid	14		–	Yes		1 to 6							Kihara (1937)
Triticum monococcum ssp aegilopoides (Link) Thel.	A-	Diploid	14		–	Yes	11.56	1.14	0.02	0.04					Sears (1941a)
Triticum polonicum (♀)	AB-	Tetraploid	28		No	No						Sterile			Oehler (1935)
Triticum polonicum (♂)	AB-	Tetraploid	28		No	No						Sterile			Oehler (1935)
Triticum polonicum CI 7498	AB-	Tetraploid	28		No	9.3% [b]						Sterile			Sando (1935b)
Triticum persicum (=*T. carthlicum Nevski*)	AB-	Tetraploid	28		No	Yes						Fertile; $2n = 21$	70% F_2 seeds had 33–45 chrom. and produced fertile plants		Nakajima (1959b Tab. 4) 1966b
Triticum didoccoides var. spontaneum	AB-	Tetraploid	28			7.2% [b]									Sando (1935b)
Triticum dicoccoides (later identified as *T. dicoccon*)	AB-	Tetraploid	28		No	Yes		0.79					Yes (by colchicine)	Yes (after crossing to "Chinese Spring")	McFadden and Sears (1947); Sears (1953, 1976). Sears (1985, personal communication); The Dv parent was a Greek ecotype. The V chromosomes in CS-DA were identified for their homoeology to wheat homoeology. groups by Sears 1982.
Triticum didoccoides	AB-	Tetraploid	28		No	Yes						Fertile; $2n = 21$	F_2 seeds $2n = 33$–45; 17% floret gave F_3 plant		Nakajima (1966a, b)
Triticum pyramidale (=*T. dicoccoides* race *pyramidale* Percival 1921)	AB-	Tetraploid	28		No	Yes						Sterile; $2n = 21$			Nakajima (1966a)
Triticum dicoccon cv khapli	AB-	Tetraploid	28			7.4% [b]									Sando (1935b)
Triticum dicoccon	AB-	Tetraploid	28					Some							Kostoff (1937a,b,c); Kostoff and Arutiunova (1937a)
Triticum dicoccon	AB-	Tetraploid	28		No	Yes									

(*continued*)

Table 4.8 (continued)

Triticeae parents[1]					F_1								Amphiploid obtained	Aneuploid derivative	Reference
Triticeae species used as seed parent (rarely used as ♂)				*Dv* ecotype	Embryo formation		Chromosome configuration at meiosis					Number of F_1 plants and phenotype			
Essential names and synonyms of the species	Genome symbol	Ploidy level (*n*)	Chrom number (2*n*)		Embryo rescue	Mature seed (% seed set)	I	II rod	II ring	III	IV				
													Yes; named *Haynatricum*; self pollinated vitreous grains		Zhukowsky (1944) (in Aase 1946)
Triticum dicoccon var. atratum	AB-	Tetraploid	28		No	Yes						Fertile; $2n = 21$			Nakajima (1966a, b)
Triticum turgidum ssp. dicoccon (=*T. dicoccum var. durum*)	AB-	Tetraploid	28		No	Yes [3]							Spontaneous chromosomes doubling; *Haynotricum* Zhuk.		Vakar (1966)
Triticum dicoccon	AB-	Tetraploid	28		No	Yes									Liu and Chen (1984)
Triticum turgidum ssp. dicoccon	AB-	Tetraploid	28		No	38 (20% seed set)		1.55				55% F_1 seeds gave plant			von Bothmer and Claesson (1990)
Triticum turgidum	AB-	Tetraploid	28		No	No									Cross made before 1900 (Tschermak 1921)
Triticum turgidum var. durum cv "Frumento nero	AB-	Tetraploid	28		No	Yes									Cross made in 1908 (Raineri 1914; Strampelli 1932)
Triticum turgidum var. durum	AB-	Tetraploid	28		No	Yes						Turgidovillosum Sterile			Cross made in 1916 (Tschermak 1929, 1930)
Triticum turgidum var. durum	AB-	Tetraploid	28		No	Yes						Triticum turidovillosum Fertile	Fertile F_2 plants due to chromosome doubling. First hexaploid amphiploid ABBVV.		Cross made in 1929 (Tschermak 1929 and Tschermak-Seysenegg 1934); Aase (1946); Tschermak (1930, Ref. 116 di Aase 1935)
Triticum turgidum var. durum	AB-	Tetraploid	28										Haynaldtricum turgido-vilosum		Tschermak-Seysenegg 1934; von Berg 1934
Triticum turgidum	AB-	Tetraploid	28		No	Yes									Bleier (1930a, b)
Triticum turgidum	AB-	Tetraploid	28		No	Yes						V-genome not homologous to A- and B-genome			von Berg (1934)
Triticum turgidum ♀	AB-	Tetraploid	28		No	6.34%						3 F1 plants			Oehler (1935)
Triticum turgidum ♀	AB-	Tetraploid	28		No	0% (47 floret)									Oehler (1935)
Triticum turgidum cv Alaska	AB-	Tetraploid	28		No	14.2% [b]						Fertile F1			Sando (1935b)
Triticum turgidum	AB-	Tetraploid	28		No	Yes						$2n = 21$; fertile	Pl. morph. similar to DV; Fertile F_2; $2n = 33–45$		Nakajima (1966a)
Triticum turgidum	AB-	Tetraploid	28	Accession GP005 Cambridge Bot. Garden, UK	No	Yes							No	Yes by backcross to bread wheat	Liu and Chen (1983, 1984); Liu et al. (1988); DA, DS and T lines were assigned to wheat homoel. group by Qi et al. (1995a)
Triticum turgidum var. aethiopicum	AB-	Tetraploid	28		No	11 (50% seed set)						79% F1 seeds gave plants			von Bothmer and Claesson (1990)
Triticum durum	AB-	Tetraploid	28		No	1.13% [b]						3 F1 plants			Oehler (1935)

Triticum durum	AB-	Tetraploid	28			0% (47 poll. florets)							Oehler (1935)
Triticum durum cv Arnautka	AB-	Tetraploid	28		No	12.7%[b]				Sterile F1			Sando (1935b)
Triticum durum cv Mindum	AB-	Tetraploid	28		No	8.3%[b]				Sterile F1			Sando (1935b)
Triticum durum cv Kubanka	AB-	Tetraploid	28		No	18.3%[b]				Sterile F1			Sando (1935b)
Tetraploid wheat	AB-	Tetraploid	28										Kihara and Nishiyama (1937)
Triticum durum	AB-	Tetraploid	28		No	Yes				$2n = 21$; fertile	Pl. morph. similar to DV; Fertile F_2		Nakajima (1959a, b)
Triticum durum	AB-	Tetraploid	28		No	Yes				$2n = 21$; fertile	Pl. morph. similar to DV; Fertile F_2; $2n = 33–45$		Nakajima (1966a)
Triticum durum cv Cappelli	AB-	Tetraploid	28							Fertile	Lines showing 28″		Alessandroni et al. (1966)
Triticum durum "Mexicali," "81086°," and "D311"	AB-	Tetraploid	28	Ecotype collected in the territory of the former USSR	YES	Yes (after colchiine treat. of F_1 calli and regenera-tion of F_1 plants with chrom. doubled secors)				Fertile		Yes, after BC the TH1 and TH3 amphiploids to several *T. aestivum* lines including CS and Wan7107	Chen et al. (1996a, b); Li et al. (2000a, b, c). The DS lines 94G22-1, and 94G33-1 were identified by Shang et al. (1997). The translocation lines were identified by Li et al. (1999)
T. durum cv "Sauwne20" (Td_S)	AB-	Tetraploid	28	Ecotype collected by researchers at CAAS, Beijing, China	YES	Yes				Fertile		Yes	Ma et al. (1997). The DS lines GN21 e GN22 were developed after crossing the Td_S × Dv_C to *T. aestivum* (see Li et al. 2002a).
Triticum turgidum var. durum cv Cappelli	AB-	Tetraploid	28		No	Yes				Rare seeds	Fertile *Haynaldoticum sardoum* Meletti and Onnis (1975)		Meletti et al. (1977), Stefani et al. (1983)
Triticum turgidum var. durum cv Cappelli	AB-	Tetraploid	28		No	Yes	21 (37%)	3		Pollen viability 4.2%, 9.5% floret set seeds			Stefani et al. (1986)
Triticum turgidum var. durum (♂)	AB-	Tetraploid	28		No	Yes						Durum alloplasmic fertile lines (durum genome and DV cytoplasm)	Sasakuma and Maan (1978)
Triticum turgidum var. durum cv. Creso	AB-	Tetraploid	28	Ecotype H07	No	Yes	13.62	3.30″	0.26	$2n = 21$; 1–2 F_2 seeds upon selfing	$2n = 40–44$		Blanco et al. (1983a, b)
Triticum turgidum var. durum cv Cappelli ph1	AB-	Tetraploid	28	Ecotype H17	No	Yes		3.30					Blanco et al. (1988b)
Triticum turgidum var. durum cv. Creso ph1 Ph1	AB-	Tetraploid	28		No	Yes		0.38				Yes by backcrossing the amphiploid to the *durum* parent	Blanco et al. (1988b); V chromosomes in wheat MA were assigned to wheat homoeologous groups by Blanco et al. (1991)

(*continued*)

Table 4.8 (continued)

Triticeae parents[1]					F_1								Amphiploid obtained	Aneuploid derivative	Reference
Triticeae species used as seed parent (rarely used as ♂)				*Dv* ecotype	Embryo formation		Chromosome configuration at meiosis					Number of F_1 plants and phenotype			
Essential names and synonyms of the species	Genome symbol	Ploidy level (*n*)	Chrom number (2*n*)		Embryo rescue	Mature seed (% seed set)	I	II rod	II ring	III	IV				
Triticum turgidum var. durum cv. Appulo ph1 Ph1	AB-	Tetraploid	28					2.78							Blanco et al. (1988b)
Triticum turgidum var. durum	AB-	Tetraploid	28		No	Yes									Liu and Chen (1984)
Triticum turgidum var. durum cv. CI 1320	AB-	Tetraploid	28		No	Yes						Fertile	Yes; $2n = 42$; 21^{II} 80% of cells		Liu and Chen (1986)
Triticum turgidum var. durum cv. Modoc	AB-	Tetraploid	28	Ecotype from Apulia (Italy)	3 culturable embryos	No						2 F1 plants	Yes colchicine		Jan et al. (1986)
Triticum turgidum var. durum	AB-	Tetraploid	28		No	9 (1.5% seed set)						78% F1 seed gave plants			von Bothmer and Claesson (1998)
Triticum turgidum var. durum cv. Modoc	AB-	Tetraploid	28	Ecotype from Bomarzo (Italy)	No	11% seed set						1.24–3.7% unreduced gametes	At frequency of 0.046%		De Pace et al. (2003), 10 th IWGS
Triticum araraticum	AG-	Tetraploid	28		No	Yes						Died at seedling stage			Piralov (1980)
Triticum timopheevi ssp araraticum	AG-	Tetraploid	28			23 (22.8% seed set)						17% of the F_1 seeds gave plants			von Bothmer and Claesson (1990)
Triticum timopheevi	AG-	Tetraploid	28		No	6.3% (1)						"			Sando (1935b)
Triticum timopheevi	AG-	Tetraploid	28		No	Yes		some	some			"			Kostoff (1937a,b,c); Kostoff and Arutiunova (1937b)
Triticum timopheevi	AG-	Tetraploid	28		No	Yes						Florets without pistil and anthers; $2n = 21$			Nakajima (1953)
Triticum timopheevi	AG-	Tetraploid	28		No	Yes						Sterile; $2n = 21$			Nakajima (1966a)
Triticum timopheevi ssp timopheevi	AG-	Tetraploid	28		No	33 (1.9% seed set)		D1 66				89% of the F_1 seeds gave plants			von Bothmer and Claesson (1990)
Triticum spelta	ABD-	Hexaploid	42												Cross made in 1929; Tschermak (1929); Tschermak-Seysenegg (1934)
Triticum spelta cv Alstroum	ABD-	Hexaploid	42		No	0 seed out of 95 florets						No mature plants			Sando (1935b)
Triticum compactum cv Coppei	ABD-	Hexaploid	42		No	Yes						No mature plants			Sando (1935b)
Triticum compactum	ABD-	Hexaploid	42		No	No									Oehler (1935)
Triticum aestivum cv Rieti	ABD-	Hexaploid	42		No	Yes						?	Yes	cv Cantore was released	Strampelli (1932)
Triticum aestivum cv Akagomughi	ABD-	Hexaploid	42		No	Yes								cv Roma after backcross to T. aest.	Strampelli (1932); Forlani (1954)
Triticum aestivum cv CI 6223	ABD-	Hexaploid	42		No	8 seeds (2.5% floret fert.)						1 plant died			Sando (1935b)
Triticum aestivum cv Hard Federation	ABD-	Hexaploid	42		No	1 seed out of 610 pollinated floret								The F_1 seed did not germinate	Sando (1935b)
Triticum aestivum cv Velvet Chaff	ABD-	Hexaploid	42		No										Sando (1935b)
Triticum aestivum cv Nittany	ABD-	Hexaploid	42		No										Sando (1935b)
Triticum aestivum	ABD-	Hexaploid	42		No	No									Oehler (1935)
Triticum aestivum cv Novinka	ABD-	Hexaploid	42		No	Yes	18–28	0–5		0–1	0–1				Kostoff (1937b)
Triticum aestivum cv C	ABD-	Hexaploid	42		No	Yes						Culm length 125.5 cm; spike length 9.7 cm; sterile			Nakajima 1962

Triticum aestivum cv C	ABD-	Hexaploid	42		No	Yes					1 F_1 plant gave 20 seeds	14 F2 mature pl. $2n = 29–52$		Nakajima 1966b
Triticum aestivum cv C	ABD-	Hexaploid	42								1 F_1 plant gave 26 seeds	10 F2 mature pl. $2n = 29–52$		Nakajima 1966b
Triticum aestivum monosomic for 5B	ABD-	Hexaploid	42		No	Yes	27.5	0.12		0.13	$2n = 28$ (has 5B)			Halloran 1966a, b
Triticum aestivum monosomic for 5B	ABD-	Hexaploid	42				9.6	3.8	1.06	0.86	0.7 $2n = 27$ (without 5B)			Halloran 1966a, b
Triticum aestivum	ABD-	Hexaploid	42		–	–								MacIndoe and Brown (1968)
Triticum aestivum	ABD-	Hexaploid	42		No	Yes					Yes	–	Yes	Chen and Liu (1982, 1986)
Triticum aestivum cv Anza	ABD-	Hexaploid	42		No	No								Jan et al. (1986)
Triticum aestivum cv Yecoro Rojo	ABD-	Hexaploid	42		No	No	22.5–27.8	0.11–2.4	0.17	0.1				Jan et al. (1986)
Triticum aestivum cv Chinese Spring	ABD-	Hexaploid	42		Yes (33 embryos out of 450 floret)	No					17 hybrid plants ($2n = 28$)	Yes (after colchicine treat. 2 F_1 plants were fertile)	No	Jan et al. (1986)
Triticum aestivum cv Chinese Spring	ABD-	Hexaploid	42										Yes	Lukaszewski (1988), Lukaszewski (2009, personal communication) *http://wheat.pw.usda.gov/ggpages/GeneticStocks/AJLukaszewski_Wheat_Stocks.doc*
Triticum aestivum cv Chinese Spring	ABD-	Hexaploid	42		Yes (12 seeds out of 600 floret)						8 hybrid plants ($2n = 28$) resmbling CS but with hairy glumes and short awns	Yes, 3 mature F_2 seeds without colchicine treat. of the F_1 plants	Yes	De Pace et al. (2001), Minelli et al. (2005). "CSxV32" and "CSxV63" were DS and DA lines for chromosome 6V. Chromosome additions for 1V and 3V telo were also obtained.
Aegilops bicornis	B-	Diploid	14		No	Yes					Sterile			Sando (1935b)
Aegilops caudata	C-	Diploid	14		No	Yes		1.18						Oheler (1933)
Aegilops cylindrica	C-	Diploid	14		No	Yes					2 F_1 sterile plants			Oheler (1933)
Aegilops squarrosa	D-	Diploid	14		No	Yes		1.24						Oehler (1935)
Aegilops squarrosa	D-	Diploid	14		No	Yes					Absence of chr. pairing			Kihara and Lilienfeld (1936)
Aegilops squarrosa	D-	Diploid	14				12		1					Zennyozi (1961)
Aegilops squarrosa	D-	Diploid	14		Yes		11.49	1.25			2 F_1 sterile plant, one treted with colchicine; The morphological differences between F_1 hybrids and their parents were significant	Yes, partially sterile		Deng et al. (2004)
Aegilops uniariatata	L-	Diploid	14		No	Yes		0.71			1 F_1 sterile plants			Oehler (1935)
Aegilops comosa	M-	Diploid	14		No	Yes					4 F1 sterile plants			Oehler (1935)
Aegilops umbellulata	U-	Diploid	14		No	Yes		1.3						Oehler (1935)
Aegilops umbellulata	U-	Diploid	14		No	Yes					Absence of chr. pairing			von Berg (1937)
Aegilops umbellulata	U-	Diploid	14		No	Yes	8		3		Treat. with colchicine, fertile			Sears (1941a, b)

(*continued*)

Table 4.8 (continued)

Triticeae parents[1]					F_1									
Triticeae species used as seed parent (rarely used as ♂)				*Dv* ecotype	Embryo formation		Chromosome configuration at meiosis					Number of F_1 plants and phenotype	Amphiploid obtained	Aneuploid derivative	Reference
Essential names and synonyms of the species	Genome symbol	Ploidy level (*n*)	Chrom number (2*n*)		Embryo rescue	Mature seed (% seed set)	I	II rod	II ring	III	IV				
													Tetraploid (sterile): 24′+2″ and 8′ +10″		
Aegilops umbellulata	U-	Diploid	14				14								Zennyozi (1961)
Aegilops umbellulata	U-	Diploid	14		No	Yes						Very low mean pairing			Lucas and Jahier (1988)
Aegilops ovata	MU-	Tetraploid	28		No	Yes						20–23 chr.			Bleier (1930a, b)
Aegilops ovata	MU-	Tetraploid	28		No	Yes						Sterile			Sando (1935b)
Aegilops triaristata	MU-	Tetraploid	28									?			Tschermak-Seysenegg (1934)
Aegilops ventricosa	DM-	Tetraploid	28		No	Yes						20–23 chr.			Bleier (1930a, b)
Aegilops ventricosa	DM-	Tetraploid	28		No	Yes									Tschermak-Seysenegg (1934)
Aegilops ventricosa	DM-	Tetraploid	28		No	Yes						3 F1 sterile plants			Oehler (1935)
Aegilops ventricosa	DM-	Tetraploid	28		No	Yes						Sterile			Sando (1935b)
Aegilops ventricosa	DM-	Tetraploid	28				8		5	1					Zennyozi (1961)
Secale cereale	R-	Diploid	14		No	No						No			Sando (1935b)
Secale cereale	R-	Diploid	14		No	Yes						Yes but died before maturity			Kostoff (1937)
Secale cereale	R-	Diploid	14		No	Yes		0–2							Nakajima (1951)
Secale fragile	R-	Diploid	14		No	35						35 plants with hairy neck; glab. Nodes			Sando (1935b)
Secale fragile	R-	Diploid	14		No	8.26% floret formed seeds		0 (79%)				Intermediate to the parents;			Nakajima (1959a)
								1–3 (21%)				Partially fertile			

[1]Essential synonyms, gene symbols, ploidy level and chromosome number from the nomenclatural synopsis of Löve (1984)
[2]Proportion of F_1 seeds formed per 100 pollinated florets
[3]Spontaneous hybridization

and Claesson (1990) made the most extensive attempts, after Sando (1935a) and Nakajima (1953), to cross *Dv* with several Triticeae species.

Current status on *Dv* hybridization to the members of the genera *Triticum*, *Secale*, *Aegilops*, and *Agropyrom*n is reported in Tables 4.7 and 4.8.

4.2.9.1 Diploid ($2n = 2x = 14$) *Triticum* ssp. × Dv Hybridization

Oehler (1933) reported the direct and reciprocal hybridization of *Dv* to *T. monococcum* with no F_1 seeds produced. Sando (1935a) succeeded in obtaining the *T. aegilopoides* × *Dv* F_1 with up to five bivalents per cell observed. Kihara (1937) repeated that hybridization and observed 1–6 bivalents at meiosis of the F_1.

Sears (1941a) crossed *T. monococcum* and *T. aegilopoides* with *Dv* and observed more bivalents (0–4 open bivalents, average 1.78; 0–1 closed bivalent, average 0.14) in the first cross than in the second (0–4 open bivalents, average 1.14; 0–1 closed bivalents, average 0.02). These numbers were lower than those observed by Sando (1935a) and Kihara (1937) for the first hybrid and higher for the second. Waines (1976) reviewed those observations and indicated that differences existed between *T. monococcum* and *T. aegilopoides* in controlling the amount of suppression of homoeologous chromosome pairing in interspecific hybrids with *Dv*. Piralov (1980) reported a cross between *T. monococcum* and *Dv* that gave rise to one F_1 seedling that died at later seedling stage. Lucas and Jahier (1988) reported 2.47 bivalent associations in *T. boeoticun* × *Dv* F_1, and 1.70 bivalent associations in *T. urartu* × *Dv* F_1 (Table 4.6). von Bothmer and Claesson (1990) reported crosses of *Dv* with *T. monococcum* ssp. *monococcum* and with *T. monococcum* ssp. *urartu* (both with A-genome) with success in the first case (5.2% seed set and nine plants obtained) and failure in the second. Up to three occasional rod bivalents (average 0.76) were observed in *T. monococcum* ssp. *monococcum* × *Dv* hybrids.

4.2.9.2 Tetraploid ($2n = 4x = 28$) *Triticum* ssp. × Dv Hybridization

The pioneer work on interspecific hybridization carried out by Nazareno Strampelli at the beginning of the twentieth century demonstrated that *Triticum* Spp. × *Dv* hybridization was possible and that it was an effective method to transfer (after careful backcrossing and selection steps) useful genes, frequently from *Dv* to both tetraploid and hexaploid wheat cultivars.

The first reported *Triticum* × *Dv* hybridization involved the tetraploid wheat *T. turgidum* var. *durum* cv. Frumento nero and *Dv* and was made by N. Strampelli in 1908 (Raineri 1914; Strampelli 1932). Since then, Strampelli used *Dv* as one parent for 27 other interspecific hybrid combinations. E. Tschermak, one of the rediscoverers of Mendel's laws, although in 1916 obtained the F_1 *T. turgidum* × *Dv* (Tschermak 1929, 1930) was rather skeptical about the success of such wide hybridization between *Dv* and both *T. durum* and *T. vulgare* (Strampelli 1944; Maliani and Bianchi 1979). In fact, Tschermak (1921) reported a detailed description on how to cross *Dv* and wheat, but no results of successfull hybridization to *durum* or *vulgare* wheat were reported. However, having attended in Rome an International Meeting of wheat specialists in 1927, he visited the Experimental Wheat Station of Rieti and was acquainted with Strampelli's wide hybridization programs involving *Dv*. On the basis of the materials and results observed in Italy, he was able in 1929 to repeat with success the *T. turgidum* var. *durum* × *Dv* and *T. spelta* × *Dv* hybridization (Tschermak 1929; Tschermak-Seysenegg 1934). The former hybrid was fertile and the progeny very stable. From the F_2 progeny, E. Tschermak in 1930 produced what Aase (1946) considered to be the first amphiploid hexaploid ever produced experimentally in the Triticeae group involving wheat (Tschermak 1930; Aase 1946). The amphiploid was named *T. turgidovillosum* ($n = 21$) because, at that time, von Tschermak considered both parents of the F_2 progeny belonging to the genusTriticum. However, he knew that the other name of *T. villosum* was *Haynaldia villosa* and he proposed another botanical name for his amphiploid: *Haynaldtricum turgidovillosum* (Tschermak-Seysenegg 1934). The cytology of this new amphiploid was studied by von Berg (1934, 1935).

A *Triticum turgidum* × *Dv* hybrid was also obtained in 1930 by Bleier (1930a, b) and in 1934 by von Berg (1934). Cytological studies on this last hybrid showed that the V-genome of *Dv* was not homologous to A- and B-genome of *Triticum* (von Berg 1935). Sometimes, the genome of *Dv* has been indicated Y (see Forlani 1954).

Von Berg (1934) reported detailed karyograms of *T. turgidum*, *Dv*, and *T. turgidovillosum* ($2n = 42$), and the same author (von Berg 1935) reported careful cytological analysis of the following material obtained by crossing his F_1 hybrids and the amphiploid described by Tschermak-Seysenegg (1934): F_1 *T. turgidum* × *Dv*; (F_1 *T. turgidum* × *Dv*) × F_6 *T. turgidovillosum* (93.3% of cells showed 21″, and the remaining cells displayed 38% 20″+2′, 20.2% 19″+14′, 6.9% 18″+6′, 1.5% 17″+8′); F_1 *T. turgidovillosum* × *T. durum*; F_1 *T. turgidovillosum* × *T. vulgare*; F_1 [(F_6 *T. turgidovillosum* × *T. vulgare*) × *T. vulgare*]; F_1 *Aegilotricum forma fertilis* × *T. turgidovillosum*, 49 chromosomes; *Aegilotricum forma fertilis* I and II ($n = 28$) were amphiploids between *Aegilops ovata* and *Triticum durum* cv. Arraseita obtained by Tschermak and Bleier (1926). According to Aase (1935), those were the first amphiploids described in the literature of Triticinae, but in those years, Sando (1935a), Oehler (1933), Kostoff (1936a, b, c, 1937), and Kostoff and Arutiunova (1937a, b) made other successful attempts in hybridizing *Dv* to tetraploid wheats (Table 4.8).

Cytological investigation on hybrids between tetraploid *Triticum* and *Dv* were carried out also by Kihara and Nishiyama (1937). McFadden and Sears (1947) produced the amphiploid *T. dicoccoides* × *Dv* by colchicine treatment. Later, it was recognized that, on the basis of the tough rachis, the female parent was *T. dicoccum* and not *T. dicoccoides*. This amphiploid was used as bridge in the hybridization to *T. aestivum* (Sears 1953); only 0.79^{II} per cell were observed.

Aase (1946) reported that Zhukowsky (1944) obtained a *T. dicoccum* × *Dv* amphiploid named *Haynatricum,* which was a self-pollinated annual with vitreous grains exceeding in length those of either of the parents.

Nakajima (1953) obtained the F_1 hybrid *T. timopheevi* × *Dv*, $n = 21$; the florets were without pistils and anthers and had 96 tillers; it also showed shorter stature and spike and lower number of spikelets per spike compared to both parents.

Nakajima (1959b) also reported data on morphological traits of two crosses involving *T. persicum* × *Dv* and *T. durum* × *Dv*. Nakajima (1966a, b) made a cytogenetical analysis of PMCs of F_1 and F_2 plants obtained from combination between each of the tetraploid wheats *T. durum*, *T. turgidum*, *T. persicum* (*T. carthlicum* Nevski), *T. dicoccum* var. *atratum, T. pyramidale*, and *T. timopheevi* with *Dv*. The F_1 plants of the above combinations had 21 chromosomes, except those involving *T. pyramidale* and *T. timopheevi*, were fertile, and resembled more closely the *Dv* parent. The number of bivalents observed during metaphase in meiosis of F_1 hybrids varied according to the *Triticum* species used as the mother plant. Trivalents and tetravalents were rarely observed. In most instances, F_2 seeds were formed on the F_1 plants (Nakajima 1966a).

Seventy percent of the F_2 plants from hybrids involving *T. persicum*, *T. turgidum*, and *T. durum* had 33–45 chromosomes and produced an average of 10–20 F_3 seeds in the first case, nine F_3 seeds in the second, and 1–10 F_3 seeds in the third (Nakajima 1966b). The F_2 from the hybrid involving *T. dicoccoides* produced only 17% of the F_3 with 33–45 chromosomes, and those plants were sterile.

Alessandroni et al. (1966) and Alessandroni and Scalfati (1975) were able to select (using the bulk method) 28^{II} lines in the progenies from 110 hybridizations of *Dv* to *T. durum*. Those lines showed (compared to *T. durum* cv. Cappelli) an average increase of 5.4% for seed yield, 10.7% for culm length, 10% for the number of tillers at sprouting stage, 23% for the straw/grain ratio, and 1.3% for test weight; and an average decrease of 47% for number of shriveled grains. *Dv* contributed to the improvement of spike fertility and winter hardiness but not to the reduction of plant height and sensitivity to rusts.

Meletti et al. (1977) developed a *T. durum* Desf. cv. Cappelli × *Dv* hybrid, which gave a rare spontaneous duplication (Stefani et al. 1983) from which fertile florets and caryopses were produced. From those caryopse sfertile amphiploids were obtained. Observations made by Stefani (1986) indicated that the frequency of fertile female gametes (unreduced gametes) from the F_1 was 9.5% as detected by pollinating F_1 spike with pollen from the *T. durum* × *Dv* amphiploid raised by Meletti et al. (1977).

Sasakuma and Maan (1978) reported the introgression (by crossing and backcrossing) of *T. durum* genomes into the *Dv* cytoplasm, and the resulting alloplasmic lines were fertile. These alloplasmic lines had more spikelets and longer spikes than lines with *Triticum* cytoplasm, earlier maturity, more spikelets, and more grains/spike than lines with *Aegilops* cytoplasm (Kofoid and Maan 1982). Piralov (1980)

obtained one F_1 *T. araraticum* × *Dv* seedling, which died at late seedling stage.

Intergeneric hybridization between *T. turgidum* var. *durum* Desf cv. Creso × *Dv* strain H07 (Blanco et al. 1983a, b) produced F_1 hybrid seeds on 5.6% of the pollinated florets. Seedlings from germination of those seeds showed a chromosome number of 21. An average of 13.62 univalents were observed and the mean chromosomal relationship was 3.30^{II} and 0.26^{III}, which was thought to be a high pairing frequency due to genes of *Dv* interacting with the homoeologous pairing suppressor allele *Ph1* on the long arm of chromosome 5B of wheat. As a matter of fact, the level of homoeologous pairing induced by the *ph1* mutant allele at meiosis of the F_1 from the *T. turgidum* var. *durum* Desf. cv. Cappelli *ph1*/*ph1* × *Dv* strain H17 hybridization (Blanco et al. 1988b) was as high (3.30 bivalents) as that reported for the hybrid indicated above. In other nine hybrids involving *T. turgidum* var. *durum* cv. Creso, Cappelli, and Appulo as *Ph1*/*Ph1* female parents, and *Dv* from six different ecotypes as the male parent, the number of bivalent associations ranged between 0.38″ in one case and from 0.60″ to 2.78″ per cell in the other eight hybrids (Blanco et al. 1988b). Considering that the number of bivalents observed by Lacadena and Ramos (1968) in euhaploid ($2n = 14$; AB) *T. turgidum* var *durum* was 0.37″, it was deduced that the frequency of *Dv* ecotypes with genes promoting homoeologous pairing in the presence of the *Ph1* allele was rather high. This result and the observation of trivalents in F_1 plants with the *ph1* allele on chromosome 5B allowed the inference that the chromosomes of the V-genome were homoeologous to those of the A- and B-genomes. Using C-banding technique on pollen mother cells of the F_1 hybrid of *T. turgidum* var. *durum* Desf. cv. Cappelli × *Dv* strain H17, Blanco et al. (1988b) were able to estimate that out of 194 bivalents observed, 82% involved A-B chromosome associations, 11.3% A-V, 1% B-V, 4.1% AA, 1.6% BB, and 0% VV, and deduced that: A- and B-genomes were closely related, the V-genome was more related to the A than to the B-genome, and that V chromosomes were involved in 12.3% of the observed bivalents. This result is in contrast with Halloran's statement (Halloran 1966b) "the genome of *Dv* behaves genetically in a similar fashion as that observed in *Aegilops longissima* and *Ae. bicornis* (Riley et al. 1961) and *Secale cereale* (Riley et al. 1959) in that it is unable to remove the inhibition upon the intergenomic pairing controlled by the gene, or genes, upon chromosome 5B of wheat." Meiotic non-reduction in the hybrids obtained by Blanco et al. (1983a, b) resulted in the production of male and female gametes with unreduced chromosome number and resulting in one or two F_2 seeds per selfed F_1 head (Blanco et al. 1983b). Phenotypically, the amphiploid plants from the F_2 seeds resembled the maternal wheat parent, and the chromosome number varied from 40 to 44 showing at metaphase 2.54^{I} and 19.63^{II} per PMC.

A *T. durum* × *Dv* amphiploid was also obtained by Liu and Chen (1986). Plants with normal fertility and characteristics from both parents, such as *T. durum* ear morphology and *Dv* immunity to *Blumeria graminis* (DC.) E. O. Speer f. sp. *tritici* Em. Marchal (syn. *Erysiphe graminis* DC. ex Merat f. sp. *tritici* Em. Marchal) (*Bgt*), the causal agent of powdery mildew, were obtained from self-pollinated or open-pollinated progenies of *T. durum* cv. CI 1320 × *Dv* F_1 hybrids. The chromosome number was $2n = 42$, with 21 bivalents in 80% of cases at metaphase I in PMCs. Observations over five generations indicated that the plants were stable in their characteristics. N-banding studies showed that they were amphidiploids with AABBVV-genomes. The plants were 100–110 cm tall, spikes 9–12 cm long, 20–24 spikelets/ear, kernel weight of 25–35 mg, and 20% grain protein concentration. In addition to immunity to *Bgt,* they had resistance to *P. striiformis* Westend. f. sp. *tritici* Eriks (*Pst*), and *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. & E. Henn. (*Pgt*).

Jan et al. (1986) obtained three culturable embryos out of 50 pollinated florets from the cross *T. turgidum* var. *durum* cv. Modoc × *Dv* (= M × *Dv*). Morphology of the spike and spike components of *T. turgidum* var. *durum* cv. Modoc, *Dv,* and the hexaploid amphiploid M × *Dv*, are displayed in Fig. 4.35. That was the first report on the use of embryo culture for raising F_1 hybrid plants *Triticum* × *Dasypyrum*. Two plants with 21 chromosomes were obtained and resembled Modoc with some traits of *Dv,* such as brittle rachis, bristles on the glume keel, and long (3 cm) glume beak. Chromosome elimination, as reported by Blanco et al. (1983a), was not observed. After colchicine treatment, both hybrid plants produced chromosome-doubled sectors with dehiscent anthers which, upon selfing, produced amphiploids with $2n = 6x = 42$ chromosomes and genomes AABBVV. The M × *Dv*

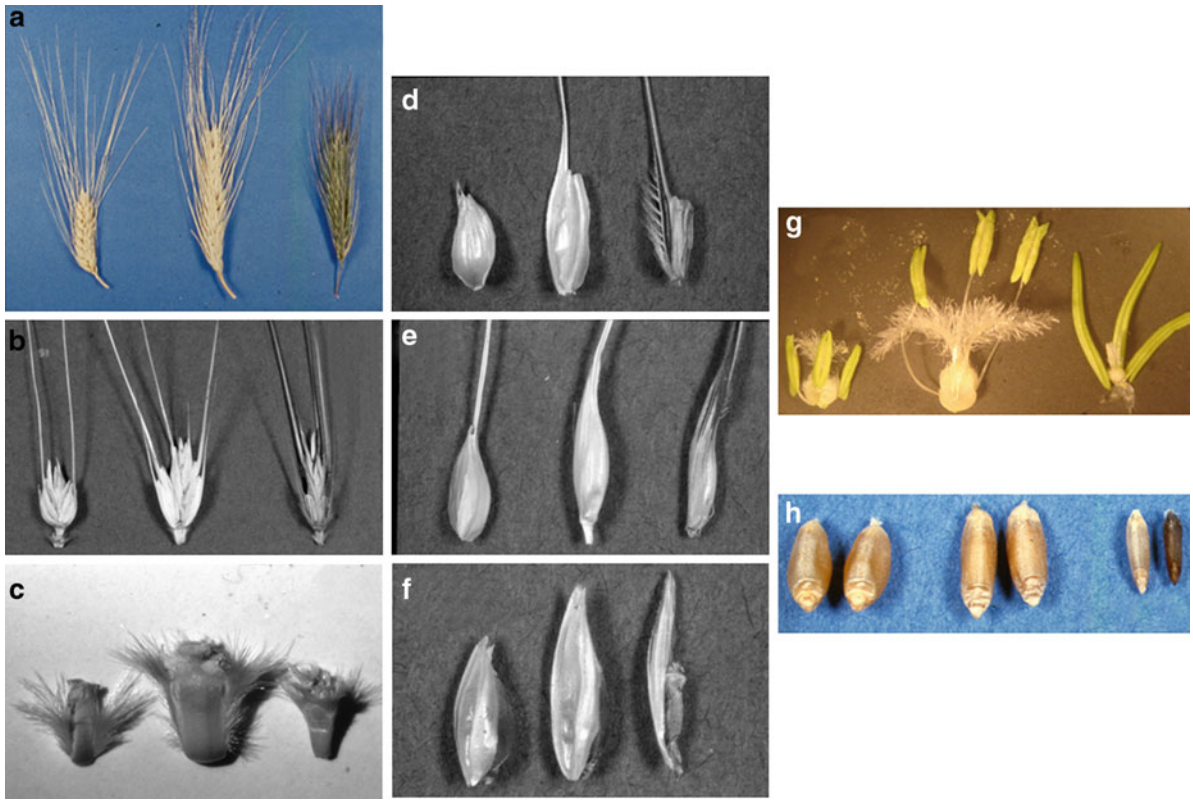

Fig. 4.35 Morphology of the spike (**a**), spikelet (**b**), rachilla (**c**), outer glume (**d**), lemma (**e**), palea (**f**), anthers and pistil (**g**), and caryopsis (**h**) of *T. turgidum* var *durum* cv. Modoc (*left*) and *Dv* (*right*) parental species and the hexaploid amphiploid MxV (*center*) obtained by their hybridization

F_1 plants did not show dehiscent anthers, and the non-doubled spikes did not set seeds. Even in the male-fertile spikes, which produced $n = 21$ gametes, anther dehiscence was poor and manual selfing was necessary to ensure seed set. The M × *Dv* amphiploid meiotic chromosome configurations did not have complete pairing but showed a mean of 1.5^{I} to 2.0^{I} per PMC. M × *Dv* had chromosome numbers from 41 to 43 and set 7 to 20 seeds per spike. It displayed semi-dwarf plant type, good seed quality, and reasonably good seed set (71%) (De Pace et al. 1985). As a primary amphiploid, the overall performance of the hexaploid (AABBVV) was equal to or better than, except for brittle rachis, to most hexaploid primary triticale. The AABBVV amphiploids deserve consideration as new crop plants much as triticale did in its early stages of development. The variation in chromosome number among the amphiploids and the frequent presence of 1–3 univalents per PMC at MI suggested there will be a frequent occurrence of aneuploids in future generations. However, this did not occur after more than 15 generations of selfing since the Mx *Dv* amphiploid still maintained $2n = 42$ chromosomes (Minelli et al. 2005). Tschermak-Seysenegg (1934), von Berg (1935), Blanco et al. (1983a, b), and Stefani et al. (1983) reported selfed seed set on *T. turgidum* var. *durum* × *Dv* F_1 plants without colchicine treatment, leading to F_2 plants with $2n = 42$. This was attributable to meiotic non-reduction producing $n = 21$ male and female gametes (Stefani 1986). The unreduced gametes formed spontaneously at meiosis of the F_1, and their union to form $2n = 42$ F_2 embryos in mature caryopsis from which mature F_2 plants (= amphiploids) were formed, are the main evidences to sustain the proposal to include *Dv* in the secondary gene pool (GP-2) of wheat. Also, Stefani et al. (1983) and Liu and Chen (1986) found that the chromosome number of their AABBVV amphiploid remained constant at $2n = 42$

through generation advancement despite meiotic irregularities. Attempts to produce alien addition lines in *Triticum durum* were also made by Pignone (1994).

Three AABBDV plants obtained by crossing M × *Dv* with the hexaploid wheat Chinese Spring and PI 178704 had means of 14.27 to 15.56^{I}, 12.75 to 14.0^{II}, and 0.35 to 0.46^{III} per PMC (Jan et al. 1986). Two of these plants had a low frequency (0.01 to 0.06) of quadrivalents. The (M × *Dv*) × CS hybridization was repeated (Minelli et al. 2005), and although some D and V chromosomes were retained in F_2 generation, the majority of the plants had $2n = 42$, AABBDD-genomes, and some had $2n = 28$, AABB-genomes, and (1 out of 40 F_2 plants) retained one pair of V chromosomes. While other workers (Blanco et al. 1983b and 1988b) have suggested that homoeologous pairing occurs between *Dv* chromosomes and those of wheat, further work is needed to determine the extent of homoeology of the *Dv* chromosomes with those of wheat deduced from observations on chromosome pairing at meiosis I.

At the Institute of Crop Breeding and Cultivation, Chinese Agricultural Academy of Sciences (CAAS), Beijing, China, calli were obtained from the in vitro culture of young inflorescences of the F_1 from the intergeneric hybridization *T. durum* "81086A" × *Dv* (TH1), and immature F_1 embryos from the intergeneric hybridization *T. durum* "D311" × *Dv* (TH2), and *T. durum* "Mexicali 75" × *Dv* (TH3) (Chen et al. 1996b). In all cases, the pollen parent was a *Dv* ecotype from the former USSR. Calli were treated with colchicine, and chromosome doubling was shown for 78.8% of the regenerated F_1 plantlets. The amphiploids TH1, TH1W (waxy glumes), TH2W (waxy glumes), TH3, and TH3W (waxy glumes) were obtained from kernels produced on F_1s with chromosome doubled sectors or tillers.

Nakajima (1960c) attempted hybridization between six tetraploid wheats and *Db*(2*x*); he obtained caryopses with F_1 embryos in all cases at the rate of 1–4 per 100 pollinated florets. However, F_1 plants were obtained only from the *T. turgidum* ♀ ($2n = 4x = 28$) × *Db*(2*x*) ♂ ($2n = 2x = 14$) cross combination. The F_1 somatic cells had $2n = 28$ chromosomes, and the spikes resembled the *Db*(2*x*) parent. Unreduced gametes in the pollen parent may have been responsible for fertilization. If the *Db*(2*x*) parent was truly $2n = 14$ chromosome plant, then that observation represents the first evidence on the possible origin of *Db*(4*x*) from caryopses developed after union of unreduced gametes in *Db*(2*x*) florets (see Sect. 4.2.8.1). At meiosis of the F_1 PMCs, 5–12 bivalents were observed. In case of PMC displaying 12^{II}, about half of them (6^{II} or 7^{II}) might have been formed by the autosyndesis among the *Db* chromosomes, and the remaining 5^{II} or 6^{II} were most likely from A-B autosyndesis. The F_1 was completely sterile and perennial.

Db(4*x*) was hybridized with *Triticum turgidum* var. *durum* cv. Creso ($2n = 28$, AABB), and F_1 seeds ($2n = 28$, ABV^bV^b) were obtained at a frequency of about 9.0% of pollinated florets by Blanco and Simeone (1995). The hybrids inherited the perennial growth habit and the awn on the glume from the male *Db* (4*x*) parent. An average of 11.50 chiasmata per ceIl with 7.86 bivalents, and 11.37 chiasmata with 7.68 bivalents and 0.07 trivalents were observed in two hybrids with and without the *Ph* gene, respectively. The high pairing mostly involved autosyndesis among the V^bV^b-genomes, thus confirming the autoploid origin of *Db*(4*x*). All of the plants were self-sterile. However, some seeds were obtained with pollination of the F_1 hybrids with durum wheat cv. Creso. The chromosome numbers in the BC_1 progeny varied around $2n = 40$ suggesting that they originated from the fertilization of functional non-reduced female gametes with 28- or near 28-chromosomes by the 14-chromosome wheat gametes. The partial amphiploid plants ($2n = 42$, $AABBV^hV^h$) showed a mean chromosome association of 0.04 trivalents, 18.37 bivalents, and 5.12 univalents, and were partly fertile (1.15 kernel per spikelet compared with 3.36 kernels per spikelet of durum wheat).

Crossability between parental species and occurrence and frequency of viable unreduced gametes in the first fertile hybrid plant provide relevant data to infer time span for the appearance of important domestication syndrome-related traits and indirectly quantify early events in wheat domestication. Such data have been collected during and after hybridization of two free-threshing and tough-rachis tetraploid wheat varieties (*Triticum turgidum* var. *durum* cv. Modoc and cv. Creso; $2n = 4x = 28$; AABB) with diploid hulled and brittle-rachis *Dv* ecotypes (pop. Bomarzo and pop. Ferento from sites near Viterbo in the Latium region of Italy), used as pollen parent (Caputi 2002; De Pace et al. 2003). Crossability was about 11%; the spikelets

of the F_1 had hulled glumes, unreduced gametes at the frequency of 1.24 and 3.7% in the male and female germline, respectively, and brittle-rachis at maturity; fertile F_2 progeny at the frequency of 0.046%, derived from selfing F_1 plants. A previously produced fertile F_2 plant (M × *Dv*, brittle-rachis) gave a phenotypically stable $2n = 6x = 42$, AABBVV brittle-rachis amphiploid and was grown for six consecutive years (F_3 in 1982 through F_8 in 1987) in plots at the Experimental Farm of University of Tuscia, Viterbo, Italy. Every year about 4,000 seeds obtained from about 15 caryopses from each of 250 plants of M × *Dv* were sown. A non-brittle-rachis mutant amphiploid plant (M × *Dv*-nb) appeared in the M × *Dv*-brittle rachis plant population grown in 1987 (De Pace et al. 2003). It was estimated that the mutation rate that led to the non-brittle rachis mutant was 4.4×10^{-5} per individual per generation. It gave a phenotypically stable $2n = 6x = 42$, AABBVV non-brittle-rachis amphiploid. Data obtained by De Pace and Jan (unpublished) showed that in the F_2 progeny of (M × *Dv*-brittle rachis) × (M × Dv, non-brittle rachis), the non-brittle rachis phenotype was dominant and that the segregation brittle-rachis: non-brittle rachis fitted the 15:1 ratio expected for segregation of alleles at two independent polymeric loci.

Because of the paucity of archeobotanical data documenting, the early stages of tetraploid and hexaploid wheat domestication, the above data provide a reasonable recapitulation of population size (few thousand plants) and time lapse required (about ten generations under cultivation) for the transition from tetraploid or hexaploid wheat with disarticulating spike to tetraploid or hexaploid domesticated types with tough rachis.

4.2.9.3 Spontaneous *durum* × *Dv* Hybridization

Meletti (1955) described a new wheat plant type occurring every year in the majority of the *durum* wheat crops in Sardinia (Italy), which he called "Denti de Cani" (this name means "dog's teeth" referring to the shape of the kernels). The chromosome number of the plant was 42 and the comparative karyograms between "Denti de cani" and *T. durum* cv. Cappelli suggested that the 28 out of 42 chromosomes were contributed by *T. durum*, other 14 chromosomes probably belonged to a diploid Triticinae species growing in or around the fields with *durum* wheat crop stands. On the basis of the low occurrence of *Aegilops* and *Agropyron* and the widespread occurrence of *Dv* species in Sardinia, it was hypothesized that *Dv* was the donor of 14 chromosomes to the genome of "Denti de cani" after natural hybridization between *T. durum* and *Dv* followed by a spontaneous chromosome duplication event in the spike primordia of the F_1 plant or by frequent non-reduced gamete formation at meiosis, which allowed high seed set in the F_1 plant. Those seeds mixed to those of *durum* wheat gave fertile "Denti de cani" plants similar to wheat plant in the following year but with higher fitness and competitive ability. The anatomy and endosperm structure of "Denti de cani" was more similar to bread wheat, and a mixture of "Denti de cani" and *durum* wheat seeds produced flour with lower quality compared to that from *durum* wheat seeds only. Therefore, "Denti de cani" became noxious in *durum* wheat fields. Two plant types were identified in "Denti de cani" (1) a "CP type" with a solid stem (= "Culmo Pieno"), winter-like habit, long seed dormancy period, and long after-dry ripening period, and (2) a "CV type" with hollow stem (= "Culmo Vuoto," CV), spring-like habit, short dormancy period, and short after-dry ripening (Meletti and Onnis 1975; Onnis 1966, 1967b, c, 1969, 1971; Stefani et al. 1986). Those plant types did not have speltoid spike, which was a trait displayed by all the experimental tetraploid *Triticum* × *Dv* F_1 and amphiploid described previously. However, Stefani et al. (1987) managed to demonstrate that in the F_{10} generation of an experimental hybrid (Meletti et al. 1977), some lines lost the speltoid character. Unfortunately, those types showed low fertility and the caryopses did not germinate.

Meletti and Onnis (1975) assigned "Denti de Cani" to the genus × *Haynaldoticum* (*Haynaldia* × *Triticum*) (Ciferri and Giacomelli 1950) with the specific name of *Haynaldoticum sardoum* Meletti et Onnis. Meletti et al. (1986) confirmed the occurrence of "Denti de cani" in Sardinia, but this new species was reported to occur spontaneously also in several durum wheat fields in southern Italy and Maremma area near Grosseto (Meletti and Onnis 1975). Galleschi et al. (1982) described the α-amylase and glutamate decarboxylase metabolism during seed life-span of ×*Haynaldoticum*. Meletti et al. (1996) defined "Denti de cani" (= ×*Haynaldoticum sardoum*) as a volunteer hexaploid wheat with good end-use grain

quality and effective resistance to the fungal agents causing Fusarium head blight (FHB) disease.

Vakar (1966) reported an amphiploid, which arose from the spontaneous cross *T. dicoccum* var *durum* × *Dv*. He assigned the amphiploid to the *Haynatricum* Zhuk genus. The amphiploid was fertile, meiosis was normal, and chromosomes formed bivalents. The seed set was low and showed hulled grains, brittle ear, and poor threshability. However, it showed resistance to *Ustilago*, *Tilletia*, *Bgt,* and some tolerance to *Puccinia recondita*.

4.2.9.4 Hexaploid (2*n* = 6*x* = 42) *Triticum* sp. × *Dv* Hybridization

In 1915, the cross *T. aestivum* cv. Rieti × *Dv* made by N. Strampelli gave a fertile F_1 from which (after a putative process of backcrossing to cv. Rieti) the "gigas" winter bread wheat cultivar Cantore was derived (Strampelli 1932). In 1927, from another hybrid that Strampelli made using *Dv* as one parent (the other parent was *T. aestivum* cv. Akagomugi, a very early-heading Japanese cultivar), the bread wheat cultivar Roma (Forlani 1954) was released. In 20 years (1908–1927) of breeding and selection in progenies derived from wheat × *Dv* hybridization, Strampelli obtained many other interesting types, such as the so-called *Triticum giganteum* (which produced large spikes and kernels as big as a coffee seed) or the lines with sweet kernels.

Sears (1953, 1976) using the species-bridging method was able to obtain plants from the (*T. dicoccum* × *Dv*) × *T. aestivum* cv. Chinese Spring hybrid. The *Dv* pollen parent was obtained from a Greek ecotype. One of those F_1 plants was backcrossed to *T. aestivum* and progenies with $7^{I} + 21^{II}$ and 7^{I}, 19^{II}, and 1^{IV} were obtained. From these materials was obtained the first set of six out of seven possible monosomic additions and five of the seven possible disomic additions of *Dv* chromosomes to the hexaploid complement of Chinese Spring (Sears 1953, 1982; Hyde 1953; see Sect. 4.4.1 and Table 4.10).

Halloran (1966a) claimed the first successful direct hybridization between *Dv* and the hexaploid wheat *T. aestivum* cv. Chinese Spring monosomic for chromosome 5B. The monosomic condition was employed for purpose of studying possible homoeologous pairing between the chromosomes of the two species in the absence of chromosome 5B. Pollination of 250 florets of Chinese Spring plants with *Dv* microspores gave 1.2% seed set. Two types of hybrids were obtained: 28-chromosome hybrid (with 5B) and 27-chromosome hybrid (without 5B). The plant morphology of the 28-chromosome hybrid was more similar to the hexaploid wheat type than to the phenotype of *Dv*. For two characters, however, the hybrid exhibited a sharp difference from the normal Chinese Spring: rachis fragility and awning. The awns of the hybrid were about 2.0 cm long and thus shorter than those of *Dv* but longer than normal CS, which has a hooded lemma. The hybrid spike expressed the brittle rachis trait of *Dv* and was in contrast to the strong rachis of Chinese Spring. The gene suppressing awn formation in Chinese Spring is dominant (Hyde 1953), and the gene/s decreasing awn length in awned wheat varieties are generally dominant in wheat (Sears 1948). Therefore, it was assumed that genes controlling awn length and rachis fragility in *Dv* express, in the F_1 hybrid to CS, strong epistatic interaction with the homoeoalleles in the wheat chromosome complement. Analysis of meiotic pairing in the 28-chromosome hybrid indicated a very low pairing affinity, if any at all, between the chromosomes of *Dv* and those of *Triticum aestivum* (27.5^{I}, 0.12^{II} open, 0.13^{II} ring). The observed pairing was attributed to autosyndesis of the wheat chromosome complement (Halloran 1966b) and it was excluded that *Dv* possesses genes that remove inhibition to homoeologous pairing due to the *Ph* gene in chromosome 5B. The 27-chromosome hybrid (without 5B) showed chromosome pairing much higher (9.6^{I}, 3.8^{II}open, 1.06^{II} ring, 0.86^{III}, and 0.7^{IV}) compared to the 28-chromosome hybrid (with 5B) (Halloran 1966b). On the basis of quadrivalent frequency at meiosis in the 27-chromosome hybrid, it was hypothesized that the chromosomes of *Dv* paired with those of *Triticum*. This observation added further scope for the wheat breeders in the use of *Dv* genetic variation for its incorporation in bread wheat through homoeologous recombination.

Studies on the effectiveness of the *ph1b* mutation in promoting wheat-*Dv* chromosome pairing have been carried out at the Crop Research Institute of Sichuan, Academy of Agricultural Sciences, Chengdu, Sichuan, China. In the *ph1b* mutants of Chinese Spring (CS), the dominant gene *Ph1* is deleted. The strongly promotes homoeologous chromosome pairing within

wheat and between wheat and alien chromosomes (Sears 1977; Fan et al. 1993).

Chromosome pairing was analyzed in F_1 hybrids of the wheat cultivar Chinese Spring (CS) and its *ph1b* mutant (CS *ph1b*) with *Dv* (Yu et al. 1998, 2001b). On average, 1.61 chromosomes per cell paired in the hybrid CS × *Dv*, but 14.43 in the hybrid CS *ph1b* × Dv. GISH revealed three types of homoeologous associations between wheat (W) and *Dv* (D) chromosomes (W-D, D-W-W and D-W-D) in PMC of the CS *ph1b* × *Dv* hybrid, and only one type (W-W) in the CS × *Dv* hybrid. Both F_1 hybrids were self-sterile. The seed set of the backcross of CS × *Dv* with CS was 6.67% and that of CS *ph1b* × *Dv* with CS or CS *ph1b* was only 0.45%. Translocations of chromosome segment or entire arms between wheat and *Dv* chromosomes were detected by GISH in the BC_1 plants from the backcross of "CS *ph1b*" × *Dv* to "CS *ph1b*." To enhance the effectiveness of wheat wide crosses and alien gene transfer, the *ph1b* variant has been transferred from CS *ph1b* mutant into J-11, a highly compatible material possessing four recessive high crossability *kr* genes, *kr1*, *kr2*, *kr3*, and *kr4*. The effectiveness of *ph1b* in J-11 *ph1b* plants on chromosome association was studied in the F_1 of J-11 *ph1b* × *Dv* hybrid (Yu et al. 1998). Chromosome pairing in F_1 PMCs at MI of that hybrid, was similar to that of the hybrid CS *ph1b* × *Dv*. In the F_1 hybrid of J-11 *ph1b* × *Dv*, 5.11 bivalents, 0.88 trivalents, and 0.62 quadrivalents were observed; in CS *ph1b* × *Dv* hybrid, 5.07 bivalents, 0.78 trivalents, and 0.65 quadrivalents were observed. However, in the F_1 hybrids of CS × *Dv* and J-11 × Dv, only 0.47 and 0.46 bivalents were observed, respectively. These results are similar to those of Halloran (1966b), and the *ph1b* variant in CS *ph1b* and J-11 *ph1b* plants greatly promoted the homeologous pairing in the hybrids of *T. aestivum* × *Dv*.

Wide hybridization of *T. aestivum* × *Dv* was performed in Australia in 1968 at the Department of Agriculture, NSW (MacIndoe and Walkden Brown 1968). Chen and Liu (1982) reported a cytogenetic study of *T. aestivum* × *Dv* F_1 plants and were able to identify *Dv* chromosomes in alien additions to *T. aestivum* genomic background (Chen and Liu 1986; see Sect. 4.4.1).

Transfer of *Dv* chromatin to *T. aestivum* was achieved through bridge-crossing by Liu and Chen (1983). They noted that the karyotype of *Dv* chromosomes (presence of satellite and arm ratios), as well as N-banding patterns (Pei and Liu 1986; Liu et al. 1988), remained unchanged in the wheat background.

Jan et al. (1986) crossed three cultivars of *T. aestivum* (Chinese Spring, Anza and Yecora Rojo) with *Dv*. Only the cross with Chinese Spring was successful. Out of 450 florets of CS pollinated with *Dv*, 33 culturable F_1 were obtained and 52% of them (17 embryos) survived to produce mature hybrid plants, all having $2n = 28$. After colchicine treatment, two of those F_1 plants showed dehiscent anthers and, upon selfing, produced progenies with doubled chromosome number $2n = 56$. The CS × *Dv* C_1 amphiploids (AABBDDVV) had chromosome numbers ranging from 54 to 56 and set from 0 to 7 seeds per spike. Except for the increased fertility, the morphology of these amphiploids was similar to that of their respective F_1 progenitors. The octoploid amphiploid had agronomically poor plant type despite the biochemical complexity for isoenzyme and seed storage proteins compared to the wheat and *Dv* parents (Delre et al. 1986; De Pace et al. 1988a, b). Several new CS × *Dv* amphiploids (*Triticum aestivum* cv. Chinese Spring × *Dv*, AABBDDVV, $2n = 8x = 56$) were produced in 1986, and their morphological and isozyme divergence was described by Mini (1987) and Mini et al. (1988).

The non-doubled F_1 plants were partially female-fertile. Upon backcrossing using pollen of Chinese Spring, Anza, and Yecora Rojo, the CS × *Dv* hybrids had an average seed set of 2.2, 0.7, and 1.0 seeds per spike, respectively. The CS × *Dv* F_1 hybrids morphologically resembled CS much more than *Dv*. The F_1 hybrids had longer, more lax spikes than CS. The brittleness of the rachis of these hybrids was not as pronounced as that reported by Halloran (1966a). The F_1 plants resembled *Dv* in the hairiness on the glume and rachis and very short awns on the glumes. The F_1 plants had reduced vigor and tillering ability compared to Chinese Spring. The amount of pairing at MI was observed in the eight CS × *Dv* F_1 plants and ranged from 27.8^{I} and 0.11^{II} rod to 22.5^{I}, 2.4^{II} rod, 0.17^{II} ring, and 0.10^{III}. Five of the eight F_1 plants had ring bivalents and trivalents, which represented more pairing than has been observed in euhaploid Chinese Spring (Miller and Chapman 1976; Dvořák 1977; McGuire and Dvořák 1982).

The greater level of pairing observed in CS × *DV* F_1 compared to the chromosome pairing in haploids of

bread wheat, suggest that there exist *Dv* genes that interact with those of wheats to produce a net promotion of homoeologous pairing in the F_1 plants. The data of Jan et al. (1986) and those of Halloran (1966a, b) imply that there is more than homoeology involved in controlling the amount of pairing, which occurs in hybrids between species of wheat and *Dv*.

Genes on the V chromosomes, which interact with the *Ph* gene to disturb the strict homologous pairing mechanism to promote a certain degree of homeologous pairing, have been proposed by Blanco et al. (1983a, b) to explain similar proportion of homoeologous pairing observed for *T. turgidum* (*ph*) × *Dv* V17 F_1 and *T. turgidum* (*Ph*) × *Dv* V17 F_1 (see also section 4.2.9.2 on tetraploid wheats × *Dv*).

Genetic variation for the promotion of heterogenetic pairing in hybrids with bread wheat has been found in two other outcrossing annual diploid species in the Triticeae, *Triticum tripsacoides* (Jaub. et Spach) Bowden (Riley 1966; Dover and Riley 1972) and *T. speltoides* (Tausch) Gren, ex Richter (Dvořák 1972; Kimber and Athwal 1972; Chen and Dvořák 1984).

Because, upon self-fertilization, the F_1 hybrid *T. aestivum* × *Dv* set caryopses on about 3% of the florets, this means that the micro- and macrospores of the F_1 hybrid produced functional, unreduced gametes, as was the case with the *T. durum* × *Dv* hybrids of Stefani (1986) and Stefani et al. (1983, 1987). These caryopses produced fertile plants, and repeated selfing allowed the establishment of wheat lines carrying V-genome chromatin as the result of addition, substitution, or recombination events, or a combination of all these karyological events. Many of these lines were found to be karyologically unstable; for example, an original disomic addition line had become a substitution line during seed multiplication. Another observation is that the morphology and banding pattern of V chromosomes may undergo changes in the hybrids. Some of these changes can be explicable only by admitting the occurrence of amplification or loss of DNA sequences in V chromosomes. As early as in 1978, B. McClintock discussed the concept that wide crosses may cause stresses capable of triggering the reorganization of the parental genomes, and it is known that many repeated DNA sequence families can show non-Mendelian inheritance even in intraspecific crosses (Rivin and Cullis 1983; Walbot and Cullis 1985). These genomic changes, together with the karyological instability of the lines, should be taken into consideration when attempting to use hybrids and derived lines in the genetic improvement of species.

Chimeric wheat-*Dv* chromosomes were also observed in the lines, and different cytological events may be involved to explain their occurrence. The monosomic stage of the V chromosomes in (*T. aestivum* × *Dv* F_1) × *T. aestivum* hybrids or in the bridge-crossing of the amphiploid *T. dicoccum* × *Dv* with *T. aestivum* (Sears 1953) may lead to translocated chromosomes as a result of misdivision of the univalents, followed by centric fusion. Another possibility is that the *Dv* strains used to produce hybrids with wheat possessed gametocidal-like chromosomes (Endo 2003), which, during the formation of unreduced gametes, induced breaks in wheat as well as in V chromosomes, followed by structural chromosomal mutations (deletions and/or translocations). Alternatively, *Dv* strains might possess genes that promote homoeologous pairing. This situation favors recombination between homoeologous chromosomes, especially between the colinear and syntenic chromosomes 1V, 1A, 1B, and 1D.

4.2.9.5 Hybridization of *Secale* × *Dasypyrum*

Sando (1935b) has been the first to attempt *Secale* × *Dv* hybridization. The first cross of *S. cereale* × *Dv* was unsuccessful (no hybrid seed set), but the cross of *S. fragile* × *Dv* produced 35 seeds from which 35 plants were raised. The culm section 2.5 cm below the head showed a cavity intermediate to those of the parents. The peduncles below the head of the F_1 plants were covered for several centimeters with hairs (less than 1 mm in length). The nodes were glabrous as *Dv* (*S. fragile* had pubescent nodes). Both sides of leaf lamina presented abundant hairs 1–2 mm. Hairs of 4 mm were present on the auricles and resembled those of *Dv* (in *S. fragile* the hairs were much shorter). The F_1 leaf blades were narrower than those in both parents. The spikes were intermediate in length compared to the parents and were extremely fragile and had 30–34 spikelets with 3–4 sterile florets. At the base of the spikelet (exterior side), there was a tuft of short bristles 0.35 mm in length. The rachis internodes were 3 mm long and usually glabrous on the exterior surfaces but were covered with numerous bristles 3.5 mm or less on the edges. The glumes were glaucous and possessed two prominent scabrid

nerves, which formed keels separated by a boat-shaped depression about 0.75 mm wide at the center (as in *Dv*). At the apex of each glume there was a 3–5.5 cm awn.

The glume shoulder was intermediate to those of *Dv* (broad shoulder) and *S. fragile* (no shoulder). The prominent keel on the glume possessed bristles 2 mm in length. The lemmas were 12–14 mm and had a 5–7 cm awn: keels of the lemmas were beset with bristles 1–4 mm or less in length throughout the entire length. In general, the F_1 plant resembled the *Secale* parent.

Kostoff (1937) attempted the direct cross of *S. cereale* with *Dv* and only one hybrid plant was obtained (in 1931), which died at an early stage of development. To avoid further failure, Kostoff (1937) attempted to cross *S. cereale* with *Dv* using *T. dicoccum* as bridge species. Through this procedure, a trigeneric hybrid (*T. dicoccum* × *Dv*) × *S. cereale* with 28 chromosomes (genome-ABVR) was produced. At metaphase, 1.6% of the cells had one bivalent, 2% had two bivalents, and 2% had one trivalent, while the rest of the chromosomes in those cells and in 90% of other cells were univalents (Kostoff and Arutiunova 1937b). On the basis of those observations, it seemed that V chromosomes were not homologous with R chromosomes, and V and R chromosomes were not homologous with those of the A- and B-genomes (Kostoff and Arutiunova 1937a; Kostoff 1936a, b, c).

The first successful hybridization of *S. cereale* with *Dv* was reported by Nakajima (1951) who observed 0–2 bivalents in only 18.7% of the PMC during metaphase I, concluding that no homoeology existed between the V- and R-genomes. Nakajima (1959a) reported also morphological and cytogenetical studies on F_1 plants obtained from crossing of *S. fragile* × *Dv*: he observed 8.26% of the pollinated *S. fragile* florets to give rise to caryopses with an F_1 embryo. The F_1 plants were morphologically intermediate between the parents; only the number of spikelets per spike was higher (35–37) than the parents (22 in *Dv* and 32 in *S. fragile*) (Nakajima 1961b). The chromosome number in root tip cells was 14. At MI, 79.25% of the PMCs showed no bivalents, 15.8% showed one bivalent, 4.64% showed two bivalents, and 0.29% showed three bivalents. Only 0.7% of the observed bivalents were ring bivalents, the majority being rod bivalents loosely conjugated end to end. These bivalents seem to be derived from autosyndesis, and there seems to be no homology between V-genome and R-genome of *S. fragile*. In the ana-telophase of MI, 37.1% of PMCs showed a 7:7 distribution of chromosomes to the poles, 25% showed a distribution of 6:8, 21.2% were 5:9, 9.1% were 4:10, and only 0.1% showed a distribution of 0:14. Most of the F_1 plants showed partial fertility upon selfing, and the percentage of seed setting per spikelet varied from 0 to 6.9%. About 78% of the F_2 caryopses gave mature plants (Nakajima 1961b). The 32 F_2 plants resembled the F_1 plants in their gross morphology and all but one plant had $2n = 28$, possibly from the union of 14-chromsome gametes produced from F_1 PMCs. The mode number of bivalents was 6^{II}, the maximum being 11^{II}. The remaining F_2 plants had $2n = 21$ chromosomes and were thought to be derived from the union of 14-chromosome F_1 female gametes and an $n = 7$ gamete from *S. africanum* (Nakajima 1961c). That F_2 plant had a modal 5^{II} at MI probably due to syndesis between R^a and R^f chromosomes, and involvement of V chromosomes in pairing was excluded by the rare trivalent formation.

Also, Nakajima had more success in crossing *Secale* with *Dasypyrum* using a bridge species such as tetraploid or hexaploid wheats (Nakajima 1959b, c, 1960a, b, 1961a, 1964, 1966d, 1968, 1969, 1970).

Where tetraploid wheats were used as bridge species, the percentage seed set was 22.27% in the cross of (*T. persicum* × *Dv*, F_1) × *S. cereale*, and 8.15% in the cross of (*T. durum* × *Dv*, F_1) × *S. cereale* (Nakajima 1959b). Thirty-seven percent of the trigeneric F_1 plants in the first case and 29.4% in the second case had 28 chromosomes. Most of the trigeneric F_1 plants resembled the wheat parent and showed some fertility that gave rise from 3.03 to 10.71% of seed set. In 12,600 chromosome configurations observed in PMCs at MI of the trigeneric F_1 plants (*T. persicum* × *Dv*, F_1) × *S. cereale* with 28 chromosome, 26.8% showed only univalents, 25.3% showed one bivalent, 29.9% two bivalents, 13.2% three bivalents, 3.9% four bivalents, 0.7% five bivalents, and 0.06% six bivalents. The majority of the bivalents were of rod type, ring bivalents representing only 11.7% of the total bivalents observed (Nakajima 1959c). It was proposed that in the 0.06% of the cases where six bivalents were observed, four were from B autosyndesis and two from V or R autosyndesis, considering that only one bivalent occur in the haploid plant of *S. cereale* (Nordenskiöld 1939) and in other trigeneric *Triticum* × *Secale* × *Dv*

hybrids (Kostoff and Arutiunova 1937b) and *Triticum* × *Secale* hybrids (Nakajima 1959c).

The distribution of chromosomes to the opposite poles at the ana-telophase in MI proceeded at random in F_1-type division, while in the case where 28 chromosomes formed the equational plate, a 0:28 distribution of the chromosomes to the poles was observed. When hexaploid wheat (*T. aestivum*) was used as a bridge species, the percentage of (*T. aestivum* × *Dv* F_1) × *S. cereale* F_1 caryopses obtained over the pollinated florets was 0.137 (Nakajima 1962), which was much lower compared to the trigeneric crossing involving tetraploid wheats.

The trigeneric hybridization was more successful when *T. aestivum* × *Dv* F_1 was the female parent in the cross to *S. cereale* than when the cross was made in the other direction. The F_1 seed germinability was poor and only four mature plants from 55 F_1 crosses were obtained. The number of somatic chromosomes varied from 31 to 36, seven were introduced from *S. cereale* (female) and the remaining 24–29 from *T. aestivum* × *Dv*, F_1 (male; $2n = 28$). Plants with 31 or 32 chromosomes showed the following percentage of bivalents in 1,000 PMCs: 45.5–61.0% 0^{II}, 22.8–33.2% 1^{II}, 1.8–14.6% 2^{II}, 28–54% 3^{II}, 9–19% 4^{II} and 4–12% 5^{II}. One plant with 35 chromosomes showed 13% PMCs with 1^{II}, 86% with 2^{II}, 19.7% with 3^{II}, 32.1% with 4^{II}, 23.2% with 5^{II}, 1.4% with 6^{II}, and 2.7% with 7^{II}. Ring bivalents were rare and 7.5% trivalents were of V-shape. According to their results and those obtained previously by the same author (Nakajima 1951, 1959a), by Kostoff and Arutiunova (1937a), and by Zennyozi (1961), it was concluded that, although a few bivalents might have arisen by autosyndesis of R or V chromosomes, most of the bivalents were formed by autosyndesis between AB- or ABD-genomes.

Aegilops squarrosa was also used as bridge species in the trigeneric cross (*A. squarrosa* × *Dv*) × *S. cereale* (Nakajima and Zennyozi 1966). However, no spikes were formed.

A tetraploid wheat or *Aegilops* bridge species were used by Jahier et al. (1988) to achieve a combination of V- and R-genomes in the same nuclear background; in fact, a direct *Dv* × *S. cereale* cross failed. Three hybrids were studied, two involved the *T. durum* × *Dv* amphiploid ($2n = 42$; AABBVV) obtained by Blanco et al. (1983a, b) as pollen parent and triticale cv. Clercal or Beagle ($2n = 42$; AABBRR) as female parents to get F_1 hybrids H_2 and H_3 with 42 chromosomes and AABBRV-genome composition. The other hybrid (H_4) was obtained by crossing two amphiploids: *Ae. uniaristata* (genome-*Un*) × *S. cereale* and *Ae. uniaristata* × *Dv* amphiploids. The resulting F_1 hybrids contained 28 chromosomes. In all the PMCs, at least 14 univalents were observed. Chromosome associations gave rise only to bivalents (14 or 15) in crosses of H_2 and H_3, and pairing between R and V chromosomes seemed absent or very low. In cross of H_4, 10–62% of the PMCs showed more than 7″; trivalents and quadrivalents were also observed. In this cross, pairing between R and V chromosomes occurred and 14.8% of the cells contained 1^{II} (0.156^{II} per cell). Trivalents were observed at a frequency of 3.5% and in 5/6th of the cases involved UnUnV pairing (0.028 trivalents per cell) and only 1/6th were of the type UnUnR (0.005 trivalents per cell). Considering that the V chromosomes derived from an amphiploid, which in turn was from selfing of an F_1 between the durum wheat cv. Creso and a *Dv* genotype that interacted with the 5B *phl* gene promoting homoeologous pairing (Blanco et al. 1983a), the R-V pairing should have been higher. Therefore, it may be concluded that when R-V allosyndetic pairing occurs, it is very rare (only 1^{II} R-V every 6 PMC). Zhukowsky (1944) reported the first *Db* × *S. cereale* cv. Vjatka hybridization experiment that gave rise to a fertile hybrid. Recently, Grądzielewska (2009) reported the occurrence of several R-V translocations in winter rye strains obtained by hybridization of "Amilo" rye cultivar with *Dv*. The identification of *Dv* genetic material introgressed in rye was conducted with RAPD method and with PCR amplification of a part of *Dv p380* repeated sequence. Each of the strains had a distinctive RAPD banding profile and in 18 out of 19 investigated strains the presence of specific *Dv* RAPD fragments was confirmed. However, the *Dv p380* repeated sequence was not amplified in the investigated strains, which suggested that *Dv* DNA introgression was from chromatin located outside telomeres of chromosomes 1V to 6V or from chromosome 7V.

4.2.9.6 Hybridization of *Aegilops* × *Dv*

Oehler (1933, 1935) reported that early hybridization of *Dv* × *Aegilops* experiments were those reported by Bleier (1928), Tschermak (1929, 1930),

and Tschermak-Seysenegg (1934). Bleier (1930a) reported a successful hybridization between *Dv* and *Ae. ventricosa* ($n = 14$) or *Ae. ovata* ($n = 14$); in both cases, the F_1 showed 20–23 chromosomes. It seems that the *Ae. ventricosa* and *Ae. triaristata* were crossed with *Dv* by Tschermak in 1927 and 1928, respectively (Tschermak-Seysenegg 1934). Oehler (1933, 1935) reported hybridization of the *Aegilops* species having $n = 7$ (*A. longissima, A. speltoides*, *A. comosa*, *A. uniaristata*, and *A. umbellulata*) and $n = 14$ (*A. ventricosa, A. cylindrica, A. varabilis, A. triuncialis, A. triaristata*, and *A. ovata*) with *Dv*. Some of the crosses were repeated and one F_1 plant of *A. uniaristata* × *Dv*, two F_1 plants of *A. cylindrica* × *Dv*, three F_1 plants of *A. ventricosa* × *Dv*, and four F_1 plants of *A. comosa* × *Dv* were obtained (Oehler 1935). These F_1s were sterile and the mean number of associations was very low indeed, in the F_1 from crossing *Dv* to *A. squarrosa* (D-genome), *A. umbellulata* (U), *A. uniaristata* (Un), and *A. caudata* (C) the modal association number was 1.24, 1.31, 0.71, and 1.18, respectively. Sando (1935a) crossed three *Aegilops* species with $2n = 28$ (*A. bicornis*, *A. ventricosa,* and *A. ovata*) with *Dv* obtaining fully self-sterile F_1 plants. Kihara and Lilienfeld (1936) crossed *Ae. squarrosa* with *Dv*, and von Berg (1937) found no pairing in F_1 of *A. umbellulata* × *Dv*. Sears (1941b) obtained three *A. umbellulata* × *Dv* F_1 hybrid plants, two of which became partially tetraploid and fertile upon repeated application to the crown of 0.5% aqueous solution of colchicine; $3^{II} + 8^{I}$ were obtained in F_1 and $2^{II} + 24^{I}$ to $10^{II} + 8^{I}$ in the sterile amphiploid. The amphiploid shed pollen 30 days later than the latest parent (*A. umbellulata*) and tended to be taller than *Dv,* the tallest parent. The rachis segment length (5.8 mm) was similar to that of *A. umbellulata* (6.8 mm) and 3.8 mm longer than that in *Dv*. Fragility of the rachis was present at the base of the spike in *A. umbellulata* and at every node above junction of spikelet with rachis in *Dv*. The amphiploid did not show fragility at every node but broke at the base of the spike after application of a certain tension. The total number of spikelets per spike was 16, intermediate to the parents. Sears (1941b) found 8^{I}–14^{I} (mean 12.7^{I}), 0^{II} –3^{II} (mean 0.64^{II}) open bivalents, no closed bivalents, 0–1^{III} (mean 0.2^{III}). These results contrast with those of von Berg (1937) who found no pairing in *A. umbellulata* × *Dv* F_1 hybrid. In the amphiploids, he found 6^{I}–26^{I} (mean 16.6^{I}), 1^{II} –11^{II} (mean 5.5^{II}), 0–1^{III} (0.08^{III}), and 0–2^{IV} (mean 0.05^{IV}).

Therefore, despite the very low pairing in F_1, the amphiploid showed a significant departure from the expected 14^{II} and an extraordinarily high frequency of univalents associated with spike infertility. This was an unusual situation considering that other *Aegilops* × *Aegilops* and *T. aegilopoìdes* or *T. monococcum* × *Dv* showed at most 2.6 univalents and seven multivalents and seed set higher than 25%. Sears (1941b) attributed the extreme excess of univalents to the consequences of some physiological upset dependent on the extreme hybridity involved.

A. umbellulata was crossed to *Dv* also by Zennyozi (1961) who produced other F_1 hybrids, such as *A. squarrosa* × *Dv* and *A. ventricosa* × *Dv*. In three crosses, the modal configurations were: 14^{I}, $12^{I} + 1^{II}$, and $8^{I} + 5^{II} + 1^{III}$, respectively.

A very low mean number of associations between chromosome arms were observed by Lucas and Jahier (1988) in the PMCs of the F_1 hybrid *Ae. umbellulata* × *Dv*. Only the *A. umbellulata* × *S. cereale* F_1 hybrid showed similar low chromosome arm associations. In other hybrids of *A. umbellulata* to *T. urartu*, *T. boeticum*, *Ae. longissima*, *Ae. squarrosa*, *Ae. uniaristata*, and *Ae. comosa,* the average chromosome pairing ranged from 6.5^{II} in the cross involving *Ae. squarrosa,* to 2.38^{II} in the cross involving *A. longissima*. In the same study, *Dv* was crossed as male parent to *Ae. squarrosa* (genome-D), *Ae. uniaristata* (genome-Un), and *Ae. caudata* (genome-C), and low chromosome arm associations of 1.24^{II}, 0.71^{II}, and 1.18^{II} were also observed in the F_1s. The cross of *Dv* × *S. cereale* was not attempted, but F_1s from crossing of *A. squarrosa*, *A. umbellulata*, and *A. uniaristata* to *S. cereale* showed a lower chromosome arm associations (0.62, 1.37, and 0.69, respectively) compared to the F_1 involving those *Aegilops* species and *Dv*. The F_1 hybrids involving a diploid *Triticum* species and a diploid *Aegilops* species always showed an average chromosome arm pairing of 2.4. From these data, Lucas and Jahier (1988) concluded that the differentiation of *Dv* and *S. cereale* from the genera *Aegilops* and *Triticum* occurred earlier than the species in the latter two genera. In a dendrogram obtained from the mean chromosome association in *Secale* and *Dasypyrum* hybrids compared to the chromosome association observed in *Aegilops* × *Dv* and diploid *Triticum* × *Dv* hybrids, it was suggested that the closest species to *Dv* is *T. boeticum* with A-genome rather than *Aegilops*. They also suggested that gene transfer from *Dv* to

wheat should be done by targeting introgression in the A-genome of wheat. Recently, Deng et al. (2004) using in vitro embryo rescue, were able to produce an F_1 from the hybridization of *A. tauschii* (= *A. squarrosa*) × *Dv*. The PMCs of the F_1 showed an average of 1.25 rod bivalents and 11.49^{I}, but the number of rod bivalents ranged from 1–5. A relatively high homology was implied between the D- and V-genome. The F_1 were self-sterile, but fertility was restored in some F_1 plants after colchicine treatment.

4.2.9.7 Hybridization of *Roegneria* × *Dv*

The intergeneric hybrid between *Roegneria kamoji* Ohwi and *Dv* was successfully obtained by means of embryo culture in vitro (Zhou et al. 1999). The seed set of the cross was 11.63%. The somatic chromosome number in root-tips of F_1 hybrids was 28. The spikes of F_1 plants were morphologically intermediate between the two parental species. The meiotic configuration was $26.72^{I} + 0.62^{II} + 0.02^{III}$, which indicated that very low homoeology between the St-, H-, Y-genomes of *R. kamoji* and the V-genome of *Dv*. The hybrid plant was sterile.

4.2.9.8 Triparental and Trigeneric Hybridization

Triparental Hybridization

Sears (1953, 1976) carried out the triparental hybridization (*T. dicoccum* × *Dv*) × *T. aestivum* cv. Chinese Spring to get the first set ever produced of the disomic addition lines of V chromosomes to CS chromosome complement (see also Sect. 4.4.1).

In the triparental hybrid of *T. durum* cv. CI 1320 × *Dv* with *T. aestivum*, Liu et al. (1996) showed that the F_1s (AABBDV) had 14 bivalents and 14 univalents per PMC and 3% self-fertility, which increased to 7–38% after backcrossing. This study suggested that the production of aneuploid wheat lines with added V chromosomes was possible when *Dv* was used as a bridge in crosses involving *durum* and bread wheat parents.

Von Bothmer and Claesson (1998) made the triparental hybridization (*T. turgidum* ssp. *dicoccum* × *Dv*) F_1 × *T. aestivum* cv. CS, which were backcrossed to either cv. Kadett or CS. Fertile BC_2 plants were obtained whose progenies were tested for agronomic performance; some of them expressed resistance to *Puccinia striiformis*, and several progenies segregated for rachis fragility and weak straw.

Triparental crosses were also performed at CAAS, Beijing, China, by crossing the TH1, TH2, and TH3 aphiploids obtained by Chen et al. (1996b) to several *T. aestivum* cultivars and lines. Homozygous materials 94G32-1, 94G33-1, 94G22-1, and 94G25-1, with the powdery mildew resistance gene from *Dv* were developed by backcrossing the amphiploids to *T. aestivum* followed by immature embryo culture or anther culture (Chen et al. 1996b). Shang et al. (1997) evidenced that the lines 94G22-1 and 94G33-1 were DS 6V(6D), and the 6V in these lines is designated 6V#5 (Table 4.10). Crossing the TH1 and TH1W amphiploids to several bread wheat lines ("CS," "Luzi 357," "84Jia-7911515," "NPFP," and "91E27"; Li et al. 2000a), followed by immature embryo in vitro culture with genome-AABBDV, allowed selection of translocation lines involving V and D chromosomes near the centromere (Li et al. 2000a). The translocation lines 97R377 and 97R282 were selected from the cross TH1W × "CS," and the translocation line 97R447 was selected from the TH1 × "NPFP" cross. Because the root-tip cells of F_1 seedlings from immature embryos did not show translocations, it was concluded that they were induced from tissue culture during regeneration of the lines (Li et al. 2000a). The young embryo and anther culture of the F_1 from crossing the TH3 amphiploid with the bread wheat cv. "Wan7107" (developed in Nanyang Institute of Agricultural Science, Henan Province, China, and susceptible to powdery mildew), followed by three backcrosses to the bread wheat parent, allowed the selection (using biochemical markers and GISH) of the disomic T 6DL·6VS lines Pm97033, Pm97034, and Pm97035 with $2n = 42$ (Li et al. 1999). RW15 is a 6V(6D) disomic substitution line derived from TH3/Wan7107*2//Jimai30*3///Shanxi859 (Chen et al. 1996b). The 6V#5S chromosome arm (Table 4.10) transferred in those lines at CAAS from a Dv ecotype from former USSR have genes for powdery mildew resistance (Li et al. 2005) but lack the alleles for WCM resistance found in T6AL·6V#4S developed at CINAU or DS 6V#6(6A) in lines GN21 and GN22 (Ma et al. 1997) developed at Guizhou Agricultural University, Guiyang, China, from the hybridization *T. durum* cv. "Sauwne20" with a *Dv* ecotype (different from the former USSR *Dv* ecotype used as sources

of 6V#5) collected by CAAS researchers (Table 4.10) (Li et al. 2002a).

The triparental hybrid derived from direct and reciprocal crossing of the [*T. turgidum* var. *durum* cv. "Modoc" (M) ($2n = 28$; genomes-AABB) × *Dv*] amphiploid (= MxV) with CS was used by Minelli et al. (2003, 2005) to study the behavior and interactions of parental genomes and the changes that parental genomes and chromosomes may undergo in the hybrids. Chromosome painting (GISH) analysis, as expected, evidenced in the parental MxV amphiploids the presence of 14 V chromosomes, 14 A chromosomes, and 14 B chromosomes. When CS was pollinated by MxV microspores, 80% of the pollinated florets produced caryopses with hybrid embryo (considered F_1). Homologous pairing and recombination between the A and between the B-genomes of M and CS and the random assortment of the chromosomes of the D- and V-genomes occurred at meiosis of the F_1 plants. This favored the arrangement of aneuploid and euploid AB, ABV, or ABD gamete configurations and various assortments of genetic blocks from the A and B parental genomes. The selfed F_1 plants were partially fertile, and the surviving F_2 embryos tended to be euploid due to the lack of viability of aneuploid gametes. In fact, F_2 plants having 14 A chromosomes, 14 B chromosomes, 7 D chromosomes, and 7 V chromosomes were rather rare (4.5%). Many progeny plants (54.6%) had the hexaploid wheat complement with 42 chromosomes and no V chromosome or chromatin was detected. Thirty percent of the plants had fewer than 42 chromosomes and about 28% of them had two to four V chromosomes. In most cases, D chromosomes were retained at higher frequency than V chromosomes (Minelli et al. 2005). About 42% of the F_2 plants were completely fertile. Root-tip chromosome counting of the resulting F_3 seeds showed a prevalence of 14 A, 14 B, and 14 D chromosome configurations. Using GISH, F_3-seedlings with one to seven V-chromosomes were recognized. Selfing, occurring from F_3 to F_4 generations, coupled to: (a) chromosome counting for selecting $2n = 42$ plants, (b) field-plot trials managed using low-input criteria, and (c) selection for spike fertility and plant yield components, allowed the identification of several euploid IBLs (Introgression Breeding Lines) with interesting plant and grain quality features (Vaccino et al. 2009).

Trigeneric Hybridization

Trigeneric hybrids involving wheat were produced since 1936 (Vakar 1936). Kostoff (1936b, c, 1937) and Kostoff and Arutiunova (1937a, b), although they did not succeed in producing *S. cereale* × *Dv* hybrid, they were able to obtain a robust trigeneric hybrid [(*T. dicoccum* × Dv) F_1 × *S. cereale* (♂)]. The 28 chromosomes of the F_1 trihybrid remained as univalents in 90% of the PMCs indicating very little homology among the ABVR chromosomes. Several trigeneric hybridization experiments involving the genera *Triticum*, *Dv*, and *Secale* were carried out in the period of 1959–1970 by Goichi Nakajima. F_1 caryopses were produced in the trigeneric hybrids (*Ae. squarrosa* × *Dv*) F_1 × *S. cereale*, but they failed to germinate (Nakajima and Zennyozi 1966). The *Ae. triuncialis*, $n = 14$, × (*T. persicum*, $n = 14$, × *Dv*, $n = 7$) trigeneric hybridization provided $2n = 35$ plants with putative genome-C^UCABV, which gave 5–6 bivalents per PMC. In the tetrageneric hybrid (*Ae. ovata*, $n = 14$, × *T. dicoccoides*, $n = 14$) × (*T. persicum*, $n = 14$, × *Dv*, $n = 7$), plants with $2n =$ 48 chromosomes C^UM^OAABBV and an average bivalent number of 12^{II} but self-sterile were found. No V chromosomes were involved in pairing.

F_1s and F_2s derived from multispecies hybrids involving *T. aestivum*, *T. persicum*, *S. cereale,* and *Dv* were carried out (Nakajima 1966b, c, 1970), and plants with chromosome number varying from 40 to 56 were found.

The number of somatic chromosomes of trigeneric triple F_1 hybrids raised from (*Triticum persicum,* $n = 14$, × *Dv*, $n = 7$) F_1 × *Secale cereale*, $n = 7$, (Tper HR F_1) and *T. durum* × *Dv* F_1 × *S. cereale* (Tdur HR F_1) varied from 25 to 32 in the first case, and from 24 to 32 in the second (Nakajima 1959b, 1960a). The F_1 plants were highly sterile, although a few F_2 caryopses were set. The $2n = 28$ TperHR F_1 plants displayed PMCs with 0–6 bivalents. In this last case, 4^{II} were attributed to AB autosyndesis and 2^{II} to V or R autosyndesis. Trivalents were rare and quadrivalents absent (Nakajima 1959c).

The chromosome number of the 89 individuals of $TperHRF_2$ hybrids varied from 38 to 58.4 (Nakajima 1960b). Some individual differences were observed in the external characteristics of the F_2 (Nakajima 1968). The external appearance of spikes of F_2 plants having

$2n = 38$–46 chromosomes revealed rye characteristics more than that of *Dv* and wheat, and those other ones having $2n = 47$–56 chromosomes resembled more closely *Dv* or wheat (Nakajima 1960b, 1966d). About ¼ of the F_2 plants were fertile, and the others showed various degree of sterility. The number of somatic chromosomes of the 16 individuals of TdurHR F_2 hybrids was found to vary from 42 to 50, and only one plant was fertile (Nakajima 1961a).

The morphology, fertility, and the meiosis in PMCs of F_1 (TperR_TperH) plants and the external characteristics, fertility and the somatic chromosome number of F_2 plants raised from *T. persicum* × *S. cereale* amphidiploid (TperR) crossed with *T. persicum* × *Dv* amphidiploid (TperH) were described by Nakajima (1964, 1969). At the meiosis I of the F_1, 12–15 bivalents were observed, the majority (14) attributed to AB chromosome autosyndesis, and only one bivalent to RV pairing. The F_1 were partially fertile, and 12 out of 35 F_2 raised individuals were sterile and the others showed various degree of fertility.

The trigeneric F_1 hybrids raised from *Triticum vulgare* × *Dv* F_1 × *Secale cereale* (TvCHR F_1) was described (Nakajima 1962). Ten F_1 plants ranged in chromosome numbers from 31 to 36. The number of bivalents at meiosis I ranged from 0 to 7, with a frequency mode at 0^{II} in F_1 plants with 31–32 chromosomes, and a mode of four bivalents in plants with 35 chromosomes. A few bivalents might have consisted of chromosomes of R- or V-genomes, most of the bivalents may have been formed by autosyndesis within the AB- or ABD-genomes from *T aestivum*, as was observed in F_1's between *Triticum* and *Dv* by Kostoff (1937).

Triticale cv. Clercal and Beagle (AABBRR) were hybridized with *T. durum* × *Dv* amphiploids to assess the level of pairing between the R- and V-genomes in the resulting AABBRV trigeneric hybrid. The R-V pairing was very low (0.156) or absent (Jahier et al. 1988). However, the allosyndetic R-V homoeologous pairing was observed in 102 out of 676 PMCs in M-I configurations in the trigeneric hybrid obtained by crossing *Ae. uniaristata* (U^nU^n) × *S. cereale* and *Ae. uniaristata* × *Dv*. Lucas and Jahier (1988) found a higher level of homoeologous pairing of V chromosomes in the hybrids *T. boeoticum* × *Dv* and *T. urartu* × *Dv*. It appears also in this case that, although low in absolute terms, the relative pairing of V chromosomes with those of wheat or *Aegilops* is higher that that between V and R chromosomes.

Octoploid *Agrotriticum* lines ($2n = 8x = 56$, AABBDDEE), TA163, Lankang, produced by S. C. Sun (*Triticum aestivum* × *Agropyron glaucum*), and hexaploid *Haynatriticum* ($2n = 6x = 42$, AABBVV) TH1663 and TH1659 (produced by X. Chen from *T. durum* × *Dv* hybridization), were used as parents in an hybridization scheme (Yuan et al. 1994, 1995). The trigeneric F_1 plants were true hybrids with $2n = 49$. The expected chromosome pairing in the hybrid was 14^{II} (7^{II} AA + 7^{II} BB) plus 21^{I} (7^{I} D + 7^{I} E + 7^{I} V), assuming that there was homologous pairing only. In fact, an average pairing per PMC observed was $18.53^{I} + 14.67^{II} + 0.32^{III} + 0.04^{IV}$. The univalents should be D, E, and V chromosomes. At the tetrad stage, some irregularities were also observed, such as micronuclei. These hybrids were partially fertile. Percentage of seed set was about 0.2%. Hybrid plants, F_2 and BC_1 were produced in greenhouse and could be used to develop wheat lines with desirable traits of *Dv* and *Agropyron*.

Octoploid *Triticale* was crossed to *T. durum* × *Dv* hexaploid amphiploid to have materials to test the efficiency of multicolor fluorescence in situ hybridization (McFISH) technique combined with C-banding for detecting and identify wheat, rye, and *Dv* chromosomes when combined in the same cell. The procedures provided a reliable approach to identify chromosome and chromosome rearrangements in trigeneric hybrids (Yuan et al. 1998).

Intergeneric crosses between *Triticum Dv* ($2n = 42$, AABBVV) and *Psathyrostachys huashanica* ($2n = 14$, N^hN^h) were made, the seed set was 1.67% (Genlou et al. 1995). Intergeneric hybrids were successfully obtained by means of embryo culture for the first time. The average chromosome pairing in the hybrid ($ABVN^h$) was 26.61% univalents and 0.69 bivalents. The chiasmata per cell were 0.69. The chiasmata number was higher than that in *Triticum durum* haploid (AB) and lower than that in *T. durum-Dv* haploid (ABV). The result indicated that the N^h-genome of *Psathyrostachys huashanica* has no homology with the V-genome of *Dv*, and with the A- and B-genomes of *Triticum durum*.

The above data confirm the very low V-R chromosome pairing ability, although it depends on the presence of the chromosomes of the third

genome: about 15% of the PMCs display V-R pairing when also the U^n chromosomes were present, but in the presence of chromosomes of the A- and B- wheat genome, the R-V pairing is almost nil as it is the pairing of V-E or V-N^h.

4.2.9.9 Autotetraploidy in *Dv*

Autotetraploid *Dv* with $2n = 4x = 28$ was obtained by Kondo (1940a, b, 1941) at the rate of 3 out of 20 colchicine-treated (0.05–0.1% w/w concentration) caryopses. One plant was a "solid" tetraploid and two were 2*x*–4*x* chimeras. The majority of chromosome configurations at meioses were $1^{I} + 10^{II} + 1^{III} + 1^{IV}$. Therefore, even in the presence of four copies of each chromosome, the tendency to form multivalents among chromosomes of the same homologous group was low. In other induced Triticineae autotetraploids, there was a tendency to form a higher number of complex configurations, such as in *S. cereale* ($3^{I} + 9^{II} + 1^{III} + 1^{IV}$), *A. squarrosa* ($4^{II} + 5^{IV}$), *A. bicornis* ($1^{I} + 10^{II} + 1^{III} + 1^{IV}$), and *A. uniaristata* ($1^{I} + 6^{II} + 1^{II} + 3^{IV}$).

4.3 Agricultural Status

Baker (1965) defined a weed as a plant that "in any specified geographical area, its populations grow entirely or predominantly in situations markedly disturbed by man." Therefore, weeds include plants that enter agricultural land (agrestals) as well as those occurring in waste places and along roadsides (ruderals). At the present time, *Dv* is prevalently found as a ruderal plant and rarely as weed in (wheat) crop fields. In the past, *Dv* may have been a more important agrestal weed, but modern farming systems have reduced weed populations in wheat and other cereal crops. Characteristics of *Dv,* such as short distance seed dispersal strategy, are typical of an agrestal weed. Harper (1977) pointed out that many of the species characteristics of disturbed habitats possess a long flowering period and a correspondingly long period of the year when seed is ripened and released. In contrast, the periods of flowering and seed production of weeds of arable land are usually concentrated within a narrow season and in some cases require the act of harvesting and threshing for full release of seeds. *Dv* has evolved a dispersal mode, mainly due to gravity, which is more similar to that of weeds of arable lands, although it now occupies mainly disturbed habitats. Somatic polymorphism for seed characters, a large buried seed bank, and phenotypic plasticity for plant size are features of *Dv* that qualify it as an ideal weed according to several of Baker's (1974) criteria and contribute to its success as a primary colonizer of disturbed sites. Generally, weeds show an annual habit and a wide environmental tolerance during growth. Plasticity in size is a response to environmental variation. Baker (1965) indicated that a breeding system based on self-pollination or even apomixis is likely to be important for a weed for building up a large population quickly whenever an opportunity presents itself after long-distance dispersal. As such, the weed population depends upon the occurrence of "general purpose" genotypes. On the other hand, many non-weed native plants having a breeding system based on outcrossing and extensive recombination can respond to natural selective forces to confer local adaptation. However, *Dv* has many weed characteristics, including predominantly outcrossing, of non-weed native plants of undisturbed communities. *Dv* populations often face drastic disturbances, such as grazing at early stages, mowing of culms before maturity of plants growing along the roadsides, or culm burning due to an accidental fire. The genotype composition of long-established roadside plant communities include forms that tolerate or are favored by these types of disturbance. Therefore, grazing, cutting, and fires are not disasters but repeated hazards that do not affect significantly the population composition in the roadside sites. In fact, the plant densities of *Dv* observed along roadsides that were subjected the previous year to weed-cutting before plant maturity were as high as the previous year before cutting. New colonization events may be excluded to explain the continued presence of *Dv* in disturbed roadsides; rather, yearly self-reseeding might occur through normal seeds formed on the spikes of the tillers produced during regrowth that followed *Dv* plant cutting, The flora associated with *Dv* are mainly composed of small, low-growing dicotyledonous weeds and tall plants (wild oats), although interspecific competition is likely to be light (Baker 1974; see also § 4.2.1). In the ruderal flora community, where *Dv* grows, interspecies competition is evident in later

growth stages when only the tall *Dv* plants are visible along road edges, while all the other small weeds remain entangled to the lower portions of the *Dv* plant. This could also indicate that *Dv* is a good competitor with other species at the seedling stage. *Dv* mimics wheat and other Triticeae plants. However, present day wheat fields do not generally harbor *Dv*, so there must be some weed characteristics in *Dv* (growth habit, reproductive habit, harvestable characteristics, etc.) that do not fit the soil environment and the timetable of wheat cultivation leading to a depletion of *Dv* seedbank in soil frequently used for wheat cultivation.

So far, *Dv* has not been used in folk medicine, or for ornamental, or herbal uses, nor has it been used extensively as a forage crop (De Pace et al. 1990), although it is grazed by sheep.

4.4 Role in Development of Cytogenetic Stocks and Their Utility

4.4.1 Addition and Substitution Lines

Sears (1953, 1976), using the species bridging method, was able to obtain plants from the triparental hybrid (*T. dicoccum* × *Dv*) × *T. aestivum* cv. Chinese Spring (CS) (see Sect. 4.2.9.4). About 200 florets of *T. dicoccum* (originally thought *T. dicoccoides*; Sears 1976) were pollinated with *Dv* yielding 25 seeds of which 19 were planted and seven grew plants. An amphiploid was produced from one of those hybrid plants by colchicine treatment (McFadden and Sears 1947), which was hybridized to *T. aestivum*. The F_1 plant had the genomic constitution AABB*DV* and the PMCs displayed the 14^{I} and 14^{II} chromosome configuration. The F_1 was backcrossed to *T. aestivum*, and progenies with $7^{I} + 21^{II}$ and 7^{I}, 19^{II}, and 1^{IV} were obtained. A second round of backcrossing to Chinese Spring followed by two generations of selfing made possible the isolation of five (CS + $1V^{II}$, CS + $2V^{II}$, CS + $3V^{II}$, CS + $4V^{II}$, CS + $6V^{II}$) of the seven possible disomic additions and six of the seven possible monosomic additions (CS + $1V^{I}$, CS + $2V^{I}$, CS + $3V^{I}$, CS + $4V^{I}$, CS + $5V^{I}$, CS + $6V^{I}$) of *Dv* chromosomes to the hexaploid complement of CS (Hyde 1953; Sears 1953, 1982). Originally, the numbering had no significance with respect to the homoeologous chromosome groups of wheat. The extra chromosome of the addition monosomic was identified by comparing the morphology of its lagging univalent at anaphase II with the *Dv* ideogram, in which the seven V chromosome pairs were ordered according to their length. The satellited chromosome was #4 in Hyde's idiogram, while it was the fifth chromosome (in the order of decreasing length) in von Berg's (1934) idiogram of *Dv*. A general observation was that the addition of individual V chromosomes or V-chromosome pairs to the CS hexaploid wheat chromosome complement modifies the morphology of wheat only slightly. It was usually difficult not only to separate the added monosomic V chromosome from CS chromosomes but also to distinguish them from each other. Only in the sixth addition monosomic, it was evident that a trait clearly controlled by a *Dv* gene was the red coleoptile of the seedling. In no case was any of the numerous and unusual *Dv* spikelet characters (such as long awns on the lemmas and outer glumes and anther extrusion from the glumes at anthesis) apparent in the disomic addition lines. Sears made further CS × *Dv* hybridizations using Greek and Italian *Dv* ecotypes as pollen parent, and the obtained DA lines were tentatively assigned to five different wheat homoeologous groups as: DA 1Ha to group 1 (black glumes); DA 2Ha to group 2 (increased awn length, narrow-leaved, slender-stemmed); DA 4Ha to group 4 (positive for alcohol-dehydrogenase isozymes of *Dv*); DA 6Ha to group 6 (positive for glutamate-oxalate-transferase isozymes of *Dv*); and DA 7Ha to group 7 (purple coleoptile or culms, not always expressed) (Sears 1982). Later, Sears (1985, personal communication), with the contribution of A. J. Lukaszewsky who applied C-banding, identified the DA 5Ha which was assigned to homoeologous group 5. This line expressed leaf hairs. The DA lines of chromosome 1V, 6V, and 7V were made using a *Dv* Italian ecotype as pollen parent, and 2V, 4V, and 5V using a Greek *Dv* ecotype as pollen parent. The set of DA lines are designated DA 1V#1 to DA 7V#1 except DA3V#1 (Tab. 4.10), which was not selected due to the lack of reliable markers (Fig. 4.36). The DA Set#1 was confirmed for homoeology to wheat chromosomes using biochemical markers (Montebove et al. 1987; De Pace et al. 1988a, b) and was used since 1987 to locate *Dv* genes on V chromosomes.

Sears (personal communication) by 1984 had already produced a new CS-*Dv* amphiploid using a *Dv*

Fig. 4.36 Set#1 (see Table 4.10) of disomic addition lines containing single V chromosome introgressed in CS chromosome complement by E. R. Sears

ecotype from Sicily (Italy). That amphiploid was used by A. J. Lukaszewsky to get a second set of CS DA lines of V chromosomes and other CS DA translocation lines and CS DA telosomic lines for single V chromosome or chromosome arm (Lukaszewski 1988). Those lines were recovered applying different methods: selfing progenies of double and triple monosomic additions derived from crossing the CS-*Dv* (Sicilian) amphiploid with CS, backcrossing the heptaploid AABBD*DV* hybrid to the octoploid AABBDDVV, and from crossing double and triple CS monosomic additions of V chromosomes with *H. bulbosum* (50 haploids were obtained and 46 were successfully doubled). The skilled experience of A. J. Lukaszewski in using C-banding, morphology, and molecular markers to assess the homoeology of the added V chromosomes to those of wheat in the CS DA lines produced by Sears allowed the proper and rapid identification of the different lines with monosomic and disomic V additions and the prompt assignment of the V chromosomes to wheat homoeologous groups in this new set of CS DA lines.

Blanco et al. (1983a, b) produced the *T. durum* cv. Creso × *Dv* F_1 ($2n = 21$, ABV) hybrid caryopses at a frequency of 5.6% of pollinated florets. A low frequency of meiotic non-reduction at the F_1 meiosis resulted in the production of male and female gametes with unreduced chromosome number. The triploid ABV F_1 was backcrossed with pollen from the Creso wheat parent, and caryopses with pentaploid embryos ($2n = 35$, AABBV) were obtained by the fertilization of unreduced female gametes (ABV) with the normal male gametes (AB). The pentaploid plants were again backcrossed with wheat pollen and six monosomic addition lines were produced (Blanco et al. 1987). In 1988, A. Blanco and coworkers started to study the homoeology of the V chromosomes in their set of monosomic addition (MA) lines to the wheat chromosomes. Blanco et al. (1988a) identified a stable and fertile ditelosomic addition line for the short arm of chromosome 6V expressing resistance to powdery mildew and additional α−β gliadins to those encoded by wheat genes. It was also ascertained that, using the glutamate-oxaloacetate-transaminase-3 (GOT-3) and phosphodiesterase-1 isozymes, and the seed-color and rachis brittleness morphological gene markers, the DA line A carried a V chromosome homoeologous to those of group 3 of wheat and was indicated 3V (Urbano et al. 1988). Simeone et al. (1990) and Simonetti et al. (1993), using RFLP molecular markers and three ditelosomic addition lines, established the homoeology relationships of the V chromosome lines to wheat homoeologous groups 3, 4, and 6. The complete set of monosomic addition lines of V chromosomes to the "Creso" durum wheat chromosome complement with homoeology definition to wheat chromosomes were available in 1991 (Blanco et al. 1991).

A hybridization program between wheat and *Dv* began in 1976 at the National Key Laboratory of Crop Genetics and Germplasm Enhancement (former Cytogenetics Laboratory), Nanjing Agricultural University (CI-NAU). F_1 hybrids of *T. monoccum*, *T. dicoccum*, *T. turgidum*, *T. durum, T. timopheevi*, *T. aestivum*, and *Ae. squarrosa* with *Dv* and backcrossed progenies of hybrids with *T. aestivum* were obtained (Chen and Liu 1982, 1986; Liu and Chen 1983, 1984). Cytogenetic analysis of some powdery mildew-resistant strains of the hybrid progeny between wheat and *Dv* were carried out by Pei and Liu (1986) and Pei et al. (1986).

Liu et al. (1988) studied several wheat aneuploid lines for V chromosomes obtained from crossing (*T. turgidum* × *Dv*) F_1 to *T. aestivum*. The *Dv* ecotype used in the hybridizations was from the Cambridge Botanical Garden, UK. The single V chromosomes that were either added to the wheat genome or substituted single wheat chromosomes were identified using a combination of karyotypic analysis and N-banding patterns and were designated V_2, V_3, V_4, V_6 and V_7 without implication for homoeology to wheat chromosome groups. Aneuploid lines,

monosomic or disomic for chromosome V_2, were immune to *Blumeria graminis* f. sp. *tritici* (*Bgt*) while lines having addition of chromosome V_4 showed resistance to *Bgt* only during the early growing stages. Lines with V_6 and V_7 chromosomes displayed clustering of bristles on the glume ridges, and line harboring V_3 carried genes for high protein content. In 1993, during the seventh International Wheat Genetics Symposium, held in Beijing, China, two seminal communications presented materials and results that are still influencing the genetic, molecular, and breeding studies of wheat lines carrying V chromosomes in China. The paper of Liu et al. (1995) reported the homoeology assignment of the V chromosomes identified by Liu et al. (1988) to the six different wheat homoeologous groups. Analyzing wheat-*Dv* substitution lines for single V chromosomes and adopting the concept that pairing and/or substitution of chromosomes from related genomes occur primarily between those of the same homoeologous groups, they suggested that the V_2, V_3, V_5, V_6, and V_7 of Liu et al. (1988), which substituted for 6A, 5D, 3D, 4D, and 2D, respectively, should be homoeologous to wheat chromosome homoeologous group 6, 5, 3, 4, and 2, respectively, and be renamed as 6V, 5V, 3V, 4V, and 2V. Chromosome V_1 was renamed 1V for the presence of a satellite, and V_4 was determined to be 7V for the expression of *Dv* SOD isozyme in the DA V_4. The presence of 4V, 6V, and 7V in the aneuploid lines was confirmed by detection of the ADH-1, GOT-2, and SOD-2 isozyme genetic markers identified by Montebove et al. (1987) in Sears' DA set.

The second influential paper was presented by Qi et al. (1995a), which confirmed that the formerly named V_2 chromosome was homoeologous to the wheat chromosome group 6, and described a 6AL·6VS wheat translocation line that carried a gene at the *Pm21* locus on the 6VS arm, which provided immunity to *Bgt* infection. Since then, following the guidelines for the nomenclature and abbreviation of the genetic stocks of wheat proposed by Raupp et al. (1995), the V chromosome-set in the aneuploid lines obtained by Liu et al. (1988) were labeled #2 and V chromosomes in the set of disomic addition (DA) lines produced by Sears (1982) were labeled #1 (Tab. 4.10). The DA #2 set was confirmed for homoeology to wheat chromosomes using RFLP markers (Qi et al. 1998a). Qi et al. (1995a) crossed the substitution line DS6V#2(6A) ($2n = 42$), described by Liu et al. (1988), with the hexaploid wheat cv. Yangmai 4 and Yangmai 5. One group of the F_1 caryopses was treated with γ-rays and the other group were grown and self-pollinated. The M_3 and F_3 progenies were grown in the field and infected with mixed strains of *Bgt* prevailing in Nanjing area. Seventeen M_3 plants resistant to *Bgt* carried a 6AL·6VS translocation (T6AL·6V#2S; $2n = 42$). Other two lines, del6V#2L-1 and del6V#2L-2 both $2n = 42$, had the terminal part of the long arm of chromosome 6V#2 missing and this deleted chromosome substituting 6A. The gene for *Bgt* resistance residing on 6V#2S was considered a new gene source that was different from previously reported ones and, following a suggestion from R. A. McIntosh, was named *Pm21*. Controlled infections in the greenhouse confirmed the resistance of those lines to *Bgt* (Qi et al. 1995b). C-banding and GISH showed that the breakpoint in the 6AL·6VS translocation obtained by Qi et al. (1995a) was near the centromere despite variation among them for the morphology and agronomic characteristics (Chen et al. 1995). RFLP analyses of the translocation and substitution deletion lines obtained by Qi et al. (1995a) and of a spontaneous deletion [DAdel6V#2S ($2n = 44$)] derived from DA6V#2 $2n = 44$ obtained by Liu et al. (1988), revealed that *Pm21* was located between the centromere and the 58% proximal portion of 6VS arm (Qi et al. 1998c) delimited by the RFLP marker Xcdo270. A large proportion (63%) of the RFLP markers detected polymorphism between set #1 and set #2 of V chromosomes. Chromosomes of the D-genome (i.e., 3D, 4D, and 5D) are more often substituted by V chromosomes (Qi et al. 1998a, 1999) than A- or B-genome chromosomes.

The translocation line T6AL·6V#2S and the F_2 progeny from the cross between cv. Yangmai 5 and T6AL·6V#2S allowed the identification of the codominant PCR-based molecular marker NAU/xibao located on 6V#2S and linked to *Pm21*. Qi et al. (1996) identified the RAPD marker $OPH17_{1900}$ specific for chromosome arm 6VS, and suggested its use as a marker for the allele at the *Pm21* locus determining resistance to *Bgt* infections. Translocations and deletions were induced by γ-irradiation to improve physical mapping of *Pm21*. Twenty small deletions occurred in different regions of 6VS (Chen et al. 2008a, b).

The DNA purified from the T6AL·6V#2S line was used for the preparation of a transformation competent

artificial chromosome (TAC) library (Sun 2007). The clone TAC15 was selected using the Xcinau15$_{902}$ (= NAU/Xibau15$_{902}$) primer set designed from the *Hv-S/TPK* gene sequence (Cao et al. 2006; Yang et al. 2008a). Using the TAC 15 clone in TAC-FISH for physical mapping, it appeared that the molecular hybridization site was located in the same region of *Pm21*, i.e., in the fraction length (FL) comprised from FL0.45 to FL0.58 of 6AL·6VS (Chen et al. 2008a), which was narrowed down in the range of FL0.566 to FL0.587 by Yang et al. (2008a).

The T6AL·6V#2S translocation line with the *Pm21* gene located on 6VS, has been transferred to different wheat backgrounds, and some elite varieties such as Nannong 9918, Neimai 9, and Shimai 14 with high yield and powdery mildew resistance were developed (Chen et al. 2002b).

The 6V#5S chromosome arm (Table 4.10) transferred in those lines at CAAS from a *Dv* ecotype collected in the former USSR, had the gene for powdery mildew resistance (Li et al. 2005) but lacked the allele for WCM resistance found in T6AL·6V#4S developed at CINAU or DS 6V#6(6A) lines GN21 and GN22 (Ma et al. 1997) developed at Guizhou Agricultural University, Guiyang, China, from the hybridization *T. durum* cv. "Sauwne20" with a *Dv* ecotype collected by CAAS researchers, which was different from the ex-USSR *Dv* ecotype used as sources of 6V#5 (Li et al. 2002a).

Eleven IBLs have been selected from a population of 150 aneuploid lines developed through [(CS × *Dv*, F_1) × CS] backcross, followed by three generation of selfing (BC_1F_1 S_1 through S_3), five generations of single-spike descent (from S_4 through S_8), and four generations of seed increase (S_{12} IBLs) (De Pace et al. 2007). S_{12} breeding lines traced to the same S_4 plant were considered "sister lines." Genetic uniformity within lines and differentiation among lines have been tested using AFLP and GISH. The most interesting lines were DA 6V#4 (= CSxV63), DS 6V#4 (CSxV32), and several early heading lines with good grain end-use quality (CSxV58, CSxV59 and CSxV60) containing cryptic *Dv*-chromatin introgressions (De Pace et al. 2001; Minelli et al. 2005; Caceres et al. 2008; Vaccino et al. 2010). It was also shown that the former DS CS 1V#1(1B) line produced by E. R. Sears was actually a Rec CS 1BL·1V#1S line (De Pace et al. 2001; Minelli et al. 2005; Vaccino et al. 2010). It should be noticed that according to chromosome nomenclature proposed in Table 4.10, 6V#4 should be recoded to 6V#7.

The above information indicates that five sets of aneuploid wheat IBLs with well characterized V chromosomes from different *Dv* ecotypes and ascertained homoeology to wheat chromosome homoeologous groups are available. On the basis of the chronology of the published reports about the identification of the homoeology relationships of the V-chromosomes in those lines to those of the hexaploid wheat homoeologous chromosome groups, the sets of aneuploid wheat IBLs should be numbered as set #1 (Sears 1982), set #2 (Lukaszewski 1988), set #3 (Blanco et al. 1991), set #4 (Liu et al. 1995), set #5 (Chen et al. 1996b; Shang et al. 1997; Li et al. 1999), set #6 (Ma et al. 1997), and set #7 (De Pace et al. 2001; Minelli et al. 2005) (Table 4.10). However, Qi et al. (1998b, 1999) had already labeled as set #1 the IBLs of Sears (1982), set #2 the IBLs of Liu et al. (1995), and set #3 the IBLs of Lukaszewski (1988). Accordingly, the IBLs set of De Pace et al. (2001) and Minelli et al. (2005) was named set #4 (Bizzarri 2009). This discrepancy should be fixed.

4.4.2 Other Forms of Introgression of V Chromatin in Wheat Chromosomes

4.4.2.1 Translocations Induced by a Gametocidal Chromosome

Chromosome 3C of *Aegilops triuncialis* was discovered with the ability to be transferred preferentially in the case of its monosomic status in wheat background, whereas those gametes without 3C would result in chromosome structural changes including deletions and translocations. This method was applied by Chen et al. (2002a) to get 4V chromosome rearrangements. *T. aestivum-Dv* substitution line 4V(4D) was crossed to *T. aestivum* cv. Norin 26-*Ae. triuncialis* 3C addition line, and the F_1 was then backcrossed to common wheat. C-banding and GISH revealed that two translocation lines, T3AS·4VL and T4DL·4VS, two telocentric chromosome lines, and the two 4VS.4VS and 4VL.4VL isochromosomes were present in either the BC_1F_2 or BC_1F_3 progenies. This result indicated that gametocidal chromosome 3C of *Aegilops triuncialis* could effectively induce structural changes of both

chromosomes 4V of *Dv*. In a similar hybridization involving a DA 4V rather than DS 4V(4D) and screening in F_2 by C-banding and FISH, a homozygous translocation line (CQZ55) was selected, which carried a T4VS.4VL-4AL and showed high resistance to wheat spindle streak mosaic virus (Chen et al. 2007).

Gametocidal chromosome 3C can also successfully induce chromosome 2V structural changes after crossing the disomic addition line harboring the gametocidal chromosome 3C of *Aegilops triuncialis* added in Norin-26, to the wheat-D*v* disomic substitution 2V(2D) (Chen et al. 2008a). Four translocations including one small segmental translocation T6BS·6BL-2VS, two whole arm translocations (designated as T3DS·2VL and T7DL·2VS), and one intercalary translocation T2VS·2VL-W-2VL, one deletion Del 2VS·2VL, one monotelosomic Mt2VS, and one isochromosome 2VS·2VS line have been developed and characterized. GISH, the wheat SSR marker $Xwmc25_{120}$ that tags 2VS, and one wheat STS marker ($NAU/STSBCD_{135\text{-}1}$) that tags 2VL were used successfully to confirm the alien chromosome rearrangements. The tufted bristles on the glume ridge appeared in lines Mt2VS, 2VS·2VS, and the parent DS2V(2D), whereas this trait did not appear in T3DS·2VL. The gene controlling the tufted bristles was located on 2VS.

4.4.2.2 Translocations Induced by γ-Radiation

An highly efficient approach for inducing small chromosome segment translocations was pre-anthesis irradiation of the female gametes of the T6AL·6VS translocation line with ^{60}CO-γ-ray at three dosages (1,600, 1,920, 2,240 rad). Anthers were removed from the irradiated florets on the same day and the florets were pollinated with normal fresh pollen from CS after 2–3 days. M_2 seeds were obtained by backcrossing 74 M_1 plants involving 146 chromosomes structural changes of 6VS. The structural aberrations in the M_1 plants were transmitted to their progenies. Therefore, irradiating mature female gametes having whole-arm translocations is a new and highly efficient approach for creation of small segment chromosome structural changes, especially for interstitial translocations (Chen et al. 2008b).

A similar approach was used to develop more wheat-*Dv* translocations involving different chromosomes and chromosome segments of *Dv*. *T. durum* × Dv amphiploid was irradiated with ^{60}Co γ-ray at doses of 800, 1,200, and 1,600 rad. Pollen collected from the spikes 1, 2, and 3 days after irradiation were transferred to emasculated spikes of the common wheat cv. "Chinese Spring." A higher translocation induction frequency was observed when pollen was collected from the spikes 1 day after irradiation than after 2 or 3 days. More than 70% of the translocations detected in the M_1 generation were transmitted to the BC_1 through the female gametes (Cao et al. 2009b). By means of ^{60}Co γ-ray irradiation, it is possible to increase the frequency of chromosome translocation in callus cells to about 8% (Li et al. 2000a).

Pollen irradiation was also attempted for the efficient induction of wheat-*Dv* chromosomal translocations. *Triticum durum-Dv* amphiploid pollen treated with 1,200 rad ^{60}Co-γ-rays was pollinated to CS (Bie et al. 2007). Ninety-eight intergeneric translocated chromosomes between *T. durum* and *Dv* were detected by GISH in 44 of 61 M_1 plants. The ratio of small segment terminal translocations (W·W–V) was much higher than that of large segment terminal translocations (W–V·V). Transmission analysis showed that most of the translocations were transmittable. Therefore, pollen irradiation is an efficient strategy for wheat improvement through the terminal translocations of V chromosomes on wheat chromosomes.

The effect of γ-ray treatment on chromosomes of *Dv* protoplasts and derived somatic hybrids with wheat protoplasts was studied in microcalli derived from asymmetric somatic hybridization between protoplasts of wheat (*T. aestivum* L. cv. Jinan 177) and protoplasts of *Dv* treated with different dosages of γ-rays (40, 60, and 80 Gy; 1 Gy = 100 rads) (Zhou et al. 2001a, b; Zhou and Xia 2005). The putative hybrids were screened by isozyme analysis and characterized for nuclear and organellar genome composition of the hybrids. GISH demonstrated that the donor *Dv* chromosome elimination in the hybrids increased with increased γ-ray dosage. Intergenomic chromosome recombination/translocations were observed in the hybrids from different dosages of γ-rays. The development of the hybrid clones was dependent on the γ-ray dosage used for the *Dv* donor protoplasts. Regenerated plants were obtained only from fusion of protoplasts treated at low (40 Gy) and intermediate (60 Gy) dose irradiation.

4.4.2.3 Translocation Induced by Tissue Culture

One of the successful example of wheat-alien chromosome translocation via tissue culture has been reported by a joint group of scientists from Australia and China (Xin et al. 1991; Banks et al. 1995). Researchers at CAAS, Beijing, China aimed, since 1996, at chromosome translocations between wheat and *Dv* by means of in vitro culture of immature 14–16-day-old embryos produced after triparental hybridization involving *T. durum*, *Dv*, and *T. aestivum* (Chen et al. 1996b; see Sect. 4.2.9.8). The desired translocations were obtained at an average frequency of 1.9% (Li et al. 2000a). Translocations existed not only in callus cells but also in regenerants. Both whole chromosome arms and small chromosome segments were involved in the observed translocations. The frequency of wheat-*Dv* translocation events increased to 8% when the in vitro grown calli from the immature embryos underwent γ-ray treatment (Li et al. 2000a, c). Those strategies coupled with cytological analysis and GISH allowed selection of stable wheat lines carrying minimal amount of translocated chromatin from 6V harboring genes that confer resistance to powdery mildew, WCM, yellow rust, and WSMV (Li et al. 2000a, b, 2002a). Translocations might be expected also when the 6V chromosome is in the monosomic condition into the wheat chromosome assemblage (Li et al. 2002b).

4.4.2.4 Cryptic Introgression

Wheat inbred lines derived from *T. aestivum* × *Dv* showed some phenotypic differences compared to the wheat parent, such as early anthesis, awnedness, higher grain yield per spike, and enriched prolamine subunits in grain seed storage proteins (De Pace et al. 2001; Vaccino et al. 2008). However, these lines had the same chromosome number and structure as *T. aestivum*, and neither chromosomes nor chromatin from *Dv* were apparently added to their chromosome complement. However, Feulgen/DNA cytophotometric determinations showed that the amount of DNA in prophase nuclei (= 4C) of the lines exceeded significantly that in the prophases of the wheat parent (Caceres et al. 2008). AFLP analysis of genomic DNAs showed that the majority of bands were shared with *T. aestivum*, but about 15% of bands were specific to *D. villosum* (Caceres et al. 2008). Moreover, PCR amplification using specific primers revealed *Dv* alleles and not *T. aestivum* alleles at two *Vrn* loci. These findings indicate cryptic introgression of V DNA in the wheat genome of the lines, i.e., the occurrence of chimeric wheat-*Dv* chromosomes where, at each site, V chromatin is so scarce that is undetectable by GISH. It is to be noted that V chromosomes or their portions were present in these hybrids after crossing. Apparently, any *Dv* DNA sequences not introgressed into the *T. aestivum* chromosomes have been progressively eliminated from the hybrid genomes; and lines got stability in this way. DNA sequence elimination from the nuclear genome has already been shown to be a rapid and reproducible response to wide hybridization in wheat (Shaked et al. 2001). The occurrence of cryptic introgression of *Dv* DNA into the genome of wheat suggests that caution is needed in assigning genes for discrete phenotypic traits to specific V chromosomes before comparing the results of cytogenetic analyses with the hybrid phenotype (Minelli et al. 2005). The presence in the hybrid genome of GISH-visible V chromatin does not entail necessarily that given traits are surely or entirely due to the expression of genes that are borne in that chromatin. On the other hand, the absence of detectable chromatin from *Dv* in the hybrids does not call for attributing *Dv*-specific traits to mutational events other than introgression of V DNA.

4.4.3 Morphological, Biochemical, Molecular and Cytological Markers to Identify Individual V Chromosomes

Since 1987, the genetic stocks described in the previous paragraphs allowed the beginning of chromosomal location of V genes for biochemical (Montebove et al. 1987), molecular (De Pace et al. 1992), and disease resistance (Yildirim et al. 1998) genes. These lines have been instrumental for the production of stable introgression of *Dv* genes in wheat chromosomes under the form of terminal or whole V chromosome-arm translocations on wheat chromosomes or interstitial and cryptic transfer of V chromatin segments into wheat chromosomes. Therefore, those lines were important materials for both locating genes and

genetic markers on V chromosomes and for transferring the useful V-chromosome alleles in wheat introgression breeding lines for wheat improvement. An alternative method was proposed to assign gene to specific chromosomes of *Dv* by using nullisomic amphiploids isolated from the progeny of *T. turgidum*-*Dv* amphidiploids (Zhong and Qualset 1990).

4.4.3.1 Morphological Markers

Sears (1982) was the first to assign genes controlling additional morphological traits in DA wheat lines carrying single V chromosomes. Two different nullisomic amphiploids involving different V-genome chromosomes were isolated by the use of the markers brittle rachis and hairy glume (Zhong and Qualset 1990).

Genes controlling the phenotype of quantitative traits were inferred from the analysis of several CS-DA lines each harboring a pair of V chromosomes. CS-DA lines were evaluated for a set of 28 complex traits scored during four consecutive growth stages (germination, seedling elongation, tillering, stem elongation-maturity) in a greenhouse (Mariani et al. 2003). It has been postulated that: (a) for a given complex trait, the pattern of phenotypic differences between individual disomic aneuploid lines and CS provide an estimate of both the composite additive effects of QTL alleles summed over the *n* loci in a "composite factor" (CF) affecting the trait and located in the V chromosome of that aneuploid line, and suggestive evidence on the location of those QTL alleles in that V chromosome; (b) a CF affects more than one trait, eliciting correlated composite additive effects among a subset of traits; (c) each V chromosome has a CF exerting effects on the same subset of traits; (d) growth stages serve as developmental landmarks and triggers for the expression of independent CFs affecting independent subsets of traits.

Factor analysis of the genetic correlation matrix showed four uncorrelated factors accounting for about 70% of the total variation for the additive effects. The number of factors detected have biological meaning and indicates that a minimum of four independent CFs on each V chromosome account for the additive variation and covariation observed for the panel of disomic aneuploid lines. Enhancing additive effects were expressed by CFs located in chromosomes 1V and 7V (earliness at tillering stage), 4V (rapid germination), and 5V (seedling robustness). The presence of gene(s) on chromosome arm 2VS for the tufted bristles along the glume keels have been inferred by Chen et al. (2008a).

4.4.3.2 Biochemical Markers

The first biochemical markers of agronomic interest that were mapped on V chromosomes were those for seed storage proteins. Chromosome 1V have loci for storage proteins similar to the wheat high molecular weight (HMW) glutenins (*Glu-V1*, homoeologous to wheat *Glu-1*), ω- and γ-gliadins (*Gli-V1*, homoeologous to *Gli-1*), and low molecular weight (LMW) glutenins (*Glu-V3*, homoeologous to *Glu-3*). Moreover, on the short arm of chromosome 6V is located the locus *Gli-V2*, homoeologous to *Gli-2*, and on the long arm of chromosome 4V the locus *Gli*-V3, which harbor genes encoding proteins similar to wheat α-type gliadins (Montebove et al. 1987; Resta et al. 1987; Shewry et al. 1987, 1991; Blanco et al. 1991; De Pace et al. 2001; Li et al. 2009).

Variation for prolamin polypeptides in *Dv* was examined by Della Gatta et al. (1984), Grilli et al. (1988), Vapa et al. (1993), and De Pace et al. (1988c, 1994). Zhong and Qualset (1993) showed that a large variability exists at the *Glu-V1* locus on chromosome 1V of *Dv*: 14 alleles at *Glu-VI* were found among 982 individuals representing 12 populations from Italy and two from Yugoslavia, with a mean of seven alleles per population. The subunits were named from *a* to *n* using the system of Payne and Lawrence (1983). Among the 14 alleles, one produced no HMW-glutenin subunit (k), 10 coded for a single subunit (alleles *a* to *j*), and three coded for two subunits (alleles *l*, *m*, and *n*). The mobility of all the subunits coded by *Dv* alleles was in the range of subunits 7 and 12 of Chinese Spring. About 7% of the total allelic variation was distributed among populations and more than 90% within populations.

From the survey of IBLs derived from *Triticum aestivum* cv. Chinese Spring (CS) × *Dv* hybridization, and studying the segregation of groups of prolamins fragments from progenies derived from four different CS × IBL backcrosses, it was possible to define specific prolamin blocks encoded at *Gli-V1*, *Gli-V2, Glu-V1*, and *Glu-V3* loci of *Dv* introgressed in the IBLs (Vaccino et al. 2010).

Two hexaploid amphiploids from *T. turgidum* var. *durum* × *Dv,* which had the genome-AABBVV, and an octoploid amphiploid from *T. aestivum* cv. Chinese Spring × *Dv,* which had the genome-AABBDDVV, were analyzed using electrophoresis for glutamate oxaloacetate transaminase [aspartate aminotransferase] (GOT; EC 2.6.1.1), alcohol dehydrogenase (ADH-1; EC 1.1.1.1), glucosephosphate isomerase (GPI; EC 5.3.1.9), superoxide dismutase (SOD; EC 1.15.1.1), lipoxygenase (LPX; EC 1.13.11.12), and esterase (EST; EC 3.2.1), isoenzyme banding patterns, and for total grain storage proteins (Delre et al. 1986; Montebove et al. 1987). The banding patterns for HMW storage proteins in the amphiploids overlapped those of their parents. The banding patterns for GOT, ADH and PGI appeared to be the result of random association of combinations of monomers coded by sets of homoeologous genes in all the genomes in the amphiploids. Analysis of five out of seven disomic addition lines of *Dv* chromosomes to Chinese Spring showed that the genes *Gpi-V1*, *Adh-V1* and *Got-V2* for GPI-1, ADH-1, and GOT-2 isozymes were located, respectively, on chromosomes 1V, 4V, and 6V of *Dv*. The *Got-V3* gene for GOT-3 isozymes was located on chromosome 3V (De Pace et al. 1988b). Genes at locus *Glu-V1* for HMV storage protein subunits are located on 1V and prolamin genes are located on 1V and 4V. There was indirect evidence that genes for GOT-3 and LPX-2 were located on 3V and 5V, respectively (Montebove et al. 1987). The *Sod-V2* gene for SOD-2 isozyme was located on chromosome 7V. Benedettelli and Hart (1988) localized the gene *Skdh-V1* for shikimate dehydrogenase (EC 1.1.1.25) isozyme on chromosome 5V. De Pace et al. (1988a) found that the gene *Per-V1* for one form of peroxidase (EC 1.11.1.7) was located on chromosome 1V, and the gene *Ndh-V1* for NADH dehydrogenase-1 (EC 1.6.99.3) was located on chromosome 4V. Four nullisomic amphiploids involving different V-genome chromosomes were isolated by the use of the isozyme marker ADH-1, GOT-2, SOD-2 and seed storage proteins (gliadin) (Zhong and Qualset 1990).

The DA 6V#4 (=CSxV63) and DS 6V#4 (CSxV32) IBLs (or 6V#7 according to Table 4.10) showed powdery mildew immunity (a putative example of non-host resistance; Bizzarri 2009), leaf rust adult-plant resistance (Bizzarri et al. 2009), 18.5% protein content, good Fe content (+27% compared to CS), and a high Zn content (+40% compared to CS) (Vaccino et al. 2010). The 6V#1 contains allele(s) at the locus *Gli-V2* causing lower gluten strength, associated to alleles for susceptibility to powdery mildew (Cenci et al. 1998; De Pace et al. 2001). Three IBLs (CSxV 58, CSxV 59 and CSxV 60) showed early heading (for the presence of cryptic introgression of a putative *Vrn-V3* gene with similar effects to those postulated by Yang et al. (2006) for the interaction of *Vrn-B3* with vernalization treatment to express heading earliness) (Caceres et al. 2008), and good grain end-use quality due to the simultaneous presence of a cryptic *Gli-V1*/*Glu-V3* introgression and a mutant *Gli-D1g*-like allele (Vaccino et al. 2010). It was shown that the Rec CS 1BL·1V#1S line had a *Glu-V1* allele that improved grain end-use quality (De Pace et al. 2001; Vaccino et al. 2010).

4.4.3.3 Allergenic Proteins

Li et al. (2009) recently reported the isolation of 32 α-gliadin clones, 16 from *Dv* and 16 from *Db*. Twelve of 32 sequences were predicted to be pseudogenes suggesting the high variation of gliadin genes in *Dasypyrum* genome. The nucleotide comparison of the entire sequence showed a high degree of homology with other α-gliadin sequences. The deduced amino acid sequence of the *Dasypyrum* α-gliadin genes represented a presumptive mature protein of 281–303 residues and a calculated molecular weight of 29–34 kDa, having the typical structure of α-gliadins. It was also reported that in some cases, the number of the cysteine residues differed from six, the condition usually reported for α-gliadin sequences in wheat, spanning from five to seven. In gliadins, the six cysteines are involved in the formation of three intramolecular disulfide bonds: the presence of extra cysteines could have implications for flour end-use quality, leading to the formation of intermolecular disulfide bonds, usually related to high quality (Gianibelli et al. 2001). Moreover, the analysis of the promoter regions (Li et al. 2009) revealed four different lengths, and the presence of part of the retrotransposon element sequences matching with LTR gyspy Wilma, indicative of the possible involvement of retroelement-like sequences in the evolution of the *Dasypyrum* α-gliadin genes.

Similar evidence was found from the analysis of *NAM-B1*, a gene involved in protein and micronutrient

accumulation, in the disomic 6V introgression line CSxV63, derived from *Triticum aestivum* cv. Chinese Spring (CS) × *Dv* hybridization, where a 260 bp fragment with 73% identity to the *Triticum durum* retrotransposon Ttd5 was identified (Vaccino, Corbellini, and Banfi R unpublished data).

The *Dasypyrum* α-gliadin genes were also searched for four well-known T-cell stimulatory epitopes (glia-α-2, glia-α-9, glia-α-20, glia-α) in celiac disease (CD) patients (Li et al. 2009). Analyzing 20 α-gliadin full open reading frames, they found that the distribution of the epitopes varied between *Dv* and *Db*: in particular, both glia-α-2 and glia-α-9 epitopes in all 20 sequences were disrupted by deletion of a glutamine, 18 sequences contained epitope *gila-α,* but all the sequences from *Dv* lacked the region containing the epitope glia-α-20. The authors conclude that *Dasypyrum* could give some opportunities to select non-toxic germplasm for celiac disease patients.

4.4.3.4 Molecular Markers

As of 10 Mar 2009, 123 nucleotide sequences of *Dv* are deposited in the GenBank database (*http://www.ncbi.nlm.nih.gov*; Table 4.6). The sequences are related to storage proteins (about 22%), comprising high- and low-molecular weight glutenins and α-gliadins; cell metabolism (21%) (sequences for acetyl-CoA carboxylase, NADH dehydrogenase, serine/threonine protein kinase); carbohydrate metabolism (4%) (sequences for β-amylase or granule bound starch synthase I); transcription and translation mechanisms (12%) (i.e., ribosomal proteins or elongation factors); and resistance genes (8%). About 32% of the clones consist of unknown species-specific sequences, such as RAPD markers or repeated sequences, which can be useful as *Dv* markers, while four sequences are related to mobile elements.

Highly repeated DNA sequences in plant chromosomes are important DNA features for studying the organization and evolution of plant chromosomes (McIntyre 1988; McIntyre et al. 1988, 1990). The available species-specific repeated sequences described below may be useful tools for identifying *Dv* and *Db* chromatin in the wheat genome and monitoring wheat improvement achieved by *Dv* or *Db* introgression.

The physical locations of some genes and sequences in *Dv* have been identified. Genes for rRNA at the complex locus *Nor-V1* were located on chromosome 1V (De Pace et al. 1988a) and were also analyzed by Gill and Appels (1988). De Pace et al. (1992), analyzing a *Dv* genomic library, identified a highly repeated DNA sequence of 396 bp from which a fragment of 380 bp was cloned in a plasmid (p380) (Table 4.11). The sequence was concluded to be specific for the *Dv*-genome: (1) it did not hybridize to the DNA from different *Triticum* species with chromosomes closely or distantly related to the A-, B-, and D-genomes of cultivated wheats; (2) cytological hybridization experiments and Southern blot analysis of the DNAs from DA set#1 demonstrated that the sequence was present in all *Dv* chromosome added to CS, except for 7V; and (3) the sequence was located by in situ hybridization at or near the telomeres of six out of seven chromosome pairs in *Dv* root-tip cell metaphase chromosomes.

Significant alignments were produced by the p380 sequence to eight different sequences in the NCBI collection (Table 4.9), five to other sequences in *Dv*, and three to sequences in *Leymus racemosus*, another Triticeae species. The 769 bp DNA tandem repeated sequence in the "pLrPstI-4" DNA clone from a *L. racemosus* genomic library has a 384 bp repeated region where 308 out of 361 (85%) nucleotides gave a Vmax score 350 bits for the identity of the corresponding pairwise alignment with "p380"; the likelihood that a sequence with a similar score will occur in the database by chance is extremely low (4e-93), indicating that it is very unlikely that the high similarity between the "p380" repeated sequences from *Dv* and the 384 bp repeated region from *L. racemosus* occur by chance. Other observations from molecular marker and specific sequence similarity analyses reinforce the idea that the *Dv* vs. *L. cinereus* similarity (see below) might not be spurious. The diploid ancestors of allotetraploid *Leymus* were initially identified as *Psathyrostachys* and *Thinopyrum* (Dewey 1984; Löve 1984). While at least one *Psathyrostachys* genome (*Ns*) has been substantiated in *Leymus,* cytogenetic and molecular data have refuted the putative genome relationship of *Thinopyrum* to the other genome of *Leymus*. Hybridization of *Leymus* DNA with repeated nucleotide sequences from other Triticeae species suggested that *Leymus* is a segmental autopolyploid, $Ns_1Ns_1Ns_2Ns_2$, derived from

Table 4.9 Other nucleotide repeated sequences sharing more than 80% identity with the 380 bp-*Dv*-genome-specific tandem repeated sequence (BLASTN 2.2.22 search using the p380 sequence in Table 4.11)

Accession	Description	Max score	Total score	Query coverage	E value	Max ident (%)	Note
AB074442.1	*Dv* repeat region, clone: pDv19	427	427	86%	2e-116	89	Centromeric dispersion of the 380 bp family containing TG repeats on *D. villosum*
AB074440.1	*Dv* repeat region, clone: pDv27	411	411	86%	2e-111	88	
AB074439.1	*Dv* repeat region, clone: pDv24	399	399	86%	4e-108	88	
AB074441.1	*Dv* repeat region, clone: pDv40	388	388	86%	8e-105	87	
AF472572.1	*Dv* tandem repeat sequence	398	398	74%	1e-107	91	Found on chromosomes 1V, 2V, 3V, 4V, 5V, 6V
AB016973.1	*Leymus racemosus* DNA, repeat sequence, clone: pLrPstI-4	350	619	89%	4e-93	87	384 bp repeative sequences in *Leymus racemosus*
AB016972.1	*Leymus racemosus* DNA, repeat sequence, clone: pLrPstI-3	279	279	59%	5e-72	88	385 bp repeative sequences in *Leymus racemosus*
AB023620.1	*Leymus racemosus* DNA, heterochromatin region	272	272	58%	8e-70	88	384 bp repeative sequences in *Leymus racemosus* heterochromatin region

two distinct *Psathyrostachys* species (Zhang and Dvorak 1991). Triticeae phenogram based on Rogers' genetic distance for RFLP molecular markers placed *Dv* equally distant from *Psathyrostachys* and *Thinopyrum* (Monte et al. 1993). The similarity between *Dv* and *L. cinereus* or *P. juncea* for the TCP1 sequence (orthologous to the *tb1*-like gene sequences of maize, which controls the proliferation and elongation of lateral branches and tillers) was greater than similarity between *Dv* and either *Avena sativa*, *Zea mays*, or *Poa fendleriana* (Wu et al. 2003).

A species-specific repeated sequence, pHvNAU62, was cloned from *Dv* (Li et al. 1995). It strongly hybridized to *Dv* chromosomes but not to wheat. In situ hybridization localized this sequence to six of seven *Dv* chromosome pairs in telomeric or subtelomeric regions. Southern hybridization to wheat-*Dv* addition lines showed that chromosomes 1V through 6V gave strong signals in ladders, while there was no signal for chromosome 7V. In addition to *Dv*, several Triticeae species were found to have a high abundance of the pHvNAU62 repeated sequence. Another *Dv* repeated sequence, cloned in pHv62 has considerable size variation and wider distribution in the Triticeae. This sequence is similar but not homologous to that cloned in p380, because p380 did not produce a hybridization signal in a dot blot of rye (De Pace et al. 1992) whereas pHv62 did (Li et al. 1995). Yuan and Tomita (2009) showed that the above-mentioned repeated sequences belong to the 350-family isolated from rye and organized in a tandem array of a 380 bp unit on the subtelomeric chromatin.

Two dispersed repetitive DNA sequences were isolated by Galasso et al. (1997) from a *Db* genomic library hybridized with *Db*- and *Dv*-labeled genomic DNA. The pDbKB45 sequence was distributed along the *Db* chromosomes but amplified in the subtelomeric regions, and no hybridization signal was observed on *Dv* chromosomes; on the other hand, pDbKB49, in both *Dv* and *Db* species, was less amplified in terminal regions and was distributed along most chromosome arms. Also, the pDb12H cloned from *Db*(4*x*)-genome-specific RAPD product was a repeated sequence strongly homologous to a long terminal repeat (LTR) *Sabrina* retrotransposon, which was not detected by FISH in *Dv*-genome (Yang et al. 2006).

A specific DNA segment of 388 bp, named *pDv848/388* (GenBank Accession No. EF411201 and EF411201), was obtained from *Dv* by using the primer UBC848. *pDv848/388* is an intersimple sequence repeat (ISSR) marker for chromosome 5V (Tang et al. 2007, *http://www.ncbi.nlm.nih.gov/nuccore/125661829*) as well as for *Db* chromosomes. Moreover, PCR analysis was carried out on *Dv*, a

Table 4.10 Genetic stocks of wheat aneuploids containing monosomic (') or disomic (") additions or substitutions of *Dasypyrum villosum* (*Dv*) chromosomes. The sign "#" followed by a number is used to distinguish the origin of the V chromosomes in wheat introgressions lines involving the same homologous V group, but traced to different *Dv* accessions, ordered according to explicit reference reports (for nomenclature and abbreviations see Raupp et al., 1996; *http://wheat.pw.usda.gov/ggpages/nomenclature.html*).DA=Disomic addition lines; DS=Disomic substitution lines; MA=Monosomic addition lines).

Recipient wheat line	Source of *Dv* donor accession	*Dv* chromosomes identified for their homoeology to wheat chromosome							Set#of V chro-mos.	Reference
		1V	2V	3V	4V	5V	6V	7V		
Triticum turgidum var. *dicoccum* was used as bridge recipient and then backcross introgression of V chromosomes in *T. aestivum* cv Chinese Spring (CS)	This subset involves chromosomes from the so-called "Italian" Dv accession	DA 1V#1″ DS 1V#1″(1A) DS 1V#1″(1D)					DA 6V#1″	DA 7V#1″ DS 7V#1″ (7A)	Set # 1	Sears (1953); Sears (1976); Sears (1985, pers. comm.); Lukaszewski (1988); Lukaszewski (2009, pers. comm.) *http://wheat.pw.usda.gov/ggpages/GeneticStocks/AJLukaszewski_Wheat_Stocks.doc*
	This subset involves chromosomes from the so-called "Greek" *Dv* accession		DA 2V#1″ DS 2V#1″(2B)		DA 4V#1″	DA 5V#1″ DS 5V#1″ (5D)				
T. aestivum cv CS	"Sicilian" Dv accession	DA 1V#2″	DA 2V#2″	DA 3V#1″	DA 4V#2″	DA 5V#2″ DS 5V#2″(5B)	DA 6V#2″ DS 6V#2″(6A) DS 6V#2″(6B) T 6BS·6V#2L	DA 7V#2″	Set # 2	Lukaszewski 1988 (7th IWGS), Lukaszewski (2009, pers. comm.) *http://wheat.pw.usda.gov/ggpages/GeneticStocks/AJLukaszewski_Wheat_Stocks.doc*. The V chromosomes of this stock were numbered #3 by Qi et al 1999.
Triticum turgidum var. *durum* cv Creso	A Dv ecotype from the Apulia Region (Italy)	MA 1V#3′	MA 2V#3′	MA 3V#2′	MA 4V#3′	5V#3′	MA 6V#3′	MA 7V#3′	Set # 3	Blanco et al. (1987): Urbano et al. (1988) established homoeology of 3V to the wheat chromosomes of homoeologous group 3, and Blanco et al. (1991) ascertained homoeology of the other V chromosomes to the other wheat homoeologous groups.
Several cultivars of *T. aestivum* including cv "Yangmai#5"	GP005 *Dv* Accession from the Cambridge Botanical Garden, UK		DA 2V#4″ DS 2V#4″	DA 3V#3″ DS 3D-3V#3″	DA 4V#4″ DS 4V#4″(4D) T4DL·4V#4S T4AL -4V#4L •4V#4S	DA 5V#4″ DS 5V#4″	DA 6V#4″ DS 6V#4″(6A) T6AL·6V#4S	DA 7V#4″	Set # 4	Liu DJ et al., 1988 (7th IWGS); Liu DJ et al.,1995 (8th IWGS); the homeology of this set of V chromosomes to the wheat chromosomes was ascertained by Qi et al. 1995a (8th IWGS). Qi et al. 1998b indicated the chromosome arms of 6V as 6V#2S and 6V#2L rather then 6V#4S and 6V#4L. The T lines were described in Chen et al. (2002a and 2007) and Zhang et al. (2005). The other V chromosomes of this stock were numbered #2 by Qi et al. 1999 in Genes Genet Syst and were developed at the Cytogenetic Institute, Nanjing Agricultural Univ, China (Abbreviation CINAU; curator Chen PD).

(*continued*)

Table 4.10 (continued)

Recipient wheat line	Source of *Dv* donor accession	*Dv* chromosomes identified for their homoeology to wheat chromosome							Set#of V chro-mos.	Reference
		1V	2V	3V	4V	5V	6V	7V		
T.durum cvs "81086A," "D311," "Mexicali 75"	Dv ecotype collected from the former USSR						T 6DL·6V#5S DS 6V#5(6D)		Set # 5	Lines developed at Chinese Academy of Agric Sciences (CAAS), Beijing, China. Chen et al. 1996a and b. Calli from immature F_1 embryos, were treated with colchicine, chromosome were doubled, and the following fertile amphiploids were regenerated: T. durum "81086A" Dv (TH1), T. durum "D311" Dv (TH2), and T. durum "Mexicali 75" Dv (TH3). The DS lines 94G22-1, and 94G33-1 were identified by Shang et al. 1997. The translocation lines Pm97033, Pm97034 and Pm97035 were obtained after crossing and backcrossing the TH3 amphiploid to T. aestivum "Wan7107" (Li et al. 1999)
T. durum cv "Sauwne20" (*Td*_S)	Dv_C, an ecotype collected by researcher at the Inst. of Crop Germ, CAAS, Beijing, China						DS 6V#6(6A)		Set # 6	Ma et al. 1997. The DS lines GN21 e GN22 were developed after crossing the Td_S x Dv_C to T. aestivum (see Li et al. 2002a). The DS lines ere resistant to powdery mildew and WCM.
T. aestivum cv "CS"	Ecotype from the Latium region, Italy			DA te13V#5L″			DA 6V#7″; DS 6V#7(6D)″		Set # 7	De Pace et al. 2001; Minelli et al., 2005; Vaccino et al. 2009. 6V#7 is in line CSxV63 (DA) and CSxV32 (DS). A small portion of 1VL from centromere up to Glu-V1 locus is retained in the Rec line (Vaccino et al. 2010). Previously (Bizzarri 2009; Bizzarri et al. 2007, 2009; Vaccino et al 2010) the chromosome 6V#7 in the DA CSxV63 and DS CSxD32 lines was coded as 6V#4.
	Sears' "Italian" accession	Rec 1BL-1V#1L·1V#1S								

Table 4.11 Nucleotide sequence of the (a) subtelomeric tandem repeated *Dv* DNA cloned in p380 with 68.5% homology to the rye 350-family and marking *Dv* chromosomes except chromosome 7V, and (b) pDbH12 homologous to a long terminal repeat (LTR) *Sabrina* retrotransposon specifically hybridizing throughout all *Db* chromosomes (terminal sequences to be used as PCR primers are underscored in blue-color)

(a) p380[(1)]

5'-CTCGTTGTTGTTGTTGTTGTCGATGTTGTAGTAAAGTCTTCGAGATCATGATTATTAGAGTCCTATCATCTCAATTC
CCCGATACCTTTCCAACACCTACGAAATTACTCAAACGGAGCTtCGTTATGACTCATGTTGATAGTCTCATTCAAT
CACATCTATATGGTGAATTTTGTCTTTTCTCTGTTCACCGATTCTTCCCTAATTGTGAGCCTAACACCATGTGACC
CAATTCTATCGTCAAGGTGCATATTTCTACAGACCATACGGATGAGGTTGTCCCATATTTTTTTGAGCTGGGAAG
TGCAAATTGTTCCTTTTTTTCAATCCTTCGGATCCATTTTTTTGAGGGAACTAAACGGCCCTGTGTTTTGGCGCTA
AGAATTTGCGCCGCATG -3'

(b) pDbH12[(2)]

5'-ACGCGCATGTCCTATTATTCTTGGTAGACCTTTCCTTAGAACTATTGGTGCAATTATTGATATGAAGGAAGGTAAT
ATTAAATTTCAGTTTCCACTAAAGAAGGCATGGAACACTTTCCTAGAAAAAGGATTAAGATGCCCTATGAATCT
ATTATGAGAGCTACTTATGGTTTGTGCACTAAAGATGACAATACCTAAAAACTATCTCCTTACGCCTAQCTAAG
GGCGTTAAATGATAGCGCTTGTTGGGAGGCAACCAAATGAATAAATTTATTTTTGCTTTTTACTTTATGTTTAGTT
GCCAAGTAGTTTTTATGCTACTGTTATGCTTGTGTTTTTTATGTTTTAATTAGTGGTTGTGCCAAGTAGAACCTTT
ATGATAGGTTGTGGTGATAGTTGTTTGATCATGCTGAAAAAGACAGAAACTTTGCGCTCACCAAATTATTTAATT
CTCCTATCCAGTACGTGAGAATGAGTTGATTCTTTTTGCCGCTGATTGATATGCAAATTGCCATAATTGTCATAAT
TTTTTCAGAATTTTTAGGATAGCAGAAGTATTCGAAATACCCAGATTGCTACAGACTGTTCTGTTTTTGACAGAT
TCTGTTTTTATTGTGTTGTTTGCTTATTTTGATGAATCCATGGTTAGTATCGGGGGGGTGCGAGCCATGGTGAAGTA
AGAATACAGTAATATAACATCAAATTAAATGGAACCCAAGTTTGCTACAGTACCTTATGAGTATAAGGGGTTTAT
TTTCTTATGCTAATGATATCACAAGTTTCTGTTGAAGTTTTGTGTTGTGAAGTTTTCAAGTTTTGGGTGAAGTTCT
TATGGACAACGGAATAAAGAGTGGCAAGAACCTAAGCTTGGGGATGCCCATGCATCCCAAGCCAAATTCAAGG
ACACCATCAAGCCTAAGCTTGGGGATCCCCCGGGAAGGCATCCCATCTTTCGTCTTCAATCCATCGGTAATTTTA
CTTGGGGCTATATTTTTATTTACCAC

(1) De Pace et al, 1992; Yuan and Tomita, 2009. (2) Yang et al, 2006.

wheat-*Dasypyrum* amphiploid, and a wheat-*Dasypyrum* partial amphiploid and its derivatives by using the primer UBC848; the results indicated that *pDv848/388* existed in all the material tested, suggesting that *pDv848/388* can be used to detect *Dv* chromosome 5V in a wheat background.

Unilocus molecular markers are also important for finer studies (i.e., gene mapping and positional cloning of *Dv* genes) on the genetic effect attributable to *Dv* chromatin introgression into the wheat genome (Table 4.4). RFLP was used by Simeone et al. (1990) and Simonetti et al. (1993) to search for differential molecular patterns between *Dv* and wheat and to identify wheat DA lines of single V chromosomes. RFLP analysis was used to define homoeologous relationships of *Dv* chromosomes with those of hexaploid wheat (Qi et al. 1998a, b, c, 1999)

Polymorphic amplicons were detected between common wheat cv. CS and *Dv* for 148 of 276 SSR primers (Zhang et al. 2006). Primers wmc49 (1BS), wmc25 (2BS), gdm36 (3DS), gdm145 (4AL), wmc233 (5DS), wmc256 (6AL), and gwm344 (7BL) amplified a specific polymorphic DNA fragment from chromosome 1V to 7V, respectively. In addition, gwm469 (6DS) detected a specific band on 2V, and gdm107 (2DS) amplified a specific band on 6V. These microsatellite markers were effective in identifying individual *Dv* chromosomes in other *T. aestivum-Dv* chromosome addition, substitution, and translocation lines involving V chromosomes from different *Dv* ecotypes and wheat backgrounds.

A polymorphic RAPD-amplified fragment from *Dv*, $OPF02_{757}$, was sequenced and two PCR primers were designed. The primers allowed the detection of a genome-specific PCR marker for *Dv*. The PCR fragment of 677 bp was localized on all the seven pairs of *Dv* chromosomes (Liu et al. 2003).

PCR amplification of 5S rDNA spacer sequences was used to identify *Dv* chromosome fragments in hybrid calli obtained after asymmetric protoplast fusion between wheat protoplasts and *Dv* protoplasts treated with different dosages of γ-rays (Zhou and Xia 2005). Other markers have been described in the previous paragraphs.

Genetic mapping of SSR loci in bread wheat done by Röder et al. (1998) and Gupta et al. (2002) and other SSR loci identified for the D-genome by Pestsova et al. (2000) provided good transferable markers for *Dv* loci.

Based on EST sequences of rice and wheat, 34 STS primer pairs were designed by Cao et al. (2009a), which amplify specific fragments that are polymorphic between cv. CS and *Dv*; 14 of them are markers of specific *Dv* chromosomes (Table 4.4).

4.4.3.5 Cytological Markers

C-Banding

In the Triticeae, C-banding is the most widely applicable method, and has been used to identify all 21 pairs of chromosomes in common wheat (Endo 1986; Gill et al. 1991). Chromosome banding techniques are powerful tools also for the identification of alien chromosomes and chromosome fragments introgressed into wheat cultivars (Gill and Sears 1988). Heterochromatin C-band patterns were used to identify individual chromosomes in *Aegilops caudata*, *Ae. umbellulata*, *Ae. mutica*, *Elymus junceus*, *Dv*, and *Agropyron spicatum* (Gill 1981). Heterocromatic bands were lightly developed in the first two *AegiIops* species, well evident in *Ae. mutica* and *Elymus*, and highly developed in *A. spicatum* and *Dv*. Since C-bands represent sites of highly repetitive DNA, a part of the mechanism of genome evolution in these grasses has been by DNA amplification. Friebe et al. (1987) provided a nice differentiation of *Dv* chromosomes in a wheat background based on C-banding. Jahier et al. (1988) studied R-V pairing in a trigeneric hybrid involving also chromosomes of the U^n-genome. C-banding has been often used in conjunction with GISH in order to identify the single alien chromosome in multiple introgressions.

N-Banding

N-banding has been used to identify each of the seven chromosomes of V-genome (Liu and Chen 1984) in *Dv* and in a *T. durum-Dv* amphidiploid. The patterns of V chromosomes differed from those of A- and B-genomes of *Triticum durum*. N-banding has been used by Pei and Liu (1986) and Liu et al. (1988) to check if the banding pattern in *Dv* chromosomes remained unaltered when they are introgressed in the wheat background. It was found that all the peculiarities of each *Dv* chromosome remained unchanged when introgressed into wheat genome (Liu and Chen 1983, 1984; Liu et al. 1988).

In Situ Hybridization

Chromosome banding patterns is a useful diagnostic tool to identify chromosomes and chromosome structural changes; however, chromosome banding patterns can often be difficult to interpret, especially when structural changes are involved in complex karyotypes, such as the translocation of small chromosome fragments. In situ hybridization using repeated DNA sequences as probes, offers an alternative approach for cytological detection. Many cloned repeated DNA sequences are available (for a review see McIntyre et al. 1990) and some of them, which are species specific, have been noted as useful molecular markers for identifying alien chromosomes in interspecific and intergenic hybrids of the Triticeae (Lapitian et al. 1986; McIntyre et al. 1990; Xu and Kasha 1992). However, often only specific regions of chromosomes can be localized, and considerable effort is required to isolate and clone these DNA sequences.

Up to 1990 few repeated DNA sequences were available for in situ hybridization research in *Dv* (McIntyre et al. 1988). The current list of V-chromosome specific or V-genome specific repeated sequences include several new categories of markers (Tables 4.4, 4.6, 4.9, and 4.11).

GISH

A more direct approach to discriminate among parental genomes and chromosomes of hybrids in plants is the use of total genomic DNA as probe, i.e., genomic in situ hybridization (GISH) (Le et al. 1989; Schwarzacher et al. 1989). GISH with *Dv* genomic DNA as a probe in the presence of unlabeled wheat DNA was used to confirm that Add 6V is a disomic wheat-*H. villosa* chromosome addition line and to evidence that sub 6V is a homoeologous wheat-*H. villosa* chromosome translocation line rather than a substitution (Chen et al. 1996a). Similarly, it was applied to characterize chromosome translocations arising from tissue culture in hybrids of *T. aestivum* × (*T durum-Dv*, amphiploid) (Li et al. 2000a). GISH

has been used to screen progenies for double monosomic plants after crossing DS 4V#4(4D) × "Yangmai #5" wheat and select T 4DL·4V#4S lines carrying resistance to WSSMV (Zhang et al. 2005).

GISH revealed three types of homoeologous associations between wheat (W) and Dv (D) chromosomes (W-D, D-W-W, and D-W-D) in PMCs of the "CS *ph1b*" × *Dv* hybrid and only one type (W-W) in the CS × *Dv* hybrid (Yu et al. 2001a).

Using GISH and *Db* repeated sequences as probes, Galasso et al. (1997) found that the pericentromeric regions of the chromosome arms of *Dv* and *Db* are similar, while distal regions show substantial differences. Clone pSc200 containing a rye repeated sequence, hybridized to the telomeric and centromeric region of *Db*, while in *Dv* it hybridized only in the centromeric region of six pairs of chromosomes. GISH of meiotic metaphases of *Dv* × *Db* hybrid using genomic FITC-labeled *Dv*-DNA and TRITC-labeled *Db*-DNA simultaneously, it was possible to show that *Dv* chromosomes are present as seven univalents, while *Db* chromosomes form bivalents (Galasso et al. 1997).

Sequential C-Banding-GISH

Characterization of *Dv* chromosomal chromatin by means of in situ restriction endonucleases, fluorochromes, silver staining, and C-banding has been done by Pignone et al. (1994, 1995). The identity of the chromosomes cannot be determined when GISH is used alone. For this purpose, a sequential C-banding-GISH procedure performs better and, when applied on the same cells of *T. aestivum-Dv* × "Yangmai 158" hybrids, permitted the unequivocal identification of *Dv* chromosomes in the wheat background (Zhong et al. 1996). All the *Dv* chromosomes showed C-bands, either in telomeric regions or in both telomeric and centromeric regions. The seven pairs of *Dv* chromosomes were differentiated as 1–7 according to their characteristic C-bands. Using the sequential C-banding and GISH technique, it was shown that somatic cells of F_3 plants from the mentioned hybrid contain V chromosome(s). One plant had three *Dv* chromosomes (2, 3, and 4); another plant ($2n = 45$) possessed one chromosome 4 and a pair of chromosome 5, and the third plant ($2n = 43$) was found to have one chromosome 6 of *Dv*. The combination of GISH with C-banding provided a direct comparison of the cytological and molecular landmarks.

4.5 Assessment of Gene Actions for Host–Parasite Interactions and Abiotic Stress Resistance

Dv has shown resistance to biotic causal agents of several wheat diseases (Table 4.12), such as powdery mildew (*Bgt;* see Sect. 4.2.9.2), stripe rust or yellow rust (*Pst;* see Sect. 4.2.9.2), leaf rust (*P. triticina* Eriks.; syn. *P. recondita* Rob. ex Desm. f sp. *triticina*) (*Pt*), stem rust (*Pgt;* see Sect. 4.2.9.2), loose smuts (*Ustilago nuda* and *U. tritici*), eyespot (*Pseudocercosporella herpotrichoides*); take-all (*Gaeumannomyces graminis*); Rhizoctonia root rot (*Rhizoctonia solani*), wheat scab (*Fusarium* spp.), Septoria tritici leaf blotch (*Septoria tritici*), wheat streak mosaic virus (WSMV), wheat spindle streak mosaic (WSSMV), soil-borne cereal mosaic virus (SBCMV), and barley yellow dwarf virus (BYDV) (De Pace et al. 1990). Also, *Db* might contribute genes for wheat disease resistance. Some details about the *Dv* response to the main causal agents of wheat diseases and the contribution of *Dv* and *Db* genes to improve resistance to those agents in wheat are reported in the following paragraphs. A summary of the known *Dv* genes conferring resistance to the reviewed causal agents of wheat biotic and biotic stresses and their chromosomal location is presented in Table 4.12.

The resistance of *Dv* to wheat stem and leaf rusts and powdery mildew was ascertained by Taylor et al. (1939) and Pasquini et al. (1978) and reported by Hyde (1953) and Panaiotov and Todorov (1979). Powdery mildew resistance originating from *Dv* has been effective against most of the pathogen populations (Chen et al. 1997).

In Italy, since the beginning of the twentieth century, gene introgressions from *Dv* into wheat were obtained to improve disease resistance and agronomic performance. Among the cultivars realized by Strampelli, cv. Roma was reported to derive from the cross Akagomugi × *Dv* (Rusmini 1961); other varieties, such as cv. Cantore, were considered to have chromosome segments translocated from *Dv* into wheat (Forlani 1950). In the subsequent years, screening for resistance of *Dv* populations, collected in different

Table 4.12 Loci in *Dv* chromosomes harboring alleles for resistance/tolerance to diseases and abiotic stresses in *Dv* ecotypes and in the wheat introgression lines of *Dv* and *Db* chromatin

Chromosome	Locus	Agent of biotic and abiotic stress in wheat	Reference
from Dv			
1V	–	Common bunt (*Tilletia tritici*)	Bizzarri (2009)
3V	–	Take-all (*Gaeumannomyces graminis*)	Huang et al. (2007)
1V,2V,3V,4V	–	Eyespot (*Tapesia yallundae*)	Uslu et al. (1998)
1V,2V,3V,5V	–	Eyespot (*Tapesia acuformis*)	Uslu et al. (1998)
4VL	*PchDv* (=*Pch3*)	Eyespot (*Pseudocercosporella herpotrichoides*)	Yildirim et al. (1997, 1998, 2000)
4VS	*Wss1*	Wheat spindle streak mosaic virus (WSSMV) (vectored by *Polymyxa graminis* "plasmodiophorids.")	Zhang et al. (2005); Chen et al. (2007)
6VS	*Pm21*	Powdery mildew (*Blumeria graminis*)	Chen et al. (1995); Liu et al. (1996); Qi et al. (1995b, 1998a)
6V	*Lr6V#4*	Leaf rust (*Puccinia graminis*)	Bizzarri et al. (2009)
6VS	*Yr26*	Stripe rust (*Puccinia striiformis*)	Yildirim et al. (2000); Li et al. (2002a); McIntosh et al. (2003)
6V	*SrHv6*	Stem rust (*Puccinia graminis* f. sp. *tritici* Eriks. & Henn., race Ug99 or TTKS)	Pumphrey et al. (2008); Xu et al. (2008)
6VS	–	Wheat curl mite (WCM) (*Aceria tosichella* Keifer). The resistance to WCM reduced the incidence of wheat streak mosaic virus transmitted by viruliferous WCM	Li et al. (2002a)
–	–	Soil-borne cereal mosaic virus (SBCMV) (vectored by *Polymyxa graminis*)	Zlatska et al. (2008)
–	–	Rhizoctonia root rot (*Rhizoctonia solani* Kühn AG-8)	Smith et al. (2003)
6VS	–	Fusarium head blight (*Fusarium graminearum* Schwabe [teleomorph *Gibberella zeae* (Schw.) Petch])	Oliver et al. (2005); see also Lu et al. (1998); Cai et al. (2008)
4V,5V,6V	–	Salt tolerance	Zhong and Dvorak (1995)
V2, V7 from *Db*	–	Zinc efficiency	Schlegel et al. (1998)
–	–	Powdery mildew (*Blumeria graminis*)	Yang et al. (2005)
–	–	Stripe rust (*Puccinia striiformis*)	Yang et al. (2005)

Italian regions (Latium, Abruzzi, Apulia), showed that *Dv* was, as a whole, quite resistant to stem rust and powdery mildew causal agents but not to those causing leaf rust. Nevertheless, the behavior of some accessions indicated the presence of specific genes for resistance also to races of *Pt* (Pasquini et al. 1978).

4.5.1 Powdery mildew

The studies carried out for many years at the Cytogenetic Institute of Nanjing Agricultural University demonstrated the complete resistance to powdery mildew of wheat-*D. villosum* addition, substitution, and translocations lines involving chromosome 6V#2. (or 6V#4 according to nomenclature in Table 4.10). One gene, named *Pm21*, was identified on the short arm of chromosome 6V and its expression and stability was studied in different wheat backgrounds (Chen et al. 1995; Liu et al. 1996; Qi et al. 1995a, 1998c). A morphological marker appeared to be always associated with powdery mildew resistance; in fact, genes coding for black awns were located on chromosome 6VS(Chen et al. 1995). Attempts to prepare a genetic map of 6V#2 and to identify molecular markers closely linked to the *Pm21* locus were initially unsuccessful. Subsequently, *Pm21* was tagged with RFLP markers (Li et al. 1995, 2005), PCR-based dominant markers, such as $OPH17_{1900}$ (Qi et al. 1996), and SCAR markers (i.e., $SCAR_{1400}$ and $SCAR_{1256}$; Liu et al. 1999). Powdery mildew resistance genes *Pm12* transferred from *Aegilops speltoides* to wheat cv. Wembley (and available in a T6BS-6SS.6SL line), and *Pm21* (in the

T6AL·6V#4S line) conferred broad-spectrum resistance to *Bgt*. Both *Pm12* and *Pm21* genes are located on the short arms of homoeologous group six, and the chromosome arms involved were 6SS and 6VS, respectively. EST–SSR marker, *Xcau127*, amplified polymorphic fragments from the chromosome arms 6AS, 6BS, 6DS, 6VS, and 6SS. Therefore, it can be used to distinguish, simultaneously, the chromosome arms containing the two resistance genes and the chromosome arms with the susceptible alleles and become a useful "one-marker-for-two-genes" for pyramiding gene for *Bgt* resistance in wheat breeding programs (Song et al. 2008). The transfer of the *Dv* gene for resistance to wheat powdery mildew was also described by Shi et al. (1996).

A serine/threonine kinase gene (Contig17515), whose expression was induced by a mixture of *Bgt* conidia budding on 3-week-old *Dv* seedlings in the greenhouse, was selected from a gene expression experiment based on microarray analysis of the marked RT RNA from induced and non-induced samples hybridized to the barley Affymetrix Gene-Chip. No information was given on the genotypes at the *Pm21* locus in the *Dv* ecotype used for the experiment (Cao et al. 2006). A pair of primers, (NAU/xibao15F and NAU/xibao15R), was designed according to the Contig17515 sequence on the Barley GeneChip. Using genomic DNA of various wheat genetic stocks containing V chromosomes and 21 nullisomic–tetrasomic and eight deletion lines of *T. aestivum* cv. Chinese Spring (CS) as templates, four amplicons specific for 6VS, 6AS, 6BS, and 6DS, respectively, were produced. F_2 individuals derived from the cross Yangmai#5 × T6VS·6AL were analyzed, and data indicated that NAU/xibao15-902 could be used as a codominant marker for selecting *Pm21* located on 6VS (Cao et al. 2006). A 448 bp amplicon that was amplified using cDNA of *Dv* as template and NAU/xibao15F and NAU/xibao15R as primers was cloned and sequenced. This 448 bp product shared 96% similarity with Contig17515. The putative protein that it encodes is also a serine/threonine kinase, and thus, the 448 bp product was designated as Hv-S/TPK (Cao et al. 2006).

According to the sequence of *Hv-S/TPK*, a pair of primers, CINAU15, was designed, and a codominant marker Xcinau15-902 (homologous to NAUxibau 15-902) linked to *Hv-S/TPK* was developed.

A TAC (transformation-competent artificial chromosome) library was constructed using a 6VS/6AL translocation line harboring the *Pm21* (Fan et al. 2000). The PCR marker Xcinau15-902 was used to screen the TAC library, and a positive clone, TAC15, containing the sequence of the *Hv-S/TPK* gene that included the serine/threonine kinase domain, was identified (Sun 2007). A 30-kb-long positive clone and a 5-kb-long subclone were obtained. Sequencing of the subclone indicated that it contains four exons and three introns. The combined sequence of the exons was completely homologous to the original cDNA sequence of *Hv-S/TPK* (Chen et al. 2008c, p. 2).

The full-length gene *Hv-S/TPK* was used to construct the recombinant vector pAHC-*Hv-S/TPK* having a *bar* gene as a selective marker. Transformation of the *Bgt*-susceptible cv. Yangmay 158 was achieved through gene-gun bombardment (Chen et al. 2008c). Plants resistant to *Bgt* were identified through T_1 and T_2 generations.

A physical map of the *Hv-S/TPK* gene was constructed by sequential FISH with TAC15 as probe and GISH with genomic DNA of *Dv* as probe in *T. aestivum-H. villosa* 6V addition, 6V(6A)substitution, and 6VS/6AL translocation lines (Fig. 1 in Yang et al. 2008a; Fig. 4 in Chen et al. 2008c). The fraction length (FL) of hybridization sites was calculated as the distance from the centromere to the hybridization signal relative to the total length of the 6VS chromosome arm (see also Sect. 4.4.1). More than 10 chromosomes were measured for each FL calculation and standard deviations were estimated. The FL position of the *Hv-S/TPK* locus identified using the TAC 15 probe, was 0.573 ± 0.033, 0.587 ± 0.040, and 0.566 ± 0.034 in *T. aestivum-Dv* 6V addition line 06R33, 6V(6A) substitution line 06R41, and 6VS/6AL translocation line 92R137, respectively (Yang et al. 2008a). Therefore, also the Xcinau15-902 marker developed from the *Hv-S/TPK* sequence mapped at the same position.

M_2 lines obtained from the treatment of mature female gametes of 6VS/6AL translocation line 92R137 with γ-ray irradiation (Chen et al. 2008c) contained an interstitial segment from the region of 6VS between FL0.45 and FL0.58 found in two heterozygous interstitial translocation lines (NJ2-1 and NJ2-2; Fig. 2 in Chen et al. 2008c). These lines were highly resistant to *Bgt* and showed the $Xcinau15_{902}$ marker. Therefore, it was deduced that the $Xcinau15_{902}$ marker, the *Hv-S/TPK* gene, and the *Pm21* locus all map to this region of the 6VS chromosome arm.

Studies on resistance to *Bgt* conferred to wheat by *Dv* genes were carried out also at the Key Laboratory of Crop Genetics and Breeding of the Ministry of Agriculture, Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing, China. Three lines, Pm97033, Pm97034, and Pm97035, expressing resistance to *Bgt* were developed from the cross of TH3, an amphidiploid ($2n = 42$, AABBVV) derived from the cross *Triticum durum* cv. "Mexicali75" ($2n = 28$, AABB) × *Dv* accession from the former USSR (Chen et al. 1996b), and the *T. aestivum* cv. "Wan7107" of wheat, followed by two backcrosses and immature embryo and anther culture (Chen et al. 1996b). The culture of immature embryos of the hybrid was used as a biological tool for the production of chromosomal breaking, telocentric chromosomes, and alien chromosomal translocations. GISH analysis showed that the studied lines hold disomic translocations T6DL.6VS because they lacked specific biochemical loci and RFLP markers located on chromosome 6DS and 6VL (Li et al. 2005).

In Italy, the earlier studies on resistance to *Bgt* conferred to wheat by *Dv* genes where carried out at the University of Tuscia Viterbo and at the University of Bari. Resistance to *Bgt* by *Dv* accessions coming from different Italian regions was reported by Blanco et al. (1987) and De Pace et al. (1988b). Blanco et al. (1987) inferred the presence of gene(s) on the short arm of the monosomic addition line "C" (later identified as monosomic addition 6V). De Pace et al. (1988b) demonstrated high resistance to *Bgt* in hexaploid (MxV, AABBVV) and octoploid (CSx*Dv*, AABBDDVV) amphiploids. Because the parental *Dv* ecotypes were susceptible to powdery mildew,the possible existence of a new pathotype of the fungus specific for *Dv* and non-pathogenic on wheat was hypothesized by means of cross inoculations on wheat with *Bgt* spores collected from *Dv* and vice versa.

Recently, studies (Bizzarri et al. 2007, 2009; Bizzarri 2009; Vaccino et al. 2010) have been carried out on a disomic addition line (CSxV63) bearing chromosome 6V#4 (according to chromosome nomenclature of Table 4.10 and Sect. 4.4.1, this chromosome should be designated 6V#7) introgressed into the CS chromosome complement from a *Dv* ecotype collected in Latium, Italy. This line is completely resistant to powdery mildew at adult and seedling stages and it has been crossed to the susceptible disomic addition line 6V#1 obtained by E. R. Sears. The progenies have been studied both by phytopathological and molecular (PCR marker) analysis to assess the genetic basis of resistance (Bizzarri et al. 2007; Vaccino et al. 2007). The F_2 segregation for powdery mildew was 3 resistant to 1 susceptible, and all the F_3 kernels from the resistant plants expressed gliadin protein subunits encoded at the *Gli-V2* locus. Because *Gli-V2* is located on 6VS and there was cosegregation with the powdery mildew response phenotypes, it was deduced that the presence on 6VS of a locus at which the 6V#4S carries the allele for resistance and 6V#1S has the allele for susceptibility. In the absence of a test for allelism between the resistance allele on 6V#4S and *Pm21*, the locus was provisionally indicated *Pm21-Vt*, the allele for resistance *Pm21-Vt#4*, and the allele for susceptibility *Pm21-Vt#1*. All the lines carrying the other *Dv* chromosomes (1V, 2V, 3V, 4V, 5V, and 7V) in the E. R. Sears' DA set#1 were susceptible to *Bgt*. The PCR molecular marker $OPH17_{1900}$, identified by Qi et al. (1996) as tightly linked to the *Pm21#2* allele for resistance to *Bgt*, was not so with respect to the *Bgt* resistant allele *Pm21-Vt#4*. Therefore, the $OPH17_{1900}$ marker failed to be a consistent molecular marker for the *Bgt*-resistant allele at the *Pm21-Vt*#4 locus (Bizzarri 2009). The molecular marker based on the NAU/xibao15_{902} PCR primers identified by Cao et al. (2006) marks only the presence of 6V, but fails to discriminate between *Pm21-Vt#4 and Pm21-Vt#1* alleles on chromosomes 6V#4 and 6V#1, respectively (Bizzarri 2009).

4.5.2 Rusts

Adult plant resistance (APR) to leaf rust has been recognized as a major component of durable rust resistance. The disomic addition lines CS + 6V (DA6V#1; $2n = 44$) and CSxV63 (DA6V#4, $2n = 44$, which is designated DA6V#7 in Table 4.10), and the disomic substitution line CSxV32 (DS6V#4, $2n = 42$), expressing different introgression events of chromosome 6V of *Dv* in the genome of CS bread wheat, showed susceptibility to several selected pathotypes of *Pt* when inoculated at the seedling plant stage (Bizzarri 2009). When controlled inoculations on the flag-leaf lamina of these lines were performed with a mixture of leaf rust pathotypes, the DA6V#4 and DS6V#4 lines evidenced strong APR (0 and 0–10 pustules, respectively), while CS was highly susceptible (40–80

pustules per flag-leaf lamina). The APR of DA6V#4 and the susceptibility of CS with respect to natural *Pt* infections were confirmed in multilocation epidemiological trials carried out in Italy during 2007 and 2008 (National Phytopathological Surveys).

Two cycles of selection within the CSxV32 disomic substitution line allowed the development of two sister lines. With GISH, it was shown that one of the sister lines was a monosomic substitution line for chromosome 6V#4 (MS6V#4; $2n = 41$), most likely substituting chromosome 6B; this line (CSxV32-R) displayed APR to *Pt* in the field in Italy (Rome and Viterbo) and Hungary (Martonvásár) when exposed to natural pathogen infections in the same fields. The second sister line, lacking the 6V#4 chromosome, was susceptible to *Pt* (CSxV32-S). These observations confirm the hypothesis that the 6V#4 chromosome (or 6V#7 according to Table 4.10) carries gene(s) controlling APR to *Pt*, which are effective also when only one 6V chromosome is present (Bizzarri 2009; Bizzarri et al. 2009).

The genetic basis of APR to *Pt* conferred by the 6V#4 chromosome was studied in the $F_{2:3}$ progenies grown in Viterbo derived from the cross between the DA6V#4 (CSxV63) and DA6V#1 lines (Bizzarri et al. 2009). Symptoms caused by air-borne LR infections in the field were scored by counting the number of uredinia on the flag leaf and the leaf below it for each plant. DA6V#4 showed an average of 4.4 small uredinia (min = 0; max = 20; St Er = 6.43) and was considered resistant (R); DA6V#1 showed an average of 80 uredinia (min 30; St Er = 30.9) and was considered susceptible (S). In a total of 236 $F_{2:3}$ plants scored, 150 showed less than 20 pustules per plant (R) and 86 expressed over 20 and up to 350 pustules per plant (S); CS showed an average of 94.7 pustules. The null hypothesis of 10R:6S ratio for the bulk of the $F_{2:3}$ plants was not rejected when the chi-square test was used; that ratio was compatible with a 3R:1S segregation ratio among the F_2 mother-plants from which the $F_{2:3}$ progenies were derived (Bizzarri et al. 2009). Further analyses are in progress in order to confirm the hypothesis that the adult plant resistance to *P. triticina* surveyed in the 6V#4-introgression lines could be controlled by a single resistance gene (temporarily designated as *Lr6V#4*), different from those already present in CS (*Lr12*, *Lr34*).

Resistance of *Dv* to *Pst*, the causal agent of yellow or stripe rust, was reported by different authors (Yildirim et al. 2000; Li et al. 2002a). In these cases, variability was observed for the reaction of the *Dv* accessions tested with respect to different *Pst* pathotypes used for artificial inoculation. The 6VS/6AL translocation line was resistant to the rust isolates and a new gene, named *Yr26*, was identified (McIntosh et al. 2003).

Two new wheat–*Db* addition lines A6-7 and Y88-15 with high resistance to stripe rust were developed, and C-banding revealed that they contained the V^b3 and V^b7 chromosomes from *Db*, respectively (Yang et al. 2008b). The new wheat-*Db* addition lines will be promising donors to produce stripe rust resistant wheat translocation lines for wheat breeding.

Sears (1953) observed marked resistance of *Dv* to *Pgt*, the causal agent of stem rust, and to leaf rust of wheat. Vallega and Zhukovsky (1956) in Argentina reported resistance to stem rust for the species *Haynatricum* obtained by a spontaneous hybridization between *T. dicoccum* and *Dv*. Also, one of the two *Haynaldoticum sardoum* or "Denti de cani" lines (see Sect. 4.2.9.3) showed high resistance to stem rust (Meletti et al. 1996).

Pumphrey et al. (2008) found that 95 accessions of *Dv* maintained in the Wheat Genetic and Genomic Resources Center collection at Kansas State University in the US were nearly immune and likely contain novel genes for resistance to North American races of *Pgt*. Selected accessions were screened also with stem rust race TTKS (similar to Ug99, a *Pgt* isolate from Uganda with virulence to *Sr31* and to 13 out of 16 genes in the 16 differential hosts used for the international coding of the infection types produced by the isolates) and maintained high levels of resistance. Screening of a set of *Dv* disomic addition stocks in CS revealed that chromosome 6V harbors one or more genes that provide "low" infection type after *Pgt* infection at the seedling stage; those genes were temporarily designated as *SrHv6*. *SrHv6* conferred resistance to all North American races tested and also to TTKS.

Wheat germplasm useful for stem rust resistance breeding is expected from the development of compensating translocation stocks from crosses between the resistant CS-*Dv* 6V disomic addition line harboring the novel *SrHv6* gene (CS-HVIL DA 6V) and CS monosomic for chromosome 6D (CS M6D) (Xu et al. 2008). The 6AL-6VS translocation line originally developed

by Chen et al. (1995) was not resistant to the TTKSK strain of *Pg* (Xu et al. 2009), suggesting that the resistance gene might be located on 6VL.

4.5.3 Fusarium Head Blight

Two translocation lines "T 6AL·6VS/Xiang 5" and "Yangmai 158/6AL·6VS//Yangmai 15" distributed by Chen PD, NAU, showed resistance to FHB at 2 and 3 weeks post-inoculation in controlled environment and visually scored for the number of diseased spikelets per spike (Oliver et al. 2005). Lu et al. (1998) and Cai et al. (2008) identified introgression lines with FHB resistance from two greenhouse screening seasons. Wheat lines containing minimal alien chromatin from *Dv* exhibited resistance levels comparable to the bread wheat cultivar "Sumai 3."

4.5.4 Loose Smut

Resistance to *Ustilago tritici* in *Dv* populations was observed by Nielsen (1978). This author reported the non-pathogenicity of *U. nuda* on *Dv* accessions but did not exclude the possibility that the *Ustilago* species observed on *Dv* spikes by Becerescu (1970) and claimed as a new fungus (*U. haynaldiae* Becerescu.) specific for *Dv* could be *U. nuda*. In fact, *U. nuda* and *U. haynaldiae* showed similar spore germination features and membrane on the sori, while in *U. tritici*., such traits were expressed differently. The resistance to *U. tritici* was maintained in the spontaneous amphiploids *Haynatricum* reported by Vakar (1966).

4.5.5 Common Bunt

Vakar (1966) reported resistance to common bunt caused by *Tilletia tritici* (*Tt*) in the spontaneous amphiploid *Haynatricum*, and Bizzarri (2009) evidenced resistance to common bunt in experimental wheat × *Dv* amphiploids. In the last case, controlled infections with *Tilletia* teliospores from CS wheat spikes were performed during 2 years of assays (2008 and 2009) on three different hexaploid amphiploids (MxV, CxV, C_rxV_b) derived from *T. turgidum* ssp. *durum* × *Dv* hybridization involving the durum cv. Modoc Capeiti and Creso respectively, and different *Dv* ecotypes. Common bunt symptoms were not observed on either the amphiploids or the *Dv* parental spikes, while the majority of CS plants showed caryopses with teliospores. The CSV11 IBL harboring 1V chromatin (as revealed by GISH analysis in Minelli et al. 2005), and one of E. R. Sear's aneuploid lines (CS-DA7V) expressed resistance to common bunt (more than 50 and 75% of plants did not show symptoms, respectively), indicating the presence of some of the *Dv* genes conferring resistance to *Tilletia* on 1V and 7V chromosomes (Bizzarri 2009).

4.5.6 Take-all and Rhizoctonia Root Rot

Dv is highly resistant to take-all, caused by *Gaeumannomyces graminis* (Sacc.) Arx & D. Olivier var. *tritici* (Scott 1981). This pathogen attacks wheat and grass species causing a root and foot rot disease. Foex (1935) already reported *Dv* as unattacked by this disease. Heun and Mielke (1983) showed the ability of Dv to respond to infection by *G. graminis* and the possible incorporation of the *Dv* resistance to *G. graminis* into wheat was envisaged by Leske (1979). Skou (1975) studied the entry and growth of the *G. graminis* fungus and significance of lignituber formation in the roots of the *Dv* hosts. Linde-Laursen et al. (1973), by testing accessions of this species and of amphiploids involving it, confirmed its resistance to this pathogen and stated that it was intermediate between that conferred by oat and rye accessions but unsurpassed within the Triticeae. In those studies, the resistance showed by the *Dv* parent was not completely maintained in the *Dv*-derived wheat lines. Similar results were obtained in *T. durum/Aegilops tauschii* amphiploids, where increased tissue blackening caused by the pathogen was observed compared with the original resistant *Ae. tauschii* parent (Eastwood et al. 1993). In recent years, studies have been carried out on the resistance to *G. graminis* expressed by the progenies from a cross between wheat and an amphiploid ($2n = 42$, AABBVV) (Huang et al. 2007). A monotelosome from *Dv*, conferring resistance to take-all, was identified by cytological and GISH analysis on the progenies

of one derived line. Molecular analysis indicated chromosome 3V as the source of the monotelosome.

The two pure lines of "Denti de cani" isolated in Sardinia and differing for the hollow or solid stem (see Sect. 4.2.9.3) showed good resistance to different pathogens causing root and foot rot diseases (*Fusarium culmorum* and *F. graminearum*) and sharp eyespot (*Rhizoctonia cerealis*) (Meletti et al. 1996).

Rhizoctonia root rot, caused by *Rhizoctonia solani* Kühn AG-8 (Anastomosis Group 8), is a yield-limiting disease of direct-seeded cereals in the Pacific Northwest region of the US, and to date, no resistant *Triticum* germplasm has been identified (Smith et al. 2003). Eleven accessions of *Dv* (from Greece and Italy) exhibited some level of seedling resistance to the isolate C1, and 73% also displayed a resistance response to the isolate D2. All *Dv*/durum amphiploids as well as CS/*Dv* addition lines DA set #1 of E. R. Sears and DA set #2 of A. Lukaszewski were susceptible to both isolates. The absence of resistance in derivatives of *Dv* was attributed to one of the following factors: (1) the accessions used to make the DA set#1 and #2 and amphiploids might have been susceptible to *R. solani* AG-8 or (2) resistance in the diploid might have been masked when hybridized with the tetraploid and hexaploid wheat parents, as it occurs for the lower resistance to take-all observed in *Dv* derivatives.

4.5.7 Eyespot

Sprague (1936) and Heun and Mielke (1983) reported *Dv* collections as highly resistant to eyespot and Murray et al. (1994) demonstrated the association of resistance to genes in chromosome 4V by analyzing the reaction of *Dv* and of the disomic addition line for chromosome 4V. The resistance conferred by the gene on 4V is more effective than that conferred by *Pch1*, a dominant gene conferring a high level of resistance derived from chromosome $7D^V$ of *Aegilops ventricosa* Tausch (Doussinault et al. 1983), and *Pch2*, a gene located on 7A of the French bread wheat cv. Cappelle-Desprez (Law et al. 1976). All the *Dv* accessions tested were resistant to eyespot: a location on chromosome 4V of the gene controlling this resistance was suggested by analyzing the F_2 progenies of a cross between the susceptible DS 4V(4D) developed at the Cytogenetic Institute, NAU, China, and the resistant DA 4V#1 line. The new gene was named *Pch3* by Yildirim et al. (1997) and *PchDv by* Yildirim et al. (1998) and was mapped to the distal part of the long arm of chromosome 4V (Yildirim et al. 1998, 2000). Uslu et al. (1998) showed *Dv* to be resistant to both *Tapesia yallundae* (telemorph of *P. herpotrichoides*) (Wallwork 1987) and *T. acuformis* compared to CS. The two pathogens were recognized according to the morphology and pathogenicity of the colonies produced by the ascospores of the fungus (Dyer et al. 1996). The level of pathogen DNA was also generally lower in *Dv* accessions than in CS. Using DA set #1, Uslu et al. (1998) showed that resistance to *T. yallundae* was determined by factor(s) on 4V#1 and also on chromosomes 1V#1, 2V#1, and 3V#1 (the identity of 3V in this line was not surely ascertained). In contrast, resistance to *T. acuformis* was not found on 4V#1 but on chromosomes 1V#1, 2V#1, 3V#1 and to a lesser extent on 5V#1. The genetic basis of *Dv* resistance to the two pathogen species was different.

4.5.8 Viral Diseases

Dv was reported as an interesting source of resistance to infestation of the wheat curl mite (WCM) *Aceria tosichella* Keifer, the vector of wheat streak mosaic virus (WSMV), one of the most serious viral disease of wheat. In this case, heterogeneity, related to the origins of *Dv* parents, was observed in the reactions of 6V substitution and 6VS translocation lines, some of which were resistant to WCM colonization, with the positive consequence of a lower incidence or a delayed expression of WSMV symptoms (Chen et al. 1996a; Li et al. 2002a). The resistance was attributed to gene(s) on 6V#6S from a *Dv* ecotype collected by researchers from CAAS, Beijing, China (Li et al. 2002a). The chromosome was contained in the DS 6V#6(6A) lines Yi80928, GN21 and GN22. Resistance to wheat spindle streak mosaic (WSSMV), a viral disease transmitted to wheat plants by the resting spores of the "plasmodiophorids" of the non-pathogenic fungus *Polymyxa graminis* present in soil, has been demonstrated in a translocation line involving chromosome 4VS of *Dv* (T4DL•4V#4S constituted at CINAU; see-Table 4.10): the resistance gene was named *Wss1* (Zhang et al. 2005). The translocation line

T4AL-4V#4L•4V#4S, obtained also at CINAU from hybridizations involving gametocidal 3C chromosome, consisted of 4VS, a part of 4VL near the centromere and distal part of 4AL, and showed high resistance to WSSMV (Chen et al. 2007). Recently, resistance to another viral disease, also vectored by *Polymyxa graminis,* soil-borne cereal mosaic virus (SBCMV) largely spread in Europe, North America, and Asia, has been observed in *Haynatricum* (*T. dicoccum* × *Dv*) and in *Dv* accessions (Zlatska et al. 2008).

4.5.9 Abiotic Stresses

Dv has been found to be a good carrier of genetic factor(s) controlling resistance to abiotic stresses. Tolerance of salt stress is an important character especially in arid and semi-arid areas of the world where salinity is a limiting factor for agricultural production. Yuan Zhong and Qualset (unpublished data) observed genetic variability for this character in accessions obtained from natural populations of *Dv*. Zhong and *Dv*orak (1995) hypothesized a similar basis for salt tolerance in species of the tribe Triticeae. They studied the rates of leaf elongation under salt stress in wheat and other grass species, including disomic addition lines with chromosomes or chromosomal arms of *Dv* added to the wheat genetic background. They observed that the chromosomes of homoeologous groups 4, 5, and 6 in *Dv*, when added to wheat genome, had positive effects in conditioning tolerance to salt stress. Tolerance to drought was also reported in *Dv* (Scarascia Mugnozza et al. 1982).

The increase of zinc use efficiency was the aim of several breeding programs in countries characterized by zinc-deficient arable lands. Schlegel et al. (1998) performed greenhouse pot experiments at Adana (Turkey) with 2.2 kg soil/plastic pots, with (10 mg Zn kg^{-1} soil as $ZnSO_4$) and without Zn application to a zinc-deficient soil from Eskisehir (Turkey) that caused severe Zn deficiency symptoms in wheat. Plant Zn efficiency was calculated as the ratio of dry matter weight without Zn fertilization (−DW) to dry matter weight with Zn fertilization (+DW). Wheat-*Dv* amphiploids and wheat-*Dv* additions were tested even though the sources of those materials were not clearly specified. The *T. turgidum persicum-Dv*, AABBVV showed high Zn efficiency (0.65) and very slight zinc-deficiency symptoms. On the contrary, *T. turgidum durum-Dv*, AABBVV, showed low Zn efficiency (0.44) and severe zinc-deficiency symptoms. The highest Zn efficiency (0.89) was shown by "Dagro" hexaploid *Triticale*, AABBRR. Among the wheat-*Dv* V2, V3, V4, V6, and V7 additions, those indicated V2 and V7 gave evidence for the presence of *Dv* genes enhancing Zn efficiency (Schlegel et al. 1998; Fig. 4.4). It is not clear if V2 and V7 refer to DA set#1 of E. R. Sears (1982) or to DA set#2 of Liu et al. (1988) as described in Sect. 4.4.1. In DA set#1, DA 1V#1 and 5V#1 were available and not missing as in the DA set used in the Zn efficiency study; the DA 2V#1 perform very poorly in good agronomic conditions (Mariani et al. 2003) and is unlikely that it has high Zn efficiency. On the other hand, the DA set#2 lacked the V1 and V5 DA lines as in the set used by Schlegel et al. (1998), and in that set the V2 was actually 6V#2 (Qi et al. 1999). Lines with the 6V#4 chromosome have 18.5% protein content, a good Fe content (+27% compared to CS), and a high Zn content (+40% compared to CS) (Vaccino et al. 2010).

The ability of *Dv* to grow in soil contaminated by heavy metals (Cd, Cr, Cu, Ni, Pb, Zn) has been studied in Apulia region (southern Italy) by Brunetti et al. (2009). Although *Dv*, like other species, has not been found to be a metal "accumulator" plant able to remediate these contaminated sites, it could be considered candidate for "phytostabilization" due to its capacity, as "excluder or tolerant species" to grow in contaminated soils and, consequently, to promote metal stabilization and soil conservation.

4.6 Role in Crop Improvement Through Traditional and Advanced Tools

Scarascia Mugnozza et al. (1982) and Blanco and Simeone (1988) envisaged the way *Dv* can contribute to durum wheat improvement. In an experiment aimed to evaluate the potential as a forage crop of the amphiploid *T. turgidum durum* × *Dv* (M × *Dv*) (see Sect. 4.2.9.2) in comparison to the parental species *T. turgidum durum* cv. Modoc and *Dv,* and to a reference species (barley cv. Barberousse), M × *Dv* showed a good biomass yield, mainly at early stages (heading), in terms of both quality (N yield) and quantity (biomass) (De Pace et al. 1990). The N content of the amphiploid

kernels was 3.2% of the dry weight similar to that of *Dv* while the Modoc parent had N content of 2.5% dry weight (De Pace et al. 1990). At boot stage, M × Dv showed 30% (3.9% N of the dry matter, DM) higher nitrogen content than Modoc (3.0% N of DM), and 50% higher N content than *Dv* (2.6% N of DM). At different growing stages, the total biomass yield of M × *Dv* plant was comparable to that of Modoc and Barberousse and was significantly higher than the *Dv* plant at the booting and heading stages. In fact, at booting stage M × *Dv* and Barberousse produced 7.8 t/ha dry matter, Modoc 8.2 t/ha, and *Dv* 4.8 t/ha. Therefore, M × *Dv* showed good potential as forage crop.

Neither increase of soil N availability above 25 ppm nor the increase of plant density up to 680 plants/m^2 had an effect on dry matter yield. However, in sandy soils, sowing depth was critical for *Dv* and M × *Dv* seedling emergence, and a depth greater than 2 cm should be avoided. Both field and glasshouse tests showed that M × *Dv* has higher plant N content and leaf/plant dry matter ratio than M, indicating that the genes in the V chromosomes of the amphiploid contributed positively to these traits. The leaf/plant dry matter ratio was more favorable under low soil N availability conditions. Although the amount of dry matter and N content of M × Dv is similar to M, the former has a high leaf/plant dry matter ratio and thus a higher nutritional value than M.

In the amphiploids, the expression of alleles for low emergence ability at deep sowing and shortness of coleoptile present on *Dv* chromosomes are masked or counterbalanced by the action of the alleles on M chromosomes, and M × *Dv* has better seedling emergence ability than *Dv*. Drought stress tolerance is not a genetic feature of all the *Dv* genotypes, and it will be difficult to improve wheat for this character using conventional gene transfer from *Dv*, unless the *Dv* genotypes are properly selected.

The effect of gene transfer from *Dv* to wheat on the small-scale tests for bread-making quality was first investigated by De Pace et al. (2001). Using *Dv* single-chromosome addition or substitution lines, the authors tested the contribution of seed storage protein components encoded by genes on chromosomes 1V, 4V, and 6V on wheat grain quality properties by means of the grain protein concentration (GPC) and SDS sedimentation volume (SSV) test. The seed protein concentration of the genetic stocks containing *Dv* chromosomes ranged from 13.9 to 17.1% and was significantly higher than the seed protein concentration of CS (12.9%) and significantly lower than the seed protein content of the *Dv* lines (20%, on average). The largest positive effect on SSV was reported for an introgression line with a chromosome chimeric for the 1V short-arm (including the *Gli-V1*/*GluV3* loci), a part of the 1V long-arm (including the HMW glutenin-encoding *Glu-V1* locus), and the terminal part of the wheat 1B long arm carrying the *Glu-B1* locus. *Dv* chromosomes 4V and 6V did not contribute to improved quality probably due to *Gli-V2* and *Gli-V3*, which, as the loci orthologous to the corresponding *Gli* loci in wheat, do not enhance wheat quality.

The role of *Dv* chromosome segments introgressed in hexaploid wheat for pre-breeding and preparing primary mapping populations for analyzing complex genetic traits was evidenced by Mariani et al. (2003). In an analysis of genotype-by-environment interaction of wheat IBLs with chromatin introgressed from *Dv,* Vaccino et al. (2007) found that those IBLs containing cryptic introgressions of *Dv* chromatin (i.e., CSxV60) were early heading, good grain yielders, and environmentally stable over the years. Vaccino et al. (2008, 2010) analyzed the same IBLs for their bread-making quality, measured through small-scale (thousand kernel weight, protein content, SDS sedimentation volume, specific SDS sedimentation volume) and large-scale (Brabender farinograph and bread test) quality analyses. They observed that prolamin genes from *Dv* and wheat were co-expressed in the IBLs. *Dv* chromatin, including genes at the *Glu-V1* locus, improves wheat bread-making quality, and chromatin from the short arm of 6V improves also protein and micronutrient content. The strongest and positive effects were evidenced when the *Glu-V1* locus was introgressed in CS, at the level of both small-scale and large-scale analyses. Moreover, the results from two pairs of lines derived from 09.CS 1B-1V, isogenic for prolamin genes and differing only for the presence/absence of the HMW glutenin component named *1v*, indicate that the absence of *1v* decreases substantially the SSV: the authors concluded that such an HMW component, as already reported by De Pace et al. (2001) and anticipated by Montebove et al. (1987), is responsible for the improved values of bread-making parameters.

The analysis of three hard-red hexaploid lines derived from [(*T. turgidum durum* × *Dv*) × *T. aestivum*] hybridization, characterized by vitreous texture/

fracture in replicated field trials revealed agronomic performance, in terms of yield, test weight, and kernel weight, comparable to the best standards. Moreover, the lines were characterized by very good bread-making quality, measured by SDS sedimentation test, Brabender Farinograph, and Chopin Alveograph (Vaccino et al. 2009).

4.7 Conservation Initiatives

4.7.1 Evaluation of Genetic Erosion at Space and Time Scale

The first account on the potential use of the *Dv* gene pool for wheat improvement was done by Qualset et al. (1981) and Scarascia Mugnozza et al. (1982), and the first comprehensive evaluation of genetic resources of *Dv* was reported by Qualset et al. (1993). During several years of observation on *Dv* populations in random sites in its distributional areas in Italy, no sign of genetic erosion was detected. In this core area of endemism, *Dv* has been found to maintain an outcrossing breeding system, is widely dispersed, and occurs at sites that are unlikely to be destroyed; therefore, in situ conservation should be sufficient. However, at the population level, there were some exceptions to the pattern of higher within-family variance component in comparison to the between-family variance component expected for outcrossers. Population 84-16 found near the "Monster Park" at Bomarzo (Viterbo-Italy) was the main exception. It showed a high outcrossing rate and the highest number of alleles per locus, but low within-family variance component for number of culms per plant, and occupied an isolated site in which a very early heading phenotype was selected (De Pace 1987).

The genetic and ecological situations encountered in population 84-16 suggest that a small number of founder plants most likely lead to the establishment of this population (Zhong and Qualset 1995), and the repeated mowing during late spring to avoid accidental fires made that population vulnerable to extinction. Also, in areas at the edges of the *Dv* distributional limits, population size is sensitive to environmental changes, and disappearence of the residual populations may be a real outcome as it has occurred in Hungary (see Sect. 4.2.1). Therefore, collection of *Dv* from areas at the limit of the distributional range should receive priority in collection or needs special attention for ex situ conservation. *Dv* ecotypes in areas with low population size and widely scattered are expected to show a high degree of inbreeding as for the ecotype from "Marsala" (Sicily, Italy) studied by Stefani and Onnis (1987). Repeated selfing for four generations gave highly fertile plants suggesting adaptation to the extreme saline environment, where the "Marsala" ecotypes was found, through changes in breeding system from outcrossing towards selfing.

The high intrapopulation variability detected for isozyme and quantitative traits of populations scattered over a wide range of geographic sites, point to a collection strategy based on within-population sampling for ex situ conservation. Sampling should be done for research and plant breeding purposes. The enzyme and storage protein results would suggest that geographic-based sampling was not too important; however, the quantitative trait data gave another picture (Zhong and Qualset 1995). Therefore, it has been concluded that geographic-based sampling will be highly desirable to identify genotypes having useful traits.

The sampling of plants within populations in ecotypes of distant sites (>20 km) and with maximized edaphic and climatic differences should be adopted. If plant communities are continuously distributed, as appears to be the case based on the populations studied by De Pace and Qualset (1995), sampling every 40–50 km would assure the collection of different gene pools. However, if different environmental conditions, connected to local geographic diversity (i.e., narrow valley surrounded by hills), are found, then it would be highly recommended to choose the sampling sites at distances less than 15 km within that particular area. For the present, the methods of storing wheat in genebanks can be adopted for *Dv* to maintain ex situ gene banks.

Population biology studies for *Db* have been prevented by the very limited knowledge on the actual distributional range of the species and by the lack of samples in the germplasm repositories. However, the few ad hoc expeditions organized to rediscover the diploid cytotypes using the available information on the passport data of the species have been successful (Table 4.13).

Table 4.13 Number of *Dv* and *Db* accessions collected from various sites and maintained in various Gene-Banks

Species	Accession ID/Total number of accessions	Collection site (number of accessions)	Postal/URL address of the Gene-Bank maintaining the accessions
D. villosum	6	Krym, Ukraine	Maintained by the Western Regional PI Station. NPGS received: 15-Aug-1999
	147	Italy-Apulia (32), ItalyBasilicata (10), Italy-Calabria (4), Italy-Sicily (23), other Italian Regions (78)	CNR – Istituto di Genetica Vegetale – Via Amendola 165/A – 70126 BARI – Italy
	48	Italy (17), Greece (12), Albania (3), Bulgaria (6), France (1), Turkey (1), unknown (8)	IPK Gatersleben/Corrensstraße 3/D-06466 Gatersleben *http://gbis.ipk-gatersleben.de/gbis_i/ergebnisliste.jsf;jsessionid=c25e8cb8ce9f829fc6654c146279c31858b9d91e07e?autoScroll=0,2*
	52	Italy (1), Greece (45), Bulgaria (1), Turkey (4), Former Soviet Union (1)	Western Regional PI Station *http://www.ars-grin.gov/cgi-bin/npgs/swish/accboth?query=Dasypyrum&si=0&start=0*
D. breviaristatum 4x	1	Morocco	USDA, ARS, National Genetic Resources Program. Germplasm Resources Information Network – (GRIN).
	1	Greece	Faculty of Intercultural Communication, Ryukoka University, Japan
	20	Morocco	Fukui Prefectural University, Matsuoka, Yoshida, Fukui, Japan (Ohta and Morishita 2001; Ohta et al. 2002)
D. breviaristatum 2x	1	Morocco	Fukui Prefectural University, Matsuoka, Yoshida, Fukui, Japan (Ohta and Morishita 2001; Ohta et al. 2002)

4.7.2 *Attempts of* In Situ *and* Ex Situ *Conservation*

Qualset et al. (1984) collected *Dv,* several *Aegilops* species, and *Hordeum bulbosum* L. in June 1984, from roadside populations at 33 sites along the Adriatic Coast of Croatia, Bosnia and Herzegovina, and Montenegro and in the interior of Montenegro. The populations sampled were found mostly on disturbed sites from 0 to 1,000 m asl. Populations at the higher elevations had not produced mature seed by 15 June and could not be sampled. Following the criteria described in Sect. 4.2.2, random samples were taken, generally of 3–5 spikes from each of 10–15 plants at each site. A distance less than 50 km between sites was maintained in order to capture as much as possible the between-population genetic variability for molecular and morphological traits. Major features of the collection sites were recorded, such as elevation and soil texture. Some characteristics of the *Dv* plants could be recorded during collection, such as amount of tillering, height, spike length, glume color, awn color, waxiness, and spikelet pubescence.

Five collection routes were followed. These routes are labeled in Fig. 4.37 along with the identification numbers for the sites where collections were made.

Route 1, along the coastal road from approximately 12 km west of Split southeast to Ulcinj (Site 1, Gradac, 25 m asl; Site 2 and 3, Split 100–200 m asl; no further *Dv* was found along this area from 200 up to 700 m asl; Site 4. Opuzen, sea level; Site 5, Komolac, sea level; Site 6, 1 km southeast of Mlini, 400 m elevation; Site 7, 300 m southeast of Gruda, 100 m elevation; Site 8, Risan, sea level; Site 9, Budva, 30 m asl; Site 10, Milocer, sea level; Site 11, Petrovac; Site 30, Buljarica; 5 m asl; Site 31, Misici; 50 m asl).

Route 2, along the road from Petrovac on the coast inland to Titograd (Site 12, 12.5 km from Petrovac turn off at 1,300 m asl; Site 23, Cemulsko Polje, 3 km from Titograd, 50 m asl).

Route 3, along the roads between Titograd and Niksic (Site 13, Ljesko Polje, 50 m asl; Site 14, Spuz, 50 m asl; Site 15 and 16, Slap, 50 m asl; Site 17, Kapino Polje 600 m asl; Site 18, Mrnostica 600 m asl; Site 19, Drenovstica, 350 m asl; Site 20, Glavica, 60 m asl; Site 21, Stologlav, 50 m asl, no *Dv*; Site 22, Lesko Polje, 60 m asl).

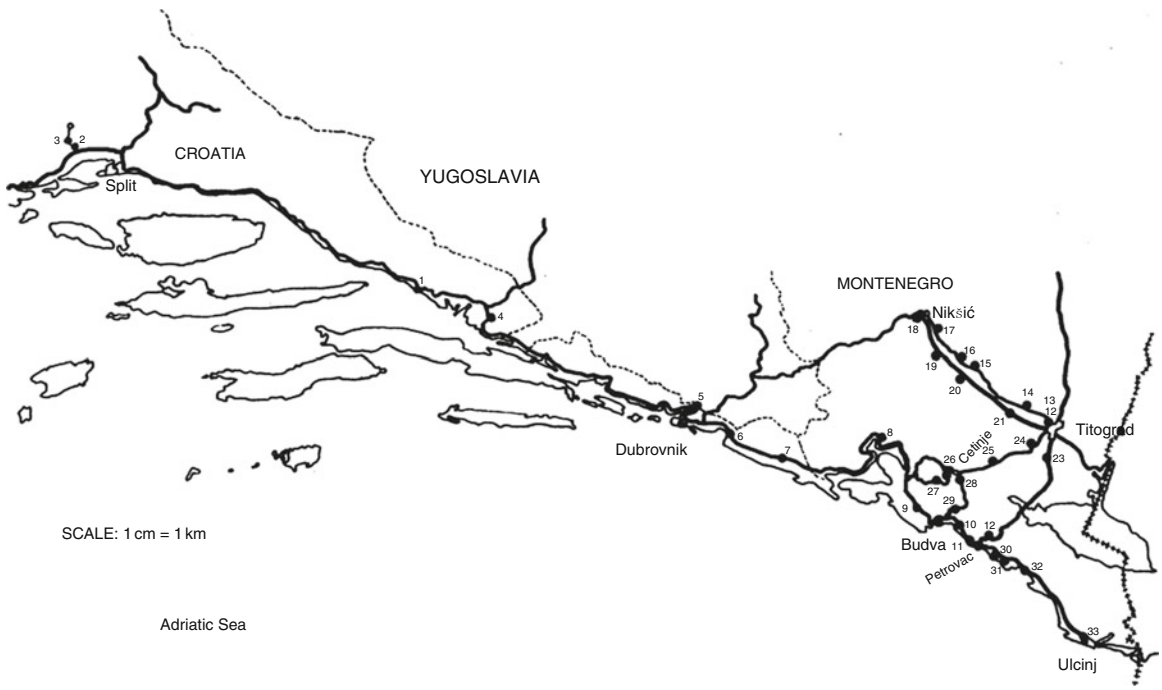

Fig. 4.37 Sites identified by number for the collection of *Dv* along the Adriatic coast of the former Yugoslavia (as presented in Qualset et al. 1984)

Route 4, along the road between Titograd and Cetinje and on toward the Njegos Mausoleum (Site 24, Farmaci, 40 m asl; Site 25, Meterizi, 200 m asl; Site 26, Cetinje 750 m asl; Site 27, Bjelosi, 900 m asl).

Route 5, along the road between Cetinje and Budva on the coast (Site 28, 500 m from Cetinje, 600 m asl; Site 29, 2 km from coast road to Budva, 150 m asl).

The seeds were collected for evaluation of genetic variability in isozyme and seed protein loci and for important agronomic traits such as drought and disease resistance. Samples were taken at most sites with one set being retained at the Institute of Biology, Novi Sad, and another set being processed at the Department of Agrobiology and Agrochemistry, University of Tuscia, Viterbo, Italy, for distribution to the University of California, Davis.

Dv has been collected in the 1980s in Greece and maintained in the USDA National Plant Germplasm System (see Table 4.13). In North Greece, *Dv* plants were taken from margins of maquis and garique, hilly, calcareous, light brown, rendzine-like loam, low stones, and good drainage, 2 km before Krini on way from St. Antonious to Petralopa (220 m asl); the *Dv* plants were associated with *Aegilops* species, *Quercus coccifera*, *Juniperus oxycedrus*, and other maquis and garique plants. *Dv* plants were sampled from margins of heavily grazed, disturbed fields with low stones, 1 km after Georgiani (470 m asl); they were associated with other grasses, mainly *Hordeum murinum*. *Dv* plants have been found growing also on hills in light brown loam and calcarous soil with medium stones and good drainage, 1 km before Metalliko, on road from Herson to Kilkis (140 m asl).

In central Greece, *Dv* plants were found in stands with *Triticum boeoticum*, *Aegilops triuncialis*, and *Rubus* on hills at margins of *Quercus* forest in loam and clay on road from Kalambaka to Grevena (560 m asl). *Dv* was found on road from Kipourio to Grevena at elevation: 560 m asl, growing with *Aegilops* species, *Quercus*, *Pyrus amygdaliformis*, annual and perennial grasses. *Dv* was also present along the road from Grevena to Kozani at 740 m asl and along the road from Kozani to Veria at 810 m asl growing among scattered shrubs of *Juniperus* and *Pyrus amygdaliformis*, annual and perennial grasses.

In central-southern Greece, *Dv* was found growing at margins of pine forests, and roadsides 2 km from Kallithea (10 m asl), at margins of cultivated fields with cereals, near Vavdos Minas (220 m asl), on plains in fallow fields near Krioneri, on road from Athens to Thessaloniki growing with *Pyrus amygdaliformis*, *Avena sterilis*, *Hordeum bulbosum*, *Daucus carrota*, and annual and perennial grasses, and 1 km before Piniada, on road from Larissa to Trikala (100 m asl) growing with *Aegilops. biuncialis* (*Ae. lorentii*), *Achillea*, *Paliurus*, *Erygium*, and *Pyrus amygdaliformis*.

In Peloponissos, *Dv* plants have been found on hills, in loam with rocks and good drainage near Nauplio (in association with *Phlomis fruticosa*, *Micromeria juliana*, *Avena sterilis* and *Phagnalen* spp.), in batha and garique (in association with *Sarcopoterium spinosum*, *Coridothymus capitatus*, *Eryngium campestre*, *Pyrus amygdaliformis*, *Asphodelas aestivus*, and *Quercus coccifera*). *Dv* plants near Korinthos (elevation 100 m), on road to Mt. Acrokorinthos, grew on margins of fallow fields and roadsides, on hills in loam with low stones and good drainage in association with *Phlomis fruticosa* and *Euphorbia*. *Dv* plants were collected from olive plantation on hills, in calcareous loam with medium stones and good drainage, halfway up mountain to town Nauplio have been found with scattered olives, annual and perennial grasses, *Micromeria juliana*, *Avena sterilis*, and *Hordeum murinum*.

In 1999, sampling of *Dv* was made in Ukraine by collectors H. Bockelman, USDA-ARS; R. Boguslavsky, National Center for Plant Genetic Resources of Ukraine; R. Johnson, USDA-ARS; V. Korzhenevsky, State Nikitsky Botanical Gardens (*http://www.ars-grin.gov/cgi-bin/npgs/html/site.pl?W*).

Collection sites were:

(1) Near Simeiz along road A-294 (habitat: South slope, rocky, dry, highly diverse calcarous. Latitude: 44° 24′ 39″ North (44.411), Longitude: 034° 00′ 15″ East (34.004); elevation: 195 m; accession W6 21717).

(2) Near Monastery and cave dwelling (hora Chufutkale) near Bakhchsarai (habitat: South slope, rocky, steep. Latitude: 44° 44′ 27″ North (44.741), Longitude: 033° 55′ 12″ East (33.920); elevation: 465 m; accession W6 21748).

(3) Road to Sevastopol (habitat: South slope, rocky, very dry. Latitude: 44° 30′ 55″ North (44.515), Longitude: 033° 33′ 23″ East (33.556); elevation: 260 m; accession W6 21757).

(4) Near coast and south of Sevastopol (habitat: Flat, along road, disturbed, old orchard area. Latitude: 44° 30′ 48″ North (44.513), Longitude: 033° 29′

32″ East (33.492) (GPS coordinates) GoogleMap it; elevation: 220 m; W6 21764).
(5) Locality: Near Black Sea, Greek and Roman ruin a Sevastrol (habitat: Flat. Latitude: 44° 36′ 34″ North (44.609), Longitude: 033° 29′ 34″ East (33.493); elevation: 20 m; W6 21769).
(6) Locality: North of Kerch (habitat: Nearly flat, open grassland, rocky, formerly mined. Latitude: 45° 24′ 11″ North (45.403), Longitude: 036° 28′ 58″ East (36.483); elevation: 80 m; W6 21866).

Collections were made in several parts of Italy in 1984 and 1988 by Qualset, McGuire, and De Pace (unpublished results). Populations were sampled at 155 roadside sites in central and southern Italy from June 2 to 12 and from June 21 to 23, 1984 and along the Adriatic coast from Ravenna to Ancona and then inland to Terni from June 29 to July 2, 1988. The sites were usually disturbed areas and ranged in elevation from 0 to 1,000 m. Populations in the higher sites had not produced mature seeds at the time of visit and could not be sampled. The seed was collected for evaluation of genetic variability in isozyme, seed storage protein loci (Zhong and Qualset 1993), ribosomal DNA sequences and for important agronomic traits that were studied by De Pace (1987), Iapichino (1988), Kotsonis (1999), De Pace and Qualset (1995), De Pace et al. (1988c, 1990, 1992, 1994a, b), Delre et al. (1988), and Zhong and Qualset (1995).

In 1988, the coastal areas of what is now Slovenia and Croatia (from Trieste to Split) and several Croatian Adriatic islands (Cres, Lošinj, Hvar, Rab, and Krk) were explored for *Dv* populations and 18 populations were sampled (McGuire and Jackson unpublished results). Sites were all at or near sea level and were typically roadsides and disturbed areas.

Several collection expeditions were organized in southern Italy islands by Laghetti et al. (1990, 1992, 2003, 2005), Diedrichsen et al. (2002), and Perrino et al. (1993). They found *Dv* in only one site on the S. Domino island of the Tremiti Islands group in the Adriatic Sea, and on both S. Pietro and S. Paolo, small islands of the Gulf of Taranto in Puglia, southern Italy.

4.7.3 Germplasm Banks

Germplasm repositories of *Dv* and *Db* ex situ collections are indicated in Table 4.13. The collections underrepresent the geographic distribution for both species (see Sect. 4.2.2) and do not embody the genetical variation expected within countries that are at the core of the geographical distribution range. In situ conservation strategies should be developed for sites on the Atlas mountains in Algeria, especially if *Db* occurs in those mountainous sites where also olive (*Olea europaea* ssp. *europeae* and ssp. *sylvestris*) groves are spread, in order to join in situ conservation for oleaster and *Db* as sources of useful gene to improve the respective related crop species, olive and wheat. For the same reason, the Hoggar mountains in the Saharan-Sahelian region of Algeria where *Olea europaea* ssp. *laperrinae* occurs (Besnard et al. 2007; Anthelme et al. 2008) should be explored for drought and cold resistant *Db* ecotypes.

4.7.4 Modes of Preservation and Maintenance

Studies on seed polymorphism indicated that spikelets rather than dehulled kernels should be used as unit of conservation. Because dark-red kernels maintain germinability for a longer period compared to the yellow kernels (De Gara et al. 1991; Stefani et al. 1998; Table 4.2), they should be given priority in conservation when are available as dehulled seeds. Dark-red caryopses will not skew the representation of diversity in the *Dv* collections for the reason that they are produced under nearly random mating, and there is no significant difference between plants derived from dark-red- and yellow-caryopses for means of morphological traits or for frequencies of biochemical traits (De Pace 1987).

4.8 Some Dark Sides and Their Addressing: Constraint as Weed, Invasive Species, and Potential for Superweed Due to Gene Flow from Transgenic Crops

The autoecology (adaptations and tolerance to ecological niches, seed rain, seed bank, spatial distribution, phenology, age, and reproductive structure) and

synecology (interspecific relationships, successional series, vegetation strata) features reported for *Dv* suggest that it will not become an invasive weed in the sense described by Colautti and MacIsaac (2004). There is little risk of it being transformed into a superweed due to gene flow from transgenic varieties of crops such as wheat, barley, or maize because there is little to no possibility of forming F_1 embryos, F_1 fertile plants, and F_2 fertile progenies following natural fertilization of *Dv* female gametes with pollen of the mentioned species (see Sect. 4.2.9). With experimental hybridization of *Dv* to other diploid Triticeae species, F_1 viable embryos were formed only when *Dv* was used as pollen parent (Lucas and Jahier 1988; Table 4.13). However, in hybridization of *Dv* to *Db* (2*x*) and *Db*(4*x*), F_1 viable embryos were formed only when *Dv* was used as female parent (Table 4.5).

Dv is the only species of the *Dv*–*Db*(2*x*)–*Db*(4*x*) species complex that is sympatric with some other Triticeae wild species and thrives in disturbed habitats in the neighborhood of the tetraploid and hexaploid wheat fields. The experiments of Sasakuma and Maan (1978) on the introduction (by crossing and backcrossing) of *T. turgidum durum* genomes into the *Dv* cytoplasm with the resulting selection of fertile alloplasmic lines indicate that experimental gene transfer from wheat to *Dv* is possible. Gene flow might also occur from *Dv* to wheat. Emasculated florets of *T. turgidum* var. *durum* pollinated with *Dv* microspores (therefore, in absence of competition with pollen from the same floret) set 5.6% (Blanco et al. 1983a) to 11% (De Pace et al. 2003) caryopses with F_1 embryos. Selfing the F_1 plants produced fertile F_2 progeny at the frequency of 0.046%, derived from the union of the unreduced gametes. However, only one report (see "Denti de cani" in Sect. 4.2.9.3) on spontaneous *durum* wheat-*Dv* hybridization gene transfer, the likelihood of spontaneous unilateral wheat pollination of *Dv* stigma, setting F_1 embryos and caryopses, F_1 development, production of fertile F_2 plants, and occurrence of repeated hybridization and introgression of wheat genes into the *Dv*-genome is unlikely due to the ploidy level difference, but further investigation is needed for use in risk assessment in view of the possible development of transgenic wheat varieties. Gene transfer is even more unlikely between *Db* and *Triticum*. Spontaneous DNA introgression from domesticated polyploid wheat into distantly related, wild tetraploid Triticeae species and the stabilization of this DNA sequence in wild populations despite not having homologous chromosomes may occur (Weissmann et al. 2005). This indicates that spontaneous outcrossing and gene introgression of tetraploid wheat genes into the wild tetraploid *Db* might occur. However, differences in habitat preferences between wheat and *Db* indicate that species sympatry, concurrent flowering, spontaneous introgression, and enrichment of the *Db* wild populations with wheat genes is not expected.

4.9 Recommendations for Future Actions

Comparisons of the SNP map of *Aegilops tauschii* with the rice and sorghum genome sequences revealed greatly accelerated genome evolution in the large Triticeae genomes (Luo et al. 2009). Comparisons of recently diverged genomes allow not only the mapping of conserved genomic elements but also the detection of lineage-specific selection or the identification of recently introgressed segments from relatives (Nordborg and Weigel 2008). The understanding of plant evolution requires comparison of genomes of close relatives as demonstrated in yeasts, *Caenorhabditis*, *Drosophila,* and primates.

Phylogeography studies sustained by (a) greater collecting activities of *Dv* an *Db* ecotypes from low and high altitude sites in Italy, Morocco, Algeria, and Greece, and (b) high-throughput sequencing strategies for the *Dv*- and *Db*-genomes will provide in the near future solid information on the evolutionary and ecological factors that determined speciation events for the *Dv*–*Db*(2*x*)–*Db*(4*x*) complex, and will contribute to the understanding of the phyletic relationship of the *Dasypyrum* genus to the other Triticeae species and the rate of evolution within *Dasypyrum* and between *Dasypyrum* and other Triticeae species. The *Dasypyrum* species complex may become an important taxon to be used as a model for studying the genomic and epigenomic events that govern expression of somatic dimorphism of caryopses and the appearance of stolon-like and rhizomatous vegetative-multiplication structures in Triticeae. The demonstrated introgression of *Dv* and *Db* genes into wheat to enhance the expression of traits adaptive to impacts of climate change such as the biotic and abiotic stress resistance genes

and the end-use grain quality in low input environments will be a stimulus to consider and intensify the use of *Dasypyrum* as an important genus to contribute genes for wheat improvement worldwide.

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Chapter 5
Fagopyrum

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5.1 Introduction

Extensive breeding programs initiated on conventionally leading crops have fuelled the promotion of the "major crops" and ensured their success across continents. However, the presence of a limited amount of germplasm of the so-called "minor crops" in gene banks and their poor representation in terms of genetic diversity (Padulosi 1999a) represent a great challenge for the successful improvement and promotion of this group of species. Amongst the existing known plant resources, the International Plant Genetic Resources Institute (IPGRI) and Consultative Group on International Agriculture (CGIAR) have identified buckwheat (*Fagopyrum* spp.), grain amaranth (*Amaranthus* spp.), and White Goosefoot (*Chenopodium* spp.) as important but underutilized nutraceutical crops, which have tremendous potential for use in crop improvement programs.

The genus *Fagopyrum*, an important non-poaceous pseudocereal belonging to the family Polygonaceae, has great potential as a forage crop and also in food and medicine (Li and Zhang 2001; Zeller 2001). The amino acid composition of 13S globulin, the main storage protein of buckwheat grains, matches closely the FAO recommended values for a protein with nutritionally balanced amino acid composition (Rout et al. 1997; Radović et al. 1999; Rout and Chrungoo 1999). Due to its short growth span, capability to grow at high altitudes, and the high quality protein content of its grains, it is an important crop in mountainous regions of India, China, Russia, Ukraine, Kazakhstan, parts of eastern Europe, Canada, Japan, Korea, and Nepal. The plant is a rich source of Zn, Cu, Mn, Se, vitamin B_1, B_2, E, and dietary proteins for gluten-sensitive individuals (Wei et al. 2003; Stibilj et al. 2004). Buckwheat leaves and flowers are a rich source of rutin, catechins, and other polyphenols that are potential antioxidants (Luthar 1992; Oomah and Mazza 1996; Watanabe 1998). Buckwheat proteins have also been reported to have anticancer activity (Kayashita et al. 1999; Park and Ohba 2004). Not much work has been carried out to tap the potential of the wild relatives of buckwheat. Lately, there have been some reports on the antitumor activity of root extracts of wild *F. Cymosum* (Gao and Meng 1993; Chan 2003).

Taxonomic investigations on the genus *Fagopyrum* have made progress mainly through morphological studies. The wild species of buckwheat were first classified by Gross in 1913. However, many species that were included by Gross (1913) within the genus *Fagopyrum* are now classified in other genera in the family Polygonaceae. On the basis of embryo morphology and achene characters, Nakai (1926) insisted that *Fagopyrum* should be separate from other genera in the family Polygonaceae. Furthermore, cytological studies revealed that all *Fagopyrum* species have the basic chromosome number 8, while the basic number is 10 for *Polygonum sensu stricto* and 11 for *Persicaria* and *Bistorta* (Munshi and Javeid 1986). While Gross (1913) had suggested that *Fagopyrum* was closely related to *Fallopia* Ronse,, Decraene and Akeroyd (1988) were of the opinion that *Fagopyrum* was more closely related to *Persicaria*. Most studies have concluded that *Fagopyrum* lies at the basal position of the tribe Polygonaceae. The genus *Fagopyrum* consists of about 19 species of which only two species,

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namely *F. esculentum* and *F. tataricum*, are cultivated. On the basis of morphological characters, the genus *Fagopyrum* has been divided into two phylogenetic groups, viz. the *cymosum* group and the *urophyllum* group. The *cymosum* group comprises the two cultivated species *F. esculentum* and *F. tartaricum* and other wild species, viz. *F. cymosum, F. homotropicum, F. lineare*, *F. pilus, F. zugongense, F. megaspartanium,* and *F. giganteum*. Plants belonging to this group have large, lusterless achenes, which are incompletely covered with a persistent perianth. The *urophyllum* group, on the other hand, comprises *F. urophyllum* and other wild species, which have small lusterous achenes that are completely covered with a persistent perianth (Ohnishi and Matsuoka 1996; Yasui and Ohnishi 1998a; Ohsako and Ohnishi 2000; Yamane et al. 2003). The genus *Fagopyrum* is comprised of both perennial as well as annual species with diploid ($2n = 2x = 16$) as well as tetraploid ($2n = 4x = 32$) cytotypes. While the genus *Fagopyrum* is comprised of both self- as well as cross-pollinated species, the occurrence of dimorphic heterostyly renders some of the species self-incompatible.

The two cultivated species of *Fagopyrum* have their own share of limitations. *F. esculentum* suffers from low yield, indeterminate growth habit, and susceptibility to frost. Even though *F. tataricum* has better yield, its flour is bitter in taste because of the presence of high content of phenolics. Attempts at improvement of *F. esculentum* through conventional breeding programs have achieved limited success due to its heteromorphic self-incompatibility (Morris 1952; Samimy 1991). The discovery of *F. homotropicum*, the homomorphic wild species of *Fagopyrum* by Ohnishi (1995), has opened up new possibilities for transfer of the homomorphic trait from *F. homotropicum* to *F. esculentum* by generating interspecific hybrids between *F. homotropicum* and *F. esculentum*. *F. homotropicum* is similar to the wild ancestor of common buckwheat, *F. esculentum* ssp. *ancestralis*, except for its homostylous and self-pollinating system. The wild species of *Fagopyrum* may also offer many other agronomic traits such as resistance to diseases and pests, resistance to frost, bigger groat, higher rutin content, lower allergen levels, etc., which could be introgressed into the cultivated species through breeding. The low rate of success in conventional buckwheat breeding programs has been ascribed to the limited information about the diversity of buckwheat gene pool (Suvorova et al. 1994; Kreft 2001). Since the knowledge of diversity patterns allows plant breeders to better understand the evolutionary relationships among accessions and to develop strategies for tailoring desirable genotypes (Arunachalam 1981; Joshi and Dhawan 1996; Betting and Widrlechner 1995), identification and characterization of genetically diverse germplasm in the genus *Fagopyrum* would be essential for initiating successful breeding programs in this genus.

Traditionally, assessment of genetic diversity has been based on differences in morphological and agronomic traits or on pedigree information for the different crops (Gizlice et al. 1996; Sneller et al. 1997; Bernard et al. 1998). Joshi and Paroda (1991) have evaluated 408 accessions of buckwheat from the Himalayan region for 31 descriptor parameters including plant height, number of branches and leaves, clusters per cyme, seed per cyme, days to maturity, seed weight, and seed color/shape/surface (Fig. 5.1). They have considered Accession No. IC-13145 on the level of a species as *Fagopyrum himalianum*. The accession has been listed as *Fagopyrum tataricum* var. *himalianum* by IPGRI (*http://www.ipgri.cgiar.org/publication*). Campbell (1997) has, however, considered this accession as *Fagopyrum esculentum*. Arora and Engels (1992) treated the two species *F. sagittatum* and *F. emarginatum* (Gohil and Rather 1981; Farooq and Tahir 1987) as conspecific with *F. esculentum*. Evaluation of genotypes for their consistency of performance under different environments is important in plant breeding programs. The occurrence of high genotype–environment (GE) interaction poses a major problem for relating phenotypic performance to genetic constitution and makes the selection of genotypes difficult. Registration of buckwheat cultivars in gene banks is mainly based on morphological and physiological characteristics. Even though these descriptors are useful, they are limited in number and may be affected by environmental factors. Molecular markers, genome size, and cytogenetic variations have become useful complements to morphological and physiological characterization of cultivars because they are plentiful, independent of tissue or environmental effects, and allow cultivar identification early in plant development. These markers have been successfully used for analysis of diversity in many crops including buckwheat (Jabornik and Kump 1993; Ohnishi 1998a;

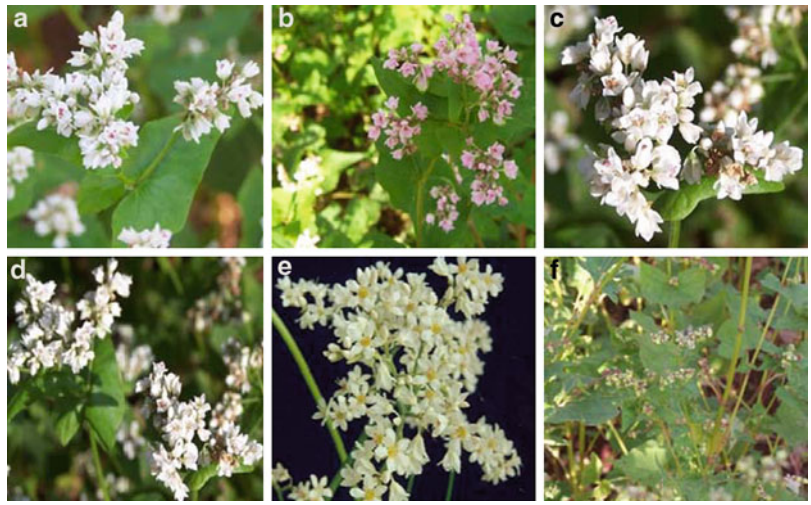

Fig. 5.1 Variations in flower color of sepals in flowers of *F. esculentum*, (**a–c**); *F. himalianum*, (**d**); *F. cymosum*, (**e**); and *F. tataricum*, (**f**)

Ohsako and Ohnishi 1998, 2000; Tsuji and Ohnishi 1998; Ohnishi and Asano 1999).

5.2 Genome Size Variation

Genome size of an organism is considered to be one of the important indicators of phylogenetic relationship and genetic diversity. Inter- as well as intraspecific variations in genome size have been correlated with cytological, physiological, and ecological characters in many groups of plants (Goin et al. 1968; Bennett 1976a, b, 1987; Price et al. 1981; Grime and Mowforth 1982; Laurie and Bennett 1985; Cullis and Cleary 1986; Bennett and Leitch 1995; Jockusch 1997; Grime 1998; Ohri 1998; Gregory 2001; Nevo 2001; Knight and Ackerly 2002). The underlying causes of genome size variation are, however, not well understood. Much of the data has been interpreted to support the concept that variation has an adaptive basis and is strongly influenced by natural selection (Sparrow et al. 1972; Cavalier-Smith 1978, 1980; Price et al. 1981). This, in turn, has led to suggestions of possible relationship between variation in genome size and speciation (Hatch 1976; Hinegardner 1976; Cavalier-Smith 1978). Although genome size is considered to be the most basic parameter for genome analysis, not much information is available on genome size in the genus *Fagopyrum*. Nagano et al. (2000) have reported a wide variation in C-values across the species in the genus *Fagopyrum*. The values ranged from 0.55 in *F. tataricum* to 1.92 in *F. urophyllum*. The DNA content of diploid *Fagopyrum* species varies from 1.08 pg in *F. lineare* to 3.83 pg in *F. urophyllum* (Table 5.1). The C-values of members of the *cymosum* and *urophyllum* groups overlapped almost across the full range of the genus, indicating occurrence of dynamic rearrangements in the genome during rather a short span of evolution. Even within the *cymosum* group, *F. tataricum* and its proposed wild ancestor, *F. tataricum* ssp. *potanini* has much smaller genome than *F. esculentum*. Nagano et al. (2000) have attributed the difficulties in generating fertile hybrids from crosses between *F. esculentum* and *F. tataricum* to large variation in the genome size between the two species. Even though available data on genome size in *Fagopyrum* may not be sufficient to interpret the results of the various interspecific crosses attempted in order to speculate the possible role of genome size in interspecific crossability, there does not seem to be a strong correlation between the amount of DNA per cell and organismal advancement or genetic complexity in the genus *Fagopyrum*. Similar observations have been made by Sparrow et al. (1972) and Price (1988). This well-documented lack of correspondence between genome size and morphological or physiological complexity of an organism has been historically termed the "C-value paradox" (Thomas 1971). Since the discovery of non-coding DNA and its impact on genome size

Table 5.1 Somatic chromosome number, C-value, and genome size variations in different species of buckwheat (*Fagopyrum* spp.) (after Nagano et al. 2000)

Species	pg	Somatic chromosome number	C-value	DNA Mbp/C
Cymosum group				
F. esculentum (2*x*)	2.77	16	1.39	1,340
*F. esculentum** (4*x*)	5.49	32	1.37	1,320
F. esculentum ssp. ancestrale	2.65	16	1.33	1,280
F. tataricum	1.11	16	0.56	540
F. tataricum ssp. potanini	1.09	16	0.55	530
F. homotropicum (*n*)	2.23	16	1.12	1,080
F. homotropicum	2.46	16	1.23	1,190
F. homotropicum (4*x*)	5.20	32	1.30	1,250
*F. cymosum** (2*x*)	2.32	16	1.16	1,120
*F. cymosum**	3.37	32	0.84	810
F. pilus	1.52	[a]	[a]	[a]
F. giganteum	1.52	[a]	[a]	[a]
Urophyllum group				
F. urophyllum	3.83	16	1.92	1,850
F. statice	1.35	16	0.68	650
F. leptopodum	1.43	16	0.72	690
F. capillatum	1.71	16	0.86	830
F. pleioramosum	3.05	16	1.53	1,470
F. gracilipes	3.35	32	0.84	810
F. lineare	1.08	[a]	[a]	[a]
F. rubifolium	3.31	[a]	[a]	[a]
F. macrocarpum	2.32	[a]	[a]	[a]
F. gilessii	1.80	[a]	[a]	[a]

[a]Information not available
*: cymosum group
No mark: uropyllium groupe (Yasui and Ohnishi 1998)

variation, "paradox" has been replaced by "enigma" in an attempt to more appropriately identify the topic as a "perplexing subject" made up of several independent components (Gregory 2004). Leitch and Bennett (2004) have pointed out that the mean genome size of polyploids in angiosperms was always lower than that of diploids. While the C-value of diploid and tetraploid *F. cymosum* has been reported to be 1.16 and 0.84, respectively, it is 1.12 and 1.3 for the diploid and tetraploid forms of *F. homotropicum*, respectively (Nagano et al. 2000). In an attempt to correlate size of the genome with seed size, Knight and Ackerly (2002) have suggested a triangular relationship between genome size and seed size in plants. They have inferred that while small genomes can be associated with either small or large seeds, plants having large genomes may not have small seeds. However, Beaulieu et al. (2007a) could not find any correlation between genome size and seed mass across 1,222 species from 139 families and 48 orders of seed plants. Their observations did, however, indicate that species with very large genome sizes never had small seeds, while species with small genome size had a large range of seed size. Interestingly, the genome size of *F. leptopodum, F. tataricum,* and its subspecies, *F. tataricum* ssp. *Potanini*, all of which produce small seeds, is much smaller than the mean genome size for the entire genus.

For long, annual life history and self-breeding behavior have been associated with smaller genome size (Stebbins 1957; Ehrendorfer 1970; Berret et al. 1997). A correlation of selfing with low genome size has been reported across 176 seed plants (Labani and Elkington 1987; Govindaraju and Cullis 1991; Albach and Greilhuber 2004). Reports on relationship between genome size and breeding behavior in buckwheat do not indicate any correlation between genome size and breeding behavior. Although *F. tartaricum* and *F. tataricum* ssp. *potanini,* which have the smallest genome size within the genus *Fagopyrum*, exhibit self-breeding behavior, *F. pleioramosum*, which has the second largest genome within the genus, also shows self-breeding habit. On the other hand, *F. statice* and *F. leptopodum*, which have comparatively smaller genomes, are outbreeders. *F. cymosum* and *F. gracilipes* have exactly the same C-value, but the former is an outbreeding species, while the latter predominantly displays selfing although some outcrossing populations have also been reported (Ohnishi 1995).The majority of species belonging to the genus *Fagopyrum* are annuals, with only *F. cymosum, F. urophyllum,* and *F. statice* being perennial. Comparison of genome size data with life cycle habit in the genus *Fagopyrum* fails to reflect any correlation, although it is interesting to note that *F. urophyllum*, which has the highest C-value within the genus, is a perennial species. While Bennett (1972) has suggested that perennials have larger genome, Knight and Ackerly (2002), Albach and Greilhuber (2004), and Jakob et al. (2004) are of the opinion that small genome size does not necessarily indicate an annual life cycle.

Isozyme profiles, interspecific crossability, and *rbc*L gene sequence analysis in buckwheat have indicated a closer relationship within the members of

either *cymosum* or *urophyllum* groups rather than between members belonging to the two subgroups (Wang 1987; Ohnishi and Matsuoka 1996; Chen 1999a, b). On the basis of morphology, *F. cymosum* appears to be more closely related to *F. esculentum* than to *F. tataricum* (Steward 1930). However, based on RFLP analysis of cpDNA, Kishima et al. (1995) suggested that *F. cymosum* was more closely related to *F. tataricum* than *F. esculentum*. This view has been supported by Ohnishi and Matsuoka (1996), Yasui and Ohnishi (1998a, b), and Rout and Chrungoo (2007). This relationship exists in spite of differences in morphological characters and breeding traits. *F. cymosum* had long been suggested to be the wild ancestor of *F. esculentum* and *F. tartaricum* until the discovery of *F. esculentum* ssp. *ancestrale* and *F. tataricum* ssp. *potanini* (Ohnishi 1991, 1998a). Yasui and Ohnishi (1998a, b) postulated that *F. esculentum* ssp. *ancestralis* differentiated from *F. cymosum* much earlier than *F. tataricum* ssp. *potanini*. Further, *F. homotropicum* is closely related to *F. esculentum* ssp. *ancestralis* and has probably evolved from *F. esculentum* ssp. *ancestralis* following shifting of the breeding system from outcrossing to self-fertilization (Ohnishi and Matsuoka 1996; Yasui and Ohnishi 1998b). If this hypothesis is true, then genome evolution within the *cymosum* group has progressed in the direction of increase as well as decrease in size. Presumably, the processes leading to the evolution of *F. esculentum* from *F. cymosum* also included decrease of genome size, while that leading to evolution of *F. tataricum* included increase in size of the genome. On the other hand, evolutionary processes within the *urophyllum* group involved only decrease in size of the genome. *F. urophyllum*, considered to be the ancestral species within the *urophyllum* group (Ohnishi and Matsuoka 1996), has the highest C-value. *F. letopodum, F. statice,* and *F. lineare* might have evolved from *F. urophyllum* by genome reduction. Downsizing of genome after polyploidization appears to be a general trend in angiosperms (Kellogg and Bennetzen 2004; Leitch and Bennett 2004). It appears that genome downsizing followed polyploidization in *F. cymosum* also. Mechanisms leading to a decrease in genome size include unequal crossing over (Wendel et al. 2002), illegitimate crossing over (Devos et al. 2002), higher overall rate of deletions than insertions (Comeron 2001; Petrov 2001, 2002a, b), and selection against transposable elements (Wright and Schoen 1999; Morgan 2001).

In the interspecific crosses made by Ohsako and Ohnishi (1998), cross between *F. statice* and *F. leptopodum* resulted in good seed set and the seeds germinated as well. However, crosses between *F. leptopodum* × *F. gracilipes, F. leptopodum* × *F. capillatum,* and *F. capillatum* × F. *callianthum* failed to set seeds. *F. leptopodum* × *F. gracilipes* must have failed due to the difference in ploidy level, although their genome sizes were not strikingly different. *F. leptopodum* and *F. capillatum* had similar genome sizes, yet the cross between them failed. Thus, genome size similarity may be required for interspecific crossability in *Fagopyrum*, but it is definitely not the sole determinant of crossability.

5.3 Molecular Phylogeny and Genetic Divergence

Phylogenetic relationships among members of the genus *Fagopyrum* have been studied using morphology, isozyme analysis, chloroplast DNA, nuclear DNA, random amplified polymorphic DNA (RAPD), and restriction fragment length polymorphism (RFLP) (Murai and Ohnishi 1996; Ohnishi and Matsuoka 1996; Yasui and Ohnishi 1998a, b; Ohsako and Ohnishi 1998). All these studies supported division of the genus *Fagopyrum* into two monophyletic groups, viz. *cymosum* and *urophyllum*. Both *F. cymosum* and *F. urophylum* are heterostylous outbreeding perennial shrubs and probably represent the primitive type of their respective groups (Ohnishi and Matsuoka 1996). One of the pioneering works on phylogenetic relationship amongst different species of the genus *Fagopyrum* based on chloroplast genome was undertaken by Matsuoka and Ohnishi (1993). They also recognized the divergence of the members into *cymosum* group and *urophyllum* group. Ohnishi and Matsuoka (1996) extended their work on phylogenetic relationships amongst *Fagopyrum* species using morphology and isoenzyme variability in addition to cpDNA. The three independently generated phylogenetic trees were fairly congruent and reflected the relationship of the earlier tree (Matsuoka and Ohnishi 1993). Heo et al. (2001) have resolved the species belonging to the genus *Fagopyrum* into three groups on the basis of pericarp anatomy. The first group comprised the cultivated *F. esculentum*, the wild

species *F. esculentum* ssp. *ancestralis,* and *F. homotropicum*, which are composed of undivided, circular sclerotic cells in the exocarp. The second group comprised the cultivated *F. tartaricum* and the wild species *F. tataricum* ssp. *potanini* and *F. cymosum* in which sclerotic cells are divided into two zonations. The third group comprised all other species with one layer of palisade sclerotic cells making up the pericarp. Thus, the pericarp anatomy was shown to be consistent with the previous study made by Ohnishi and Matsuoka (1996) wherein the genus was classified into three groups based on molecular data. Nonetheless, the grouping into two monophyletic groups by Ohnishi and Matsuoka (1996) is a widely accepted point of view.

5.3.1 Genetic Divergence Within Cymosum Group

To clarify the genetic diversity of a wild species as compared to its related cultivated species, it is important to use the potential of wild species as a genetic resource. The *cymosum* group consists of *F. esculentum, F. tataricum, F. eculentum* spp. *ancestrale, F. tataricum* ssp. *potanini, F. cymosum, F. homotropicum, F. pilus,* and *F. zuogongence*. There exists a wide range of variation with respect to geographical distribution, morphology, and breeding system among the members of this group. *F. cymosum* is the only perennial species and the rest are annuals. *F. tartaricum* and *F. homotropicum* are homostylous self-pollinating species, but the rest are distylous and self-incompatible. While most of them occur in nature as diploids with $2n = 16$, some species like *F. cymosum, F. homotropicum,* and *F. zuogongense* are tetraploids.

Ohnishi and Asano (1999) have studied 19 natural populations of *F. homotropicum*, a self-fertilizing close relative of common buckwheat from the Yunnan and Sichuan provinces of China, for their chromosome number and allozyme variation at 16 loci of 11 enzymes. They reported diploid ($2n = 16$) as well as tetraploid ($2n = 32$) populations. Three populations, "Deqin," "Zhongdian," and "Xiancheng," were revealed to be allotetraploid. Judging from allozyme constitution of the tetraploids and their possible progenitors, Ohnishi and Asano (1999) have suggested that the diploid progenitors were probably the diploid population of *F. homotropicum* from Lijiang and a natural population of *F. esculentum* ssp. *ancestralis*. On the basis of nucleotide sequence variation in cpDNA and the internal transcribed spacer (ITS) region of nuclear ribosomal RNA in two tetraploid accessions of *F. cymosum* and the diploid progenitor of the tetraploid cytotype, Yasui and Ohnishi (1998a, b) suggested that *F. tataricum* had speciated from a population of *F. cymosum* in the maternal line and that the tetraploid population of *F. cymosum* from Kathi, India, probably had arisen allopatrically and independently from tetraploid populations in China and Thailand. Further, ITS1 and ITS2 regions were shown to have 7.4 and 3.7 times faster rates of evolution, respectively, than the 5.8S subunit of rRNA. Since their study did not cover the entire diversity of *F. tataricum*, it may be assumed that the assertions need further confirmation. Moreover, phylogenetic relationships within *F. cymosum* showed poor resolution in their tree with a low level of bootstrap values. Matsui et al. (2003) have suggested that one of the events in the domestication process in the genus *Fagopyrum* was a mutation at the *Sht1* locus, which eliminated brittle pedicels, a trait exclusive to wild relatives of the genus (Fig. 5.2). The brittle pedicel was suggested to be the outcome of the presence of two complementary genes *Sht1* and *Sht2;* the *sht1* locus was further shown to be linked to the *S* locus with a recombination frequency of $5.46 \pm 1.18\%$.

Allozyme analyses of 20 populations of *F. cymosum* also supported the hypothesis of multiple origins for tetraploid *F. cymosum* (Yamane and Ohnishi 2001). Tsuji et al. (1999) had earlier reported a diploid sample of *F. cymosum* with seed morphology similar to that of wild tartary buckwheat in the Yaruzampu river

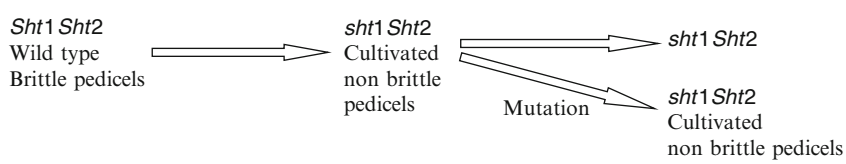

Fig. 5.2 Possible mutation events in *Sht* locus during domestication of buckwheat

valley of eastern Tibet. This form of *F. cymosum*, reported under the name of *F. pilus* by Chen (1999a), has been suggested by Yamane et al. (2003) to be a strong candidate for the diploid ancestor of the tetraploid cytotypes in India and Nepal. In an attempt to derive conclusions about the allopatrical multiple origin of tetraploid cytotypes of *F. cymosum* and the speciation of *F. tataricum* from *F. cymosum,* Yamane et al. (2003) investigated 3′ end of *rbcL, accD,* and associated intergenic spacer region, the *trnC* (GCA)–*rpoB* spacer region, the *trnK* (UUU) intron, and the *matK* region from 12 natural populations of *F. cymosum*, including a diploid population from eastern Tibet, which covered almost the whole range of the species and one cultivated and three natural populations of *F. tataricum*, which represented wide range of variability within *F. tataricum*. Two morphologically different accessions, YC9806 a and b of *F. cymosum* from Tongmai, had exactly the same sequences in all the three regions studied. Yamane et al. (2003) detected intraspecific variation of chloroplast DNA sequences in all the three regions studied. The number of nucleotide substitutions within *F. cymosum* was 12, 12, and 30 in the 3′-*rbcL–accD* region and associated spacer, the *trnC–rpoB* spacer region, and the *trnK/matK* region, respectively. On the other hand, no informative site was found in the 5′ intron of the *trnK* region. Further, all the coding regions of the *rbcL, accD*, and *matK* genes had constant lengths in *F. cymosum*, except for a 6-bp insertion in *matK*-coding region of the accession C8927. While the 3′ intron of *trnK* gene showed the highest value of 0.00502 for nucleotide diversity, the 5′ intron of the gene showed the lowest value of 0.00113. The nucleotide diversity (π) values for the coding regions in the *accD* and *matK* genes were higher than the corresponding value for the associated spacer regions. On the other hand, the total number of polymorphic and informative sites within individuals belonging to *F. tataricum* was only four with no polymorphic character in the 1.1 kbp *rbcL–accD* region and its associated spacer. *F. tataricum* showed lower values for nucleotide diversity than *F. cymosum*. On the basis of their results, Yamane et al. (2003) divided *F. cymosum* into a Tibet–Himalayan clade and a Yunnan–Sichuan clade, the two clades having diverged about 0.7 million years ago. It was suggested that the genetic divergence in *F. cymosum* might have been caused by geographical and climatic interruption due to Hengduanshan Mountains. Yamane et al. (2003) suggested that the autotetraploid populations of *F. cymosum* had arisen allopatrically from a diploid progenitor at least twice, once in the Tibet–Himalayan area and once in the Yunnan–Sichuan area. Results of the investigations carried out by Yamane et al. (2003) also revealed that *F. tataricum*, a close relative of *F. cymosum*, belonged to the Tibet–Himalayan clade. This suggests that *F. tataricum* speciated from *F. cymosum* in the Tibet–Himalayan area. The nucleotide diversity between the cultivated species and their wild ancestors was less than the corresponding values between two wild populations of the same species. This is possible because the domestication of buckwheat is more recent compared with the long history of the evolution of *Fagopyrum* species. Chen et al. (2004) have made a detailed study on cytology, isozyme profiles, and interspecific crossability within the members of the *cymosum* group. Analysis of glutamate oxaloacetate transaminase (GOT) revealed marked variations between different species of *Fagopyrum*. Besides having similar morphological characteristics and intercrossability, *F. esculentum, F. homotropicum*, and *F. zuogongense* showed identical isozyme profiles for GOT. Chen et al. (2004) postulated that *F. zuogongonse* had originated and speciated from hybrids of *F. esculentum* and *F. homotropicum*. The GOT isozyme profile of *F. giganteum* showed bands, which were common to *F. pilus, F. megaspatanium, F. cymosum,* and *F. tartaricum*. This could be ascribed to the fact that *F. giganteum* is a hybrid between perennial species of the *cymosum* complex (including *F. pilus, F. megaspatanium, F. cymosum*) and *F. tartaricum.*

5.3.2 Genetic Divergence Within Urophyllum Group

While *cymosum* group comprises both cultivated as well as wild species of *Fagopyrum*, the *urophyllum* group is comprised of only the wild species. *F. urophyllum* and *F. statice* are the only two perennial species within this group. *F. urophyllum,* a heterostylous cross-pollinating species, is considered to be the ancestral species among the members of *urophyllum* group. Phylogenetic analyses based on molecular data revealed a striking divergence between the members of *F. urophyllum* from Kunming in eastern Yunnan

and from Dali in western Yunnan (Yasui and Ohnishi 1998a, b; Ohsako and Ohnishi 2000). Phylogenetic trees developed on the basis of morphological data as well as nucleotide sequence data of the *trn*C–*rpo*B intergenic spacer region of cpDNA revealed clustering of the members of *F. homotropicum* into two groups, viz. the Kunming group and the Dali group. Ohsako and Ohnishi (2000) have suggested a polyphyletic origin of *F. urophyllum*. Even though nuclear and cpDNA analysis has yielded much information about interspecific relationships within members of the *urophyllum* group, several issues on intraspecific variations still remain to be resolved. *F. statice*, an outcrossing perennial species, has been shown to be paraphyletic to an annual species, *F. leptopodum,* in molecular phylogeny (Yasui and Ohnishi 1998a, b). The origin and intraspecific variation within *F. gracilipes,* a tetraploid self-fertilizing species, has not yet been clarified. Intra- and interspecific variations in *F. pleioramosum, F. macrocarpum,* and *F. callianthum,* the three newly reported species, are also unsolved problems.

The phylogenetic relationship in *Fagopyrum* revealed by the nucleotide sequences of the ITS region of nuclear rRNA gene (Yasui and Ohnishi 1998b) is also reflected in variations in nucleotide sequence of *trn*K region of cpDNA (Ohsako and Ohnishi 2001). Ohsako and Ohnishi (2001) have investigated inter- and intraspecific phylogenetic relationships among 37 accessions belonging to 10 species of the *urophyllum* group based on nucleotide sequences of two non-coding regions in the cpDNA, i.e., the *trnK* (UUU) gene intron and an intergenic spacer between the *trnC* (GCA) and *rpoB* genes. The *trn*K intron comprises *mat*K gene, which is an internal open reading frame (ORF) that is thought to encode a muturase-like protein (Neuhaus and Link 1987; Johnson and Soltis 1995) and two non-coding regions flanking both sides of the *mat*K gene. Ohsako and Ohnishi (2001) detected 104 nucleotide substitutions, 12 indels out of which eight were duplications, i.e., direct tandem repeats of short sequences and 34 structural mutations in the two non-coding cpDNA regions having a combined length of 2.3 kbp. Twenty-two out of the 34 structural mutations were potentially phylogenetically informative. The accessions also displayed an inversion in nucleotides, which varied in length from 31 to 78 bp. These results indicate a high rate of variability, including nucleotide substitutions, insertion/deletions, and inversions within members of the group. Based on the nucleotide sequence variations, the members of *urophyllum* group have been segregated into three monophyletic clades, viz. the *F. gracilipes–F. capillatum–F. rubifolium* clade, the *F. callianthum–F. pleioramosum–F. macrocarpus* clade, and the *F. statice–F. leptopodum* clade (Ohsako and Ohnishi 2000). *Fagopyrum urophyllum* was basal to all other species. These relationships are consistent with the results of previous molecular systematic studies by Ohnishi and Matsuoka (1996), Yasui and Ohnishi (1998a, b), and Ohsako and Ohnishi (1998). Members of each of the three groups share several morphological characters. *F. leptopodum* and *F. statice* are characterized by lustrous and hairless leaf surfaces. These two species also share such characters as waxy stem, leafless flower-bearing branches, and equal size of upper and lower perianth, although they are not synapomorphies because these characters have evolved in parallel to other species. Characters shared by *F. gracilipes, F. capillatum,* and *F. rubifolium* include heavy pubescence on the stems and stipules (Ohnishi and Matsuoka 1996; Ohsako and Ohnishi 1998). *F. pleioramosum, F. macrocarpum,* and *F. callianthum* share heterostylous self-compatibility and larger achenes than the other two groups. However, the achenes of *F. urophyllum* are as large as those of *F. macrocarpum* and *F. callianthum*, indicating that the achenes might be plesiomorphic in the *urophyllum* group. Even though *F. lineare* appears to be very close to *F. urophyllum* in the molecular phylogeny, the two species are morphologically quite different. *F. lineare* is rather similar to *F. leptopodum* in such characters as slender branches, small white flowers, and small achenes. The apparent resemblance between *F. lineare* and *F. leptopodum* could be ascribed to parallelism in evolution. Parsimonious analysis of the combined data presented by Ohsako and Ohnishi (2000) revealed the distant relationship between the *F. leptopodum–F. statice* group and *F. lineare*. It appears that *F. lineare* might have accumulated autoapomorphic characters at both the morphological and molecular levels since divergence from its ancestor. The nuclear substitution rate of plant cpDNA is reported to be lower than that of nuclear DNA (Wolfe et al. 1987; Clegg 1993). The wide range of variation in the non-coding region of chloroplast DNA in members of the *urophyllum* group observed by Ohsako and Ohnishi (2000) indicates high level of divergence among members of the group.

On the basis of overlapping of distribution *F. gracilipes–F. capillatum* clade across the Yunnan province of China, Ohnishi (1995) had earlier postulated that the two species might have had concurrent evolution. Ohnishi (1995) also postulated that *F. gracilipes* (4*n*) must have arisen by tetraploidization of *F. capillatum*. The divergence after tetraploidization must have involved loss of self-incompatibility. This is evidenced from the existence of both selfers and outbreeders in populations of *F. gracilipes*. This view is also supported by Ohsako and Ohnishi (2000). Ohsako and Ohnishi (2000) also suggested that *F. pleioramosum, F. macrocarpum,* and *F. callianthum,* the three newly discovered species endemic to the upper Min River valley of China, had evolved from a common ancestor.

5.3.3 Genetic Divergence at Intraspecific Level

Being a cultivated species, *F. esculentum* has received much of the research attentions in comparison to other species of the genus. The main regions of common buckwheat cultivation across the globe include China, Russia, Ukraine, Kazakhstan, parts of eastern Europe, Canada, Japan, Korea, Nepal, and India. Buckwheat is grown throughout a large area of Asia and Southeast Asia as a crop that fits the farming system in marginal and fairly unproductive land. According to Vavilov (1926), buckwheat originated in the Chinese center. Komarov (1938), Stoletova (1958), and Krotov (1960) suggested the Himalayas or western Tibet as the original birth place of buckwheat. In an expedition to China and the Himalayan Hills, in search of the wild ancestor of common buckwheat, Ohnishi (1991) discovered a wild buckwheat species, *F. esculentum* ssp. *ancestralis*, in the Yongsheng district of Yunnan province. Later, population of *F. esculentum* spp. *ancestralis* was also discovered in the Yanyuan and Muli district of Sichuan province, in the Lijiang and Deqing districts of Yunnan province, and in the Mangkang district of eastern Tibet (Ohnishi and Konishi 2001; Ohnishi 2002).

Allozyme analyses on *F. esculentum* and its wild progenitor *F. esculentum* ssp. *ancestralis* from northwestern Yunnan revealed that both the species had "*F*" as well as "*S*" alleles at the *adh* locus. The allelic frequencies of the *F* and *S* alleles between populations decreased gradually as the distance from northwestern Yunnan increased (Ohnishi 2004). Ohnishi (2004) has proposed that the northeastern part of Yunnan was the center of origin and diversification of common buckwheat. This hypothesis is further strengthened by the RAPD analysis of cultivated common buckwheat (Murai and Ohnishi 1996). Allozyme and amplified fragment length polymorphism (AFLP) analyses (Ohnishi 2004; Konishi et al. 2005), however, indicate that among the natural populations of wild ancestors, the population of eastern Tibet and the Adong population of northwestern Yunnan are most closely related to cultivated common buckwheat. Chen (2001b) has attributed the low level of variation between cultivated and wild common buckwheat to the short history of cultivation of common buckwheat. On the other hand, the high degree of intraspecific variation has been attributed to allogamous nature, large population, pronounced pollen flow between fields by insects, and artificial randomization during harvest and sowing (Ohnishi and Nishimoto 1988). Rout and Chrungoo (2007) found significant intraspecific variations in the endosperm protein profile of different accessions of *F. esculentum*. The variations were ascribed to a high degree of grain protein polymorphism within members of this species. Most of the variations were observed in the protein bands ranging in size between 21 and 74 kDa. Interestingly, most of the protein bands in the 26–54 kDa category belong to the legumin-type family of seed proteins (Rout and Chrungoo 1996; Bharali 2002). Accessions of *F. tataricum* and *F. cymosum*, on the other hand, did not show any significant intraspecific variations in the SDS-PAGE profile of grain endosperm proteins. These results are consistent with the observations of Nishiyama et al. (1991) and Zeller et al. (2004). One of the most important features of the profile was the presence of a 42 kDa band in accessions of *F. esculentum* having winged grains and a 31 kDa band in grains having prominent strips. Dendrograms generated on the basis of variations in the SDS-PAGE profile of endosperm proteins of different species of the genus *Fagopyrum* clustered the accessions into three broad groups. While Cluster 1 included all the accessions of *F. esculentum*, clusters 2 and 3 included accessions of *F. tataricum* and *F. cymosum*, respectively. Based on the unique RAPD profiles generated for different accessions of common buckwheat, Rout and Chrungoo (2007) have suggested that

F. esculentum had a broader genetic base than other species of the genus *Fagopyrum*.

F. esculentum is similar to *F. homotropicum* in many respects, except for heterostylar self-incompatibility and seed shattering habit. It is possible that the non-shattering nature of *F. esculentum* played an important role in its domestication in preference to *F. homotropicum*. *F. homotropicum* also displays both diploid and tetraploid cytotypes; populations of diploid cytotypes showed more genetic differentiation than the tetraploids (Ohnishi and Asano 1999). While the tetraploid cytotype of *F. homotropicum* has been suggested to be an allotetraploid, the diploid *F. homotropicum* and diploid *F. esculentum* ssp. *ancestralis* are considered to be the diploid progenitors of tetraploid *F. homotropicum* (Ohnishi and Asano 1999). The authors have argued that in the long history of these species, they have had a chance to cross with each other, and hybrids became tetraploid through doubling of their genomes.

Besides *F. esculentum*, the only other cultivated species of the genus *Fagopyrum* is *F. tataricum*. Compared to *F. esculentum, F. tataricum* has better yield performance, better tolerance to frost, and higher rutin content. *F. tataricum* ssp. *potanini,* which was discovered by the Russian botanist G.N. Potani in Gansi province of China, has been proposed to be the immediate wild ancestor of cultivated *F. tartaricum* (Ohnishi 1998b). Since the distribution of natural populations of tartary buckwheat (*F. tataricum*) ranges from China in the east to northern Pakistan in the west, there has been no consensus on the center of origin of the species. Tsuji and Ohnishi (2001a, b) have reported a wild tartary buckwheat from eastern Tibet, which showed the same AFLP and RAPD profiles as the cultivated tartary buckwheat. They proposed eastern Tibet as the center of origin of tartary buckwheat. Allozyme analysis of populations of wild and cultivated tartary buckwheat carried out by Ohnishi (2000, 2002) supported this hypothesis. Evidence for close similarity between *F. tataricum* and *F. cymosum* exists (Kishima et al. 1995; Ohnishi and Matsuoka 1996; Yasui and Matsuoka 1998), and this fact has driven researchers to consider *F. cymosum* to be the ancestor of tartary buckwheat. The phylogenetically closest accession to *F. tataricum* was found to be diploid *F. cymosum* from Tongmai in Tibet (Yamane et al. 2003), and this led to the suggestion that tartary buckwheat speciated from *F. cymosum* in Tibet–Himalayan region (Yamane et al. 2004).

Genetic diversity correlation analysis on 96 accessions of tartary buckwheat from different parts of India, US, Italy, Russia, Japan, Nepal, and Hungary showed a wide range of variability for all the traits studied (Rana 1998). The accessions clustered into 13 heterogenous groups across the range of genotypes and geographic zones, thereby indicating a low degree of genetic divergence in this species. Nagarajan and Prasad (1980) have emphasized that absence of a clear relationship between genetic diversity and geographical location could indicate that forces other than geographical origin, such as exchange of genetic stocks, genetic drift, spontaneous variation, and natural and artificial selection were responsible for genetic diversity within the genus *Fagopyrum*. Compared to the cultivated species, the wild varieties of tartary buckwheat showed 12-fold higher nucleotide diversity (Tsuji and Ohnishi 2000). The high nucleotide diversity amongst wild varieties of tartary buckwheat could be ascribed to the differentiation of the wild subspecies during their longer evolutionary history. Even amongst the wild subspecies, populations from northwestern Yunnan and eastern Tibet showed higher nucleotide diversity than the populations in other regions (Tsuji and Ohnishi 2000). This low diversity in cultivated tartary buckwheat may be attributed to the process of domestication and conscious selection. Except for mutant alleles at a few loci, buckwheat landraces from different parts of the world have been found to be fixed for common allele at all enzyme loci (Tsuji and Ohnishi 1998). In contrast to the results obtained by Tsuji and Ohnishi (2000), RAPD and endosperm protein SDS-PAGE profiles of accessions of wild as well as cultivated tartary buckwheat showed clear clustering according to geographical distribution (Kump and Javornik 2002; Sharma and Jana 2002; Rout and Chrungoo 2007). A striking observation was the clustering of *F. tataricum* ssp. *potanini*, the putative wild ancestor of cultivated tartary buckwheat, as a separate group. The low level of variations in the endosperm protein SDS-PAGE profiles within accessions of tartary buckwheat reported by Rout and Chrungoo (2007) had also been reported earlier by Nishiyama et al. (1991) and Zeller et al. (2004). These results clearly indicate the low level of genetic diversity in tartary buckwheat. Comparative analysis of molecular profiles of accessions of *F. tataricum* and *F. cymosum* from different geographical regions of East Asia have lead Ohnishi and Matsuoka (1996)

and Yasui and Ohnishi (1998a, b) to suggest that *F. tataricum* had speciated from a population of *F. cymosum*. As mentioned earlier, the perennial *F. cymosum* is considered to be the ancestral form of the monophyletic *cymosum* group. If it is true, one can expect *F. cymosum* to have great diversity, which, with successive divergence, has given rise to several species within the group. *F. cymosum* comprises both diploid as well as tetraploid cytotypes (Campbell 1976). While the diploid cytotypes are distributed only in the Sichuan and Yunnan Province of China, the tetraploid forms have been reported from southern China, Thailand, and Himalayan Hills of Bhutan, Nepal, and India (Ohnishi 1998a). It is generally believed that polyploidization has the potential to increase the genetic variability, evolutionary potential, and ecological amplitude of species (Hunziker and Schaal 1983). Higher genetic diversity in polyploid population than their diploid progenitor populations have been reported in many plants (Soltis and Rieseberg 1986; Lumaret and Barrientos 1990). This is also true in the case of *F. cymosum*.

Yamane and Ohnishi (2001) have observed that natural populations of *F. cymosum* maintained a high degree of variability both within a population as well as within a species; the average heterozygosity being higher in tetraploids than the diploids. The genetic differentiation (G_{ST} Value) of populations in *F. cymosum* has been reported to be higher than the corresponding values for other insect pollinated, allogamous species (Hamrick and Godt 1989). Yamane and Ohnishi (2001) reasoned that the high G_{ST} was due to the complete reproductive isolation between diploid and tetraploid populations.

Yamane and Ohnishi (2001) suggested that tetraploidization in *F. cymosum* occurred twice during the course of its evolution. While it occurred once it Yunnan, it probably occurred once in eastern Tibet. It was assumed that the tetraploid *F. cymosum* spread to warmer southern Yunnan and Thailand in one direction and the cooler Tibet and the Himalayan hills in the other. The polyphyletic origin of *F. cymosum* was also suggested earlier by Yasui and Ohnishi (1998a) and has later been confirmed by Yamane and Ohnishi (2003) on the basis of analyses of intraspecific nucleotide sequence variations in *rbc*L–*acc*D and associated intergenic spacer region, the *trn*C–*rpo*B spacer region, the *trn*K intron, and *mat*K region of cpDNA. It is now clearly established that *F. cymosum* has the widest distribution among the wild species of *Fagopyrum* (Table 5.2).

Table 5.2 Total genetic diversity (H_T), genetic diversity within populations (H_S), among populations (D_{ST}), deviations of genotype frequencies from Hardy–Weinberg expectations over all populations (F_{IT}) and within individual populations (F_{IS}), and proportion of total genetic diversity partitioned among populations (C_{ST}) of *Fagopyrum* (Huh et al. 2001)

Species	H_T	H_S	D_{ST}	F_{IS}	F_{IT}	C_{ST}
F. gracillipes	0.390	0.379	0.010	0.410	0.429	0.033
F. tataricum	0.556	0.197	0.359	0.093	0.697	0.658
F. leptopodum	0.553	0.548	0.005	0.250	0.256	0.012
F. esculentum	0.263	0.248	0.015	0.252	0.283	0.048

Crops and their wild progenitors show marked phenotypic differences that have been referred to as the "domestication syndrome" (Hammer 1984). Despite phenotypic differences that have originated from the domestication process, in general, crops and their wild progenitors belong to the same biological species (Harlan and de Wet 1971). Thus, while wild and domesticated populations are sympatric (i.e., grow in close proximity) and share a similar phenology (i.e., their flowering periods overlap), hybridization is expected to occur and result in fertile progeny. With the possible exception of *Arachis hypogea*, this is indeed what has been observed in both allogamous and autogamous species in various studies that have used different methods and approaches (Ellstrand et al. 1999). Since gene flow between wild and domesticated populations is likely to play an important role in the evolution and domestication process, distribution mapping and characterization of wild progenitors of domesticated crops assume great significance. Although a great deal of information is available on the origin, the distribution of the various species of buckwheat, as well as the interspecific diversity within the genus *Fagopyrum*, several issues on intraspecific differentiation remain to be resolved. Further information on distribution of the wild progenitors and their diversity is scanty. The origin and intraspecific differentiation of *F. gracilipes,* a tetraploid self-fertilizing species, have not yet been clarified. Intra- and interspecific differentiation of three recently discovered species, *F. pleioramosum, F. macrocarpum,* and *F. callianthum,* is also an unsolved problem. Hence, there is a need to lay greater emphasis on the collection and characterization of wild species of the genus from diverse geographic regions. This will not only facilitate

their use in breeding programs but will allow a clearer understanding of the site of origin and the differentiation in the genus *Fagopyrum*.

5.4 Future Research Prospects

Crop improvement programs on buckwheat have, to a large extent, focused exclusively on the cultivated common buckwheat (*F. esculentum*). However, renewed interest in use of wild relatives of the genus *Fagopyrum*, for introgression of genes of agronomic interest into common buckwheat, has led to greater efforts towards prospection of new species of buckwheat from the wild. Identification of the self-compatible wild species, *F. homotropicum*, has been an important finding in this direction. The finding of "rice" tartary buckwheat, which has a non-adhering hull and therefore dehulls very readily, has been reported in Nepal, Bhutan, and southern China. This allows the use of it as a rice replacement in the staple diet in these production areas. It is surprising that this interesting trait, although reported as desirable, has not resulted in increased production of this type of tartary buckwheat. Perhaps this has been due to lack of crop improvement efforts that must address yield as well as dehulling capabilities.

This calls for greater effort toward collection and documentation of buckwheat genetic resources from different regions of the globe for putative progenitor speciesthat possess some of the agronomically desirable traits such as low shattering, nutritive quality, hypoallergenicity, and self-compatibility.

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Chapter 6
Hordeum

Peter L. Morrell and Michael T. Clegg

6.1 Basic Botany of the Species

Wild barley (*Hordeum vulgare* ssp. *spontaneum*), the progenitor of cultivated barley (*H. vulgare* ssp. *vulgare*), is an annual, diploid grass species with a chromosome number of $n = 7$ and an estimated rate of self-fertilization of 98%. *Hordeum* belongs to the *Triticeae* tribe of the grass family that also includes wheat (*Triticum*), and together, these two grasses account for roughly 34% of the world's cereal production, although barley accounts for only about 6% compared to 28% for wheat. Both species are believed to be among the world's first cereal species to be domesticated by Neolithic humans.

The principle uses of barley are for malting in beer and whiskey production and for animal feed. According to a 2007 FAO report, Russia is the largest producer of barley followed by Canada: *http://faostat.fao.org/site/339/default.aspx*.

6.2 Taxonomy and Distribution

6.2.1 Taxonomy

The genus *Hordeum* includes roughly 30 species that could be considered as a potential resource for genetic improvement of barley. Among these species, only *H. bulbosum* is generally capable of producing fertile progeny when crossed to the domesticated species, *H. vulgare* ssp. *vulgare* (Bothmer et al. 1983). Thus, *H. bulbosum* is regarded as the only species in the secondary gene pool of cultivated barley (Pickering and Johnston 2005). *Hordeum bulbosum* is an obligately outcrossing (self-incompatible) (Lundqvist 1962) species with a wide geographic distribution.

Wild barley, *H. vulgare* ssp. *spontaneum*, is the progenitor of cultivated barley and is fully interfertile with *H. vulgare* ssp. *vulgare* (hereafter referred to as cultivated barley or simply as "barley"). As indicated by the nomenclature, they are members of the same biological species.

6.2.2 Distribution

The natural distribution of wild barley ranges from the Mediterranean portion of the Middle East, across the Zagros Mountains, and into adjacent Southwest Asia, a distance east to west of ~3,500 km. The eastern and western portions of the species range are relatively low-elevation regions; wild barley has limited cold tolerance and is rare above 1,500-m elevation (Zohary and Hopf 2000). Wild barley populations are abundant in the western portion of the range, but the species is rare at higher elevations (e.g., parts of the Zagros and the continental plateau in Turkey and Iran) and sporadic in the eastern portion of the range (Zohary and Hopf 2000). The Zagros Mountains, together with a series of smaller mountain ranges, trending northwest to southeast, roughly bisect the range; many mountain peaks rise above 3,000 m and the tallest single peak is 4,500 m. Thus, the Zagros Mountains represent a significant disruption in the natural range of wild

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barley, and the mountains present a substantial barrier to the movement of animals that serve as potential seed dispersers. Seed dispersion is facilitated by the spikelets of wild barley that have long, barbed lemma awns, well suited for attachment to animal fur (Bothmer et al. 1995). Moreover, a number of large animal species occur natively within the range of wild barley, including goats, boars, deer, and gazelles, providing a means for the dispersion of wild barley propagules.

Hordeum bulbosum is a perennial grass that occurs in a variety of habitats in the Mediterranean region (Bothmer et al. 1995). The geographic range of *H. bulbosum* is larger than that of *H. vulgare* ssp. *spontaneum*, extending around the Mediterranean basin, including North Africa and southern Europe, and east into western Central Asia, including present day Afghanistan and Tajikistan (Bothmer et al. 1995; Pickering and Johnston 2005). *Hordeum bulbosum* occurs as both an $n = 7$ diploid and $n = 14$ autotetraploid. Diploid forms of *H. bulbosum* predominate west of Greece and Egypt, while tetraploid forms are more common to the east (Bothmer et al. 1995).

6.3 Utilization of Transpecific Genetic Resources

Hordeum bulbosum has many desirable traits that could be used for barley improvement (Johnston et al. 2009). These include disease and pest resistance and tolerance for abiotic stress: traits that have immediate value in current barley breeding programs. Other traits from *H. bulbosum* such as perenniality and self-incompatibility have the potential to alter the crop for more sustainable production and maintenance of genetic diversity (Johnston et al. 2009).

Although direct crosses between *H. vulgare* and *H. bulbosum* are possible, most successful crossing schemes involving *H. bulbosum* include complex combinations of diploid, triploid, and tetraploid progeny (reviewed in Pickering and Johnston 2005, also see Johnston et al. 2009). The difficultly of hybridization between these species appears to be reflected in limited evidence of natural hybridization between *H. bulbosum* and *H. vulgare* ssp. *spontaneum*. In unpublished results, using a *H. bulbosum*-specific molecular marker, Johnston and Pickering found no evidence of introgression from *H. bulbosum* into *H. vulgare* ssp. *spontaneum* in almost 500 accessions from the Near East and Far East where the two species grow sympatrically (Pickering and Johnston 2005).

The largest impact of *H. bulbosum* on barley improvement occurred subsequent to the discovery that crosses of diploid *H. bulbosum* with *H. vulgare* could result in progeny containing a single haploid *H. vulgare* genome (Kasha and Kao 1970). This resulted in the widespread use of *H. bulbosum* for the production of doubled haploid barley lines. The "*Hordeum bulbosum* method" has been widely deployed to produce genetically identical progeny from barley crosses, which permits the testing of individual accessions across a large number of environments (Tinker et al. 1996). Highly replicated, doubled haploid barley populations have been used by many investigators to test new analytical methods for quantitative trait loci (QTL) mapping (e.g., Xu 2003; Zhang et al. 2005; Xu and Jia 2007).

Gametophytic self-incompatibility (GSI) occurs in diverse grass species, including *H. bulbosum* (Baumann et al. 2000). GSI in the grasses has been characterized as a two-locus system with two independent, multiallelic loci identified as the *S* and *Z* loci. The loci and genetic mechanism responsible for GSI in the grasses have yet to be isolated (Yang et al. 2008). Genetic mapping and gene expression-based approaches have been used to attempt to isolate causative loci in *H. bulbosum* (Gudu et al. 2002; Kakeda et al. 2008). Genetic maps and annotated expressed sequence tag (EST) sequence libraries for barley permit comparisons of genetic map positions for the *H. bulbosum S* locus to the barley genome, with higher marker density, physical map positions (Kunzel et al. 2000), and higher resolution genetic maps than would be available for many other self-incompatible grasses (Kakeda et al. 2008). However, the lack of a barley genome sequence means that actual gene-level identification of putatively causative loci must be based on comparison to the rice genome sequence (Kakeda et al. 2008), where gene order may not be conserved.

6.4 Genome Characteristics and Resources

The size of the barley genome has been estimated as 5.5 Gb, or roughly twice the size of the human genome. Despite the large genome size, the diploid

barley genome is more accessible and less complex than the genomes of most other cultivated species in the Triticeae tribe of the grass family, which also includes wheat and rye. Comparative sequence data from bacterial artificial chromosomes (BACs) from rice and barley suggest that colinear portions of the rice and barley genomes differ by large numbers of transposable elements (Dubcovsky et al. 2001). Much of the genome size of barley (and wild barley) can be attributed to the large number of transposable elements, including the copia-like retroelement BARE-1 (barley retroelement 1) (Manninen and Schulman 1993; Suoniemi et al. 1996), which is extremely abundant in the barley genome. Copy number of BARE-1 varies by >2-fold within *H. vulgare* ssp. *spontaneum* and ssp. *vulgare* and is a major contributor to variation in genome size (Vicient et al. 1999; Kalendar et al. 2000) with copy number being associated with eco-geographic variation within the habitat occupied by populations of *H. vulgare* ssp. *spontaneum* (Kalendar et al. 2000).

Currently, approximately ~24,000 EST sequences from *H. vulgare* ssp. *spontaneum* have been deposited in GenBank. An additional ~400,000 EST sequences are reported for *H. vulgare* ssp. *vulgare*. Much of the EST data for barley was retained as original sequence trace files, which were organized into database-driven software known as HarvEST that permits EST clustering *http://harvest.ucr.edu/*. Thus, barley EST data, including EST sequences from wild barley, can readily be browsed, visually inspected, and used for BLAST searches and single nucleotide polymorphism (SNP) identification.

Planning for barley genome sequencing is underway, but currently, neither a genome sequence nor a complete physical map of the genome is available (Schulte et al. 2009).

6.5 Conservation Initiatives

A number of germplasm repositories maintain collections of wild barley and related species for ex situ preservation. The *Gramene* website provides access to these centers at *http://www.gramene.org/species/hordeum/barley_germplasm.html*. Among the larger collections are those maintained at the United States Department of Agriculture (USDA) at Aberdeen, Washington, USA and by the International Center for Agricultural Research in the Dry Areas (ICARDA) in Aleppo, Syria. The *Hordeum vulgare* ssp. *spontaneum* collection at the USDA lists 1,152 accessions from 16 countries, and 192 *H. bulbosum* accession from 20 countries as currently available. The ICARDA collection currently reports 1,799 accessions of *Hordeum vulgare* ssp. *spontaneum* from 23 countries and 60 *H. bulbosum* accessions from 12 countries. Other large collections such as Plant Gene Resources of Canada include large number of accessions (e.g., 533 *Hordeum bulbosum* accessions); however, repositories often include duplicates of accessions in other repositories. In heavily collected regions, it is possible that multiple accessions derive from either the same or adjacent local plant populations.

All of the major repositories maintain online databases where "passport" data for each accession is available. This information includes geographic region of origin, generally including latitude and longitude and inferred elevation. This information is searchable online through the Germplasm Resources Information Network at *http://www.ars-grin.gov/npgs/index.html* and similar resources (see the *Gramene* link above for other repository websites). For many accessions, phenotypic traits, including disease and insect resistance or susceptibility, are recorded for individual accessions. For *Hordeum bulbosum*, the passport data includes ploidy, which is especially important for utilization of individual accessions of this species. However, as with other passport data, ploidy has not been assessed for all accessions. Many repositories have moved toward a system based on core collections (cf. Brown 1989), which have relatively complete phenotypic and collection data.

6.6 Role in Classical and Molecular Genetic Studies

Because wild barley is predominantly inbreeding, individual plants grown from seed provided by genetic stock centers have very low heterozygosity and thus constitute sets of naturally, partially inbred lines. This accelerates the creation of inbred lines that can be genotyped once and then used repeatedly for phenotypic analysis of traits of interest. Large projects such

as the *Arabidopsis thaliana* 1,001 genomes resequencing effort are applying this approach on a massive scale; resequencing whole genomes from many lines so that the resequencing data can then be used in a variety of studies designed to more directly link genotypes and phenotypes (Weigel and Mott 2009).

Similar efforts have been initiated in wild barley, though initially on a smaller scale. One example is the Wild Barley Diversity Collection (WBDC), developed at the University of Minnesota (Steffenson et al. 2007). The WBDC includes 318 samples representing the entire geographic range of wild barley. The WBDC has been genotyped using 558 DaRT markers and with >3,000 SNPs on the Illumina Golden Gate Genotyping platform (Close et al. 2009).

Another research group has reported establishment of a collection of wild barley lines from Israel. The Barley1K collection, as described by Hübner et al. (2009) is a hierarchical sample of 1,020 wild barley accessions from 51 populations from diverse ecogeographic locations within Israel. For each collection locality, climatic data, soil conditions, etc. are reported. Accessions were also genotyped at 42 microsatellite loci. The high-density of sampling in the Barley1K collection will be used to examine allelic variation relevant to the traits important to agriculture.

Wild barley introgression lines, which contain small portions of the wild barley genome in the genetic background of a cultivated line, provide an opportunity to introgress favorable alleles from wild barley lines into breeding populations. A number of papers have reported successful production of wild barley introgression lines. The design of these populations varies, but all have the goal of introducing small chromosomal segments from the wild barley genome in an effort to introgress favorable alleles into domesticated barley that may improve biotic or abiotic stress response, yield, or contribute to other favorable agronomic traits.

Many of the studies take the form of advance backcross QTL (AB-QTL) experiments (Tanksley and Nelson 1996) in early generations (e.g., Pillen et al. 2003; von Korff et al. 2008) and transition toward the creation of recombinant inbred lines (RIL) or similar populations in later generations (e.g., Baum et al. 2003; Grando et al. 2005; Yun et al. 2005, 2006; Inostroza et al. 2007).

Recently, a set of 110 diploid *Hordeum bulbosum* introgression lines has also been reported (Johnston et al. 2009). The introgression lines cover all but one chromosome arm in the *Hordeum* genome. A set of 46 sequence-based markers were used to identify introgression. The lines were derived from three different barley cultivars (Emir, Golden Promise, and Morex) and four *H. bulbosum* accessions. The use of multiple *H. bulbosum* lines is interesting; perhaps because of the effort involved in creating AB-QTLs and RIL populations, most wild barley introgression projects have sampled a single wild barley accession. Much additional diversity within the *H. vulgare* ssp. *spontaneum* and *H. bulbosum* remains unexplored.

While wild barley has frequently been used as a parent in QTL mapping experiments, intercompatibility with domesticated barley obviates the need for independent genetic maps specific to wild barley. A restriction fragment length polymorphism (RFLP)-based genetic map for *H. bulbosum* has been reported (Jaffe et al. 2000; Salvo-Garrido et al. 2001). Comparison with genetic maps from *H. vulgare* suggests that the genomes of the two species are largely colinear. The recombination rate within barley centromeric regions is higher than in *H. bulbosum* while the opposite is true for telomeric regions. The creation of a *H. bulbosum* genetic map should facilitate the isolation of loci of interest in *H. bulbosum*, including loci that contribute to self-incompatibility and genes that regulate haploid formation and intercompatibility in crosses with barley (Salvo-Garrido et al. 2001).

6.7 Role in Crop Improvement Through Traditional and Advanced Tools

Efforts to introgress favorable alleles from wild barley into barley breeding populations have been reported by many different research groups (Baum et al. 2003; Matus et al. 2003; Pillen et al. 2003, 2004; Grando et al. 2005; von Korff et al. 2005, 2006; Gyenis et al. 2007; Inostroza et al. 2007; Schmalenbach et al. 2008, 2009; von Korff et al. 2008). The majority of these studies report AB-QTL experiments, where wild barley is used as a donor parent. A minimum of two to three generations of backcrossing to the cultivated barley recurrent parent is generally required to recover agronomic phenotypes (cf. Pillen et al. 2004). Barley variety development typically involves crossing of

parents with favorable alleles, followed by multiple generations of selection for favorable agronomic traits prior to the development of a new inbred line. A number of independent studies have reported that wild barley lines were found to contribute favorable alleles in QTL mapping experiments, with the proportion of favorable alleles contributed by wild barley ranging from ~25 to 40.9% (von Korff et al. 2006; Inostroza et al. 2009; Schmalenbach et al. 2009). In a number of cases, the favorable alleles contributed by wild barley are for yield and yield component traits, such as grain number per spike and ear length (Schmalenbach et al. 2009). Another study reported that 37.5% of favorable alleles for the very complex phenotype, malting quality, were contributed by the wild barley parent in an AB-QTL population (von Korff et al. 2008). These studies suggest that while wild barley may be particularly valuable as a source of disease resistance alleles that can be readily introgressed into barley breeding programs (cf. Yun et al. 2006), there are a number of other phenotypes where wild barley can contribute favorable alleles. However, in general, it has been difficult to develop lines resulting from introgression that exceed the productive capability of existing cultivars (Inostroza et al. 2009).

QTL mapping based on large numbers of RILs derived from crosses with wild barley also permits the identification of genomic regions where alleles contributed by the cultivated parent are required in order to recover favorable agronomic phenotypes, an approach Inostroza et al. (2009) describe as a form of "genetic knockdown" experiment.

6.8 Distribution of Genetic Diversity in Wild Barley

A number of studies have examined the nature of adaptations that contribute to ecotypic variation in wild barley (e.g., Volis et al. 2002, 2004; Verhoeven et al. 2004, 2008). These studies have included comparison of potentially adaptive traits in wild barley populations from the extremes of the range, including populations from Israel and Turkmenistan (Volis et al. 2000) and comparison of ecotypic variation within single regions (Volis et al. 2002). Wild barley ecotypes from desert, semi-steppe batha (shrubland-like plant community), Mediterranean grasslands, and mountains have been compared under common garden conditions to identify the extent of differential response to water stress and other environmental factors (Volis et al. 2000, 2002). A density experiment using the various barley ecotypes found that plants from the Mediterranean grassland ecotype was the superior competitor, with the greatest yield and reproductive output (Volis et al. 2004). There is a complex set of traits associated with local adaptation in wild barley, involving subtle differences in factors such as drought and frost tolerance, plant resource allocation, and timing of reproduction (Volis et al. 2004). Examination of the genetic basis of differentiation between wild barley populations in QTL populations (Verhoeven et al. 2004, 2008; Poorter et al. 2005) showed that QTLs putatively associated with adaptation, and increased fitness in one ecological environment does not necessarily diminish fitness in another. This suggests a complex basis for local adaptation dependent on variation at a large number of underlying traits (Verhoeven et al. 2004, 2008; Poorter et al. 2005).

There is a long history of work on biochemical and molecular diversity in barley that is well reviewed in Bothmer et al. (2003). A particularly comprehensive early study examined more than 1,500 accessions of wild and cultivated barley on a worldwide scale, but based on just four esterase encoding loci. This study found substantial levels of genetic diversity and also that most wild alleles had been incorporated into cultivated barley (Kahler and Allard 1981), indicating little loss of genetic diversity following domestication. Interestingly, Kahler and Allard (1981) noted some genetic differentiation among collections between East Asian and Middle South Asian accessions and European and North American collections. Later, isozyme surveys tended to assay many more loci, but often with a more restricted geographic focus. An exception can be found in Nevo et al. (1986) where 27 loci were analyzed in 2,125 individuals from portions of Israel, Turkey, and Iran. While this work uncovered hints of geographic differentiation, the main focus was on environmental genetic correlations. Clear evidence for geographic patterns of differentiation did not arise until the beginning of DNA-based resequencing studies. Resequencing studies are especially informative because they reveal all mutations in a gene sample and the mutations can easily be classified into amino acid substitutions and

synonymous changes. In addition, indel mutations are easily resolved. Finally, complete haplotype data can be resolved, thereby yielding the linkage phase of mutations within a gene. Haplotype data contain valuable information on linkage disequilibrium (Watterson 1975) and they allow the investigator to reconstruct the temporal sequence of mutational differences between haplotypes.

Various statistics can be calculated from these data that relate to effective population size and that can potentially be used to test for selection at a locus. One useful statistic is θ, a function of the number of polymorphic sites in a gene; for neutral genes, θ is approximately equal to $4N_e\mu$, where N_e is the effective population size and μ is the mutation rate per nucleotide site (Watterson 1975). Another common statistic is π, a function of the pairwise frequency of site variants (Tajima 1983). A statistic known as Tajima's D is a function of the difference between θ and π. In populations at equilibrium between genetic drift and mutation, the difference is expected to be zero for neutral loci (Tajima 1989). Nonzero values of Tajima's D may arise owing to demographic changes or owing to natural selection. Some of these statistics will be referred to below.

Wild barley, along with *Arabidopsis thaliana*, was among the first plant species in which extensive resequencing data were collected (Wright and Gaut 2005). The most comprehensive survey to date was based on a sample of 45 accessions carefully selected to span the entire geographic range of wild barley. The first locus examined in wild barley, alcohol dehydrogenase 1 (*Adh1*), had relatively modest levels of diversity with θ for synonymous sites of 0.005. An excess of low frequency nonsynonymous substitutions was observed in the sample, but there was no evidence of recombination among sampled haplotypes, and no evidence of population structure (Cummings and Clegg 1998). These results were largely consistent with expectations for a self-fertilizing species. Resequencing data based on a subset of 25 accessions from two additional *Adh* loci identified higher levels of diversity and evidence of recombination at *Adh2* (Lin et al. 2002). In sharp contrast, a resequencing study of *Adh3* (Lin et al. 2001) revealed extensive divergence between haplotypes, marked by population structure between accessions collected east and west of the Zagros Mountains (Lin et al. 2001) and evidence of recombination between haplotypes (Lin et al. 2002). Estimates of diversity for synonymous sites for *Adh2* and *Adh3* were 0.0159 and 0.0325, respectively; dramatically higher than the level of diversity observed at *Adh1*. Thus, both the geographic distribution of haplotypes and level of diversity for the other two independently segregating *Adh* loci bore little resemblance to that identified at *Adh3*, even though the same 25 accessions comprised the sample. Indeed, sampled haplotypes at *Adh1* and *Adh2* seemed to be distributed almost at random across the range of the species, suggesting that the rate of migration was at least of the order of the temporal history of the coalescent process. The distribution of haplotype diversity at *Adh3* implied a barrier to gene flow across the range of wild barley, while the broad geographic distribution of some haplotypes at *Adh1* and *Adh2* implies migration sufficient to distribute all haplotypes across the range. Because all genes in a genome move together as diploid individuals or, in the case of pollen flow, as haploid gametes, the results for the *Adh* loci presented a paradox. To ask whether the unusual pattern was unique to *Adh*3, an expanded survey of additional loci was undertaken.

The subsequent study was designed to investigate the strength of migration across the geographic range and to characterize heterogeneities in spatial patterns of genetic diversity. The number of loci resequenced was expanded by six additional loci based on the same sample of 25 accessions used for the *Adh* genes (Morrell et al. 2003). Owing to small sample size, the study focused on broad-scale geographic patterns and examined levels of diversity and rate of migration between the eastern, western, and intermediate "Zagros" portion of the range of wild barley. Using a maximum-likelihood, coalescence-based approach, average migration rate per locus among regions was estimated as slightly greater than one migrant per generation. This rate of migration should be sufficient to result in a homogeneous geographic distribution of haplotype variation. However, geographic structure was evident at roughly half of the sampled loci, with the *G3pdh* locus showing evidence of extensive haplotype divergence, similar to that reported for *Adh3*. As noted above, wild barley has long, barbed awns that promote dispersal of disarticulated spikelets and thus is well adapted for dispersal by both small and large mammals. Thus, a relatively high rate of migration among wild barley populations is plausible for the species. Why do haplotypes at some loci in wild barley

disperse across the entire range on a time scale of roughly N_e while other loci exhibit strong patterns of genetic differentiation? The answer to this question is still unresolved, but it seems likely that some kind of geographic selection is affecting patterns of diversity at a substantial subset of loci.

At first sight, it appears surprising that large isozyme surveys, such as those cited above, failed to detect clear genetic differentiation east and west of the Zagros Mountains. Put differently, why should resequencing, based on a relatively small sample of accessions, reveal sharp patterns of geographic differentiation not apparent at the isozyme level? The answer can probably be found in the power of haplotype data. The mutational distance among haplotypes is readily apparent in haplotype data and this allowed the detection of divergent haplotype classes by geographic region. There is no comparable measure of mutational distance between isozyme alleles; so haplotype data are much more powerful for the detection of geographic structure.

6.9 Linkage Disequilibrium

Linkage disequilibrium (LD) in wild barley was estimated from the same 25 accessions discussed above based on resequencing data from 18 loci (Morrell et al. 2005). Three loci with very marked population structure contributed to strong admixture LD, but when the preponderance of the data is considered without these loci, the decay of LD in wild barley is relatively rapid, falling to half the initial value in roughly the first 1,000 bp. Relatively rapid decay of LD was also observed in an independent sample of four loci in 34 accessions from across the range of wild barley (Caldwell et al. 2006). Resequencing of individual loci, such as the *Ppd-H1* related to flowering time adaptation, has identified large numbers of recombination events, again suggestive of rapid decay of LD (Jones et al. 2008). Surprisingly, the estimated decay of LD is comparable to that found in the outcrossing crop species maize (Morrell et al. 2005). This result presents a paradox because predominantly self-fertilizing species are expected to be characterized by extensive LD. The reason for this expectation is that inbreeding leads to high levels of homozygosity, thus suppressing effective recombination (Nordborg 2000). It is not clear why LD is so limited in wild barley, although one plausible hypothesis is that wild barley evolved self-fertilization relatively recently in its evolutionary history (perhaps within the past 20,000–40,000 years). Some support for this hypothesis can be found in the fact that *H. bulbosum*, the sister species of wild barley, has a self-incompatible breeding system, and molecular clock calculations indicate that the two species diverged from a common ancestor roughly seven million years ago (Blattner 2004).

Admixture LD presents both a problem and an opportunity for those who wish to use association mapping to locate major genes of interest. The problem arises because the pooling of haplotypes across heterogeneous subpopulations induces LD in the pooled sample independent of map distance. Thus, LD is not a reliable indicator of map distance, and one must first determine the extent of population substructure before making inferences based on LD. The opportunity arises because when properly characterized, admixture LD can be employed to look for gene-trait associations.

6.10 Recombination

Recombination is composed of two processes: meiotic crossing-over involving a symmetrical exchange between sister chromatids and gene conversion where short tracks of a few hundred base pairs are asymmetrically exchanged between synapsing chromatids. Resequencing data permit detailed investigation of recombinational processes because the coalescent history represented in a sample includes many thousands of meioses and therefore provides much greater statistical power than even large genetic crosses. This feature of resequencing data was exploited by Morrell et al. (2006) to estimate the frequency of gene conversion events relative to crossover events in wild barley and several other species. The result showed that gene conversion events are at least as frequent as crossover events in barley recombination. This finding adds an additional complication for association mapping, because the effect of gene conversion is to recombine adjacent sequences while not affecting more distantly linked genes. LD may not be a simple linear function of map distance because of this effect, so LD is likely

to be a biased predictor of gene-trait distances because LD may be lower at short distances than at intermediate distances. This will clearly confound association mapping and lead to some errors.

6.11 Genetic Evidence and the Origins of Domesticated Barley

The question of how and why Neolithic humans began to domesticate plants and animals is certainly one of the great historical mysteries of all time. There is clear archeological evidence of domestication of barley by 10,500 BP in the Fertile Crescent region of the Middle East (Bothmer et al. 1995; Willcox 2005). Evidence of human utilization of barley may extend to 19,000 years BP (Zohary and Hopf 2000). As one of the first plant species to be domesticated in the Fertile Crescent region, barley sits at the nexus of this historical conundrum. There has been a long debate about whether barley was domesticated once in the Fertile Crescent and spread east into Asia and north into Europe or whether barley might have been domesticated multiple times (Zohary 1999; Zohary and Hopf 2000). For many years, the idea of a unique domestication was appealing because the transition to agriculture must have been associated with many changes in human culture that, among other things, required abandoning a nomadic existence for an existence tied to particular plots of land. Moreover, it seems parsimonious to argue that the genetic changes induced by such a transition occurred just once. Archeological evidence clearly supports an origin of barley domestication in the Fertile Crescent (Willcox 2005), but there have been intriguing hints of a second origin largely based on genetic data.

One of the strongest and most intriguing pieces of evidence for multiple origin of a crop comes from "domestication traits" (Sang 2009; Zohary 1999). Wild barley is characterized by a brittle rachis that disarticulates at seed maturity. This facilitates the dispersion of barley seed and is therefore thought to be adaptive for the wild plant. Domesticated barley has a mutation that produces a nonbrittle rachis, so that the ears of grain are retained on the stalk at maturity, thus facilitating seed collection by humans (hence the term "domestication trait"). It is intriguing that the nonbrittle phenotype is actually controlled by two distinct genetic loci, either of which can produce the nonbrittle phenotype (Takahashi and Yamamoto 1949; Takahashi 1964). Why would human domesticators have selected for a second mutation when one serves the purpose? The best explanation for this puzzle is that the two mutations were selected independently in two geographically separate populations, thus implying a second independent domestication. There is strong evidence for multiple origins of another key domestication trait, the two-rowed versus six-rowed phenotype (Komatsuda et al. 2007). However, another domestication trait, "naked" or hulless barley grains, appears to have a single origin (Taketa et al. 2008).

The extensive genetic differentiation east and west of the Zagros Mountains afforded an opportunity to apply a different test for multiple domestications of barley. The reasoning is that if landraces of barley were domesticated more than once, they should be genetically similar to their geographic area of domestication and subsequent cultivation. Morrell and Clegg (2007) applied this reasoning by resequencing seven loci that exhibited geographic differentiation in wild barley in a set of 32 cultivated barleys. A genetic assignment approach, implemented in the program Structure (Pritchard et al. 2000; Falush et al. 2003), was applied to the data to ask whether there was concordance between wild and landrace barley from particular geographic regions. The data clearly showed strong concordance, supporting a multiple domestication hypothesis.

The cultivated sample also included a few modern cultivars of barley. Cultivars of European or North American origin tended to show a Fertile Crescent origin while cultivars from Asia indicated an origin east of the Zagros Mountains. The sample mesh was insufficient to ask whether population substructure exists on a finer scale. It also did not pinpoint the geographic location(s) of domestication events, although the data hint that the origin of eastern landraces was in the western foothills of the Zagros or points farther east. Locations of early Neolithic agropastoral settlements suggest three general regions in which the secondary domestication could have taken place; in the foothills of the Zagros, at such sites as Ali Kosh and Jarmo at Mehrgarh (in present day Pakistan) or in the piedmont zone between the Kopet Dag mountain range and Kara Kum Desert (east of the Caspian Sea, in present day Turkmenistan).

The discovery of multiple domestications is of more than academic interest. Multiple origins imply that unique genes of agricultural value may be uncovered in different ecogeographical regions. This places a premium on maintaining genetic resource collections from different ecogeographical regions and on conducting more detailed surveys with a much finer sample mesh to uncover the finer details of both genetic substructure and domestication history. Ultimately, it will be possible to trace the geographic origins of many major adaptive haplotypes and to ask how their introduction into otherwise adapted cultivars may improve barley quality and yield.

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Chapter 7
Oryza

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7.1 Introduction

Rice (*Oryza sativa* L.) is the primary food source for more than a third of the world's population. It accounts for 35–60% of the calories consumed by 2.9 billion Asians. Rice is planted on almost 150 million ha, with annual production of 650 million tons. It is the only major cereal crop that is consumed almost exclusively by humans. More than 90% of rice is grown and consumed in Asia. Within Asia, China is the largest producer of rice (185.49 m tons) and India has the largest area under rice production (Table 7.1). It is grown under a wide range of agroclimatic conditions between 55°N and 35°S latitudes from sea level to altitudes of 2,500 m or even higher. Rice cultivation is classified into four major ecosystems (1) irrigated, (2) rainfed lowland, (3) rainfed upland, and (4) deep water. The area under each ecosystem is variable: irrigated (55%), rainfed lowland (25%), upland (12%), and deep water (8.0%).

During the last few decades, major advances have been made in increasing rice productivity. World rice production has more than doubled from 257 million tons in 1966 to 650 million tons in 2007. This was achieved mainly through the application of principles of classical Mendelian genetics and conventional plant breeding. The current world population of 6.1 billion is expected to reach 8.0 billion by 2030, and rice production must increase by 25% to meet the growing demand. Further, rice productivity is continually threatened by several diseases (bacterial blight, blast, tungro virus, yellow mottle virus, sheath blight, etc.), insects (planthoppers, stem borer, and gall midge), and many abiotic stresses (drought, salinity, submergence, cold, heat, soil toxicities, etc.). The major challenge is how to overcome these constraints particularly in the context of global climatic changes and newly emerging diseases and pathogen types. The other major concern is how to produce more rice with less land, water, chemicals, and labor. To overcome these constraints and meet challenges to increase productivity, there is a need to develop rice varieties with higher yield potential and durable resistance to diseases, insects, and abiotic stresses.

Recent advances in cellular and molecular biology, particularly genomics, have provided new opportunities to develop improved germplasm with new genetic properties and to understand the function of rice genes (see Khush and Brar 2001). Rice has, in fact, become a model plant for genetic and breeding researches. Several factors have contributed to this (1) diploid species ($2n = 24$) with smallest genome size (390 Mb) among cereals, (2) self-pollination and short growth duration, (3) most extensive germplasm collection comprising more than 100,000 accessions of rice and wild species, (4) a large number of mutant markers, well characterized cytogenetic stocks (primary trisomics, secondary trisomics, monosomic alien addition lines), (5) a dense molecular map consisting of more than 4,000 DNA markers, (6) extensive synteny with genomes of other cereals, (7) ease in genetic transformation and to produce transgenics with new genetic properties, (8) availability of genomics resources such as expressed sequence tag (EST), bacterial artificial chromosome (BAC) libraries both in cultivated and wild species, T-DNA insertion

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Table 7.1 Area, production, and yield of rice paddy in different countries in 2007

Region/Country	Area (000 ha)	Production (000 tons)	Yield (t/ha)
Asia	140,036	590,170	4.21
Bangladesh	11,200	43,504	3.88
Cambodia	2,542	5,995	2.36
China	29,230	185,490	6.35
India	44,000	141,134	3.21
Indonesia	12,166	57,049	4.69
Iran	630	3,500	5.56
Japan	1,678	10,970	6.54
Korea, DPR	575	2,165	3.77
Korea, Rep	950	5,960	6.27
Laos	820	2,870	3.50
Malaysia	660	2,231	3.38
Myanmar	8,200	32,610	3.98
Nepal	1,440	3,681	2.56
Pakistan	2,600	8,300	3.19
Philippines	4,250	16,000	3.76
Sri Lanka	796	3,131	3.93
Thailand	10,360	27,879	2.69
Vietnam	7,305	35,567	4.87
South America	4,806	21,402	4.45
Africa	9,386	23,483	2.50
Australia	47	340	7.40
USA	1,112	8,956	8.05
World	156,688	650,193	4.15

mutants, retrotransposons, deletion mutants, and above all, (9) availability of whole genome sequence of both *indica* and *japonica* rices.

The genus *Oryza,* to which cultivated rice (*O. sativa* L.) belongs, has 22 wild species ($2n = 24$, 48 chromosomes) representing ten genomes: AA-, BB-, BBCC-, CC-, CCDD-, EE-, FF-, GG-, HHJJ-, HHKK- (Vaughan 1989, 1994, 2003; Aggarwal et al. 1996, 1997; Ge et al. 1999). These wild species are an important reservoir of useful genes for rice improvement (Table 7.2). Wide hybridization, involving crosses among rice and its wild species, is one of the important plant-breeding methods to broaden the gene pool for tolerance to major biotic and abiotic stresses, cytoplasmic diversification, and to introgress yield-enhancing genes or quantitative trait loci (QTLs). Low crossability, increased sterility, and restricted recombination between chromosomes of rice and wild species limit the transfer of genes from wild species into rice. We are using embryo rescue, anther culture, molecular markers, and genomic in situ hybridization (GISH) techniques to overcome incompatibility barriers and to track alien introgression. As a result, several successful gene transfers from wild species into rice have been made and varieties released for commercial cultivation (Brar and Khush 2002, 2006).

7.2 Taxonomy

The cultivated rice (*O. sativa* L.) belongs to the tribe Oryzeae, subfamily Oryzoideae of the grass family Poaceae (Gramineae). The tribe has 11 genera, of which genus *Oryza* is the only one with cultivated species. The genus *Oryza*, to which cultivated rice belongs, originated at least 130 million years ago and spread as a wild grass in Gonwanaland, the super continent that eventually broke up and drifted apart to become Asia, Africa, the Americas, Australia, and Antarctica. Of the two cultivated species, *O. sativa* ($2n = 24$, AA), the Asian rice is high yielding and grown worldwide, but *O. glaberrima* ($2n = 24$, AA), the African rice, is low yielding and limited to some parts of West Africa. The genus has 22 wild species ($2n = 24$, 48), with ten genomic types. Cultivated varieties of *O. sativa* were initially grouped into three types: *indica*, *japonica*, and *javanica* (tropical *japonica*). Glaszmann (1987) later classified rice varieties into six groups based on isozyme polymorphism.

Roschevicz (1931) published a comprehensive study of 19 species that provided a basis for later taxonomic investigations of the genus *Oryza*. Chatterjee (1948) and Sampath (1962) listed 23 species, and Tateoka (1962a, b) listed 21 species. At present, two cultivated and 22 wild species are recognized in the genus *Oryza*. The species of the genus have been characterized into several groups on the basis of morphological characteristics, primarily the spikelet structure.

The genus *Oryza* has been divided into four species complexes (1) *sativa* complex, (2) *officinalis* complex, (3) *meyeriana* complex, and (4) *ridleyi* complex (Table 7.2). Two species, *O. brachyantha* and *O. schlechteri* cannot be placed in any of these groups (Vaughan 1989, 1994).

7.2.1 O. sativa *Complex*

This complex consists of two cultivated species, *O. sativa* and *O. glaberrima,* and six wild taxa

Table 7.2 Chromosome number, genomic composition, and distribution of *Oryza* species and their useful traits

Species	2*n*	Genome	Number of accessions[a]	Distribution	Useful traits
O. sativa complex					
O. sativa L.	24	AA-	93,318	Worldwide	Cultigen
O. glaberrima Steud.	24	A^gA^g-	1,562	West Africa	Cultigen; tolerance to drought, acidity, iron toxicity; resistance to blast, RYMV, African gall midge, hoppers, nematodes; weed competitiveness
O. nivara Sharma et Shastry	24	AA-	1,260	Tropical and subtropical Asia	Resistance to grassy stunt virus, BB
O. rufipogon Griff.	24	AA-	858	Tropical and subtropical Asia, tropical Australia	Resistance to BB, BPH, tungro virus; moderately tolerant to Shb, tolerance to aluminum and soil acidity; source of CMS and yield-enhancing loci
O. breviligulata A. Chev. et Roehr. *O. barthii*	24	A^gA^g-	218	Africa	Resistance to GLH, BB; drought avoidance
O. longistaminata A. Chev. et Roehr.	24	A^lA^l-	203	Africa	Resistance to BB, nematodes, stemborer, drought avoidance
O. meridionalis Ng	24	A^mA^m-	56	Tropical Australia	Elongation ability; drought avoidance
O. glumaepatula Steud.	24	$A^{gp}A^{gp}$-	54	South and Central America	Elongation ability; source of CMS
O. officinalis complex					
O. punctata Kotschy ex Steud	24, 48	BB-, BBCC-	71	Africa	Resistance to BPH, zigzag leafhopper
O. minuta J.S. Presl. ex C.B. Presl.	48	BBCC-	63	Philippines and Papua New Guinea	Resistance to BB, blast, BPH, GLH
O.officinalis Wall ex Watt	24	CC-	265	Tropical and subtropical Asia, tropical Australia	Resistance to thrips, BPH, GLH, WBPH, BB, stem rot
O. rhizomatis Vaughan	24	CC-	19	Sri Lanka	Drought avoidance
O. eichingeri A. Peter	24	CC-	30	South Asia and East Africa	Resistance to BPH, WBPH, GLH
O. latifolia Desv.	48	CCDD-	40	South and Central America	Resistance to BPH, high biomass production
O. alta Swallen	48	CCDD-	6	South and Central America	Resistance to striped stemborer; high biomass production
O. grandiglumis (Doell) Prod.	48	CCDD-	10	South and Central America	High biomass production
O. australiensis Domin.	24	EE-	36	Tropical Australia	Resistance to BPH, BB, blast; drought avoidance

(*continued*)

Table 7.2 (continued)

Species	2*n*	Genome	Number of accessions[a]	Distribution	Useful traits
O. meyeriana complex					
O.granulata Nees et Arn. ex Watt	24	GG-	24	South and Southeast Asia	Shade tolerance; adaptation to aerobic soil
O. meyeriana (Zoll. et (Mor. ex Steud.) Baill.)	24	GG-	11	Southeast Asia	Shade tolerance; adaptation to aerobic soil
O. ridleyi Complex					
O. longiglumis Jansen	48	HHJJ-	6	Irian Jaya, Indonesia, and Papua New Guinea	Resistance to blast, BB
O. ridleyi Hook. F.	48	HHJJ-	15	South Asia	Resistance to blast, BB, blast, stemborer, whorl maggot
Unclassified					
O. brachyantha A. Chev. et Roehr.	24	FF-	19	Africa	Resistance to BB, yellow stemborer, leaf folder, whorl maggot; tolerance to laterite soil
O. schlechteri Pilger	48	HHKK-	1	Papua New Guinea	Stoloniferous
Hybrids or unidentified species	–	–	941	–	–
Related genera					
Chikusiochloa Koidz.	24	–	1	China, Japan, Indonesia	–
Hygroryza Ness	24	–	4	Asia	–
Leersia Soland	24,48,60,96	–	8	Worldwide	–
Luziola Juss.	24	–	1	North and South America	–
Prosphytochloa Schweickerdt	Unknown	–	0	Southern Africa	–
Rhynchoryza Baill.	24	–	1	South America	–
Zizania Gronov. ex Linn	30,34	–	0	Europe, Asia, North America	–
Zizaniopsis Doell. & Aschers	24	–	1	North and South America	–
Porteresia Tateoka	48	–	1	South Asia	–
Potamorphila R. Br.	24	–	1	Australia	–

Modified from Brar and Khush (2006)
BPH brown planthopper; *GLH* green leafhopper; *WBPH* white-backed planthopper; *BB* bacterial blight; *Shb* sheath blight; *CMS* cytoplasmic male sterility; *RYMV* rice yellow mottle virus
[a]Accessions maintained in rice genebank at IRRI, Philippines

(*O. nivara*, *O. rufipogon*, *O. breviligulata* (*O. barthii*), *O. longistaminata*, *O. meridionalis*, *and O. glumaepatula*) (Table 7.2). All of these are diploid with AA-genome and form the primary gene pool for rice improvement. *O. sativa* (Asian rice) is widely cultivated whereas *O. glaberrima* (African rice) is cultivated on a limited scale in western Africa. *O. nivara* is widespread over the Deccan Peninsula and in the middle Gangetic valley of India. It also grows in the plateau regions of Myanmar, Southeast Asia, and southern and southwestern regions of China. *O. nivara* has been referred in the literature as *O. fatua*, *O. sativa* var. *fatua*, and *O. sativa ssp. spontanea*. *O. nivara* is closely related to *O. sativa* and *O. rufipogon* with which it may grow sympatrically.

O. rufipogon, in the literature, has been described as *O. perennis* var. *balunga* distributed in tropical and subtropical Asia including northern territory and

Queensland of Australia (Vaughan 1994). *O. longistaminata*, a native of Africa, is closely related to *O. barthii* with which it sometimes grows sympatrically in West Africa. It is quite tall (~2 m), erect, rhizomatous, outcrossing in nature, and is partially self-incompatible. O. *meridionalis* is distributed in tropical Australia whereas *O. glumaepatula* is distributed in South America. Their taxonomic affinity is towards *O. rufipogon* and *O. nivara*. Wild species closely related to *O. sativa* have been variously named. The species name *O. perennis* has been widely used for the perennial wild relative of rice found in Asia, Africa, and Latin America. Tateoka (1963) considers *O. rufipogon* a taxonomically valid name for this species.

Sharma and Shastry (1965a) divided *O. rufipogon* of Asia into two categories, recognizing a new species, *O. nivara,* as an annual form of wild rice and retaining *O. rufipogon* for perennial populations. The three taxa – *O. sativa, O. nivara,* and *O. rufipogon* – together with the weedy race (*O. sativa* f. *spontanea*) form a large species complex. Recent literature seems to agree on giving the perennial wild relatives of rice separate species names, *O. rufipogon* and *O. nivara,* to the annual forms.

The weedy types of rice have been given various names, such as *fatua* and *spontanea* in Asia and *stapfii* in Africa. Depending on the location, these weedy rices may be more closely related to *O. rufipogon* and *O. nivara* in Asia and *O. longistaminata* or *O. breviligulata* in Africa. The *O. sativa* complex includes two other species. The one distributed across tropical Australia is called *O. meridionalis* (Ng et al. 1981). This species has many characteristics similar to those of *O. nivara* described by Sharma and Shastry (1965a, b). However, it has longer awns, narrower spikelets, and a more compact panicle. This species is often sympatric with *O. australiensis* in Australia.

The species closely related to the West African cultivated rice, *O. glaberrima,* are somewhat easier to distinguish from each other. The perennial and annual relatives of *O. glaberrima* are *O. longistaminata* and *O. breviliguiata,* respectively. Previously, O. *longistaminata* was called *O. barthii*; this species is easily distinguished from the annual wild species of this complex by its strong rhizomes and long anthers. *O. longistaminata* differs morphologically from its Asian counterpart primarily in its panicle branching and short ligule. *O. glaberrima* is distinguished from *O. sativa* by its short, rounded ligule, panicle-lacking secondary branches, and almost glabrous lemma and palea. *O. glaberrima* is not as variable as *O. sativa.* However, in some areas of West Africa, local people prefer the taste of *O. glaberrima,* and the species is better adapted to upland habitats than the introduced common rice from Asia. *O. longistaminata* is more widely distributed in Africa than *O. glaberrima.*

7.2.2 O. officinalis *Complex*

The largest complex in the genus is *O. officinalis* complex. It consists of nine species, also called the *O. latifolia* complex by Tateoka (1962a, b). The complex comprises five diploid and four allotetraploid species viz. *O. minuta*, *O. latifolia*, *O. alta*, and *O. grandiglumis* (Table 7.2). Species of this complex can be easily identified by virtue of their truncated ligules, straight rachilla, small sized-spikelets, and linearly arranged tubercles on the surface of lemma and palea. Some species grow in partial shade or moist soil. Others are adapted to swamps and seasonal pools of water and open habitat. This complex has related species groups in Asia, Africa, and Latin America. In Asia, the most common species is *O. officinalis,* widely distributed in South and Southeast Asia and South and Southwest China. *O. officinalis* thrives in partial shade or full sun. In Philippines it is called bird rice.

The tetraploid species *O. minuta* grows in Philippines. *O. minuta* is sympatric with *O. officinalis* in the central islands of Bohol and Leyte. It grows in shade or partial shade along stream edges as a minor member of the flora. Only a few populations of *O. minuta,* also called *O. malampuzhaensis,* have been found localized in neighboring parts near the town of Malampuzha of Kerala and Tamil Nadu in South India. A new species from Sri Lanka belonging to this complex was described by Vaughan (1989) as *O. rhizomatis*. A form of the *O. officinalis* complex from Sri Lanka was named *O. collina* (Sharma and Shastry 1965b). Another species of this complex, *O. eichingeri,* grows in the forest shade in Uganda (Tateoka 1965). It was found distributed in Sri Lanka as well (Vaughan 1989).

In Africa, two species of the *O. officinalis* complex are *O. punctata* and *O. eichingeri*. Both have been reported as having diploid and tetraploids forms

(Tateoka 1965). The American species of this complex (*O. latifolia, O. alta* and *O. grandiglumis*) are allotetraploids with a CCDD-genome. *O. latifolia* is widely distributed in Central and South America as well as in the Caribbean islands. *O. alta* and *O. grandiglumis* grow only in South America, primarily in the Amazon basin, except for one population of *O. alta* reported from Belize (Oka 1961). These species are all allotetraploid. A diploid species of this complex, *O. australiensis,* occurs in northern Australia in isolated populations.

7.2.3 Oryza meyeriana *Complex*

Species of *O. meyeriana* complex differ from other two complexes in that they are small-sized plants with lanceolate leaves and the panicle is a raceme. This complex has two diploid species with GG-genome: *O. meyeriana* and *O. granulata*. The most common and widespread species of the complex, *O. granulata* grows in South Asia, Southeast Asia, and Southwest China. *O. meyeriana* is found in Southeast Asia. Ellis (1985) described another species, *Oryza indandamanica,* from Andaman Islands, India. However, Khush and Jena (1989) consider it to be a subspecies of O. *granulata*. This is the only group of species in the genus *Oryza* that is not found in or near permanently or seasonally standing or flooded water. All members of this species complex grow in the shade or partial shade of degraded primary or well-established secondary forests, often on sloping terrain. *O. granulata* is called forest rice by tribal people in Kerala, South India, peacock rice in parts of Vietnam, and bamboo rice in Philippines. Members of the *O. meyeriana* complex grow at higher elevation than other wild species, as high as 1,000 m. All species of this complex have unbranched panicles with small spikelets.

7.2.4 Oryza ridleyi *Complex*

The plants of this complex are tufted erect, 1–2-m tall, with truncate ligule and straight rachilla of spikelet. This complex comprises two tetraploid species, O. *ridleyi* and *O. longiglumis,* which usually grow in shaded habitats beside rivers, streams, or pools. The *O. ridleyi* complex is primarily found in the lowland forests of insular Southeast Asia and New Guinea. *O. longiglumis* is known only from a few sites along the Koembe River, Irian Jaya, Indonesia, and in Papua New Guinea. *O. longiglumis* is related to *O. ridleyi* but has much longer sterile lemmas in relation to the palea and lemma length. *O. ridleyi* grows across Southeast Asia and Far East in Papua New Guinea. *O. ridleyi* and *O. longiglumis* are very similar in morphology and ecology except for minor differences in quantitative characters. For example, *O. longiglumis* has shorter ligule and shorter spikelets but longer awns and longer setaceous sterile lemmas. The F_1 hybrids from crosses of *O. ridleyi* with *O. minuta*, *O. officinalis*, and *O. australiensis* have been produced at IRRI.

7.2.5 Unknown Complex

7.2.5.1 *Oryza brachyantha*

Oryza brachyantha is a diploid species ($2n = 24$, FF) distributed in the African continent. Annual tufted grass is up to 60-cm tall, thin leaves, 15–20-cm long, 6–7-mm wide, ligule flat or acute not split. Of all the species, it is the most closely related to the genus *Leersia*. This species has a small, narrow spikelet with long awns (6–17 cm). Features of the awn, such as its coriaceous, rigid structure served with a single vascular bundle, ally this species with *Oryza* rather than with *Leersia* (Launert 1965). This species grows in the Sahel zone and in East Africa in small temporary pools, often in laterite soils. It is often sympatric with *O. longistaminata*.

7.2.5.2 *Oryza schlechteri*

Oryza schlechteri, a tetraploid species ($2n = 48$), is the least studied species in the genus. Richard Schlechter first collected it in 1907 from Northeast New Guinea. Vaughan and Sitch (1991) recollected it as living material from the same location. Naredo et al. (1993) found that the presence of a sterile lemma and a striated spikelet epidermal (abaxial) surface lacking siliceous triads in *O. schlechteri* ally this species with other *Oryza* species rather than with *Leersia*. It is tufted perennial, 30–40-cm tall, with an erect, 4–5-cm panicle and small, unawned spikelets. Roots

with short underground shoots are covered by broad-scale like leaves. It is tetraploid, but its relationship to other species is unknown. It is a stoloniferous species of unstable stony soil, such as riverbanks, and grows in full or semi-shade.

7.2.6 Related Genera

Besides *Oryza,* the tribe *Oryzeae* contains ten other genera. Vaughan (1989, 1994) has given brief description of these genera. These genera include *Chikusichloa, Hygrooryza, Leersia, Luziola, Prosphytochloa, Rhynchoryza, Zizania, Zizaniopsis, Potaomophila,* and *Porteresia*. *P. coarctata* ($2n = 24$ HHKK) is commonly found in coastal areas of South Asia. It has unusual anatomy, including glands to secret salts. It has rough, erect leaves and occurs in brackish water. The species is characterized by large caryopses with a somewhat bent apex, a large embryo relative to the endosperm, and a short petiole attachment at the base. The leaf blade is coriaceous with prickly tuberculate margins and has a peculiar arrangement of vascular bundles; each rib contains one smaller vascular bundle and below it a larger one. Earlier, many workers classified *P. coarctata* as *O. coarctata*. Later it was included under *Porteresia,* but now the species has again been classified as *O. coarctata* (Ge et al. 1999).

7.3 Genomic Relationships in *Oryza*

Various approaches involving morphological differentiation, meiotic chromosome pairing in F_1 hybrids, molecular analysis, and seed protein analysis have been used to determine genomic relationships in *Oryza* species.

7.3.1 Meiotic Pairing in F_1 Hybrids

Ogawa (2003) gave detailed description on genomic relationship based on meiotic pairing in F_1 hybrids involving different crosses between diploid × diploid, diploid × tetraploid, and tetraploid × tetraploid wild species of *Oryza*.

Morinaga (1939), based on chromosome pairing in F_1 hybrids, concluded that *O. glaberrima, O. breviligulata, O. perennis, O. cubensis, O. sativa* var. *fatua*, and *O. sativa* var. *spontanea* have the genomic constitution designated as the AA-genome. Nezu et al. (1960) studied chromosome pairing in F_1 hybrids of *O. sativa* with related diploid species such as *O. perennis, O. glaberrima, O. stapfii*, and *O. breviligulata*. The hybrids invariably showed 12 bivalents at meiosis, indicating that these species also have the AA-genome. Lu et al. (1998) observed normal chromosome pairing in hybrids of four A-genome species, *O. rufipogon, O. nivara, O. glumaepatula*, and *O. meridionalis*.

On the basis of meiotic chromosome pairing in F_1 hybrids, *O. sativa, O. officinalis, O. minuta,* and *O. latifolia* genomes were designated as AA (Morinaga 1943), CC (Morinaga and Kuriyama 1959), BBCC, and CCDD (Morinaga 1943), respectively. Li et al. (1963) studied F_1 hybrids of *O. sativa* × *O. australiensis* and *O. minuta* × *O. australiensis* and suggested the E-genome for *O. australiensis*. Katayama (1967) reported that diploid *O. punctata* has the BB-genome. Li et al. (1961) and Wuu et al. (1963) designated the F-genome for *O. brachyantha*. On the basis of chromosome pairing in F_1 hybrids, various authors have assigned the genome symbol AA for the *sativa* complex; BB, CC, BBCC, CCDD, and EE for the *Officinalis* complex; and FF for *O. brachyantha* (see Nayar 1973; Khush and Brar 2002; Vaughan 2003).

7.3.2 Molecular Approaches

Molecular markers have been used in several studies to determine divergence among different genomes and species of *Oryza* (Aggarwal 2003). Isozymes and other molecular markers such as restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), simple sequence repeat (SSR), inter simple sequence repeat (ISSR), transposable elements (TE), and rDNA including chloroplast DNA and mitochondrial plasmid-like DNAs have been used in evolutionary studies in *Oryza*. Second (1982) studied electrophoretic patterns of 13 different enzymes and found that alcohol dehydrogenase (ADH) locus was monomorphic in 1,948 strains. The mean diversity index was high (0.23) in *O. sativa*, medium (0.14) in *O. breviligulata* (*O. barthii*), and

low (0.06 and 0.03) in *O. glaberrima*. African species formed a genetic group distinct from *O. sativa*. Grover and Pental (1992) studied ADH profile of 141 accessions of 19 *Oryza* species. Based on ADH isozyme patterns, the genus was classified into six groups.

Wang et al. (1992) carried out RFLP analysis of 93 accessions belonging to 21 species. Classification of *Oryza* species based on RFLPs matched remarkably well with the classification based on morphological and hybridization studies. Four species complexes were identified, which corresponded to those proposed by Vaughan (1989). Within the *O. sativa* complex, accessions of *O. rufipogon* from Asia and Australia clustered together with *O. sativa*. Two groups of cultivated rices, *indica* and *japonica*, showed close affinity with different accessions of *O. rufipogon*. The CCDD tetraploid species (*O. latifolia, O. alta,* and *O. grandiglumis*) are of ancient origin and show closer affinity to each other than to any known diploid species. Their C-genome donor may be *O. eichingeri,* and the D-genome donor may have been related to the E-genome of *O. australiensis*.

In several cases, due to strong incompatibility barriers, hybrids are difficult to produce between divergent species. For example, the hybrids between species of the *O. ridleyi* complex and *O. meyeriana* complex with other species could not be produced, and genome analysis based on meiotic pairing in F_1 hybrids could not be carried out. Under such situations, molecular approaches have been used to determine genomic relationships. Aggarwal et al. (1997) used molecular divergence analysis based on total genomic DNA hybridization. Genomic DNA (after restriction digestion) of 79 accessions of 23 *Oryza* species, six related genera, and five outgroup taxa (two monocots, three dicots) was hybridized individually with 32p-labeled total genomic DNA from 12 *Oryza* species: *O. ridleyi, O. longiglumis, O. granulata, O. meyeriana, O. brachyantha, O. punctata, O. officinalis, O. eichingeri, O. alta, O. latifolia, O. australiensis,* and *O. sativa*. The labeled genomic DNAs representing the *O. ridleyi* and *O. meyeriana* complexes cross-hybridized best to all the accessions of their respective species, less to those representing other genomes of *Oryza* and related genera, and least to the outgroup taxa. In general, the differential hybridization measured in terms of signal intensities was more than 50-fold under conditions that permit detection of 70–75% homologous sequences, both in the presence and absence of *O. sativa* DNA as competitor. In contrast, when total DNA representing other *Oryza* genomes was used as a probe, species of the *O. ridleyi* and *O. meyeriana* complexes did not show any significant cross-hybridization (<5%). The results revealed that the genome(s) of both of these complexes are highly diverged and distinct from all other known genomes of *Oryza*. Based on molecular divergence analysis, new genomic designations GG for the diploids of the *O. meyeriana* complex and HHJJ for the allotetraploids of the *O. ridleyi* complex were proposed (Aggarwal et al. 1997).

Ge et al. (1999) used two nuclear genes, *Adh1* and *Adh2,* and one chloroplast gene (*matK*). Based on sequences of these genes and clades, it was concluded that *O. schlechteri* and *O. coarctata* share the same genome-HHKK. The EE-genome species is most closely related to the DD-genome progenitor. Based on *Adh* phylogenies, *O. sativa* is most closely related to *O. nivara* and *O. rufipogon* supporting the previous hypothesis of Asian origin of *O. sativa* (Seconds 1982; Khush 1997).

Aswidinnoor et al. (1991) isolated genome-specific repeated sequences of *O. minuta* and *O. australiensis*. Southern hybridization showed strong hybridization to *O. minuta* and no cross-hybridization with the genomic DNA of *O. sativa*, indicating divergence among AA and BBCC-genomes. Joshi et al. (2000) used ISSR markers and analyzed 42 genotypes including 17 species. The analysis showed that *O. brachyantha* is the most divergent species and *O. australiensis* does not fall under the *O. officinalis* complex.

Shim (2007) developed *O. minuta* ($2n = 48$, BBCC)-specific clones comprising 99 clones (300–600 bp) from the initial library composed of 1,920 clones against *O. sativa* by representational difference analysis (RDA), a subtractive cloning method and validated through Southern blot hybridization. Chromosomal location of *O. minuta*-specific clones was identified by hybridization with the genomic DNA of eight monosomic alien additional lines (MAALs). The 38 clones were located either on chromosomes 6, 7, or 12. Different hybridization patterns between *O. minuta*-specific clones and wild species such as *O. punctata*, *O. officinalis*, *O. rhizomatis*, *O. austranliensis*, and *O. ridleyi* were observed, indicating conservation of the *O. minuta* fragments across *Oryza* spp. A highly repetitive clone, *OmSC45* hybridized with *O. minuta* (BBCC) and *O. australiensis* (EE) and was found in

6,500 and 9,000 copies, respectively, suggesting an independent and exponential amplification of the fragment in both species during the evolution of *Oryza*. Hybridization of 99 *O. minuta* specific clones with BB and CC-genome wild *Oryza* species resulted in the identification of six BB-genome-specific and 15 CC-genome-specific clones. *OmSC45* was identified as a fragment of *RIRE1* and long terminal repeat (LTR) retrotransposon (RT). Furthermore, the clone was introgressed from *O. minuta* into the advanced breeding lines of *O. sativa*.

7.3.3 Seed Protein Analysis

Sarkar and Raina (1992) studied profiles of soluble protein of cultivated rice and wild species belonging to different groups. Protein analysis confirmed the previous classification based on morphological and cytological criteria. The information from diverse sources confirms the genome designations of various *Oryza* species as shown in Table 7.2. Four species complexes are clearly known. *O. brachyantha* and *O. schlechteri* do not belong to any of the four complexes and are not related to each other.

7.4 Genome Size and Karyotype of *Oryza* Species

Kuwada (1910) was first to report chromosome number of rice as $2n = 2x = 24$. Since then, various workers have confirmed this chromosome number for rice and wild species with $2n = 24$, 48 chromosomes (Table 7.2). The karyotype of rice has been studied at the somatic prometaphase as well as at the pachytene stage of meiosis and individual members of the chromosome complement have been identified.

The genome size of cultivated rice *O. sativa* (AA) is 389 Mb whereas the genome size in wild species ranges from 362 to 1,283 Mb. Ammiraju et al (2006) summarized available information on nuclear DNA content as estimated by flow cytometry. The amphidiplod species with CCDD (1,008 Mb) and HHJJ (1,282 Mb) genomes have more nuclear DNA content than diploid species. Among diploid species, *O. australiensis* (EE) has the largest genome size (965 Mb) and *O. brachyantha* (FF) has smallest (362 Mb). Another diploid wild species, *O. granulata* (GG) also has relatively larger genome size (882 Mb) as compared with diploid AA-genome species, *O. rufipogon* (439 Mb).

7.4.1 Somatic Chromosomes

Rice chromosomes at somatic metaphase are small in size, 0.7–2.8 μm (Nandi 1936) to 2.0–5.0 μm (Yasui 1941). Pachytene chromosomes, however, are fairly long (12–79 μm) in size (Shastry et al. 1960). Rau (1929) reported that out of 12 pairs of the complement, five were large, four were medium, and the remaining three were small. Nandi (1936) hypothesized that *O. sativa* is a secondarily balanced allotetraploid; it may indeed have undergone genomic duplication. Sen (1963) reported that of the 12 chromosome pairs, two were metacentric, nine submetracentric, and one subtelocentric. Kurata and Omura (1978) reported that length of various chromosomes at prometaphase ranges from 1.5 to 4.1 μm, and at metaphase, it ranged from 1.0 to 1.9 μm. The karyotype consisted of five metacentric, five submetracentric, and two subtelocentric chromosomes.

Hu (1961) compared chromosome morphology among several *Oryza* species and reported the karyotypes were almost identical among those species. However, the chromosome size of *O. australiensis* (EE) and *O. officinalis* (CC) were larger, while that of *O. brachyantha* (FF) were smaller. Kurata and Omura (1982) constructed karyotype of four species of *O. sativa* (AA), *O. punctata* (BB), *O. officinalis* (CC), and *O. brachyantha* (FF). No obvious differences were detected in chromosome morphology at mitotic prometaphase. Rapid chromosome contraction in prometaphase causes difficulty in comparing chromosome size. However, several small differences could be observed on some part of individual chromosomes.

Fukui et al. (1994) identified ribosomal RNA gene (rDNA) loci in *Oryza* species by FISH. The rDNA loci were located on one-to-three chromosomes (two–six sites) within the eight diploid *Oryza* spp. The chromosomes containing the rDNA loci were identified as 9, 10, and 11 in descending order of the copy number of

rDNA. Kurata and Fukui (2003) reviewed various modifications in the cytological techniques on chromosome preparations including C-banding. It is now recognized that *Oryza* karyotype comprises five metacentric, five submetracentric, and two subtelocentric chromosomes. Chromosome 7 and 9 are highly heterochromatic along the whole length. *Japonica* types have a nucleolar organizing region (NOR) in the distal portion of the short arm of chromosome 9, but in some *indicas*, the NOR is located at the distal end of chromosome 10.

7.4.2 Pachytene Karyotype

The pachytene chromosome complement of rice was first studied by Shastry et al. (1960). Each chromosome was identified on the basis of length, arm ratio, and presence or absence of dark-staining knobs. The chromosomes were numbered in decreasing order of length – the longest being chromosome 1 and the smallest chromosome 12. The karyotype consisted of eight submetacentric, two metacentric, and two subtelocentric chromosomes. The centromeres of all chromosomes were flanked by darkly stained heterochromatic chromomeres, although the centromere position was not unequivocally clear in all cases. Kurata et al. (1981) analyzed the pachytene chromosome complement of *japonica* cultivar Nipponbare. The chromosome designation agreed in these two studies except for chromosomes 11 and 12, which were interchanged.

The pachytene-based numbering system of Shastry et al. (1960) is now adopted by the rice community. Pachytene chromosomes are 15–45-μm long, with a total length of 240–270 μm (Kurata and Fukui 2003). Image analysis has provided valuable information on the detailed structure of individual rice chromosomes. Khush et al. (1984) identified an extra chromosome in each of the 12 primary trisomics of rice at pachytene following the chromosome designation of Shastry et al. (1960). Thus, the trisomic for chromosome 1 (longest chromosome) was designated as triplo 1, and so on. Segregation pattern in trisomics were studied for 18 genes, and each trisomic showed trisomic segregation for at least one gene. Thus, the associations were determined between the 12 linkage groups and 12 cytologically identifiable pachytene chromosomes.

Cheng et al. (2001b) constructed karyotype of pachytene chromosomes in *japonica* cultivar Nipponbare. A set of 24 arm-specific BACs was established. An ideogram depicting the distribution of heterochromatin in rice genome was developed based on the staining patterns of 4, 6-diamidono-2-phenylindole (DAPI) of pachytene chromosomes. The majority of heterochromatin is distributed in the pericentric regions with some rice chromosomes containing a significantly higher proportion of heterochromatin than other chromosomes. Yu et al. (2002) reported the sequence of chromosome 10, which contains considerable heterochromatin with an enrichment of repetitive elements on 10S and an enrichment of expressed genes on 10L. Multiple insertions from the organelle genome were also detected.

7.5 Origin of Rice

O. sativa is a highly variable species and is distributed worldwide. The Chinese scientists have recognized two rice varietal groups, Hsien and Keng. They correspond to *indica* and *japonica* classification. Kato et al. (1928) expressed the opinion that *indica* and *japonica* rice originated independently from a wild ancestor. Ting (1957), on the other hand, proposed that *japonicas* were derived from the *indicas*. Morinaga (1954) proposed a third group to include bulu and gundil varieties of Indonesia under the name *javanica*. Several authors have ranked *javanicas* at the same taxonomic level as *indicas* and *japonicas*. Earlier studies primarily focused on *indica–japonica* differentiation. However, the so-called *indicas* are such a diverse group that several morphological types can be recognized that correspond to Glaszmann's classification based on isozymes.

Glaszmann (1987), on the basis of genetic affinity using isozyme analysis, reported that *javanica* varieties fall within the *japonica* group and are now referred to as tropical *japonicas*, and the so-called typical *japonicas* are referred to as temperate *japonicas*. A set of 1,688 genotypes was analyzed from a wider geographical distribution for allelic frequencies at 15 isozyme loci by multivariate analysis. On the basis of this classification, 95% of the cultivars fell into six distinct groups (I to VI), the remaining 5% being scattered over intermediate positions. This

classification involved no morphological criteria. Group I corresponded to the *indica* and group VI to the *japonica*, including the bulu and gundil varieties, or the so-called *javanica*s. Groups II, III, and IV were atypical but also classified as *indica*s in the conventional classification. Group V includes aromatic rices of the Indian subcontinent. Khush et al. (2004) analyzed 25,519 varieties originating from different Asian countries using 20 loci for 11 enzymes. The vast majority of varieties belonged to group I (73.4%) and group VI (23%). Less than 1% of the varieties could be assigned to each of groups II, III, IV, and V.

Information from genetic affinity (isozyme analysis), isolation barriers (F_1 sterility), and morphological classification suggests that the six groups may have been domesticated from different populations of *O. nivara* at different locations and on different time scales. Rayada rices (group IV) of Bangladesh adapted to deepwater conditions, for example, might have been domesticated only in recent times when some of the deepwater areas were brought under cultivation.

The *O. nivara* origin accounts for the morphological and physiological similarity between cultivated rice and the wild progenitor, cultivars that are grown in deep water conditions or have relatively strong photoperiod sensitivity resembling *O. rufipogon*. Conversely, with the realization of the large phenotypic gap between *O. sativa* and *O. rufipogon*, authors who advocated the *O. rufipogon* origin did not rule out the possibility of rice domestication from populations intermediate between *O. nivara* and *O. rufipogon* (Oka 1988).

Domestication of rice has been debated for several decades now (Oka 1958, 1974, 1988; Oka and Morishima 1982; Chang 1976, 2003; Khush 1997; Morishima 2001), especially after *O. nivara* was recognized as an independent species (Sharma and Shastry 1965a). Some recent reviews present a detailed discussion on possible domestication routes of rice (Sang and Ge 2007; Sweeny and McCouch 2007; Izawa et al. 2009). Two major hypotheses have been proposed on the origin of rice (1) monophyletic origin and (2) multiple, geographically independent (diphyletic) domestications from its wild ancestor. Archeological evidence, based on ancient rice grains and some genetic markers support a single domestication of *O. sativa indica* from *O. rufipogon* populations. In this scenario, *O. sativa* ssp. *japonica* was later developed in upland growing regions, selected from the *indica* rice (Oka and Morishima 1982).

The alternative hypothesis, of at least two separate domestication events leading to *indica* and *japonica* rice, has been suggested by genetic distance studies (Cheng et al 2003; Yamanaka et al 2003; Vitte et al. 2004; Garris et al 2005; Londo et al. 2006). The genetic distance data reveal that *japonica* and *indica* are genetically distinct from each other, raising the possibility that the two major rice types may have arisen from different ancestral gene pools. The hypothesis of an origin from *O. nivara* was based on the phenotypic similarity between *O. nivara* and *O. sativa*, including annuality, self-fertilization, and high reproductive allocation (Chang 1976; Khush 1997; Sharma et al. 2000). The hypothesis of an origin from *O. rufipogon* emphasized the benefit of higher genetic diversity of the outcrossing progenitor (Oka 1988). However, either hypotheses does not seem to explain the considerable diversity of rice cultivars (Sang and Ge 2007).

Several studies have examined evolutionarily neutral DNA markers at genome wide loci and domestication-related QTLs in several collections, including various cultivars of Asian rice *O. sativa* and its wild relative *O. rufipogon* (Izawa et al. 2009). Genome-wide analyses of retroelement insertion in rice have provided clues as to when the *indica* and *japonica* groups were established. On the basis of the mutation rates of the LTRs of several retroelements, the insertions of common retroelements (as an indicator of the timing of divergence of the *japonica* and *indica* groups from common ancestors) are estimated to have occurred at least 200,000 years ago (Ma and Bennetzen 2004). Therefore, this divergence is probably unrelated to rice domestication processes and strongly suggests at least two domestication processes in Asian rice. Londo et al. (2006) used 203 cultivars of *O. sativa* including landraces and recently released cultivars and 129 populations of *O. rufipogon*, which span the entire geographic range of *O. rufipogon* and sequenced three gene regions, chloroplast *atpB-rbcL* gene, nuclear *S*-adenosyl methionine synthetase (*SAM*) gene region, and evolutionarily neutral nuclear pseudogene, V-ATPase B-subunit (*p-VATPase*). DNA sequence variation in these three gene regions indicated that India and Indochina may represent the ancestral center of diversity for *O. rufipogon*. Their data also suggested that cultivated rice was domesticated at least twice from different *O. rufipogon* populations and that the products of these two independent

domestication events are the two major rice varieties, *O. sativa* var. *indica* and *O. sativa* var. *japonica*. Based on phylogeographical analysis, it was proposed that *O. sativa* var. *indica* was domesticated within a region south of the Himalaya mountain range, likely eastern India, Myanmar, and Thailand, whereas *O. sativa* var. *japonica* was domesticated from wild rice in southern China. These authors, however, did not differentiate *O. rufipogon* and *O. nivara*, rather considered these as the same ancestral gene pool.

Yamanaka et al. (2003) used 23 accessions each of *O. rufipogon* and *O. nivara* and one accession each of sativa and *japonica* and analyzed these for distribution of the retrotransposon *p*-SINE1-r2, a short interspersed nuclear element (SINE) at the waxy locus, chloroplast DNA polymorphism at ORF100, nuclear DNA polymorphism at the 300 bp fragment amplified with CMN-A32. They concluded that *O. sativa* has diphyletic origin based on markers of the three regions. Their study had two major limitations (1) sampling of the wild species accessions did not represent the whole geographical region of their distribution. It represented only a small region in Thailand, Cambodia, and Vietnam, leaving out the vast regions in China and India where *O. rufipogon* and *O. nivara*, respectively, are widely distributed, and (2) the amplification patterns overlapped in both the forms for all the three regions.

Aggarwal et al. (1999) used amplified fragment length polymorphism (AFLP) markers to study phylogenetic relationships in *Oryza* species. Seventy-seven accessions of 23 *Oryza* species, five related genera, and three outgroup taxa were fingerprinted using AFLPs. A total of 1,191 polymorphic markers were obtained using five AFLP primer combinations. Species relationships were studied using different clustering algorithms. The results showed common ancestry to the genus *Oryza*. Further evolution in *Oryza* has followed a polyphyletic path wherein multiple lineages underwent independent divergence after separation from a common ancestor/pool of related taxa. In another study, Joshi et al. (2000) using ISSR markers suggested that the genus may have evolved following polyphyletic pathway.

Representational differential analysis (RDA) was employed to characterize genomic differentiation in rice (Panaud et al. 2002). In this study, rice was used as the tester and millet as the driver. The RDA clones were used as probes in Southern hybridization experiments with genomic DNAs of several species from the family Poaceae. The results suggest that the genomic differentiations associated with the activity of transposable elements are of relatively recent origin. Comparison of the hybridization patterns obtained for several *Oryza* species suggests that several independent amplifications of these transposable elements might have occurred within the genus.

Cheng et al. (2003) determined polyphyletic origin of cultivated rice based on the interspersion pattern of *SINEs*. The retrotransposon, *p-SINE1,* which shows insertion polymorphism in the *O. sativa–O. rufipogon* population, was identified and used to bar code each of the 101 cultivated and wild species based on the presence or absence of the *p-SINEI* members at the respective loci. The phylogenetic tree showed that *O. sativa* strains fall into two groups, corresponding to *japonica* and *indica*, whereas *O. rufipogon* strains were in four groups, in which annual *O. rufipogon* strains formed a single group, differing from the perennial *O. rufipogon* strains of the other three groups. *Japonica* strains were closely related to the *O. rufipogon* perennial strains of one group, and the *indica* strains were closely related to the *O. rufipogon* annual strains, indicating that *O. sativa* has been derived polyphyletically from *O. rufipogon.*

Cheng et al. (2002) identified new *p-SINEI* members showing interspecific insertion polymorphisms from representative strains of four species (*O. barthii, O. glumaepatula, O. longistaminata,* and *O. meridionalis*) with the AA-genome. Some of these members were present only in strains of one species, whereas the others were present in strains of two or multiple species. Phylogenetic analysis based on the *p-SINEI* insertion patterns showed that the strains of each of the five wild rice species formed a cluster. The strains of *O. longistaminata* appear to be distantly related to those of *O. meridionalis.* The strains of these two species appear to be distantly related to those of three other species, *O. rufipogon, O. barthii,* and *O. glumaepatula.* The latter three species are closely related to one another, with *O. barthii* and *O. glumaepatula* being most closely related. A phylogenetic tree including a hypothetical ancestor with all loci for *p-SINEl* insertion showed that the strains of *O. longistaminata* are related most closely to the hypothetical ancestor. This indicates that *O. longistaminata* and *O. meridionalis* diverged early on, whereas the other species diverged relatively recently.

A number of genes such as shattering (*qSH1, sh4*), plant stature (*PROG1*), red pericarp (*RC, Rd*), grain filling (*GIF1*), flowering time (*Ghd7*), grain number per panicle (*Gn1a*), and taste/texture of cooked grains (*Wx*) contributed to rice domestication process (Izawa et al. 2009). Recently, some evolutionary mechanisms and genetic factors controlling a few rice domestication-related traits, such as grain shattering (Konishi et al 2006; Li et al. 2006; Lin et al 2007), plant architecture (Jin et al. 2008; Tan et al 2008), pericarp color (Sweeny et al. 2006; Furukawa et al. 2007), and grain filling (Wang et al. 2008) have been cloned (Table 7.3) and their fingerprints used in elucidating the events for domestication of rice. Two groups (Jin et al. 2008; Tan et al. 2008) simultaneously cloned prostrate growth gene, *PROG1*, which determines the growth habit in rice. Both the studies convincingly showed that all the *O. sativa* ssp. *indica* and *O. sativa* ssp. *japonica* lines carry identical mutations, including single nucleotide polymorphisms (SNPs) and indels, in the *prog1* coding region that may have become fixed during rice domestication.

Tan et al. (2008) further sequenced the coding regions of prostrate-growth in 30 accessions of wild rice, including 25 perennial *O. rufipogon* and five annual *O. nivara* and found that 11 *O. rufipogon* and five *O. nivara* harbored the same or similar nucleotide sequences to *PROG1*. However, the remaining 14 *O. rufipogon* contained identical or similar mutations to *prog1* in *O. sativa*. Though a single gene mutation that transformed the wild rice into cultivated rice has been identified, even this could not answer whether *O. nivara* or the *O. rufipogon* or both are the immediate progenitors of cultivated rice *O. sativa*. Red pericarp, which is ubiquitously present in all the wild species, is controlled by a dominant gene, *Rc*. It has been cloned from *O. rufipogon*, and 14 bp deletion was shown to be responsible for truncated protein in cultivated rice that otherwise quote for proanthocyanidin synthesis (Sweeny et al. 2006; Furukawa et al. 2007). *O. nivara* accessions have variation for the pericarp color. The white pericarp accessions might be a result of the mutation similar to *O. sativa* or introgression from *O. sativa*. Likewise, a number of *O. sativa* lines have red pericarp, meaning that the mutation might have occurred after domestication.

The controversy whether *O. rufipogon* or *O. nivara* or both are the ancestral progenitors of rice still remains, though some groups prefer treating these two ecotypes of the same species rather than different species (Zhu et al. 2007), while others strongly advocate their independent species structure (Grillo et al. 2009). Phylogenetic and population studies using a variety of molecular markers as summarized by Sang and Ge (2007) seemed to have reached the following consensus. First, the sampled accessions of the wild progenitors, *O. rufipogon* and *O. nivara*, did not form monophyletic groups within each species. Second, cultivated rice did not show a clearly closer relationship with one wild species compared with the other. Third, the major types of cultivars, such as subspecies *indica* and *japonica*, tended to form monophyletic groups separated by a relatively large genetic distance thereby supporting diphyletic origin.

Table 7.3 Some examples on cloning and functional analysis of QTLs related to evolutionary traits and yield components

Trait	QTL	Chromosome	Diagnostic marker
Yield related QTL			
Yield	*Yld 1*	1S	SSR
Grain number	*qGn1a*	1S	Functional
Grain length	*qGS3*	3 (pericentric)	Functional
Submergence tolerance	*qSub1A*	9S	Functional
Grain weight	*qGW2*	2S	Functional
Spikelet/spike	*qSSP7*	7 (pericentric)	SSR
Grain size	*qSW5*	5S	Functional
Heading date	*qGhd7*	7 (pericentric)	Functional
Grain incomplete filling	*qGIF1*	4L	Functional
Domestication-related QTL			
Seed dormancy	*qSdrl*	3S	CAPS
Shattering	*qSH1*	1L	Functional
Red pericarp	*qRc*	7 (pericentric)	Functional
Plant growth	*qPro11*	7S	Functional
Internode elongation	*Snorkel1* and *Snorkel2*	12L	Functional

7.6 *Oryza* Map Alignment and Evolutionary Relationships in *Oryza*

Although rice is considered a model plant and placed at the center of the cereal crop syntenic circle (Moore et al. 1995; Devos 2005), only a few genome wide

comparative analyses as yet have been performed using the rice genome sequence as a reference. Various rearrangements between rice and other cereals have been reported in sequence-level comparisons using the rice genome sequence as the reference (Chen et al. 1998; Goff et al. 2002; Salse et al. 2004). With the availability of whole genome sequence of rice, studying functions of all the putative genes and also the structural similarities/variations of *O. sativa* with its wild relatives are the two major areas of research in rice genomics. *Oryza* Map Alignment Project (OMAP) was initiated for studying structural and functional similarities of *O. sativa* with its wild relatives. Major progress has been made in developing physical maps of different wild species of *Oryza* at the Arizona Genomics Institute, Tucson, USA (*http://www.omap.org*).

Ammiraju et al. (2006) reported the construction and analysis of a comprehensive set of 12 BAC libraries for various wild species. Each library represents a minimum of 10x genome equivalent and an average insert size ranging between 123 and 161 kb. The BAC library comprised of variable number of clones: *O. nivara* (AA), 55,296; *O. rufipogon* (AA) 64,512; *O. glaberrima* (AA) 55,296; *O. punctata* (BB) 36,864; *O. officinalis* (CC) 92,160; *O. minuta* (BBCC) 129,024; *O. alta* (CCDD) 92,160; *O. australiensis* (EE) 92,160; *O. brachyantha* (FF) 36,864; *O. granulata* (GG) 73,728; *O. ridleyi* (HHJJ) 129,024; *O. coarctata* (HHKK) 147,456. A preliminary analysis of BAC end sequences of clones from these libraries indicated that LTR-RTs are the predominant class of repeat elements in *Oryza* and roughly linear relationship of these elements with genome size was observed.

The considerable diversity of genome sizes in higher eukaryotes, particularly the lack of correlation between genome size and biological complexity, first formulated as the C-*value paradox* (Thomas 1971), was one of the most disturbing discoveries in the early days of structural genomics. It has since been established for many organisms that transposable elements (TEs) are the main components of complex genomes and that transposition can be regarded as the predominant force driving their structural changes, besides polyploidy (Bennetzen et al. 2005). In this regard, a particular class of TEs, the retrotransposons, is considered an important factor of genomic inflation in both plants and animals because of their propensity to increase their copy number during transposition. This is well documented in grasses, where LTR-RTs can compose more than half of the genome of some species. Panaud et al. (2002) characterized seven kinds of rice transposable elements. These represent *gypsy*-like retroelements (*Retrosat1, RIRE3, RIRE8*), *hipa* (a CACTA-like transposon), *houba* (a copia-like retroelement), *hopi*, and *dagul* (two gypsy-like retroelement) retrotransposons-the mobile elements, which transpose via a "copy and paste" mechanism, thus increasing their copy number while active. The accumulation is considered to be the main factor of increase in genome size.

Furthermore, fine-scale sequence comparisons have illustrated that the genes in grass genomes are generally colinear, with occasional small rearrangements (inversions, duplications, and deletions) that appear to be associated with unequal homologous or illegitimate recombination and rarer gene movements from unlinked chromosomes sites without a known mechanism for mobility.

Piegu et al. (2006) reported that *O. australiensis*, a diploid wild species, has undergone recent bursts of three LTR-RT families. This genome has accumulated more than 90,000 retrotransposon copies during the last three million years, leading to a rapid twofold increase of its size. Three LTR-RT families (*RIRE1, Kangourou* and *Wallabi*) compose 60% of the *O. australiensis* genome. In addition, phenetic analyses of these retrotransposons clearly confirm that the genomic bursts occurred posterior to the radiation of the species. This provides direct evidence of retrotransposon-mediated variation of genome size within a plant genus. Based on dot-blot assays, *Kangourou* and *Wallabi* were estimated to contribute a total of 90 $\pm$ 9 Mbp and 250 $\pm$ 25 Mbp, i.e., 9% and 26% of the genome of *O. australiensis*, respectively.

Ammiraju et al. (2007) discovered LTR-RT family named RWG in the genus *Oryza*. Comparative analysis of transposon content (approximately 20–27,000 copies) and transpositional history of this family across the genus revealed a broad spectrum of independent and lineage-specific changes. The basal GG-genome of *Oryza* (*O. granulata*) has expanded by nearly 25% by a burst of the RWG lineage *Gran3* subsequent to speciation. *Gran3* is closely related to two other LTR-RT families previously characterized in *O. sativa* (AA) and *O. australiensis* (EE), i.e., *RIRE2* and *Wallabi*, respectively. The causes for massive RWG family proliferation in some lineages

(i.e., *O. granulata* and *O. australiensis*) and almost sudden death or attenuation in others (i.e., *O. brachyantha* and O. coarctata) are still not clearly understood. Such differential fates of the *RWG* family could mechanistically result from lineage-specific differences in the regulation and/or suppression of transposition. The differential amplification of the *RWG* family is mainly regulatory, at transcriptional or posttranscriptional level.

Kim et al. (2007) constructed a comparative physical map of *O. sativa* (AA) and *O. punctata* (BB) by aligning a physical map of *O. punctata*, deduced from 63,942 BAC end sequences (BESs) and 34,224 fingerprints, onto the *O. sativa* genome sequence. The alignment result suggests more divergence of intergenic and repeat regions in comparison to gene-rich regions. The alignment identified 16 locations containing expansions, contractions, inversions, and transpositions. The genome size of *O. punctata* was estimated to be 8% larger than that of *O. sativa* with individual chromosome differences of 1.5–16.5%. The sum of expansions and contractions observed in regions >500 kb were similar, suggesting that most of the contractions/expansions contributing to the genome size difference between the two species are small, thus preserving the macro-colinearity between these species, which diverged approximately two million years ago. Kim et al. (2008) have summarized comprehensive analysis of genus wide comparative framework composed of 12 BAC fingerprints and end-sequenced physical map of ten genomic types of *Oryza* aligned to *japonica* reference genome sequence. Over 932 Mb of end sequence were analyzed for repeats, miRNA, and single nucleotide variations.

Zuccolo et al. (2008) analyzed 12 random sheared genomic libraries representative of 12 *Oryza* species and ten distinct genome types and identified the Ty3-Gypsy element *Atlantys*. *Atlantys* is an ancient and ubiquitous component of the genus *Oryza* and has made significant contributions to genome size variation across the genus. Several studies have shown that LTR-RTs are the principal components responsible for genome size expansion in the grasses, accounting for as much as 50–90% of the barley, maize, and wheat genomes. Subsequent to speciation, both *O. australiensis* (EE) and *O. granulata* (GG) diploid genomes increased by 100% and 50%, respectively, by bursts of a few LTR-RTs families in a relatively short period of time, subsequent to speciation. *Atlantys* accumulated differentially across the *Oryza*. Although differential accumulation of LTR-RTs families has already been demonstrated in the *Oryza*, for *Wallabi* (Piegu et al. 2006) and *Gran3* (Ammiraju et al. 2007) in *O. australiensis* and in *O. granulata*, respectively, *Atlantys* has attained extremely elevated copy number levels in several species, especially in the "*Officinalis* complex" where it represents a significant fraction of these genomes: 8.31%, 7.90%, 7.93%, 5.93%, and 7.41% of the *O. alta* (CCDD), *O. minuta* (BBCC), *O. officinalis* (CC), *O. punctata* (BB), and *O. australiensis* (EE) genomes, respectively. *Atlantys* element-related sequences make up a significant fraction of the genomes of species from the *Officinalis* complex as well as the *O. ridleyi* and *O. granulata* genomes. The proliferation of *Atlantys* elements, in many cases, took place after respective speciation events occurred. Most of the retrotranspositional events occurred within the last three million years.

Ma et al. (2007) based on comparative genomics analysis reported that *Cen8* was formed at its current location at least seven to nine million years ago and was physically shifted by a more recently inversion of a segment spanning centromeric and pericentromeric regions. It provides the first molecular description of the positional shift of any higher eukaryotic centromere caused by a small chromosomal inversion. This study also demonstrates the value of physical mapping with BAC contigs from comparative and evolutionary analysis of complex genomic regions recalcitrant to other analytical approaches (e.g., sequencing and assembly of repetitive DNA). Centromere-specific histone H3 variant, *CENH3*, plays a key role in recruiting other centromeric proteins; thus, it is the central component in kinetochore formation and centromere function. Hirsh et al. (2009) analyzed the expression and evolution of the *CENH3* genes in two allotetraploid rice species (*O. minuta*, *O. alta*) as well as their representative diploid progenitor species (*O. punctata*, *O. rhizomatis* and *O. australiensis*). Both copies of the *CENH3* genes were transcribed in the two allotetraploid species and showed a non-preferential expression pattern. Contrasting positive and stabilizing selection of *CENH3* genes associated with different diploid *Oryza* species. This lineage-specific adaptive evolution of *CENH3* was maintained in the two allotetraploid species. The allopolyploidization events did not alter the expression or evolutionary pattern of the *CENH3* genes in the *Oryza* species.

Lu et al. (2009) analyzed a large genomic region surrounding the MONOCULM1 (*MOC1*) locus in 14 *Oryza* species, including ten diploids and four allotetraploids. Sequencing and annotation of 18 BAC clones for these species revealed highly conserved gene colinearity and structure in the *MOC1* region. In this region, transposons were only conserved between genomes of the same type (e.g., AA or BB). In addition to the conserved gene content, several apparent genes have been generated de novo or uniquely retained in the AA lineage. The allotetraploids *O. alta* and *O. minuta* were found to be products of recent polyploidization, less than 1.6 and 0.4 Mya, respectively. In allotetraploids, pseudogenization of duplicated genes was common, caused by large deletions, small frame-shifting insertions/deletions, or non-sense mutations. Thus, the physical maps of the wild oryza species conform to the cytological maps generated based on somatic or pachytene chromosomes.

7.7 Rice Genetic Resources: Exploration and Conservation

Rice genetic resources comprise landrace varieties, old and modern varieties, genetic stocks, and the wild rices (Table 7.2). The International Rice Gene Bank at the International Rice Research Institute (IRRI) in Philippines conserves the largest and most diverse collection of rice germplasm. The facilities of the gene bank ensure the long-term conservation of this valuable gene pool.

7.7.1 Exploration and Collection of Germplasm

Records of early occasional exploration and collection for wild rice samples in different countries can be found in several publications. Specimens of wild *Oryza* species can be found in many herbaria, particularly in India, China, Indonesia, Thailand, Singapore, and Malaysia. Efforts for collection and conservation of wild *Oryza* species were initiated in the late 1950s by many National Agricultural Research and Extension Systems (NARES) on small scales, along with conservation programs for cultivated rice varieties and landraces. India and China are the world's two largest rice producing and consuming countries, and wild *Oryza* species are found abundantly in both these countries. Lu and Sharma (2003) have reviewed the exploration and collection of wild *Oryza* species in different countries/regions.

Two workshops organized by IRRI and the International Board for Plant Genetic Resources on genetic conservation, in 1977 and 1983, and the "Third International Workshop on Rice Germplasm: Collection, Preservation, and Use," held at IRRI, were important to international cooperation and conservation activities of wild *Oryza* species. Since then, collection of wild *Oryza* species has gradually received more attention by the NARES, particularly in Asian countries, and more intensive and systematic collecting activities have been conducted in different countries. Based on reports by several NARES programs, a nominal number of seed samples of wild *Oryza* specks were collected in the early 1980s, except in Thailand, where more than 100 accessions of wild *Oryza* species were collected.

Much of the germplasm exploration for rice was completed by the early 1990s. By the end of 1962, the IRRI varietal collection contained 6,867 accessions from 73 countries. By 1972, the collection had grown to 14,600 accessions (Chang 1972), and by the early 1980s, the number of accessions in the IRRI gene bank reached 49,027, and presently, it is holding more than 93,000 accessions (Table 7.2). More than 200 accessions of rice were collected during the second half of 1995 from Lao People's Democratic Republic (PDR). The IRRI gene bank now contains 3,265 accessions of 21 wild species of *Oryza*, besides 1,562 accessions of African cultivated rice (*O. glaberrima*).

7.7.2 Genetic Erosion

Land races and wild species of *Oryza* are important reservoir of useful genes for rice improvement. Through millions of years of evolution and genetic adaptation to variable environments, wild species have accumulated abundant diversity. Genetic erosion or loss of biodiversity of rice varieties has

been recognized as a problem since the 1960s. Factors such as the adoption of high-yielding rice varieties, farmers' increased integration into the markets, changes in farming systems, industrialization, human population increases, and cultural change have significantly accelerated continual erosion of the rice gene pool (Bellon et al. 1998). A similar situation has also been observed for wild *Oryza* species. In many places of Asia, populations of wild *Oryza* species are becoming extinct or are threatened because their natural habitats are seriously damaged by extension of cultivation areas, expansion of communication systems such as road construction, and urban pressures. According to unpublished data collected by the Chinese Academy of Sciences in 1994, nearly 80% of the common wild rice (*O. rufipogon*) sites recorded during the 1970s have already disappeared (cited by Lu and Sharma 2003). The size of some surviving *O. rufipogon* populations was also found to be significantly reduced. A similar situation has been observed in other countries such as Philippines, Vietnam, Thailand, Nepal, Indonesia, Malaysia, India, and Bangladesh.

The problems of genetic erosion are severe, but international efforts to conserve rice genetic resources, in which IRRI has taken a leading role, have led to the establishment of several gene banks in Asia. These joint efforts between national, regional, and international organizations ensure the long-term conservation of the biodiversity of the rice gene pool.

7.7.3 Conservation Strategy

For many plant species, ex situ conservation of seeds is safe and cost-efficient, provided proper attention is paid to seed drying and storage conditions. Fortunately, rice seeds exhibit orthodox storage behavior and can be dried to a low moisture content of ca. 6% and stored at −20°C, retaining their viability for decades, if not longer. Vaughan (1994) has elaborated on Herbarium specimens of various wild *Oryza* species preserved in many herbaria of different countries. Jackson (1997) has summarized the strategies on conservation of genetic resources. There are two basic approaches to germplasm conservation (ex situ and in situ conservation).

7.7.3.1 Ex Situ Conservation

In this approach, genetic resources are actually removed from their original habitat or natural environment. Ex situ conservation provides efficient means for germplasm preservation, utilization, exchange, and information generation through effective management and value-added research of the conserved wild rice species. However, seed samples placed under ex situ conservation in a gene bank become isolated from the ecosystem where they originated and grow. The expected microevolution of these species in their original environment is blocked, particularly the adaptive variations that could occur during change in environmental conditions. Therefore, in evolutionary terms, ex situ conservation is static (Bellon et al. 1998). Concerns have been raised following the observation that static conservation may reduce the adaptive potential of wild *Oryza* species and their populations in the future. Thus, ex situ conservation cannot be considered the only approach for conserving biodiversity of wild *Oryza* species. Complementary dynamic approaches such as in situ conservation are also necessary.

The long-term conservation of rice genetic resources is the principal aim of the International Rice Gene Bank (IRG). The gene bank has operated since 1977, although genetic conservation activities started in the early 1960s. For several countries, including Sri Lanka, Cambodia, Lao PDR, and Philippines, the germplasm conserved in the IRG represents a more or less complete duplicate of their national collections. For other countries, such as India and the People's Republic of China, only parts of their national collections are duplicated at IRRI. Nevertheless, the IRG has provided an important safety net for national conservation efforts. On several occasions, it has been possible to restore rice germplasm that had been lost in national genebanks with accessions already conserved at IRRI. IRRI maintains an active collection for medium-term storage and distribution of rice germplasm, at +2°C in sealed laminated aluminum foil packets, and long-term (50–100 years) conservation, at −20°C, each with two vacuum-sealed aluminum cans.

The germplasm collection is held in trust by IRRI under the auspices of FAO in an International Network of ex situ collections. Duplicate storage of the IRG collection is maintained at the National Seed Storage Laboratory (NSSL), Fort Collins, USA, and about

75% of the collection is currently stored under black-box conditions. Duplicate storage of African rices is shared between IRRI, the International Institute of Tropical Agriculture (IITA) in Nigeria, and the Africa Rice Center (earlier named as WARDA) in Benin, Africa.

7.7.3.2 In Situ Conservation

This method attempts to preserve the integrity of genetic resources by conserving them within the evolutionary dynamic ecosystems of their original habitat or natural environment. Under in situ conservation, local control of traditional rice varieties will ensure that benefits accrue to farmers and communities that have developed them. For long-term and dynamic conservation, the in situ approach has great value. However, for some reason, in situ conservation has, in general, received the least attention and even been rejected. Limited scientific and financial inputs are constraints in in situ conservation and its design and management for wild *Oryza* species.

7.8 Cytogenetic Stocks

A series of cytogenetic stocks including haploids, triploids, tetraploids, primary trisomics, secondary trisomics, telotrisomics, translocations, alien addition lines, etc., have been produced in rice and used for various genetic and cytogenetic studies.

7.8.1 Translocations

Radiation-induced chromosome translocations have been studied by many workers. Nishimura (1961) identified individual chromosomes involved in translocations from intercrosses of translocation stocks. The 12 chromosomes were arbitrarily numbered (I to XII) but not related to the number assigned to individual chromosomes on the basis of cytological observations. These translocation stocks have been used to a limited scale to locate marker genes on chromosomes.

7.8.2 Haploids, Triploids, and Aneuploids

Haploids of spontaneous origin and through anther culture have been reported. Haploids are characterized by complete sterility and show reduced height and smaller leaves. Tetraploids have been induced through colchicine treatment and also occur spontaneously. Tetraploids, in general, have reduced seed fertility and are inferior to disomics in agronomic performance; no commercial release has been made. Triploids also occur spontaneously but have also been produced through crosses of $4x \times 2x$. Triploids have proved to be the best source of primary trisomics.

7.8.3 Primary, Secondary and Telotrisomics

Khush et al. (1984) established a complete series of 12 primary trisomics in rice by crossing a spontaneous triploid to diploid rice cv. IR36. The series has been used for mapping several mutant markers on rice chromosomes. Singh et al. (1996a) isolated secondary trisomics and telotrisomics representing the 12 chromosomes from the progenies of primary trisomics. Plants showing variation in gross morphology compared to the primary trisomics and disomic sibs were selected and analyzed cytologically at diakinesis and pachytene. Secondary trisomics for both arms of chromosomes 1, 2, 6, 7, and 11 and for one arm of chromosomes 4, 5, 8, 9, and 12 were identified. Telotrisomics for short arms of chromosomes 1, 8, 9, and 10 and for long arms of chromosomes 2, 3, and 5 were isolated. Genetic segregation of 43 marker genes was studied in the F_2 or backcross progenies. On the basis of segregation data, these genes were delimited to specific chromosome arms. Correct orientation of ten linkage groups was determined and centromere positions on nine linkage groups of the classical linkage map were approximated. In another study, Singh et al. (1996b) used secondary and telotrisomics to assign RFLP markers to specific chromosome arms and thereby to map the positions of centromeres. More than 170 RFLPs were assigned to specific chromosome arms through gene dosage analysis using secondary and telotrisomics, and the centromere positions were mapped on all 12 linkage groups of molecular linkage map. The

orientations of seven linkage groups were reversed and the corrected map of all the 12 linkage groups was presented.

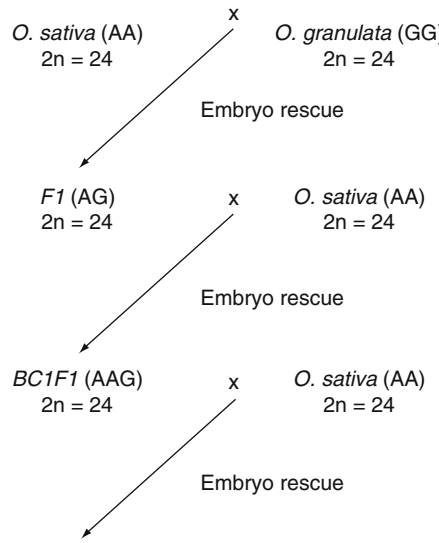
Cheng et al. (2001a) developed a complete set of telotrisomics covering all the 24 chromosome arms. In general, the telotrisomics for short arm had stronger resemblance to the diploid counterparts, whereas the telotrisomic with long arm had greater resemblance to the corresponding primary trisomic. The telocentric nature of the extra chromosomes in these stocks was verified by FISH using a rice centromeric BAC clone as a marker probe.

7.8.4 Monosomic Alien Addition Lines

MAALs refer to the presence of one extra chromosome from the wild species in addition to the normal chromosome complement of the cultivated species. MAALs are useful cytogenetic stocks in mapping genes introgressed from wild species and also serve as an important source of alien genetic variation. MAALs have been extensively studied in polyploid species such as wheat, oats, tobacco, and cotton (Khush 1973). MAALs representing extra chromosomes from wild species in the genetic background of *O. sativa* have been reported. MAALs are produced during backcrossing of wide cross progenies (BC_1F_1, BC_2F_1, etc) with the recurrent parent (Fig. 7.1).

MAALs are characterized based on plant morphology and through isozyme and molecular marker analysis. MAALs representing 6–12 chromosomes have been obtained in *O. officinalis* ($2n = 24$ CC), *O. minuta* ($2n = 48$ BBCC), *O. latifolia* ($2n = 48$ CCDD), *O. australiensis* ($2n = 24$ EE), *O. brachyantha* ($2n = 24$ FF), *O. granulata* ($2n = 24$ GG), and *O. ridleyi* ($2n = 48$ HHJJ) (Shin and Katayama 1979; Jena and Khush 1989; Brar and Khush 2002, 2006).

The MAALs show characteristic features of changes in morphology like primary trisomics of rice. In general, MAALs show reduced plant height and low seed setting. MAALs in *O. sativa* having single chromosome of *O. officinalis* were established by Shin and Katayama (1979). However, a complete series representing each of the individual chromosomes of *O. officinalis* was established by Jena and Khush (1989). The female transmission of the extra



BC2F1 (2n = AA, AA+1G,.....AA + 7G)

2n = 24	AA (introgression lines)
2n = 25	AA + 1G (monosomic alien addition line)*
2n = 26-31	(AA + 2G ..... + 7G)

*Characterization of MAALs based on plant morphology, cytology and molecular markers.

Fig. 7.1 Scheme showing production of monosomic alien addition lines ($2n = 25$) and alien introgression lines ($2n = 24$) from crosses of rice and distantly related wild species of *Oryza* (modified from Brar and Khush 2006)

chromosome varied from 6.6 to 26.8%. Four MAALs also transmitted the extra chromosome through male gametes. The female transmission rates of the alien chromosome could vary from 4.4 to 35.5% in MAALs of *O. latifolia* (Multani et al. 2003). MAALs are always unstable due to segregation of extra chromosome and need to be identified in each generation either based on morphological traits and or cytological analysis. The disomic addition lines cannot be produced for all the chromosomes due to reduced transmission through pollen.

7.9 Gene Transfer from Wild Species into Rice

Advances in tissue culture, genetic engineering, molecular cytogenetics, comparative genetics, and genomics, particularly the rice genome sequencing, have opened new opportunities to develop improved rice germplasm with novel genetic properties and in understanding the function of rice genes. Breeders have successfully used conventional breeding methods and exploited the rice (*O. sativa*) gene pool to develop high yielding improved rice varieties resistant to pests and abiotic stresses with improved quality characteristics. The major emphasis has been on utilizing *indica*, *japonica*, and *javanica* germplasm through intraspecific hybridization (*indica* × *indica*, *japonica* × *japonica*, *indica* × *japonica*). More recently, *indica* × tropical *japonica* (*javanica*) crosses have been used to develop new plant type rices and the commercial high yielding hybrids. In several cases, genetic variability for target agronomic traits is limited in the cultivated rice gene pool. Under such situations, interspecific hybridization is an important plant breeding approach to introduce novel genes for different agronomic traits from wild species into rice.

IRRI is maintaining more than 3,000 accessions of wild species and 1,500 accessions of cultivated African rice (*O. glaberrima*) in the rice genebank. These wild species are reservoirs of many useful genes, particularly for resistance to major biotic and abiotic stresses (Table 7.2). However, these wild species are associated with several weedy traits, such as grain shattering, poor plant type, poor grain characteristics, and low seed yield. Besides, several incompatibility barriers also limit the transfer of useful genes from wild species into cultivated species (Brar and Khush 1986, 1997; Khush and Brar 1992). The major consideration in alien gene transfer is to selectively transfer agronomically important genes from wild species avoiding linkage drag. To achieve precise transfer of genes from wild to cultivated species, strategies involving a combination of conventional plant breeding methods with tissue culture and molecular approaches have become important (Brar and Khush 2002, 2006).

At IRRI, useful genes for resistance to bacterial blight (BB), blast, tungro virus, grassy stunt virus, and tolerance to soil toxicity (acid sulfate, iron toxicity) and cytoplasmic male sterility have been transferred from wild species of rice (Brar and Khush 2002, 2006). Some of the alien introgression lines developed at IRRI have been released as varieties by several NARES centers. A few examples include MTL98, MTL103, and MTL110 (BPH resistant) in Vietnam, Matatag 9 (tolerant to tungro) and NSIC Rc112 (high yielding and BB resistance) in Philippines, and AS996 (moderately tolerant to acid sulfate conditions and early maturity) in Vietnam.

7.9.1 Strategies for Alien Gene Transfer

Strategy to transfer genes from wild species into rice depends on the nature of the target trait(s), relatedness of the wild species, and incompatibility barriers. Several protocols are available to overcome such barriers (Brar and Khush 2002). Some of the steps involved in alien gene transfer are described below in this section:

7.9.1.1 Search for Useful Genetic Variability for Target Traits

This involves screening wild species germplasm to identify particular accession(s) possessing useful variability for specific target trait(s). In a number of cases, the desired genetic variability is present only in selected accessions of a particular species. Emphasis should be placed on identifying variability in the

closely related (AA-genome) species, followed by CC-genome species, and later, screening distantly related species such as *O. brachyantha* (FF), *O. granulata* (GG), and *O. ridleyi* (HHJJ), which show limited homoeologous pairing with the AA-genome.

7.9.1.2 Production of Hybrids MAALs and Introgression Lines

Interspecific hybrids are produced between elite breeding lines with the wild species accessions carrying useful genes for target traits of immediate interest to the breeder. Such hybrids are produced through direct crosses between rice and AA-genome wild species. However, embryo rescue is required to produce hybrids and backcross progenies (alien introgression lines, AILs) between rice and all the wild species of *Oryza* except AA-genome species. Hybrids have been produced between rice (AA-genome) and wild species of *Oryza,* representing all ten genomes (AA-, BB-, CC-, BBCC-, CCDD-, EE-, FF-, GG-, HHJJ-, HHKK-) through direct crosses or through embryo rescue (Brar et al. 1991; Brar and Khush 2002, 2006). MAALs are isolated from the wide cross progenies (Fig. 7.1). A large number of alien introgression lines have been produced through direct crosses as well as through embryo rescue (Table 7.4). In most of these wide crosses, useful genes like resistance to BPH, BB, blast, tolerance to tungro disease, acid sulfate and iron toxicity, and cytoplasmic male sterility have been transferred into rice (Table 7.5). Some of the genes introgressed from wild species have been tagged with molecular markers (Table 7.6).

7.9.1.3 Construction and characterization of Chromosome Segment Substitution Lines

Chromosome segment substitution lines (CSSLs) are valuable cytogenetic stocks for mapping of genes governing agronomic traits. Molecular markers have made it possible to develop well-defined CSSLs. Doi et al. (2003) constructed a series of *O. glaberrima*, *O. glumaepatula*, and *O. meridionalis* CSSLs in the background of *japonica* rice Taichung 65. These lines cover most parts of the genome of donor species. We are using microsatellite markers to identify and develop CSSLs of *O. glaberrima*, *O. rufipogon,* and *O. longistaminata* in the background of high-yielding *indica* cv. IR64 and *japonica* cv. Ilpumbyeo. Such CSSLs would be an important genetic resource for mapping genes/QTLs and for functional genomics of rice. The availability of a dense molecular map of rice comprising SSR markers has facilitated large-scale

Table 7.4 Interspecific hybrids and wide-cross progenies produced at IRRI from crosses of rice and wild species of *Oryza*

Cross combination	F_1	Mapping population(s)	Alien introgression lines (AILs) ($2n = 24$)	Monosomic alien addition lines (MAALs) ($2n = 25$)
O. sativa × *O. rufipogon* (AA)	+	+	+	−
O. sativa × *O. glaberrima*[a] (AA)	+	+	+	−
O. sativa × *O. longistaminata* (AA)	+	+	+	−
O. sativa × *O. punctata* (BB)	+	−	−	−
O. sativa × *O. officinalis* (CC)	+	+	+	+
O. sativa × *O. minuta* (BBCC)	+	+	+	+
O. sativa × *O. latifolia* (CCDD)	+	+	+	+
O. sativa × *O. alta* (CCDD)	+	+	−	−
O. sativa × *O. australiensis* (EE)	+	+	+	+
O. sativa × *O. brachyantha* (FF)	+	+	+	+
O. sativa × *O. granulata* (GG)	+	+	+	+
O. sativa × *O. ridleyi* (HHJJ)	+	−	−	+
O. sativa × *O. coarctata* (HHKK)	+	−	−	−

Modified from Brar and Khush (2006)
MAALs from A-genome wild species are not recovered because of homologous genomes
[a]cultivated African rice species; + progenies available; − not available

Table 7.5 Genes transferred at IRRI from wild species of *Oryza* including *O. glaberrima* into rice

Trait transferred	Donor species (wild species)
Grassy stunt resistance	*O. nivara* (AA)
Bacterial blight resistance	*O. rufipogon* (AA), *O. officinalis* (CC), *O. minuta* (BBCC), *O. latifolia* (CCDD), *O. australiensis* (EE), *O. brachyantha* (FF)
Blast resistance	*O. glaberrima* (AA), *O. minuta* (BBCC), *O. australiensis* (EE),
Brown planthopper resistance	*O. glaberrima* (AA), *O. officinalis* (CC), *O. minuta* (BBCC), *O. latifolia* (CCDD), *O. australiensis* (EE)
Whitebacked planthopper resistance	*O. officinalis* (CC)
Tungro tolerance	*O. rufipogon* (AA)
Tolerance to acid sulfate conditions	*O. rufipogon* (AA)
Cytoplasmic male sterility	*O. perennis* (AA), *O. glumaepatula* (AA)
Iron toxicity tolerance	*O. rufipogon* (AA), *O. glaberrima* (AA)
Elongation ability (deep water)	*O. rufipogon* (AA)

Modified from Brar and Khush (2002, 2006)

analysis to determine the extent and process of introgression and characterize the alien chromosome segment substitution into rice genome. Molecular markers facilitate in the construction of CSSLs representing segments of wild species chromosomes in the genetic background of cultivated rice (*O. sativa*).

7.9.1.4 Evaluation of AILs for Transfer of Target Traits

Advanced introgression lines generated through backcrossing are evaluated for the transfer of target traits. This involves extensive laboratory screening, greenhouse, and field testing using various screening and inoculation protocols and testing in hot spot-nurseries for major biotic and abiotic stresses. Evaluation could also be carried out in target environments in different rice-growing countries in collaboration with National Agricultural Research and Extension Systems (NARES).

Table 7.6 Genes/QTLs introgressed from wild species into rice tagged with molecular markers

Trait	Gene	Species
BPH resistance	*Bph10*, *Bph18*(t)	*O. australiensis*
	Bph14, *Bph15*	*O. officinalis*
	bph11, *bph12*(t)	*O. eichingeri*
	Bph20(t), *Bph21*(t)	*O. minuta*
BB resistance	*Xa21*	*O. longistaminata*
	Xa23	*O. rufipogon*
	Xa27	*O. minuta*
	Xa29(t)	*O. officinalis*
	Xa30(t)	*O. nivara*
Blast resistance	*Pi-9*(t)	*O. minuta*
	Pi-40	*O. australiensis*
Tungro resistance	RTSV	*O. ruftpogon*
Yellow stemborer resistance	QTL	*O. longistaminata*
Drought tolerance	QTL	*O. glaberrima*
Aluminum toxicity tolerance	QTL	*O. ruftpogon*
Yield-enhancing loci	QTL	*O. ruftpogon*

Modified from Brar and Khush (2002, 2006)

7.9.1.5 Mapping of Introgressed Alien Genes/QTLs

MAALs are used to locate the introgressed alien gene(s). Different types of mapping populations are generated through wide crosses such as recombinant inbred lines (RILs), doubled haploid (DH), and near-isogenic alien introgression lines, including segregating population F_2, F_3, and BC populations (alien introgression lines × recurrent parent). Introgressed alien genes and QTLs are mapped and tagged with molecular markers for use in marker-assisted selection (MAS). One of the major advantages in using alien introgression lines for mapping QTL is the reduced background noise because of the higher level of recipient parent background.

7.9.1.6 Characterization of Wide Cross Progenies Using GISH

GISH has become popular in characterization of parental genomes in interspecific progenies and for locating introgressed segments on rice chromosomes. Total genomic DNA of wild species is used as a probe in GISH experiments. FISH techniques are used on both mitotic and meiotic chromosomes. Centromere-specific probes and BAC clones are also used to map the introgressed alien segments/genes on rice chromosomes using FISH.

7.10 Introgression from AA-Genome Species

The A-genome species, which form the primary gene pool of rice, are easily crossable with cultivated rice *O. sativa*. However, the F_1s and subsequent generations in several interspecific crosses show variable levels of sterility due to genic interactions. A number of useful genes that have been transferred from related cultivated and wild AA-genome species are presented below.

7.10.1 Introgression from Wild AA-Genome Species

Crosses between cultivated rice (*O. sativa*, $2n = 24$, AA) and the AA-genome wild species can be easily made and the genes transferred through conventional crossing and backcrossing procedures. Hybrids between *O. sativa* and *O. rufipogon* are partially fertile; however, *O. sativa* × *O. barthii* and *O. sativa* × *O. longistaminata* F_1s are highly sterile. Among the classical examples are the introgression of a gene for grassy stunt virus resistance from *O. nivara* to cultivated rice varieties (Khush 1977), and the transfer of a CMS source from wild rice, *O. sativa* f. *spontanea*, to develop CMS lines for commercial hybrid rice production (Lin and Yuan 1980). Other useful genes, such as *Xa21* for bacterial blight resistance, were transferred into rice from *O. longistaminata*, and new CMS sources from *O. perennis* and *O. glumaepatula*. More recently, genes for tungro virus tolerance and tolerance to acid sulfate soil conditions have been transferred from *O. rufipogon* into *indica* rice cultivar. Two new genes for bacterial blight resistance *Xa23* from *O. rufipogon* (Zhang et al 1998) and *Xa30*(t) from *O. nivara* (Cheema et al 2008a) have been transferred into rice. Three varieties (Matatag 9, AS996, NSIC Rc112) have been released from crosses of *O. sativa* × *O. rufipogon* and *O. sativa* × *O. longistaminata*.

7.10.1.1 Introgression of Gene(s) for Resistance to Grassy Stunt Virus

The grassy stunt virus is a serious disease transmitted by the vector brown planthopper (BPH) *Nilaparvata lugens*. The diseased rice plants are severely stunted and either produce no panicles or produce only small panicles with deformed grains. Severe yield losses or even total loss may occur under epidemic conditions. Of the 6,000 accessions of cultivated rice and several wild species screened, only one accession of *O. nivara* (accession 101508) was found to be resistant (Ling et al. 1970). A single dominant gene *Gs* confers resistance in *O. nivara*. It segregates independently of *Bph1* (Khush and Ling 1974). Following four backcrosses with improved rice varieties, the gene for grassy stunt resistance was transferred into cultivated germplasm (Khush 1977). The first set of grassy stunt resistant varieties, IR28, IR29, and IR30, was released for cultivation in 1974. Subsequently, many such varieties, for example, IR34, IR36, IR38, IR40, IR48, IR50, IR56, and IR58, have been released, some developed at IRRI, others by NARES. This is an important example of a gene present in a few obscure plants of diploid wild species (*O. nivara*), which has been incorporated in the large number of improved rice germplasm/breeding lines developed at IRRI and elsewhere.

7.10.1.2 Introgression of Gene(s) for Resistance to Tungro Disease

Rice tungro virus disease (RTD) is another most serious viral disease in South and Southeast Asia. It is transmitted by the vector green leafhopper (GLH) *Nephotettix virescens*. The disease is caused either by a single infection or by a double infection with two viral particles, the rice tungro bacilliform virus (RTBV), a double-stranded DNA virus, and the rice tungro spherical virus (RTSV), a single-stranded RNA virus. There is limited variability in cultivated rice germplasm for resistance to RTBV, the main cause of tungro symptoms. *O. longistaminata* and *O. rufipogon* have shown tolerance to RTBV. Kobayashi et al. (1993) found 15 accessions of eight wild species resistant to RTBV. Three accessions of *O. rufipogon* (IRGC accessions 105908, 105909, and 15910) showed a low or moderate level of antibiosis to GLH. From the crosses of IR64 with *O. rufipogon*, many tungro-tolerant lines have been developed. One of the elite breeding lines, IR73885-1-4-3-2-6, resistant to tungro has been released as a variety (Matatag 9) for cultivation in tungro-prone areas of Philippines. Many tungro-tolerant breeding lines have been selected

from crosses with *O. rufipogon* and *O. longistaminata* (Khush et al. 2004). Ramos (2005) analyzed F_2/F_3 populations derived from the cross of IR64 (susceptible to tungro) × Matatag 9 (introgression line derived from *O. rufipogon* resistant to tungro). The results showed resistance in *O. rufipogon* to be controlled by two genes.

7.10.1.3 Introgression of Gene(s) for Resistance to Bacterial Blight

The bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* is one of the most destructive diseases of rice in Asia. As many as 30 genes for BB resistance have been identified from the cultivated and wild species. The genes *Xa21, Xa23, Xa27*, *Xa29, Xa30*(t) have been derived from wild species. At IRRI, we screened 662 *O. rufipogon* accessions, of which 13.6% were resistant and 7.9% were moderately resistant to PXO61 strain (race 1), while against strain PXO99 (race 6), 5.7% and 6.8% were found resistant and moderately resistant. Two *O. rufipogon* accessions (Accn. 104423 and 80671) were found to be resistant to all the strains in Philippines and India (Ram personal communication), making them highly promising as donors for broad spectrum and durable resistance to BB. Many wild species such as *O. longistaminata, O. officinalis, O. latifolia, O. brachyantha,* and *O. ridleyi* have been found to be highly resistant to BB.

Through backcrossing with the recurrent rice parent, a gene for resistance to bacterial blight was transferred from *O. longistaminata* and designated *Xa21* (Khush et al. 1990). This is another classical example of gene transfer with a wide spectrum of resistance into rice cultivar. In subsequent studies, *Xa21* has been transferred through MAS and also cloned using map-based strategy. Zhang et al. (1998) transferred bacterial blight resistance from *O. rufipogon* (RBB16) into another rice cultivar. The resistance gene was mapped on chromosome 11 and designated as *Xa23*(t). This gene also conferred a very wide spectrum of resistance and showed a highly resistant reaction to all the nine races of bacterial blight of Philippines. *Xa30*(t) has been introgressed recently from *O. nivara* into rice (Cheema et al 2008b). Five genes *Xa21*, *Xa23, Xa27, Xa29*, and *Xa30*(t) have been transferred into rice from *O. longistaminata, O. rufipogon, O. minuta, O. officinalis*, and *O. nivara*, respectively (Khush et al. 1990; Zhang et al. 1998; Cheema et al. 2008a). Two new genes, one each from *O. glaberrima* and *O. barthii,* have been identified and transferred to *O. sativa* (Mahajan 2007) (Table 7.5 and Table 7.6).

The *Xa21* has been transferred through MAS in several other *indica* lines, such as IR64 and PR106, Pusa Basmati and Sambha Mahsuri, including elite breeding lines of new plant type (NPT) rice (Sanchez et al. 2000; Singh et al. 2001). *Xa21* has been pyramided through MAS into high yielding genetic backgrounds. Many NARES have used isogenic lines IRBB21 (*Xa21*) and IRBB60 (*Xa4 + xa5, xa13 + Xa21*). New rice varieties have been released; 2 in India (Improved Sambha Mahsuri and Improved Pusa Basmati 1), 2 in Philippines (NSICRc 142 and NSICRc 154), and 8 commercial hybrids in China (Xieyou 218, Zhongyou 218, Guodao 1, Guodao 3, Neizyou, Ilyou 8006, Ilyou 218, ZhongbaiYou 1) (Table 7.8).

Xa21 has also been cloned (Song et al. 1995) and is shown to be developmentally regulated. Transgenic

Table 7.7 Varieties released through wide-hybridization in rice

Key trait	Wild species	Varieties released	Country
Grassy stunt virus resistance	*O. nivara*	Many rice varieties	Rice growing countries in Asia
BPH resistance	*O. officinalis*	MTL 98, MTL 103 MTL 105, MTL 114	Vietnam
Acid sulfate tolerance	*O. rufipogon*	AS 996	Vietnam
Tungro resistance	*O. rufipogon*	Matatag 9	Philippines
Bacterial blight resistance	*O. longistaminata*	NSICRc 112	Philippines
Blast resistance	*O. rufipogon*	Dhanrasi	India
Blast resistance	*O. glaberrima* (African rice)	Yun Dao	YAAS, China
High yield, earliness, weed competitive ability	*O. glaberrima*	Many Nerica rices	African countries

Table 7.8 Rice varieties released through MAS carrying *Xa21* gene from *O. longistaminata*

Inbreds/hybrids	Year	BB resistance gene(s)	Institute/Country
NSICRc 142 (Tubigan 7)	2006	*Xa4 + Xa21*	PhilRice, Philippines
NSICRc 154 (Tubigan 11)	2007	*Xa4 + Xa21*	Philippines
Improved Sambha Mahsuri	2007	*xa5+xa13+Xa21*	India
Improved Pusa Basmati 1	2007	*xa5+xa13+Xa21*	India
Xieyou 218	2002	*Xa21*	China
Zhongyou 218	2002	*Xa21*	China
Guodao 1	2002	*Xa4+xa5+xa13+Xa21*	China
Guodao 3	2004	*Xa4+xa5+xa13+Xa21*	China
Neizyou	2004	*Xa4+xa5+xa13+Xa21*	China
Ilyou 8006	2005	*Xa4+xa5+xa13+Xa21*	China
Ilyou 218	2005	*Xa21*	China
ZhongbaiYou 1	2006	*Xa21*	China

rice lines carrying *Xa21* have been field-tested at IRRI and Philippine Rice Research Institute (PhilRice). These lines have shown high level of field resistance to BB.

7.10.1.4 Incorporation of CMS Sources from Wild Species

The A-genome wild species have been an important source of CMS, the major tool to breed commercial rice hybrids. A number of CMS sources have been developed in rice. However, the most commonly used CMS source in hybrid rice breeding is derived from the wild species *O. sativa* f. *spontanea* (Lin and Yuan 1980). The cytoplasmic source has been designated as wild abortive (WA), which refers to a male sterile wild rice plant having abortive pollen. About 90% of the male sterile lines used in commercial rice hybrids grown in China and other counties have the WA type of cytoplasm.

A new CMS source from *O. perennis* (Accn. 104823) was transferred into *indica* rice (Dalmacio et al. 1995). Crosses of 46 accessions of *O. perennis* and two accessions of *O. rufipogon* as female parents were made with two restorers (IR54, IR64) of WA sterility. Several hybrids were backcrossed with the respective recurrent parents. Of these, one line having the cytoplasm of *O. perennis* Accn.108423 and nuclear background of IR64 was found to be stable for male sterility. The male sterility source of the new line (IR66707A) is different from that of WA. Southern hybridization using mtDNA-specific probes showed an identical banding pattern between IR66707A (recipient) and *O. perennis* (donor), indicating that CMS may not be caused by any major rearrangement or modification of mtDNA. Another CMS line (IR69700A) having the cytoplasm of *O. glumaepatula* (A-genome species) and the nuclear genome of IR64 has been developed (Dalmacio et al. 1996). No good restorer could be identified for both the CMS sources. Many laboratories have transferred CMS from other AA-genome wild species; however, due to lack of good restorers, none of these sources except WA are used in commercial hybrid rice breeding.

Extensive efforts were made at the Directorate of Rice Research, Hyderabad, India to develop CMS lines with cytoplasm from several wild species. However, none of these CMS lines could become popular for developing commercial varieties.

7.10.1.5 Introgression for Tolerance to Abiotic Stresses

A limited work has been carried out for the transfer of genes for tolerance to abiotic stresses from wild species into rice. We have evaluated several introgression lines derived from the crosses of *O. sativa* × *O. rufipogon* and *O. sativa* × *O. glaberrima* at hot-spots under field conditions for tolerance to abiotic stresses at Iloilo, Philippines. Elite breeding lines with good agronomic traits and moderate tolerance to iron toxicity, aluminum toxicity, and acid sulfate conditions have been identified. One of the accessions of *O. rufipogon* that grows under natural conditions in acid sulfate soils of Vietnam was used in crosses with IR64. Three promising lines were selected and tested through the

yield-testing network of Cuu Long Delta Rice Research Institute (CLRRI), Vietnam. Of the three breeding lines, IR73678-6-9-B has been released as a variety (AS996) for commercial cultivation in Mekong Delta, Vietnam. This variety has become popular and occupies 100,000 ha (Bui Chi Buu personal communication). It is a short-duration (95–100 days) semi-dwarf variety with good plant type suitable for moderately acid sulfate soils and is tolerant to BPH and blast.

A set of advanced introgression lines derived from *O. sativa* × *O. rufipogon* have been tested for elongation ability under deep water conditions in the Philippines and India. One of the breeding lines in the All India Coordinated Rice Improvement Project testing has shown good promise (Mallik personal communication).

7.10.1.6 Identification and Introgression of Yield-enhancing Loci/QTLs

Wild species are phenotypically inferior to the cultivated species. However, transgressive segregation for yield in crosses of cultivated and wild species suggests that, despite inferior phenotypes, wild species contain genes that can improve quantitative traits, such as yield. Molecular markers have made it possible to identify and introgress desirable QTL from wild species into elite breeding lines. Tanksley and Nelson (1996) proposed advanced-backcross QTL (AB-QTL) analysis to discover and transfer valuable QTL alleles from unadapted germplasm, such as wild species into elite breeding lines.

QTLs from AA-genome wild species of rice for increased yield have been identified. Xiao et al. (1996) analyzed 300 BC_2 test-cross families produced from the cross of *O. sativa* × *O. rufipogon*. *O. rufipogon* alleles at two marker loci, RM5 (*yld1-1*) on chromosome 1 and RG256 on chromosome 2 (*yld2-1*), were associated with enhanced yield. Both alleles, *yld1-1* and *yld2-1,* were associated with a significant increase in grains per plant. In another experiment, Xiao et al. (1998) identified 68 QTLs. Of these, 35 (51%) had trait-improving alleles derived from the phenotypically inferior wild species. Nineteen of these beneficial QTL alleles had no deleterious effects on other characters.

Moncada et al. (2001) followed an advanced-backcross breeding strategy and analyzed BC_2F_2 populations derived from the cross involving an upland *japonica* rice cultivar, Caiapo, from Brazil and an accession of *O. rufipogon* from Malaysia. The populations were tested under drought-prone acid soil conditions. Based on analyses of 125 SSR and RFLP markers, two putative *O. rufipogon*-derived QTLs were detected for yield, 13 for yield components, four for maturity, and six for plant height. Septiningsih et al. (2003) used advanced-backcross QTL analysis to identify and introduce agronomically useful genes from *O. rufipogon* into the cultivated gene pool. A total of 165 markers consisting of 131 SSRs and 34 RFLPs were used to construct the genetic linkage map. Despite its inferior performance, 33% of the QTL alleles originating from *O. rufipogon* had beneficial effects for yield and yield components in the IR64 background. Twenty-two QTLs (53.4%) were located in similar regions as previously reported rice QTLs, suggesting the existence of stable QTLs across genetic backgrounds and environments. Additionally, several QTLs for plant height, grain weight, and flowering time detected in this study corresponded to homoeologous regions in maize containing previously detected maize QTLs for these traits. Cheema et al. (2008a) also analyzed advanced-backcross progenies from the same *O. rufipogon* accessions (IRGC105491) and identified the genomic regions that are associated with yield increase.

Our preliminary results at IRRl of advanced-backcross progenies derived from the crosses of an elite breeding line of NPT rice, with *O. longistaminata* and IR64 × *O. rufipogon,* also support transgressive segregation for yield and yield components (Reintar 2007). These findings show that genes from wild species can increase the yield of elite rice lines, even though wild species are phenotypically inferior to cultivated rice. Introgression lines with yield-enhancing QTLs, "wild species alleles," need to be transferred into high-yielding genotypes and validated in well-designed field experiments.

At the Punjab Agricultural University, Ludhiana, India, several A-genome wild species (*O. nivara, O. rufipogon, O.barthii, O. longistaminata, O. glumaepatula*) including *O. glaberrima* have been crossed with two high-yielding *indica* varieties, PR114 and Pusa 44. The F_1s were backcrossed to recurrent parents to generate BC_1F_1. In each backcross, at least 400–500 plants were generated. Phenotypically desirable plants were again backcrossed

to the recurrent parents to generate BC_2F_1 progeny. Here too, more than 100 BC_2F_1 plants were generated in each progeny. In BC_2F_1, phenotypically desirable plants were selected leaving out the ones with undesirable traits like awns, shattering, etc. The selected BC_2F_1 plants were carried forward as BC_2F_2. In this generation, agronomically desirable plants were selected and continued up to BC_2F_4 and later generation as single seed descend method. With this approach, about 2,000 introgression lines utilizing more than 40 accessions of different species in the background of PR114 and Pusa 44 have been generated and are being evaluated for yield and yield components and are also being analyzed for introgression of wild species segments using SSR markers. Four promising progenies, designated as IET21544 (Pusa44/*O. glaberrima* Accn. IRGC101800//2* Pusa 44), IET21545 (Pusa 44/*O. nivara* Accn. CR100113//2*Pusa 44), IET21546 (Pusa44/*O. nivara* Accn. CR100113//2*Pusa44), and IET21566 (PR114/*O. nivara* Accn. CR100142// 2*PR114), have been evaluated in national trials.

7.10.2 Introgression from AA-Genome Cultivated (O. glaberrima) Species

Cultivars of Asian rice *O. sativa* are high yielding, whereas those of African rice, *O. glaberrima,* are low yielding. However, *O. glaberrima* has several desirable traits, such as resistance to rice yellow mottle virus (RYMV), African gall midge, and nematodes, and tolerance to drought, acidity, and iron toxicity. Another important feature of *O. glaberrima* is its strong weed competitive ability. Thus, the interspecific hybridization among Asian and African species offers tremendous potential for combining the high productivity of *O. sativa* with tolerance to biotic and abiotic stresses of *O. glaberrima*. The F_1 hybrids between *O. sativa* and *O. glaberrima,* in spite of complete chromosome pairing, are highly sterile. Backcrossing is used to restore fertility and derive agronomically desirable lines. Molecular analysis has revealed frequent exchange of segments between *O. sativa* and *O. glaberrima*.

Efforts have been made at the West Africa Rice Development Association (WARDA-now called Africa Rice) to introgress genes for weed competitive ability from *O. glaberrima* into elite breeding lines of *O. sativa* (Jones et al. 1997). WARDA has used extensively *O. glaberrima* to develop commercial rice cultivars. A large number of elite breeding lines and "Nerica" rices have been released for commercial cultivation in African countries. These varieties are early, high yielding, and exhibit weed competitive ability (Dingkuhn et al. 1998). Nerica rices have become popular with the farmers in Africa.

At IRRI and other NARES centers, a large number of advanced introgression lines have been produced from crosses of *O. sativa* and several accessions of *O. glaberrima*. These progenies are being evaluated in collaborative projects with WARDA and NARES for introgression of tolerance to RYMV, African gall midge, and abiotic stresses. Elite breeding lines tolerant to iron toxicity have been identified (IR75870-5-8-5-B-5-B, IR80340-23-B-12-6-B, IR80314-4-B-1-3-B). These lines have been field-tested for iron toxicity in Iloilo, Philippines.

O. glaberrima has been found to be highly tolerant to root knot nematode (Soriano et al. 1999). Bimpong (2005) identified two lines (IR80311-9-B-1-2 and IR80311-2-B-1-2) derived from *O. sativa* × *O. glaberrima* showing tolerance to nematode, *Meloidogyne graminicola* based on gall midge rating and Pf/Pi ratio. Ramos et al. (2009) analyzed AILs derived from *O. sativa* × *O. glaberrima.* Of the 525 AILs tested at IRRI in the blast nursery, 42 from IR64 × *O. glaberrima* and 35 lines from IR69502-SRN-N-UBN × *O. glaberrima* showed introgression from *O. glaberrima* into two *indica* rice varieties.

Bimpong (2009) analyzed backcross progenies derived from crosses of two *indica* rice varieties (IR64, IR55423-01) with *O. glaberrima*. Mapping populations were evaluated under both low land and upland drought stress. A number of QTLs were identified for drought related traits. *O. glaberrima* contributed 50–67% of the alleles to the newly identified QTLs. Two QTLs for grain yield per plant (*ypp2.1* and *ypp4.2*) were new and two others (*yld1.1* and *yld8.1*) were common in two locations. In IR55423-01 × *O. glaberrima*, 11 new QTLs for biomass were identified, of which one QTL (*bm8.1*) was common in two locations. Nine QTLs for yield, of which two were new (*ypp3.1* and *ypp8.2*) were identified. Three QTLs (*bm2*, *dth2* and *dth4*) were common in two populations derived from IR64 and IR55423-01. QTLs *ypp6.1, bm6.1, hi6.1,* and *ps6.1* associated with an increase

in grain yield were identified in the same region on chromosome 6 at locus RM 275.

7.11 Introgression of Genes from Distantly Related Genomes

AILs have been produced from crosses of *O. sativa* with distantly related species with CC-, BBCC-, CCDD-, EE-, FF-, GG-, and HHJJ-genomes. However, gene transfer has been achieved only from CC-, BBCC-, CCDD-, EE-, and FF-genomes (Table 7.6). So far, no introgression could be achieved from GG- and HHJJ-genomes.

7.11.1 Introgression from the CC-Genome Species

Jena and Khush (1990) produced several AILs from the cross of *O. sativa* × *O. officinalis*. A number of AILs were found to be resistant to three BPH biotypes of Philippines including resistance to BPH populations in India and Bangladesh. One of the most successful examples on transfer of genes from C-genome wild species of rice is that of BPH. Four genes, *Bph10, bph11*, *bph12*, and *Bph18*, have been transferred from wild species to rice. Four IRRI breeding lines have been released as varieties (MTL95, MTL98, MTL103, MTL110) for commercial cultivation in the Mekong Delta, Vietnam. Hirabayashi et al. (2003) analyzed RILs from the cross between Hinohikari (susceptible *japonica*) and the *indica* introgression line derived from *O. sativa* × *O. officinalis*. Two genes for BPH resistance, *bph11*(t) and *bph12*(t), were identified and mapped to chromosomes 3 and 4 of rice. Huang et al. (2001) also transferred BPH resistance from *O. officinalis* into Zhensheng 97B. Tan et al. (2004b) mapped two genes. *Wbph7*(t) and *Wbph8*(t), using RILs derived from B5 introgression lines from *O. officinalis*.

BB resistance genes, *Xa27* and *Xa29*, have been transferred from *O. minuta* and *O. officinalis*, respectively (Gu et al. 2003; Tan et al. 2004a). *Xa27* has been cloned and involves induction by the pathogen (Gu et al. 2005). At IRRI, we have produced advanced-backcross progenies from crosses of an elite breeding line of NPT rice (IR65600-81-5-3-2) with two accessions of *O. officinalis* and another with tetraploid species *O. minuta*. The NPT line is highly susceptible to bacterial blight. More than 1,053 BC_2F_3 progenies derived from NPT × *O. officinalis* (Accn. 101399) were screened after inoculation with race 1. Several progenies resistant to bacterial blight have been identified.

7.11.2 Introgression from the BBCC-Genome Species

Interspecific hybrids have been produced between *O. sativa* and the tetraploid wild species *O. minuta* (BBCC). Following backcrossing and embryo rescue, AILs showing resistance to bacterial blight and blast were identified (Amante-Bordeos et al. 1992). The introgressed gene conferring resistance to blast has been designated as *Pi9*(t) (Liu et al. 2002). It has resistance to several isolates of blast. The AIL showed resistance to all the 43 blast isolates from 13 countries. High resolution genetic map was constructed. BAC contig covering about 100 kb was constructed, and the BAC library was screened with one of the markers. *Pi-z* and *Pi9* were physically tightly linked or could be allelic.

Introgression lines produced from the cross of NPT × *O. minuta* were evaluated for resistance to ten Philippine races of bacterial blight. The NPT parent was susceptible to each of the ten races. One of the families, derived from *O. sativa* × *O. minuta* cross, was found to have a broad spectrum of resistance to all the ten races tested. The genes introgressed from *O. minuta* seem to have a wide spectrum of resistance, and also the number of genes introgressed could be more than one. Similarly, BPH resistance from *O. minuta* has been transferred to rice (Brar et al. 1996). These lines have shown a wide spectrum of resistance to BPH in Philippines and Korea. Two AILs (IR71033-62, IR71033-121-15) have consistently shown resistance to BPH.

Rahman et al. (2009) conducted genetic analysis of BPH resistance using an F_2 population derived from a cross between an introgression line, "IR71033-121-15," from *O. minuta* (Accn. 101141) and a susceptible Korean *japonica* variety, "Junambyeo." Resistance to

BPH (biotype 1) was evaluated using 190 F_3 families. Two major QTLs and two significant digenic epistatic interactions between marker intervals were identified for BPH resistance. One QTL was mapped to 193.4-kb region located on the short arm of chromosome 4, and the other QTL was mapped to a 194.0-kb region on the long arm of chromosome 12. These major QTLs are new BPH resistance loci and have been designated *as Bph20*(t) on chromosome 4 and *Bph21*(t) on chromosome 12.

7.11.3 Introgression from the CCDD-Genome Species

A number of workers have produced hybrids between rice and CCDD-genome species (Sitch 1990; Brar et al. 1991). Several lines derived from *O. sativa* × *O. latifolia* cross have been evaluated for introgression of useful traits (Multani et al. 2003). Of the 2,295 disomic BC_3F_3 progenies, 309 showed resistance to BPH and 188 each for WBPH and BB resistance. Four plant progenies that were resistant to both BPH and WBPH were also resistant to BB race 2 of the Philippines. Introgression for ten allozymes of *O. latifolia*, such as *Est5, Amp1, Pgi1, Mdh3, Pgi2, Amp3, Pgd2, Est9, Amp2*, and *Sdh1*, located on eight of the 12 chromosomes were observed. Alien introgression was also detected for morphological traits such as long awns, earliness, black hull, purple stigma, and apiculus. Abnormal plants with many wild species traits suddenly appeared in normal disomic progenies. These plants showing instability and abnormal segregation behavior are being investigated for the activation of transposons.

Yoon et al. (2006) reported yield-enhancing alleles from *O. grandiglumis* (CCDD). QTL for yield components have been mapped using advanced-backcross population derived from crosses of introgression line of *O. glaberrima* and *japonica* cultivar Hwaseongbyeo. A total of 39 QTLs and one gene conferring resistance to blast isolate were identified. Phenotypic variation associated with each QTL ranged from 4.2 to 30.5%. For 18 QTLs, the *O. grandiglumis*-derived alleles contributed desirable alleles. Results demonstrate the usefulness of such alleles to improve the yield of rice cultivars.

7.11.4 Introgression from the EE-Genome Species

Multani et al. (1994) produced hybrids between colchicine-induced autotetraploids of rice and *O. australiensis* ($2n = 24$ EE). Introgression was detected for morphological traits, such as long awns and earliness, and for *Amp-3* and *Est-2* allozymes. Of the eight MAALs, only MAAL 12 segregated for resistance to BPH. The data on BPH segregation in $2n$ progenies and MAAL 12 plants ($2n = 25$) suggested that the gene for BPH resistance is located on chromosome 12. Of the 600 BC_2F_4 progenies, four were resistant to BPH. The introgression lines in BC_2F_2 resembled the recurrent parents. The rapid recovery of the recurrent parent could be due to restricted recombination. BPH resistance was found to be controlled by a recessive gene in two of the four lines, but was controlled by a dominant gene in the other two lines. Two of these lines (IR65482-4-136, IR65482-7-216) have proven resistant to the Korean BPH population (Jena personal communication). One of the lines (1R65482-4-136-2-2) carried the *Bph10* gene.

Jena et al. (2006) identified a major resistance gene, *Bph18*(t), in an introgression line (IR65482-7-216-1-2) that has inherited the gene from the wild species, *O. australiensis*. Genetic analysis revealed the dominant nature of *Bph18*(t) and identified it as non-allelic to *Bph10* that was earlier introgressed from the same accession of *O. australiensis*. *Bph18* (t) gene was initially located on the subterminal region of the long arm of chromosome 12 flanked by the SSR marker, RM463, and the STS marker, S15552. The *Bph18*(t) locus was further localized within a 0.843-Mb physical interval. The marker allele of 1,078 bp completely cosegregated with the BPH resistance phenotype. STS marker, 7312.T4A, was validated from two temperate *japonica* backgrounds (Jena et al. 2006). *Bph18* has been transferred into *japonica* cultivars through MAS.

Jeung et al. (2007) identified *Pi40* introgression from *O. australiensis* with broad spectrum of resistance to several isolates of blast. Suh et al. (2009) further confirmed durable resistance based on <3% disease leaf area, which was significantly below the threshold level of 40% considered durable resistance. *Bph18* derived from *indica* introgression line, IR65482-7-216-1-2, has been transferred through

MAS into a susceptible Korean cultivar Junambyeo. The converted *japonica* line designated as Suweon 523 shows resistance to BPH population and resembles the recurrent parent in agronomic performance (Jena personal communication).

7.11.5 Introgression from the FF-Genome Species

A series of introgression lines has been derived from the cross of *O. sativa* cv. IR56 and the wild species *O. brachyantha* ($2n = 24$, FF). IR56 is susceptible to bacterial blight races 1, 4, and 6 from Philippines, whereas *O. brachyantha* was resistant. Of the 149 backcross progenies analyzed, 27 showed introgression for resistance to bacterial blight races 1, 4, and 6 (Brar et al. 1996). Further, introgression for awning and growth duration has also been obtained. Gene transfer for BB resistance was not associated with any undesirable traits of *O. brachyantha*.

7.11.6 Introgression from the GG-Genome Species

Hybrids have been produced from the cross of *O. sativa* and *O. granulata* (Brar et al. 1991). Advanced progenies have also been produced; however, none of the lines tested has shown introgression of traits from *O. granulata* into rice.

7.11.7 Introgression from the HHJJ-Genome Species

The tetraploid *ridleyi* complex comprises two species: *O. ridleyi* and *O. longiglumis*. *O. ridleyi* shows strong resistance to all the ten Philippine races of BB. Hybrids between rice cv. IR56 and *O. ridleyi* (Accn. 100821) have been produced. However, the cross shows strong necrosis. Thus, only a few introgression lines (BC_3F_3) from this cross have been produced, but no introgression could be detected.

7.11.8 Introgression from the HHKK-Genome Species

Intergeneric hybrids between *O. sativa* and *O. coarctata* (syn. *P. coarctata*) have been produced both through sexual crosses following embryo rescue (Brar et al. 1997) and protoplast fusion (Jelodar et al. 1999). The hybrid ($2n = 36$) is sterile and shows no chromosome elimination of either parent. Due to strong incompatibility barriers, no backcross progenies could be obtained.

BC_2 progenies derived from crosses of *O. sativa* with *O. officinalis* (CC), *O. australiensis* (EE), *O. brachyantha* (FF), and *O. granulata* (GG) resembled the recurrent rice parent in most morphological traits. This suggested that only a limited recombination occurs between the A-genome of *O. sativa* and C-, E-, F-, and G-genomes of wild species. Progenies recovered in BC_2 of *O. sativa* × *O. officinalis* were so similar in morphology to *O. sativa* that they were evaluated in field trials and released as varieties for commercial cultivation in Vietnam. Most introgressed segments were detected via single RFLP and SSR markers; the flanking markers were negative for introgression. This also supports the conclusion regarding limited recombination and the possible cause for the rapid recovery of the recurrent parent phenotype.

7.12 Molecular Mapping of Introgressed Alien Genes

Some of the genes introgressed from wild species have been mapped via linkage to molecular markers (Table 7.6). We have carried out extensive molecular analysis of alien introgression lines derived from crosses of *O. sativa* × *O. glabberima*, *O. sativa* × *O. rufipogon*, and *O. sativa* × distantly related genomic types (CC-, BBCC-, EE-, FF- and GG-). Results indicate frequent introgression from AA-genome wild species including from *O. rufipogon*. However, limited introgression occurs from distant genomes of *Oryza*. It is interesting to note small segments introgressed from wild species through crossing over: a possible cause of clean gene transfer and rapid recovery of

recurrent phenotypes in wide-crosses of rice. There may be a mechanism that induces double crossing over in the adjacent region of chromosomes.

7.12.1 Mapping of the Genes for Bacterial Blight Resistance

The *Xa21* gene, introgressed from *O. longistaminata*, was among the first genes to be tagged with DNA markers. *Xa21* was mapped to chromosome 11 close to the RFLP marker, RG103 (Ronald et al. 1992). Wang et al. (1995) used BAC library and isolated 12 BAC clones that hybridized with three DNA markers linked to the *Xa21* locus. Jiang et al. (1995) used BAC clones and FISH and physically mapped the *Xa21* locus to chromosome 11 of rice. This gene has been transferred through marker-assisted breeding (MAB) into many rice cultivars including IR64, PR106, Pusa Basmati, Sambha Mahsuri, and NPT rice (Sanchez et al. 2000; Singh et al. 2001). *Xa21* gene has been isolated (Song et al. 1995) via positional cloning. The transgenic plants carrying the cloned *Xa21* gene show high level of resistance to bacterial blight pathogen. Field testing of transgenic rice at PhilRice and IRRI showed resistance to bacterial blight.

Cheema et al. (2008b) mapped a new gene, *Xa30*(t), introgressed from *O. nivara* on long arm of chromosome 4. This gene showed wide spectrum of resistance to seven *Xoo* pathotypes prevalent in the northern states of India.

Min (2009) found that *O. rufipogon* Accn. 106407 carries two dominant BB resistance genes. Based on STS marker analysis, *O. rufipogon* did not show any of the four BB resistance genes (*Xa4, xa5, xa13*, *Xa21*); however, it has a resistance allele *Xa7*. Phenotypic and genotypic data indicated that *O. rufipogon* has other unidentified dominant genes besides *Xa7*. A subset of 225 SSR markers for IR64 × *O. rufipogon* and a subset of 204 for Ilpumbyeo × *O. rufipogon* were used in bulk segregant analysis (BSA). Results showed 15 markers with *O. rufipogon* alleles in the resistant bulks. Of these, five markers, RM6569 (chromosome 1), RM227, RM3586 (chromosome 3) RM1111, and RM404 (chromosome 8), showed *O. rufipogon* alleles only in the resistant bulks.

Hechanova (2008) characterized several wild species and the derived introgression lines for the presence of known BB resistance genes. *Xa4*, *Xa7*, and *Xa21* using previous linked marker to these genes. The putative genes identified were: *Xa4, Xa7,* and *Xa21* in *O. longistaminata*; *Xa4* and *Xa7* in *O. officinalis* Accn. 100896 and *O. rufipogon* Accn. 106157; *Xa7* in *O. officinalis* Accn. 101399; and *Xa4* in *O. brachyantha*. However, *O. minuta* did not show any of the five genes (*Xa4, xa5, Xa7, xa13, Xa21*).

7.12.2 Mapping of Genes for Tungro Tolerance

We have produced a number of tungro-tolerant elite breeding lines with genes introgressed from *O. rufipogon*. One tungro tolerant introgression line (IR73385-1-4-3-2.1-6) derived from IR64 × *O. rufipogon* was analyzed with molecular markers. Of the 181 SSR markers analyzed, 11 showed introgression of *O. rufipogon* alleles. On average, 6% of the *O. rufipogon* genome was introgressed into *O. sativa*. No marker could be associated with tungro resistance. We are now analyzing backcross progenies derived from the cross of tungro-tolerant introgression lines × IR64 using the 11 introgressed SSR markers to map the gene(s) for tungro tolerance introgressed from *O. rufipogon* into rice.

7.12.3 Mapping of Genes for BPH Resistance

A gene conferring resistance to three BPH biotypes from Philippines was introgressed from *O. australiensis* into rice (Multani et al. 1994). MAAL analyses showed that the gene for BPH resistance is located on chromosome 12 of *O. australiensis*. Ishii et al. (1994) mapped *Bph10*(t) introgressed from *O. australiensis* on chromosome 12 using RFLP markers. All the 14 probes were polymorphic in recurrent parent and wild species; however, only RG457 detected introgression from *O. australiensis* into the introgression line. Cosegregation for BPH reaction and molecular markers showed a gene for BPH resistance linked

to RG457, with a distance of 3.68 cM. *Bph10*(t) confers resistance to three biotypes (1, 2, 3 of Philippines). Hirabayashi et al. (2003) located *bph11*(t) and *bph12* (t) introgressed from *O. officinalis* into rice on chromosomes 3 and 4, respectively. Tan et al. (2004b) mapped two QTLs, *Qbp1* and *Qbp2*, for BPH resistance from *O. officinalis* on chromosomes 3 and 4, respectively. QTL analysis in RILs confirmed that white-backed planthopper resistance genes *Wbph7*(t) and *Wbph8*(t) were mapped on the same loci as *Qbp1* and *Qbp2* genes. *Wbph7*(t) was located within an 1.1 cM region between R1925 and G1318 on chromosome 3, and the other designated *Wbph8*(t) was within a 0.3 cM region flanked by R288 and S11182 on chromosome 4.

Rahman et al. (2009) analyzed a mapping population derived from a cross between an introgression line, IR71033-121-15 from *O. minuta,* and a susceptible Korean *japonica* cultivar Junambyeo. Two major QTLs and two significant digenic epistatic interactions between the markers were identified for BPH resistance. These major QTLs have been mapped and designated as *Bph20*(t) on chromosome 4 and *Bph21* (t) on chromosome 12.

7.12.4 Mapping of Genes for Blast Resistance

A gene for blast resistance, *Pi9*t, was introgressed from *O. minuta* (BBCC) into rice (Amante-Bordeos et al. 1992). A backcross population produced by crossing the introgression line and the susceptible parent IR31917-45-3-2 was analyzed. Three RAPD markers were found to be linked to *Pi9*(t). *Pi9* shows resistance to several blast isolates (Lu et al. 2004). High resolution mapping indicated that *Pi-z* and *Pi9* are physically tightly linked or could be allelic.

Ramos et al. (2009) tested AILs with introgression for blast resistance from *O. glaberrima*. SSR analysis showed introgression from chromosome 1, 6, 8, and 11. Two putative markers, RM208 and RM406, on chromosome 2 linked to blast resistance were identified. Jeung et al. (2007) located a major gene, *Pi40*(t), derived from *O. australiensis* on the short arm of chromosome 6. The DNA marker, 9871T7E2b, was identified to be linked to *Pi40*(t) gene at 70 Kb chromosomal region and differentiated the *Pi40*(t) gene from the monogenic differential lines possessing *Pi-z, Piz5, Pizt,* and *Pi9*.

7.12.5 Mapping QTL for Tolerance to Aluminum Toxicity

Nguyen et al. (2003) mapped QTLs for tolerance to aluminum toxicity introgressed from *O. rufipogon* (Accn. 106424) into IR64. A population of 171 F8 RILs (F_6) derived from IR64 × *O. rufipogon* was evaluated using a nutrient solution. Nine QTLs were identified including one for root length under non-stress condition, three for root length under stress, and five for relative root length (RRL). *O. rufipogon* contributed favorable alleles for each of the QTLs for RRL, an important parameter for tolerance to aluminum toxicity, and individually explained 9.0–24.9% of the phenotyped variation. A major QTL with 24.9% phenotypic variation for RRL was found on chromosome 3 of rice, which is conserved across cereal species.

7.13 Molecular Characterization of Alien Introgression Lines

Once the introgression lines are developed from interspecific and intergeneric crosses, characterizing these at molecular level helps not only in identifying the lines with minimum linkage drag but also in understanding the mechanism of transfer as to whether the gene transfer occurred through recombination or translocation. In rice, both RFLP and SSR markers have been used to detect introgression from different wild species.

7.13.1 Introgression from O. glaberrima

We have produced a large number of advanced-backcross progenies from the crosses of elite breeding lines of *O. sativa* with different accessions of *O. glaberrima* and analyzed them for introgressions using microsatellite markers. Introgression was detected from all the

12 chromosomes of *O. glaberrima,* suggesting occurrence of recombination among AA-genomes of *O. sativa* and *O. glaberrima*. Analysis of DH populations derived through anther culture of *O. sativa* × *O. glaberrima* showed frequent exchange of chromosomal segments between *O. sativa* and *O. glaberrima*. Distorted segregation, however, was observed in these crosses. Marker analysis of introgression lines has revealed introgression from centromeric regions of *O. glaberrima* chromosomes, and also the hot spots for recombination have been identified. Bimpong (2005) analyzed advanced introgression lines derived from *O. sativa* × *O. glaberrima* for tolerance to *M. graminicola*. Two introgression lines, IR80311-9-B-B-1-2 and IR80311-2-B-B-1-2, were found to be resistant to the nematode. Ramos et al. (2009) analyzed blast-resistant backcross progenies derived from the crosses with *O. glabberima*. Of the 134 SSR markers tested, eight detected introgression from *O. glaberrima* on chromosomes 1, 2, 6, 8, and 11. BSA showed RM208 and RM406 located on chromosome 2 as putative markers linked to blast resistance. Of the 525 AILs tested in the blast nursery, 42 from IR64 × *O. glaberrima* and another 35 lines from IR69502-SRN-B-UBN × *O. glaberrima* showed introgression for blast resistance from *O. glaberrima* into *O. sativa*.

Bimpong (2009) evaluated a set of 1,574 BC_2F_3 AILs, produced from crosses of *O. sativa* (IR55423-01) and *O. glaberrima* for drought-related traits in upland nurseries at two locations in the dry season of 2008. Of the 1,574 AILs evaluated in drought nurseries, 22 showed higher yield per plant than the recurrent parent. Marker analysis showed an average of 5.7–8.1% of the *O. glaberrima* genome introgressed into *O. sativa* in all the selected groups. Some AILs outyielded the recurrent parent under drought stress, and 36 new QTLs for different agronomic traits were identified. Two QTLs (*ypp3.1* and *ypp8.2*) had a positive effect on yield under stress, accounting for 25% of the genetic variation.

7.13.2 Introgression from Distant Genomes of Oryza

Jena et al. (1992) analyzed 52 introgression lines (BC_2F_8) derived from crosses of *O. sativa* × *O. officinalis*. Of the 174 informative RFLP markers, 28 (16.1%) identified *O. officinalis* segments introgressed in one or more of the lines. Individual introgression lines contained 1.1 to 6.8% introgressed segments from *O. officinalis*. Introgressed segments were found on 11 of the 12 rice chromosomes. In most cases, *O. sativa* alleles were replaced by *O. officinalis* alleles due to recombination. Introgressed segments were smaller in size and similar in plants derived from early and later generations. Single RFLP markers detected most introgressed segments, and the flanking markers were negative for introgression. Brar et al. (1996) analyzed 29 derivatives of *O. sativa* × *O. brachyantha* cross and 40 derivatives of *O. sativa* × *O. granulata* cross. Extensive polymorphism between rice and wild species was observed. Of the six chromosomes surveyed, no introgression was detected from chromosomes 7, 9, 10, and 12 of *O. granulata* and chromosomes 10 and 12 of *O. brachyantha*. For each of the remaining chromosomes, one to two RFLP marker(s) showed introgression in some of the derived lines. Although the level of introgression was low, the results showed possibilities of introgressing chromosome segments even from distantly related genomes into cultivated rice, and thus the feasibility of transferring useful genes from distant *Oryza* species.

7.14 Characterization of Parental Genomes, MAALs, and Homoeologous Pairing in *Oryza* Through GISH

In situ hybridization is a powerful technique to characterize parental genomes in interspecific hybrids, identify alien chromosome(s) in MAALs, locate the introgressed segments, and detect homoeologous pairing. Total genomic DNA was used as a probe in in situ hybridization to detect parental genomes in interspecific hybrids and to characterize homoeologous pairing in *Oryza*. GISH was used successfully to characterize parental genomes and extra chromosome in MAALs and homoeologous pairing among several genomes involving crosses of AxC, AxE, AxBC, AxF, AxHJ and AxG. Parental chromosomes have been identified through GISH in wide hybrids (F_1, BC_1) involving *O. sativa* (AA) × *O. officinalis* (CC), *O. sativa* (AA) × *O. brachyantha* (FF), *O. sativa*

(AA) × *O. australiensis* (EE), *O. sativa* (AA) × *O. granulata* (GG), and O. *sativa* (AA) × *O. ridleyi* (HHJJ). The alien extra chromosome in MAALs and introgressed segments could also be identified. Asghar et al. (1998) applied FISH for characterizing the chromosomes of *O. sativa* and *O. officinalis* and located rDNA loci on somatic chromosomes of both *O. sativa* and *O. officinalis*. Yan et al. (1999) used FISH to characterize A- and C-genome chromosomes in the F_1 and BC_1 of *O. sativa* × *O. eichingeri* cross.

Abbasi et al. (1999) used total genomic DNA of *O. australiensis* as a probe and hybridized it with the meiotic chromosomes of the F_1 hybrid (*O. sativa* × *O. australiensis*). Both autosyndetic and allosyndetic pairing among A- and E-genomes could be detected. Meiotic analysis using GISH showed three types of pairing (1) between A- and E-genome chromosomes, (2) within A-genome chromosomes, and (3) within E-genome chromosomes. Of the paired chromosomes, 78.8% involved A- and E-genomes, 16.8% showed pairing within A-genome chromosomes, and 4.3% within E-genome chromosomes. Similarly, autosyndetic and allosyndetic pairing have been characterized among chromosomes involving different genomic combinations such as AA × CC, AA × BBCC, AA × EE, AA × W, AA × GG, and AA × HHJJ (Hue 2004). Pairing was observed between BC- and HJ-genomes in F_1 hybrids of *O. sativa* × *O. minuta* and *O. sativa* × *O. ridleyi*, indicating sufficient homoeology between unknown H- and J-genomes. Thus, over estimation of homoelogous pairing through classical cytogenetic techniques needs to be considered while predicting alien introgression based on chromosome pairing.

FISH can be used precisely for characterizing homoeologous pairing and highlighting the overestimation resulting from autosyndetic pairing, which is otherwise difficult to detect through conventional cytogenetic analysis. FISH revealed one or two bivalent(s) resulting from pairing among A-genome chromosomes, indicating duplication of chromosome segments, and thus the possibility of rice being a secondary polyploid proposed as early as 1936 by Nandi. Variability in rDNA loci has been detected though FISH in *Oryza* species (Fukui et al. 1994). Jiang et al. (1995) mapped the *Xa21* gene derived from *O. longistaminata* to rice chromosomes, using FISH and BAC clones. Shishido et al. (1998) used multicolor FISH to characterize A-, B-, and C-genomes in somatic hybrids of rice.

7.15 Enhancing Alien Introgression Through Somaclonal Variation

The technique of somaclonal variation appears to be particularly important in enhancing variation in interspecific crosses, specifically where the parental genomes of the two species show little or no homoeology. Under such situations, chromosome breakage and reunion could result in new combination and in the transfer of alien chromosome segments into the cultivated species. In *Hordeurn vulgare* × *H. jubatum* cross, enhanced variation in isozyme pattern and chromosome was observed in contrast to the original hybrid, which was asynaptic.

Larkin et al. (1989) reviewed the usefulness of cell culture to enhance alien introgression in wide crosses. Tissue culture of wheat–rye monosomic addition lines showed introgression of cereal cyst nematode resistance from rye to wheat. Similarly, tissue culture of MAALs of wheat–*Thinopyrurn intermedium* showed introgression of barley yellow dwarf virus (BYDV) resistance into wheat. The technique appears equally promising to obtain chromosomal exchanges and derive progenies with introgression of useful genes from somatic hybrids produced through protoplast fusion among widely divergent species.

A tissue culture cycle of the wide hybrids of rice (F_1, monosomic alien chromosome addition lines, somatic hybrids) could enhance the frequency of genetic exchange, particularly among distant genomes (AA- × FF-, AA- × GG-, AA- × HHJJ-, AA- × HHKK-) of *Oryza* species, which otherwise lack or show very limited homoeologous pairing, and where gene transfer through conventional methods is difficult. However, so far, the technique has not been employed for enhancing exchanges among cultivated rice and distant genomes of *Oryza*.

7.16 Somatic Cell Hybridization in *Oryza*

It has been possible to produce sexual hybrids between rice and each of the ten genomic types of *Oryza* following direct crosses and embryo rescue (Brar and Khush 2006). Somatic cell hybridization

involves isolation, culture, and fusion of protoplasts from different species and regeneration of somatic hybrid plants. The technology provides the potential to develop cybrids and organelle recombinants and to (1) produce somatic hybrid novel plants between otherwise sexually incompatible species, (2) produce cybrids and exploit the cytoplasmic variability (mitochondrial recombination), (3) transfer CMS into adapted cultivars through fusion of X-irradiated protoplasts of the CMS parent, (4) study compatibility between nuclei and cytoplasms of different species, and (5) in the uptake of chloroplasts and isolated chromosomes into protoplasts. Since the first production of somatic hybrids in tobacco in 1972, many laboratories worldwide have produced somatic hybrids in many species through protoplast fusion but with limited success in applied breeding.

Hayashi et al. (1988) produced 250 somatic hybrid plants through electrofusion of protoplasts of rice with four wild species. Somatic hybrids have also been produced through protoplast fusion of *O. sativa* and *Porteresia coarctata* (Jelodar et al. 1999). Liu et al. (1999) produced a highly asymmetrical and fertile somatic hybrid through protoplast fusion of *O. sativa* and *Zizania latifolia*. Southern blot analysis of hybrid plants ($2n = 24$) showed intergenomic exchange between rice and *Zizania*.

Somatic cell hybrids were produced through protoplast fusion between rice and carrot (Kisaka et al. 1994) and between rice and soybean; however, most of the hybrid cells failed to regenerate plants. Terada et al. (1987) attempted protoplast fusion of rice and barnyard grass (*Echinochlon oryzicola* Vasing). A total of 166 calli were identified as hybrid by isozyme and chromosome analyses. Of the 44 regenerated shoots, nine grew to plantlets. Hybrid plant through electrofusion of rice and barley protoplast was produced (Kisaka et al. 1998). The selection of somatic hybrids was based on the low rate of cell division of barley protoplasts and lack of generative ability of rice protoplasts. Only one plant regenerated, which, upon Southern hybridization with the *trpB* gene of rice, showed bands specific to rice (9.0 kb) and barley (3.0 kb). It showed novel bands of both mtDNA and ctDNA, which were not detected in either of the parents.

7.17 Screening Wild *Oryza* Species for Apomixis

Apomixis refers to asexual reproduction through seed. It is a method of reproduction in which the embryo (seed) develops without the union of the egg and sperm. Apomixis is being explored as a new frontier project to exploit hybrid vigor and develop true breeding hybrid rice varieties. Apomixis is widespread and more than 300 plant species are apomictic. It occurs mainly in polyploid species. Apospory, diplospory, and adventitious embryony are the most common types of apomixis. Various cytological, histological, and genetic tests are available to screen germplasm for apomixis. Genetic studies indicate that the switch between sexual reproduction and apomixis is controlled by one or two major genes. Khush et al. (1994) have reviewed various aspects of apomixis, such as occurrence, types, inheritance, screening techniques, and potential for exploiting hybrid vigor and cultivar development. Three approaches have been proposed to obtain apomictic rice (1) searching for apomixis in the wild *Oryza* germplasm, (2) mutagenesis to induce apomixis, and (3) molecular approaches to engineer apomixis.

Rutgers (1992) screened 547 accessions of closely related wild species of rice with AA-genomes through the pistil-clearing technique. Results were negative. Apomixis is rare in diploid species but is common in polyploid relatives of crop plants. Hence, tetraploid wild species of *Oryza* and other related genera were screened using two techniques (1) pistil clearing and (2) callose detection using fluorescence microscopy. We used the pistil-clearing technique to detect diplospory. We examined several accessions of diploid and tetraploid wild species of rice, including related genera (Table 7.9) for the possible occurrence of apospory based on multiple embryo sac development. None of the accessions however, showed any evidence of apospory (Brar et al. 1995; Khush et al. 1998).

We have also examined several accessions of diploid and tetraploid wild species of *Oryza* and five accessions of related genera for diplospory through callose detection using fluorescence microscopy. Callose is deposited in the cell walls of megaspore mother cells during megasporogenesis in sexual

Table 7.9 Wild species of *Oryza* screened for apospory (multiple embryo sac development) and diplospory (callose fluorescence) types of apomixis

Species	Genome	Number of accessions analyzed	
		Apospory	Diplospory
Diploid			
O. sativa	AA-	3	3
O. nivara, O. longistaminata, O barthii, O. glumaepatula	AA-	12	6
O. punctata	BB-	2	2
O. officinalis	CC-	5	3
O. australiensis	EE-	6	3
O. brachyantha	FF-	3	3
O. granulata	GG-	4	2
Tetraploid			
O. punctata, O. minuta, O. malampuzhaensis	BBCC-	51	45
O. alta, O. latifolia, O. grandiglumis	CCDD-	47	33
O. ridleyi, O. longiglumis	HHJJ-	10	8

Modified from Khush et al. (1998)

species, but this deposition is nearly absent in the cell walls of apomictic embryo sacs. None of the accessions of *Oryza* species and related genera examined however, showed a diplospory type of apomixis (Brar et al. 1995; Khush et al. 1998).

We used physical and chemical mutagens to explore the possibility of inducing mutations for apomixis in rice. Identifying mutants with the asexual mode of reproduction in a large population of mutagenized individuals is problematic. We employed a genetic male sterile line (*ms*/*ms*) of rice cv. IR36. Male sterile plants were pollinated with pollen from fertile (*Ms*/*Ms*) plants. Fertilized egg cells (*Ms*/*ms*), 8–16 h after pollination, were treated with 1.5 mM *N*-methyl-*N*-nitrosourea (MNU) for 1 h. We used a dominant marker, purple leaf mutant of rice, for identifying apomictic mutants following mutagenesis. Two approaches were followed (1) emasculated panicles of IR36 were irradiated with gamma rays and immediately pollinated with the pollen of purple leaf mutant and (2) fertilized eggs from the cross of IR36 × purple leaf mutant were mutagenized with MNU. More than 30,000 M_2 progenies were screened to identify any true breeding purple leaf progeny for dominant mutations for apomixis. None of the mutagenized population showed any evidence of apomixis.

7.18 Genomics Resources

Rice probably is the richest among all crop plants in terms of genomics resources. High density linkage maps, comparative maps, whole genome sequence of *O. sativa* ssp. *japonica* cv. Nipponbare (International Rice Genome Sequencing Project 2005) and *O. sativa* ssp. *indica* cv. 93-11 (Yu et al. 2002): (*http://rgp.dna.affrc.go.jp/E/IRGSP/Build5/build5.html*, *http://rice.genomics.org.cn/rice/index2.jsp*) are already available in public databases. Once the whole genome sequence became available, the next quest was to identify and annotate the genes (functional genomics) and identify differences among genotypes at base pair level (SNPs) to be used as markers for gene cloning and molecular breeding. Khush and Brar (2001) reviewed the progress made in rice genetics from Mendel to functional genomics. Since then, major progress has been made in structural (Yu et al. 2005) and functional (Zhang et al. 2008) genomics of rice. Sequencing of *indica* and *japonica* genomes by public and private organizations has ushered in the era of functional genomics. This will lead to understanding the functions of estimated 30,000 rice genes and for the intergenic DNA that plays an important but poorly understood role in gene expression, DNA replication, chromosome organization, recombination, speciation, and evolution. The activator–dissociator (AC–DS) maize transposable elements, retrotransposons, miniature inverted repeat transposable elements (MITES), and T-DNA insertions will continue to provide a wealth of genetic resources for functional genomics.

With the availability of high-throughput technologies using microarrays or gene chips, it has become possible to identify candidate genes for biotic and abiotic stress pathways and other agronomic traits and to assess gene function at a genomic level. The combination of forward genetics and reverse genetics offers new potential to apply modern tools of genomics and cell biology for precise understanding of the complexity of the genetic architecture of the rice genome. Identification of genes through functional genomics and their manipulation would be another

major step in advancing frontiers of rice genetics and breeding.

7.18.1 Comparative Genetic Maps

The development of comparative molecular genetic maps has been of great value in understanding the homoeologous relationship between the genomes of various crop plants. Comparative genetic maps can be generated for various species of the same genus and even across the genera. Jena et al. (1994) constructed a comparative RFLP map of *O. sativa* (AA) and *O. officinalis* (CC). The linkage order of mapped RFLP loci on different chromosomes of *O. officinalis* was mostly conserved relative to cultivated rice, but some rearrangements were detected. Nine of the 12 chromosomes of *O. officinalis* were homosequential to those of *O. sativa*. The order of RFLP markers on chromosome 1 was rearranged by large inversions at both terminal regions of chromosome. Two small inversions were detected on chromosome 3 and 11. Several translocations were detected between markers on chromosomes 11 and 12.

Ahn and Tanskley (1993) found extensive homoeology in several regions of the genomes among rice and maize. Later studies in rice and wheat have shown that many wheat chromosomes contain homoeologous genes and genomic DNA fragments in an order similar to those found in rice. Comparative genome mapping in rice, maize, wheat, barley sorghum, foxtail millet, and sugarcane into a single synthesis demonstrates that gene content and orders are highly conserved at both the map and megabase level between different species within the grass family, but the amount and organization of repetitive sequences have diverged considerably (Devos and Gale 1997; Gale et al 2001). Comparative genomics reveal that cereal genomes are composed of similar genomic building stocks (linkage blocks). The genomes of major cereals have been aligned by dissecting the individual chromosomes segments and rearranging these blocks into highly similar structures. Based on comparative mapping, gene location in one species can be used to predict presence and location of orthologous loci in other species. Comparative mapping is accelerating map-based cloning of orthologous genes. Moore et al. (1995) described colinearity for cereal genomes, which led to the hypothesis of the single ancestral chromosome for all cereal genomes.

7.18.2 Single Nucleotide Polymorphism

The SNP may be considered the ultimate genetic marker as they represent the finest resolution of a DNA sequence (a single nucleotide) and are generally abundant in populations. Comparison of Nipponbare and 93-11 genome sequences led to identification of 1,703,176 SNPs and 479,406 insertion/deletions (InDels) (*http://shenghuan.shnu.edu.cn/ricemarker*). This comparison identified approximately one SNP in every 268 bp and one InDel every 953 bp in rice genome. This rice DNA polymorphism database could be a valuable resource and important tool for map-based cloning of rice genes. The *Oryza* SNP Consortium has generated a large fraction of SNPs present in cultivated rice through whole-genome comparisons of 20 rice genomes, including cultivars, germplasm lines, and landraces (*http://www.oryzasnp.org*). Using high-throughput genotyping through whole genome sequencing, Huang et al. (2009) constructed a genetic map for 150 rice RILs with a genotype calling accuracy of 99.94% and a resolution of recombination breakpoints within an average of 40 kb. In comparison to the genetic map constructed with 287 PCR-based markers for the rice population, the sequencing-based method was ~20x faster in data collection and 35x more precise in recombination breakpoint determination. Using the sequencing-based genetic map, they were able to locate a quantitative trait locus of large effect on plant height in a 100-kb region containing the rice dwarfing gene.

7.18.3 Full Length cDNA

Full-length complementary DNA (FLcDNA) clones are important for functional analysis of genes, gene annotation, and determination of transcriptional start sites, so they serve as a resource for functional genomics. *Indica* and *japonica* are two subspecies of Asian cultivated rice. *Indica* varieties have dispersed throughout the tropics and subtropics from eastern

India, whereas *japonica* varieties moved northward from southern China and developed into temperate ecotypes. Since *indica* and *japonica* types have diverged several million years ago, several unique genes are expected in both the subspecies. Transcriptome comparison between *indica* and *japonica* types will lead to the discovery of molecular mechanism for phenotypic difference and will make impact on rice molecular breeding. Full length cDNA (FLcDNA) resources have been developed in *japonica* cv. Nipponbare (Kikuchi et al. 2003) and indica cvs Gangluai4 and Minghui 63 (Lu et al. 2008). More than 90% of the Gangluai 4 and 96% of Minghui 63 FLcDNAs showed similarity with Nipponbare FLcDNAs. In Nipponbare, more than 25,000 protein-coding loci with FLcDNAs have been identified. Of these, 664 are identical to known rice protein, 7,562 are similar to known protein, 13,632 are InterPro domain-containing protein, 6,954 are conserved hypothetical protein, and 1,380 are hypothetical protein.

7.18.4 Mutant Resources

Insertion sequences comprising the endogenous *Tos17* retrotransposon, maize *Ds*/*dSpm* transposons and *Agrobacterium* T-DNA, and physical and chemical mutagens have been used in rice for generating a large number of mutants for assigning functions to the genes. The insertion sequences function as enhancer trap (ET), gene trap (GT), or activation tags (ATs). The analysis of mutants by forward and reverse genetics approaches is an effective way to study gene function. Knockout (KO) mutations, which abolish gene expression and display a phenotype, provide a direct causal relationship between the gene sequence and its biological function. However, not all gene mutations display a KO mutant phenotype, primarily due to gene redundancy. The use of molecular tags or DNA insertions, such as transposons or T-DNA, is favored for mutations because their genome positions can be easily monitored to determine the correlations between tagged genes and phenotypes (Krishnan et al. 2009). A library of 29,482 T-DNA enhancer trap lines has been generated in rice cv. Nipponbare (Sallaud et al. 2004), and insertion sites to at least one position in the rice genome has been assigned in 6,645 (88.8%) trap lines.

Using chemical agents, such as ethylmethane sulfonate (EMS), methyl nitrosourea, and diepoxybutane, and physical methods such as fast-neutron, gamma-rays, and ion beam irradiation mutant populations have been generated in rice in which the point mutations can be screened by TILLING and larger deletions by PCR-based screens (Wu et al. 2005; Till et al. 2007). The IR64, Nipponbare (Till et al. 2007), and Nagina 22 populations offer different backgrounds and mutation spectrum. Using the reverse genetics approach, however, has resulted in elucidating the functions of only a limited number of genes so far. Structural and functional genomics studies in rice have led to cloning and characterization of a number of QTL of agronomic importance (Table 7.3). For these QTLs, closely linked markers have been identified and their functions characterized. Han et al. (2007) have described in detail cloning and functional analysis of nine QTLs cloned in different studies.

7.19 Utilization of Wild Relatives of *Oryza*: Future Priorities

- Collection of new wild species germplasm from different countries particularly in hotspots for biotic and abiotic stresses is emphasized to enhance genetic diversity in rice genepool.
- Identify new source of resistance to major biotic (sheath blight, stem borer, false smut, neck blast) and abiotic (drought, heat, etc.) stresses. Transfer such genes into high yielding rice genotypes. Emphasis should be given to use genes with wide spectrum of resistance.
- Identify and pyramid new genes/QTLs to enhance tolerance to major biotic and abiotic stresses.
- Identification and introgression of yield-enhancing "wild species alleles" into elite breeding lines need to be explored to further increase the yield potential of *indica* and *japonica* rice cultivars.
- Use BAC libraries and CSSL to map and isolate genes/QTLs for various agronomic traits and in functional genomics. Use CSSLs to identify heterotic gene blocks/chromosome regions and transfer such regions to develop high yielding heterotic hybrids.
- Search for genes controlling homoeologous pairing to promote recombination so as to transfer genes

from distant genomes of wild species into rice, which at present is difficult to achieve.
- Understanding evolutionary mechanisms in genus *Oryza* using new tools of genomics.
- Intensify exploratory research on C4-ness in wild species.
- Exploratory research to identify endophytes in wild species as novel source for nitrogen fixation is emphasized.
- Explore production of haploids particularly in *indica* rice similar to existing chromosome eliminating system for production of haploids in wheat and barley.
- Extinction of wild species is a threat to genetic diversity and international efforts are needed to overcome the trend in the loss of valuable biodiversity. Integration of new tools of genetics to accelerate alien gene introgression is emphasized.

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Chapter 8
Secale

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8.1 Introduction

Rye (*Secale* spp.) is a member of the grass family, Poaceae (syn. Gramineae), in the tribe Triticeae, which also includes wheat (*Triticum* spp.) and barley (*Hordeum* spp.). Over time, the genus *Secale* has widely radiated from its probable origins in southwestern Asia (Bushuk 2001) to the Mediterranean, the Balkans, and Central Asia. An isolated population also appeared in South Africa (Sencer and Hawkes 1980).

Secale is a small but important cereal genus that includes cultivated rye (*S. cereale* L.), weedy rye, and several wild species. Capable of producing higher yields than wheat under adverse conditions, rye has become a staple food grain at higher elevations and in regions with poor soils and severe winters (Fageria 1992). The wild and weedy rye species constitute a vast source of genetic diversity for the improvement of the cultivated species (Meier et al. 1996). *Secale* spp. contain genes associated with high protein content, high lysine, resistance to many cereal diseases, sprouting, drought, winter hardiness, and other morphological and biochemical traits that can be transferred to closely related cereal crops (Molski et al. 1985).

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8.1.1 Basic Botany of the Species

8.1.1.1 Distribution, Geographical Locations of Genetic Diversity

Among cereals, the global distribution of the genus *Secale* is broader than other taxa of the Triticeae (Stutz 1972; Lorenz 1991; Bushuk 2001). Rye is adapted to a wider range of environmental and climatic conditions than are wheat or barley. *Secale* spp. inhabit the temperate zones of six continents, extending into dry and colder regions, and have been cultivated at higher elevations in tropical and subtropical latitudes (Brink 2006). The species are found on sandy, rocky soils and will yield under conditions too marginal or stressful for the other cereals.

The main distribution of the genus *Secale* is in Southwest Asia, with the main regions of diversity in Turkey, Lebanon, Syria, Iran, Iraq, and Afghanistan (Sencer and Hawkes 1980). *Secale strictum* subspecies *strictum* is found mainly in dry mountain areas, in oak scrub, along the edges of the cultivated fields, and along road sides, and was found from southern Spain in the west to the Caspian Sea in the east. While *S. strictum* subspecies *africanum* (Stapf) K. Hammer is a South African endemic, found in only one location. *Secale sylvestre* (also spelled *silvestre* in the literature) grows in sandy regions of rivers, shores, and steppe ecosystems, ranging from Hungary in the west to Mongolia in the east (Stutz 1972). *Secale cereale* subspecies *ancestrale* Zhuk. inhabits roadsides and stony fields of Central Asia between Turkey and Kazakhstan. The cultivated species, *S. cereale* L. subspecies *cereale*, is chiefly grown in northern Europe; however, 58 countries produce 18 million metric tons of rye annually (FAOSTAT 2004).

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8.1.1.2 Morphology

The members of the *Secale* genus are herbaceous plants with culms solitary, up to 150 cm high. The culm nodes are glabrous, and culm internodes hollow. Leaves are auriculate with leaf blades linear, broad, or narrow, 2.5–20 mm wide, without cross venation, persistent, rolled in the bud, hairy on the lower surface otherwise glabrous, and sometime with a wax coating on leaf blade and nodes. The ligules are truncate with an unfringed membrane. The spike is composed of several spikelets, one at each node, with a hollow rachis, and the rachis segments are densely covered with white hairs on the edges. The spikelet is comprised of two hermaphroditic florets and occasionally a male sterile floret. The rye genus is mostly an obligate outbreeder with exposed-cleistogamous or chasmogamous. The glumes are narrow, 8–18 mm long, and awned. The lemmas are 10–19 mm long, keeled with a row of curved bristles and gradually tapering into an awn. A palea is present; relatively long and apically notched; not indurated (hyaline); 2-nerved and 2-keeled. Two lodicules are present, free, membranous, and ciliate. There are three stamens. The anthers are 2.3–12 mm long and not penicillate. The ovaries are hairy. The styles are free to their bases. There are two white stigmas. The fruit is not attached to either the lemma or palea, and is ellipsoid, longitudinally grooved, with hairs confined to a terminal tuft (Frederiksen and Petersen 1998).

S. strictum (syn. *S. montanum* Guss.) consists of all perennial *Secale*. In the literature, these include, *S. montanum* Guss., *S. kuprijanovii* Grossh., *S. dalmaticum* Vis, *S. ciliatoglume* (Boiss.) Grossh., *S. daralagesi* Tumanian, *S. anatolicum* Boiss., and *S. africanum* Stapf. The perennial forms have a fully fragile rachis. *S. strictum* was grouped into two subspecies, *S. strictum* subsp. *africanum*, an inbreeding subspecies, and subsp. *strictum*, an outbreeding subspecies. The *S. strictum* subsp. *africanum* differs from all others in having a smaller bristle on the lemma keel and smaller anthers. *S. strictum* subsp. *strictum* is grouped into two varieties, var. *strictum* and var. *ciliatoglume*, which are distinguished by leaf blades, sheaths, and culms densely covered with short hairs (Frederiksen and Petersen 1998).

S. cereale L. consists of the annual species *S. cereale* L., *S. vavilovii* Grossh., *S. dighoricum* Vavilov, *S. afghanicum* Roshev., *S. ancestrale* Zhuk., and *S. segetale* (Zhuk.) Roshev. They have either a tough or partly fragile rachis, but rarely are they fully fragile. This species has been grouped into two subspecies, *S. cereale* subsp. *cereale* and subsp. *ancestrale*. *S. cereale* subsp. *cereale*, the cultivated crop, has a tough, non-shattering rachis, to which the grains adhere when they are ripe, making harvesting easier. *S. cereale* subsp. *ancestrale* has a more or less fragile rachis, from which the grains fall at maturity, enabling it to thrive as a wild or weedy plant (Frederiksen and Petersen 1998).

S. sylvestre Host, Tibetan rye (syn. *S. fragile*), is an annual inbreeder. It has low growth habit, slender culms 25–50 cm, and narrow dark green leaves, smaller spikelets with very long awns, which are 6–7 times as long as the lemma or 6–7 cm in length, and the shortest anthers of all the species. The rachis is completely fragile. The glume awn is 15–33 mm, whereas the lemma awn is long (20–65 mm). Bristles on the lemma keel average 0.5–0.7 mm. The anthers are 2.5–3.5 mm in length. The pollen is nearly spherical, 37–42 μm in diameter. The caryopsis is black-brown, ca 5 mm, with a white hairy apex. It is found along riversides, shores, steppes, and semi-deserts (Frederiksen and Petersen 1998).

8.1.1.3 Taxonomic Position and Phylogenetic Studies

The members of the *Secale* genus are monocots and true grasses (Poaceae, syn. Gramineae), belonging to the Pooideae Subfamily in the Triticeae tribe. Among the *Secale* relatives are found the major small grain cereals and significant forage genera including wheat (*Triticum* spp.) and barley (*Hordeum* spp.), goatgrass (*Aegilops* spp.), wildrye (*Leymus* spp.), ryegrass (*Elmyus* spp.), tall wheatgrass (*Thinopyrum* spp.), and wheatgrass (*Amblyopyrum* spp.). The *Secale* genus includes perennial or annual, self-incompatible or self-compatible, and cultivated, weedy or wild species (Vences et al. 1987). According to the taxonomic system adopted by the Germplasm Resources Information Network (GRIN), *http://www.ars-grin.gov/*, the genus *Secale* includes four species, consisting of the annual outbreeder *S. cereale* L., the annual autogamous species, *S. sylvestre* and *S. vavilovii*, and the perennial outbreeder *S. strictum* (syn. *S. montanum*).

Eight subspecies are noted for *S. cereale* and five for *S. strictum* (GRIN 2008).

Early rye monographs distinguished as many as 14 species of *Secale*, seven of which were perennial forms and the others annuals (Chikmawati et al. 2005). With the exception of *S. sylvestre,* both annual and perennial forms were highly similar in morphological characters. They were differentiated by their rachis, perennial forms having a readily disarticulating rachis and annual forms, a persistent rachis. *S. sylvestre* was readily distinguished from the rest of the genus in spike morphology as well as in general vegetative structure. *S. cereale*, the domesticated species under cultivation was selected for non-brittle rachis (Jaaska 1983), distinguishing it from the weedy and wild species, which exhibit more spike fragility.

Sencer and Hawkes (1980) and Jaaska (1999) classified the *Secale* genus as consisting of three biological species, the outcrossing perennial *S. strictum* Presl., the outcrossing annual *S. cereale* L. s.l, and a cleistogamous annual *S. sylvestre* Host. Recent revisions of the genus *Secale,* based on morphologic, genetic (Frederiksen and Petersen 1998), and amplified fragment length polymorphism (AFLP) studies (Chikmawati et al. 2005), recognize only three species.

Over the years, taxonomic revisions of the *Secale* genus have expanded and contracted the number of species and subordinate taxa with expected regularity. Mistaken identities (Meier et al. 1996), the addition of cryptic criterion, such as cytological information, and ad-hoc determinations based on a variety of experimental methodologies have added to the uncertainty of species names. This shifting foundation generates some difficulty in comparative analysis of experimental results and in collecting, classifying, and distributing germplasm accessions. A simplification of species classification for the sake of readability is warranted (Table 8.1).

Researchers have also studied phylogenetic relationships among *Secale* species using different approaches including morphological analyses (Frederiksen and Petersen 1997), isozymes (Vences et al. 1987; Persson and Bothmer 2000, 2002), thin-layer chromatography patterns (Dedio et al. 1969), ribosomal DNA spacer lengths (Reddy et al. 1990), genomic in situ hybridization (GISH) (Anamthawat-Jónsson and Heslop-Harrison 1993; Anamthawat-Jónsson et al. 1996), AFLP (Chikmawati et al. 2005), random amplified polymorphic DNA (RAPD) techniques (Ma et al. 2004), microsatellite markers (Shang et al. 2006), and organellar genome analysis (Isik et al. 2007), as well as the internal transcribed spacer (ITS) sequences of the 18S–5.8S rDNA region of cultivated and wild species (De Bustos and Jouve 2002). A majority of studies have focused on cultivated varieties and landraces of *S. cereale*. Isozyme systems (ACO, DIA, GPI, MDH, PGD, and PGM) were used to estimate the genetic variation and diversity of improved varieties and landraces of *S. cereale* from Sweden and northern Europe (Persson and Bothmer 2000, 2002). The results obtained in these studies indicated that (1) most of the genetic diversity was found within the accessions and was very small between accessions; (2) the landraces from Germany and Norway have a low genetic variation compared to other landraces; and (3) the Finnish landraces and 11 of the Swedish ones were grouped together with a very small diversity index. These results may assist in setting priorities for conservation of cultivated *Secale* accessions and reveal something about how the cultivated rye diffused throughout northern Europe. In recent decades, molecular methods have been used to investigate genetic diversity and phylogenetic relationships of the genus *Secale*. AFLP data used to analyze the phylogenetic relationships among 29 accessions of weedy/wild rye and cultivated rye, representing 14 of the most commonly recognized ranked species or subspecies in the genus *Secale* (Chikmawati et al. 2005), indicated that (1) *S. sylvestre* was the most distantly related to all other ryes; (2) annual forms grouped together as did the perennial

Table 8.1 Abridged *Secale* genus and character traits

Species	Subspecies	Variety	Synonyms	Life cycle	Breeding habit	Rachis	Status
strictum	*africanum*		*S. africanum*	Perennial	Autogamous	Shattering	Wild
strictum	*strictum*	*strictum*	*S. montanum*	Perennial	Allogamous	Shattering	Wild, Cultivated
strictum	*strictum*	*ciliatoglume*	*S. montanum*	Perennial	Allogamous	Shattering	Wild
cereale	*cereale*			Annual	Allogamous	Non-shattering	Cultivated
cereale	*ancestrale*			Annual		Semi-shattering	Wild or Weedy
sylvestre (*silvestre*)			*S. fragile*	Annual	Autogamous	Shattering	Wild

forms; (3) *S. sylvestre* was considered to be the most ancient species, whereas *S. cereale* appeared to be the most recently evolved; (4) the 29 accessions clearly separated into only three major species groups: *S. sylvestre*, the perennial forms (*S. strictum* syn. *S. montanum*), and the annual forms (*S. cereale*). Shang et al. (2006) investigated the genetic diversity and phylogenetic relationships of 47 cultivated ryes (*S. cereale* subsp. *cereale*) accessions and 30 *Secale* accessions, consisting of five cultivated ryes and 25 non-cultivated ryes representing four species and 10 subspecies of the rye genus using simple sequence repeat (SSR) markers. Their results showed that (1) the mean genetic similarity (GS) index in *Secale* was lower than that in cultivated rye; (2) the highest within-species GS index was observed for *S. sylvestre* and the lowest for *S. strictum*, whereas the highest between-species GS index was found between *S. cereale* and *S. vavilovii* and the lowest between *S. sylvestre* and *S. cereale*; (3) there was no obvious difference in GS levels in the cultivated rye accessions from Asia, Europe, North America, or South America; (4) the *S. sylvestre* accessions were obviously divergent from the accessions of other species and that the *S. vavilovii* accessions were closely related to the *S. cereale* accessions. Most reports agree that *S. sylvestre* is highly divergent from the accessions of other species (Reddy et al. 1990; Del Pozo et al. 1995; De Bustos and Jouve 2002; Chikmawati et al. 2005; Shang et al. 2006). By investigating the distribution of SSRs, Cuadrado and Jouve (2002) concluded that *S. sylvestre* separated early from the other species of *Secale* genus. Most of the studies mentioned above supported the notion that *S. sylvestre* is the most ancient species. Most genetic analyses of rye have focused on the nuclear genome. Organellar genome analysis might further explain the genetic diversity of rye. Isik et al. (2007) investigated the organellar genome diversity and relationships of 96 accessions of rye using chloroplast and mitochondrial DNA PCR-RFLPs. They found the level of organellar polymorphism to be low among the cultivated rye genotypes used in their studies.

8.1.1.4 Cytology, Karyotype, and Genome Size

The *Secale* genome has been designated R (Wang et al. 1996), with each species having a three-letter designation, for example, R^{sec} describes *S. cereale*. The basic chromosome number of all members of the genus is seven, $x = 7$, $2n = 14$ (Jain 1960; Bowden 1966; Petersen 1991). In a flow cytometric study, Lee et al. (2004) found a mean diploid 2C DNA value for the RR-genome of 16.8 pg, a total chromosome length of 101.4 μm, and mean chromosome length of 7.24 μm. The Plant DNA C-values Database *http://www.rbgkew.org.uk/cval/homepage.html* records 1C DNA content of *S. Fragile*, *S. Africanum*, *S. Montanum*, and *S. cereale* as 7.23, 7.43, 8.20, and 8.28 pg, respectively (Bennett and Leitch 2003). The rye genome contains a very high percentage (92%) of repeated sequences (Flavell et al. 1974). About 60% of the rye genome consists of short repeated sequences (50–2,000 bp long) interspersed with either different or related repetitive sequences (Rimpau et al. 1978). The repeated sequences in the genus *Secale* have been widely investigated (Bedbrook et al. 1980a; Appels et al. 1986). Six repetitive sequence families have already been described, which include the 630-, 610-, 480-, and 120-bp families (Bedbrook et al. 1980b), and the 350–480 bp family (Appels and McIntyre 1985), and the $5.3H_3$ family (Appels et al. 1986). Furthermore, some rye-specific and retrotransposon-like elements such as pAWRC.1 (Francki 2001), pSc10C, and pSc20H (Ko et al. 2002) have been discovered. The sequence of pAWRC.1 is centromere-specific. The sequence of pSc10C was detected predominantly in the pericentromeric regions of all rye chromosomes, whereas the sequence of pSc20H was dispersed throughout the rye genome except at telomeric and nucleolar organizing regions. A new repetitive DNA, $pSaD15_{940}$, was isolated from the genome of *S. africanum* using RPAD analysis (Liu et al. 2008). $pSaD15_{940}$ disperses throughout all rye chromosomes arms except for the terminal regions. Repetitive sequences in the genus *Secale* are useful tools for the study of rye evolution and for use in rye and wheat breeding programs.

The seven chromosomes of rye are considered to be partially syntenous with the equivalent chromosomes of the related genomes of the Triticeae and even more distantly related Poaceae (Devos et al. 1993). The study of the nature and degree of pairing of rye chromosomes in interspecific and intergeneric F_1 crosses was used as a cytological tool for comparative genome analysis (Singh and Röbbelen 1977). Karyotypes of various *Secale* species are distinguished by a number

of translocations. *S. montanum*, *S. africanum*, and *S. sylvestre* differ from each other by one translocation; whereas *S. vavilovii* differs from *S. montanum* by one large translocation, from *S. africanum* by one large and one small translocation, and from *S. sylvestre* by one large and one small translocation. *S. cereale*, the cultivated species, differs from all wild species by two translocations. Another small translocation may differentiate *S. cereale* and *S. sylvestre* (Khush and Stebbins 1961). A translocation configuration of supernumenry B chromosomes was observed in a few cells of the hybrid of *S. cereale* × *S sylvestre* (Khush and Stebbins 1961). Schlegel and Weryszko (1979) examined the frequency of wheat–rye pairing using the divergent wild *S. sylvestre* and *S. strictum* (syn. *montanum*), finding that meiotic I bivalents ranged from 0.0% in crosses with *S. sylvestre* to 2.4% per PMC in *S. montanum*.

Geimsa C-banding reveals the chromosomes of the *Secale* genus to be generally characterized by large telomeric heterochromatic bands and a number of weaker interstitial bands. The chromosomes of *S. sylvestre* differ from those of the other *Secale* species by the low number of interstitial bands and the small size of the telomeric bands. *S. cereale*, the cultivated species, exhibits great variability in the Giemsa banding pattern of the chromosomes, which is consistent with observations of such patterns in other allogamous species (Linde-Laursen et al. 1980).

In situ hybridization (ISH) showed that the self-pollinated annuals, *S. sylvestre* and *S. vavilovii*, have considerably fewer repeated DNA sequences than the remaining taxa (Cuadrado and Jouve 1997). Using Giemsa C-banding combined with the seed storage protein electrophoresis, Yang et al. (2000, 2001a) showed that the heterochromatin content of *S. africanum* was lower than that of *S. cereale*. Zhang and Ren (1998) observed a distinctly different heterochromatin constitution between rye native to China and rye introduced into China, and the C-banding patterns indicated that genetic differentiation had occurred during migration into new ecological environments in China.

Using GISH, Zhang and Ren (1998) found that the rye chromosome fraction of a wheat–*S. africanum* introgression line could not be probed by the genomic DNA of *S. cereale*, suggesting a significant genomic divergence of *S. africanum* from *S. cereale*.

8.1.2 Agriculture Status

Most annual *Secale* are weedy rye types, including *S. afghanicum* (Vav.) Roshev, *S. dighoricum* (Vav.) Roshev, *S. segetale* (Zhuk.) Roshev, *S. ancestrale* Zhuk., *S. vavilovii* Grossh., and *S. sylvestre* Host., and are found in grain fields, along ditchbanks, roadsides, and other disturbed habitats. E.H. Wilson (1914), a naturalist and plant explorer, who traveled extensively in China, observed a species of rye that he identified as *S. fragile* (syn. *S. sylvestre*) being cultivated on small scale on rugged cliffs in the eastern Sichuan Province of China.

Some perennials are weedy rye types including *S. ciliatoglume* (Boiss.) Grossh., *S. daralagesi* Thum., and *S. anatolicum* Boiss., while others are wild rye types (*S. montanum* Guss., *S. dalmaticum* Vis., *S. kuprijanovii* Grossh., and *S. africanum* Stapf. (Stutz 1972)), found in natural, undisturbed environments. *S. montanum* (syn. *S. strictum*) has been transformed from a wild perennial plant into a perennial grain (Oram 1996).

8.2 Conservation Initiatives

8.2.1 Evaluation of Genetic Erosion

Wild relatives of *Secale* have infrequently been collected except in conjunction with wider, more complete collections of the wild relatives of *Triticum*, *Hordeum*, and the other Poaceae. The number of wild *Secale* germplasm accessions lags far behind in the global germplasm banks and institutional collections. Passport information is often very limited. Reported herbaria deposits are sometimes difficult to locate. Stutz (1972) deposited specimens from his extensive collecting efforts in 1967 in three herbaria. Two *S. montanum* specimens collected in Lebanon were deposited at the Post Herbarium of the American University of Beruit and are currently locatable. Two *S. anatolicum* specimens, collected by Stutz in Turkey, may be found on Tropicos (2009), the Missouri Botanical Garden Database for nomenclature, bibliographic, and specimen data (*http://www.tropicos.org/*). This database contains 3.4 million specimen records, 108 specimen of *Secale*, and the two weedy species collected by Stutz (1972). These were originally deposited

at Brigham Young University's M.L. Bean Herbarium of Vascular Plants. Frederiksen and Petersen (1998) examined type specimens and images from 17 European herbaria for their extensive revision of the *Secale* taxonomy.

The loss of biodiversity and degradation of biomass and ecosystems globally has led to an intensification of efforts to conserve, collect, and catalog all plant species (Hammer 2004). This, in turn, has led to a tangle of interlocking and overlapping networks of organizations dedicated to a wide variety of conservation activities. It is challenging to find the small numbers of *Secale* species featured in the range of endeavors and initiatives; however, there are some notable projects and efforts.

8.2.2 *Extinction in the Wild:* S. africanum, *A Situation to Ponder*

Khush and Stebbins (1961) reported *S. africanum* Stapf (= *S. strictum* subsp. *africanum*) to be endemic to a single mountain range in South Africa. It was last documented (Hoare 2003) in discontinuous patches on a single farm in the remote town of Sutherland in the cold and arid highlands of Northern Cape province, some 1,458 m (4,780 ft) above sea level (Khush and Stebbins 1961; Frederiksen and Petersen 1997). *S. africanum* has been designated by the South African Red Data List (October 2007) as critically endangered (CR), indicating that this species (i.e., subsp.) faces an extremely high risk of extinction in the wild.

Currently, *S. africanum* plants are grown in the Botanical Society Conservatory at the Kirstenbosch National Botanical Garden, Cape Town, South Africa, where it is labeled a "rare endemic from the Roggeveld escarpment." In addition to the living collection of seeds and growing plants, this institution is studying and recording the germination requirements of the species. According to Hoare (2003) in his entry on *S. africanum* in The Grasslands Index – GBASE, this once abundant species offered relatively good pasturage in its namesake region, the Roggeveld or "rye land," where it is no longer to be found. A special report by the Critical Ecosystem Partnership Fund project, *Succulent Karoo*, entitled: "Investment in the Succulent Karoo Biodiversity Hotspot," announced ongoing efforts to reestablish *S. africanum* in protected areas in the Roggeveld (CEPF 2008).

8.2.3 Germplasm Banks

The European *Secale* Database (*http://www.ihar.edu.pl/gene_bank/secale/secale.html*) is the most extensive and targeted effort to coordinate information about the genus and available germplasm. In 1982, the first effort to coordinate the *Secale* germplasm collections in Europe by the European Cooperative Program for Plant Genetic Resources (ECP/GR) resulted in the appointment of the Polish Gene Bank as the center for the collection of passport information on the *Secale* genus. With the support of the International Plant Genetic Resources Institute (IPGRI, renamed "Biodiversity International"), the European Secale Database was expanded and updated. Currently, the database includes passport information for 13,707 *Secale* accessions held in 35 contributing European institutions. The database is managed by Wieslaw Podyma National Centre for Plant Genetic Resources, Plant Breeding and Acclimatization Institute, Radzikorv, 05-870 Blonic, Poland (ECP/GR 2008). The *Secale* collection of the Botanical Garden of the Polish Academy of Sciences (status in 1996) consists of 62 wild *Secale* accessions represented by 13 different species/subspecies classified according to Hammer (1990).

Chinese Crop Germplasm Resources Information Network (CGRIS 2009) (*http://icgr.caas.net.cn*) is a central repository for all types of plant genetic resources information. It mainly consists of six subsystem: the management system of the National Crop Gene Bank (NCGB), the management system of the long-term storage in Qinghai, the management system of National germplasm Resources Nursery, crop characterization and evaluation database system, the database system for germplasm exchange at home and abroad, and the management system of the medium-term storage in Beijing. At present, there are over 2,000 megabytes of data on 160 kinds of crops, 350,000 accessions of germplasm stored in CGRIS. This database with 20 million data items was established by computerizing the passport data, characterization data, and evaluation data of all germplasm resources stored in the genebank (*http://icgr.caas.net.cn*). Besides those species of rye genus stored in the National Genebank and conservation centers, some rye species and accessions collected, including Chinese local rye (*S. cereale*), are held in provincial academic and breeding organizations to ensure their

effective utilization in crop improvement projects. The searchable database linking these genebanks is currently available only in Chinese.

The Gene Bank information system of the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK Gatersleben 2006) *http://gbis.ipk-gatersleben.de/gbis_i/ GBIS/I* lists approximately 3,400 Secale accessions, including 72 accessions of *S. strictum* (and three *S. montanum* derivatives), 22 accessions of *S. sylvestre* (and three *S. sylvestre* derivatives), and 21 *S. vavilovii* and *vavilovii* derivatives (IPK Gatersleben 2006).

Secale is listed among the mandate species for collection by the Nordic Gene Bank (NGB) at Alnarp, Sweden *http://www.nordgen.org/index.php/en/content/view/full/293/*, and for Svalbard Global Seed Vault backup collection in the arctic circle. The NGB reports nine *Secale* accessions originating from the 1940s Haslund-Christensen Expedition to Afghanistan. The NGB database SESTO (*http://www.nordgen.org/index.php/en/Vaexter/Innehaall/Sesto*) is a genebank documentation system providing full passport information for plant species. In addition to 395 accessions (representing 94 cultivars) of *S. cereale*, the wild taxon include one accession each *S. cereale* subsp. *segetale*, *S. fragile*, *S. montanum*, and *S. vavilovii* (NGB/SESTO 2007).

The N.I. Vavilov All-Russian Scientific Research Institute of Plant Industry (VIR) database *http://www.vir.nw.ru/data/dbf.htm* lists passport information for 2,930 *Secale* accessions, of which eight are *S. montanum* and is housed in The National Seed Store at the Kuban Experiment Station.

The USDA-ARS National Small Grains Collection, in Aberdeen, Idaho, lists 2,086 *Secale* accessions available for distribution through GRIN *http://www.ars-grin.gov*, of which 84 accessions are wild or weedy species. The USDA National Plant Germplasm Center (NPGC/GRIN) lists 2,389 accessions of *Secale* spp. with full passport information and availability (GRIN 2008).

8.2.4 Modes of Preservation and Maintenance

Strategies for preserving germplasm include collecting, accessioning, and maintaining seeds, whole plants, tissue culture, microproagation, cryopreservation, and in situ and ex situ conservation and restoration. *Secale* seed accessions are preserved, dessicated, and maintained at low temperature or cyrogenically frozen. The National Genebank of the Institute of Chinese Germplasm Resource of the Chinese Academy of Agricultural Sciences (ICGR-CAAS), for example, is the long-term conservation center for all crop genetic resources in China and one of the largest in the world. The capacity of the Genebank is more than 400,000 accessions, and the seed is stored at −18°C with less than 57% relative humidity. In 1985, a National Genebank of Triticeae was constructed at Sichuan Agriculture University in China with a storage area of 325 m^2 at a temperature range of 0°C to −10°C for medium-term storage in addition to a field genebank. By 1998, a total of 310,000 accessions of germplasm resources had been stored in the Triticeae genebank. From 1989 to 1991, ICGR collected "weedy-type" rye resources in Xinjiang, in western China. Weedy rye growing in the Mulei areas of the eastern Xinjiang belonged to *S. cereale* subsp. *segetale*, an annual allogamous plant of winter habit. One of its distinct character was rachis fragility, particularly during cold weather. About 147 accessions (131 accessions from China and 16 from other countries) belonging to the genus *Secale* (two species from China, seven from other countries) were collected and evaluated for agronomic characters, grain quality, stress resistance, disease and pest resistance, and cell biological characters.

8.3 Role in Elucidation of Origin and Evolution of Allied Crop Plants

8.3.1 Related Crop Plants

Jaaska (1999) and Hillman (1978) recounted several scenarios for the domestication of *Secale* proposed by Vavilov, who held the progenitor to be a wild perennial form, namely *S. montanum* (= *S. strictum*). Vavilov thought the first stage of domestication occurred in the Near East where rye became a secondary, weedy crop before introduction to Europe as a crop. According to Stutz (1972) and Hammer (1990), all annual rye species evolved from the perennial *S. strictum*. Zohary and Hopf (2000) noted that non-shattering weedy ryes

commonly infested wheat fields. Farmers tolerated weedy rye, because traditional harvesting could not separate the grains of rye from wheat. In addition, during the years of extreme cold or drought, the weedy rye probably constituted the only yield. In some regions, this fortuitous crop was called the "wheat of *Allah*."

Hillman et al. (2001) postulated the pre-domestication cultivation of an annual wild rye such as *S. cereale* subsp. *vavilovii* (Grossh.) Zhuk. or *S. iranicum* Kobyl. Based on archeological remains in the Abu Hureyra site, wild type rye continued to be used during a period of increasing aridity, which would have eliminated previously abundant wild stands. After a period of about 400 years, domesticated grains similar to *S. cereale*. subsp. *cereale* Zhuk. appeared. There is no evidence indicating transitional stages between the wild type rye and domesticated rye. Furthermore, the species cited in this study, though all annuals and interfertile, were not all allogamous. On the other hand, Frederiksen and Petersen (1997) and Chikmawati (2003) found no evidence for separating these species or subspecies biologically from the cultivated species. While various morphological, cytogenetic, and molecular studies have implicated almost all of the wild or weedy species as possible progenitors at one time or another, it is quite possible that hybridization and introgression as well as dispersal and isolation have fully obscured and perhaps eliminated the true progenitor of cultivated rye.

8.3.2 Application of Morphotaxonomy, Chemotaxonomy, Biochemical, and Molecular Markers

Data generated from many different approaches have been used to determine the origin of cultivated rye. *S. sylvestre* appears to be the most divergent species (Cuadrado and Jouve 2002; Petersen et al. 2004; Chikmawati et al. 2005), while the ancestor to the cultivated rye still remains controversial. N.I. Vavilov, P.M. Zukhovsky, and other botanists concluded that cultivated rye evolved from an annual weedy rye (Khush 1962; Cuadrado and Jouve 1997). Stutz (1957) suggested that cultivated rye resulted from the hybridization between wild perennial rye, *S. montanum* (syn. *S. strictum*), and the wild annual rye, *S. sylvestre*. Others have noted that morphological and cytological changes in *S. montanum* might account for the evolution of *S. cereale* through an intermediate weedy subspecies (*S. cereale* subsp. *segetale*) (Khush and Stebbins 1961), which exhibits similarity in size and plumpness of grain and a non-shattering rachis (Khush 1962, 1963). However, the direct progenitor of the cultivated species is not clear.

Puchalski and Molski (1981) demonstrated that the isoenzyme variation in the wild *Secale* population was affected by the breeding system of the rye species. The perennial *Secale* species were more polymorphic than the annual ones. *S. sylvestre,* an autogamous species, was monomorphic for two different enzyme systems, non-specific esterases and peroxidase. *S. vavilovii,* also autogamous, exhibited a very low esterase polymorphism. Vences et al. (1987), analyzing several isozyme systems, were able to distinguish between the self-pollinated species (*S. vavilovii* and *S. sylvestre*) and open-pollinated species (*S. cereale* and *S. montanum*). Isozyme data was in agreement with the hypothesis that *S. montanum* was the oldest species. *S. cereale* and *S. montanum* differed only slightly in isozyme characteristics.

Meier et al. (1996) demonstrated the existence of wide variability in biochemical characteristics within *S. sylvestre*. Their results supported the biological species designations of *Secale*. *S. sylvestre* lacked a 480-bp repeated nucleotide sequence (RNS) in all telomeres, supporting the hypothesis of an early separation of *S. sylvestre* and the clear distinction of this species from the rest of the *Secale* genus (Cuadrado and Jouve 1997). *S. cereale, S. montanum*, and *S. kuprijanovii* showed amplification and complex organization of RNS families in the telomeres, interstitial formation, and a tendency towards the doubling of loci for the 120- and 480-bp sequences and were considered the most evolved species. The appearance of a new locus or 5S rRNA in *S. cereale* and *S. ancentrale* supported the opinion that cultivated ryes evolved from the weedy species. Based on the ITS sequences of the 18S–5.8S rDNA (ITS-1) region of cultivated and wild species, de Bustos and Jouve (2002) found similar results to those observed by Cuadrado and Jouve (1997); however, they could not distinguish between the weedy forms of *S. cereale* and cultivated rye. Recent studies using 24 *S. cereale* microsatellite (SCM) markers demonstrated that *S. sylvestre* accessions were divergent from the accessions of other species and that *S. vavilovii*

accessions were closely related to *S. cereale* (Reddy et al. 1990, Del Pozo et al. 1995; Frederiksen and Petersen 1997; De Bustos and Jouve 2002; Chikmawati et al. 2005; Shang et al. 2006).

8.4 Role in Development of *Secale* Species Cytogenetic Stocks and Their Utility

Because *Secales* are naturally diploids, the creation of monosomics and other deletion aneuploids is not feasible. However, rye has been successfully incorporated as alien addition, substitution, and translocation lines into polyploid species. Chapman and Miller (1978) and Miller (1984) reported additions and substitutions of wild perennial rye *S. montanum* chromosomes in hexaploid wheat, "Chinese Spring." Gustafson et al. (1976) identified additions of *S. montanum* to hexaploid wheat "Kharkov M.C.22." Using this material, Shewry et al. (1985) showed that gamma secalin proteins were located on $6R^{mon}$ addition and substitution lines in contrast to the $2R^{cer}$ location in *S. cereale*, indicating one translocation of *S. montanum* with respect to *S. cereale*. Related proteins are found on group 6 chromosomes of wheat. Montero et al. (1986) found a presumed wheat–rye $5BS/5RS^{mon}$ translocation that disrupted the pairing control mechanism (*Ph* gene) on 5BS of wheat. In addition, the alpha amylase phenotype (influencing dormancy) of the translocated line was found to be identical to wheat 5BL ditelosomic lines. Cuadrado and Jouve (1995), in a follow-up study on the translocated material using FISH and genome-specific probes, determined that the *S. montanum* donor chromatin was not from $5RS^{mon}$ but more likely $2RS^{mon}$, $2RL^{mon}$, or $7RL^{mon}$.

Early cytological studies included the synthesis of interspecific and intergeneric amphiploids by chromosome doubling of sterile or semi-sterile F_1 hybrids. These synthetic lines enabled scientists to observe the expression of genes introduced by alien chromatin into another genus. Wheat and rye can be hybridized at all ploidy levels, and allopolyploids can tolerate aneuploidy. The discovery of "diploidizing genes" in wheat and the creation of the *Ph* mutant (lacking wheat chromosome 5B, arm 5BL, or suppression of the *Ph* gene by other means) further enabled the determination of homoeology between chromosomes of different genera by allowing some degree of non-homologous pairing (Okamoto 1957; Sears and Okamoto 1958; Riley and Chapman 1958). The introduction of homoeologous pairing by manipulating the homoeologous pairing suppressor gene (*Ph*) in wheat was reported by Riley et al. (1968). Lukaszewski and Gustafson (1982) used hybrids between hexaploid triticale × common wheat to introduce rye chromosome into wheat. Another efficient method was used to produce homoeologous translocations between wheat and rye chromosomes (Ren et al. 1990a, b) by first creating amphiploid between the target variety and a suitable population of the donor species. The amphiploid is then backcrossed once or twice with the parental wheat variety and the backcross progeny selfed until the chromosome number returns to 42. New monosomic addition plants can also be selected. This method is simple and is not restricted to genotypes for which aneuploids exist. Furthermore, the genotypes will have a more stable genetic background than those produced by triticale × wheat crosses. Jiang et al. (1994) reviewed the techniques and advances in alien gene transfer from distantly related species into wheat. Subsequently, Jauhar and Chibbar (1999) also reviewed the methods by which alien gene have been introduced into wheat. They all indicated that the synthesis of hybrids between wheat and alien species is the single most important step for transferring a targeted alien gene into wheat followed by induced chromosome pairing between wheat and alien species.

8.5 Role in Classical and Molecular Genetic Studies

8.5.1 Use in Classical Genetic Studies

Stutz (1957) reported that *Secale* hybrids between various perennial forms all showed regular meiotic chromosome behavior as did the hybrids between the annual species belonging to *S. cereale*. However, in all hybrids between *S. cereale* and perennial forms a translocation was observed at metaphase I in the pollen mother cells (PMC). This was presented as evidence for a translocation between the short arm of a group 2 chromosome and the short arm of a group 6

chromosome (a 2RS/6RS translocation) in the genome of *S. cereale* relative to those of wheat and *S. montanum* (Shewry et al. 1985).

Wang (1989, 1992) and Petersen (1991) created intergeneric hybrids of *Hordeum* and *Secale* species of various diploid perennial species, including *S. montanum*, to assess genomic relationships by studying meiotic pairing. To discriminate between closely related Triticeae species, including *S. strictum* subsp. *africanum* (syn. *S. africanum*), Anamthawat-Jónsson et al. (1990) developed species-specific probes, using genomic DNA. Cytogenetic and molecular tools were used to locate and identify *S. africanum* segments on wheat chromosomes.

Yang et al. (2001b) created an amphiploid between *Aegilops tauschii* and *S. sylvestre* to study the cytology and gene expression of endosperm storage proteins and disease resistance.

Katto et al. (2004) designed a PCR-based marker based on pSc20H, a rye-specific RAPD marker previously reported by Ko et al. (2002). The sequence detected rye chromatin in wheat–rye addition, substitution, and translocation lines and all *Secale* species tested including one *S. sylvestre* (syn. *S. fragile*) line. The sequence was found to be homologous to a partial Ty3/gypsy type retrotransposon, which is abundant in both rye and wheat. However, wheat accessions with high homology to the pSc20H sequence showed sequence diversity near the 3′ terminus of the primer pairs. This PCR-based marker has the ability to detect very small rye chromosome segment that might easily be overlooked by GISH. Liu et al. (2008) developed a genome-specific DNA sequence, pSaD15940 that amplified an 887-bp fragment in *S. africanum* and other rye species. This high copy, rye-specific DNA derived from the *S. africanum* genome had no significant homology to known nucleotides, which was considered a new repetitive sequence of *Secale*. In situ hybridization showed hybridization in a number of *Secale* spp., throughout all rye chromosome arms except in the terminal regions. The probe will be useful as a molecular marker for the introgression of *S. africanum* and other rye chromatin into wheat. Jia et al. (2009) identified a repetitive sequence of 411 bp, pSaO5411, in *S. africanum* by RAPD analysis of wheat and wheat – *S. africanum* amphiploids. GenBank BLAST search revealed that the sequence of pSaO5411 was highly homologous to part of a Ty1-*copia* retrotransposon. Fluorescence in situ hybridization (FISH) analysis showed significant hybridization dispersed along the *S. africanum* chromosome arms except in the terminal regions and no hybridization to wheat chromosomes in a wheat–*S. africanum* amphiploid. A pair of sequence-characterized amplified region (SCAR) primers were designed from the probe, and the resultant SCAR marker was able to target both cultivated rye and the wild *Secale* species. This diagnostic PCR-based marker together with FISH can detect the *Secale* chromatin introduced into a wheat background, particularly in the early generations of wheat – *Secale* hybridization.

In addition, some repetitive DNA sequences isolated from rye can be used as markers to investigate genomic diversity of rye and variation of chromosome structure. For example, centromere-specific repetitive DNA can be used to display the centromeric structures of rye chromosomes (Fig. 8.1). Telomeric tandem repetitive DNA sequence, pSc200, can be used to display divergence of different rye genomes (Figs. 8.2 and 8.3).

8.5.2 *Use in the Construction of* Secale *Species Linkage Maps*

There are currently more than 2,000 biochemical, molecular, and morphological markers available in

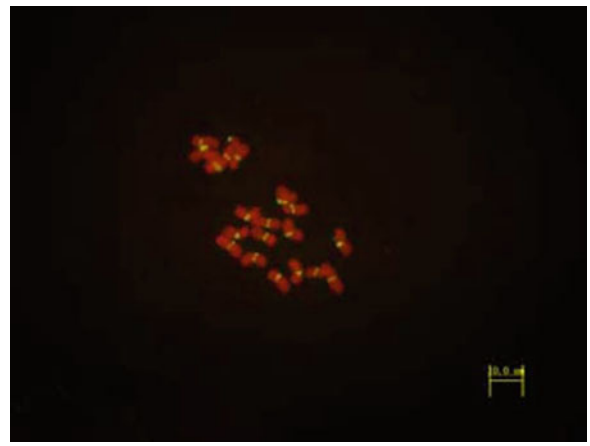

Fig. 8.1 Rye-specific centromeric sequence pMD-CEN-3 (GeneBank Accession No. EF165548) was used as probe to hybridize to metaphase chromosomes of *S. sylvestre*. The *yellow-green fluorescence* indicates the signals of pMD-CEN-3. Scale bar: 10 μm

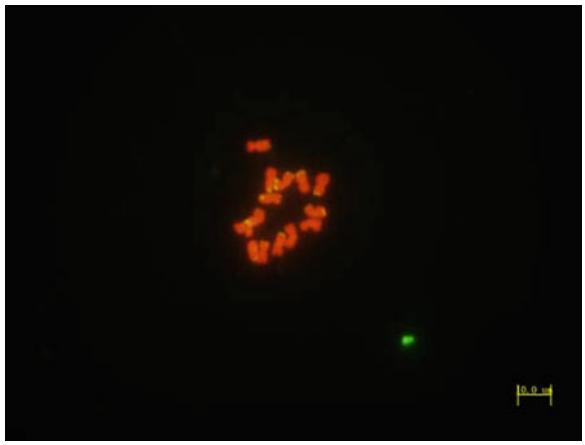

Fig. 8.2 Telomeric repetitive sequence pSc200 was used as probe to hybridize to metaphase chromosomes of *S. vavilovii*. The *yellow-green* fluorescence indicates the signals of pSc200. Scale bar: 10 μm

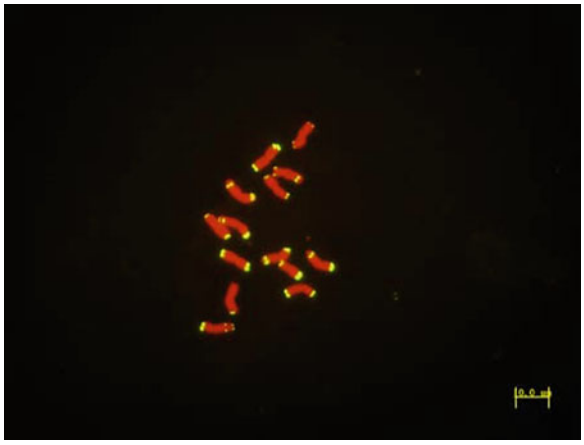

Fig. 8.3 Telomeric repetitive sequence pSc200 was used as probe to hybridize to metaphase chromosomes of Qingling rye (*Secale cereal* L.) (Chinese rye). The *yellow-green* fluorescence indicates the signals of pSc200. Scale bar: 10 μm

cultivated *S. cereale*. Of this number, about 80% are molecular markers, 12% biochemical markers, and 8% mapped morphological features. The genetic linkage maps of rye are based on restriction fragment length polymorphism (RFLP), SSR, AFLP, RAPD, sequence-specific amplified polymorphic (SSAP) markers, EST-derived *S. cereale* microsatellites (SCM), intermicrosatellites (SCIMs) or intersimple sequence repeat (ISSR), isozyme loci, quantitative trait loci (QTL), and gene loci representing individual mapping populations (see Appendix 1 for references). The first linkage map of all seven rye chromosomes was based on RFLP markers, which was performed using a population of 120 F_2 plants, or their F_3 derivatives, from the cross between the inbred rye lines DS2 × RXL10 (Devos et al. 1993). This RFLP-based genetic map provided evidence for multiple evolutionary translocations in the rye genome relative to that of hexaploid wheat (Devos et al. 1993).

A consensus map was constructed for all seven rye chromosomes by combining published data from 12 individual linkage maps (see Appendix 1) consisting of RFLP, isozyme, and gene loci (Börner and Korzun 1998). The Börner and Korzun (1998) consensus map contained 374 RFLP, 24 isozyme, and 15 gene loci determining the traits reduced plant height, self fertility, male sterility restoration, vernalization response, resistance against powdery mildew, chlorophyll deficiency, hairy leaf sheath, hairy peduncle, waxy endosperm, waxless plant, and absence of ligules (Gustafson et al. 2009).

A comprehensive map of genes, markers, and linkage data of all seven rye chromosomes has been constructed and updated by Schlegel and Korzun (2009), and these data can be obtained from the web site *http://www.desicca.de/Rye%20gene%20map/index.html*. At present, no mapping of the various wild species has been attempted.

8.6 Role in Crop Improvement Through Traditional and Advanced Tools

8.6.1 *Traditional and Molecular Breeding Efforts*

Although an important source of calories in human and animal diets and a reservoir of desirable agronomic characters, the *Secale* genus has been underresearched and undertilized when compared with other cereal grains. The *Secale* spp. pose a number of problems for both conventional and molecular breeding and have been among the most resistant to genetic transformation. There is no shortage of potential value-added traits to be found in the wild *Secale* spp. In Poland, where rye is a major cereal crop, scientists of the Botanical Garden of the Polish Academy of

Sciences in Warsaw assessed the *Secale* germplasm collection (Kubiczek et al. 1981; Molski et al. 1981) for total protein content and amino acid composition. Kubiczek et al. (1981) examined four wild perennial species (*S. chaldicum* Fed., *S. kuprijanovii* Grossh., *S. anatolicum* Boiss., and *S. montanum* Guss.) and five wild annuals (*S. sylvestre* Host., *S. ancestrale* Zhuk., *S. afghanicum* Vav., *S. dighoricum* Vav., and *S. segetale* Roshev.) as well as the high protein *S. cereale* cv. "Boney," the low protein cv. "Dankowskie Zlote," and the interspecific amphiploids. The wild *Secale* spp. were found to contain higher total protein and higher percentages of the amino acids that are limiting in rye cultivars and in cereals, in general. In particular, it was discovered that the wild species had a higher percentage of sulfur-containing amino acids and therefore, methionine was not limiting. Other limiting amino acids, lysine, isoleucine, and threonine, were less so in the wild rye species. Cytoplasmic male sterility was induced in cultivated rye by interspecific crosses with *S. montanum* by Lapinski (1972).

Deliberate wide hybridization and introgression are traditional tools for crop improvement; however, relatively a few studies have involved gene transfer from the wild *Secale* spp. to *S. cereale* because of problems caused by linkage drag associated with various wild characters such as small kernal size and brittle rachis. According to Stutz (1957), the likelihood of introgression from natural hybridization between the outbreeders, *S. strictum* (syn. *montanum*) and *S. cereale* in areas where the species coexist, would explain some of the variability found in *S. cereale* and the weedy species. However, useful introgression between S. *cereale* and *S. montanum* is hampered by at least two translocations involving three chromosomes, which separate the genomes (Stutz 1957; Devos et al. 1993). On the other hand, improvement of *S. montanum* for forage, reclamation, and other uses has relied on introgression of traits such as increased kernal weight, nutritional values, vigor, and earliness from selected *S. cereale* cultivars. In spite of the difficulties, Reimann-Philipp (1986) praised the potential of "perennial rye" (*S. cereale* × *S. montanum*) as an alternative crop for low-input farming systems, and a number of efforts to improve the quality of pasturage have involved crosses between *S. cereale* and *S. montanum*.

The agronomic importance of *S. montanum* is restricted by its relatively short life span, fragile rachis (and associated difficulty of seed production), and sparse vegetation. The potential to improve the forage quality of *S. montanum* through induced mutations and through the production of autotetraploids was considered by Akgün and Tosun (2004, 2007). In a cytogenetic study, Akgün and Tosun (2007) induced tetraploidy using colchicine in order to correlate seed set with meiotic behavior. The most vigorous plants were selected for seed set over three generations with the result that seed production progressively increased as did meiotic regularity, and the differences in seed set between and among generations was statistically significant ($P < 0.01$), indicating practical breeding applications for autotetraploid rye.

Mountain rye (*S. strictum* syn. *montanum* Guss.) is a cool temperature, perennial (Andersen et al. 1992) known to be frost- and drought-resistant and yet the intergeneric amphiploid with wheat was found to be tolerant to waterlogging (Taeb et al. 1993). *S. montanum* is an important species endemic to the nickel-rich Serpentine soils of northwestern Iran (Hajiboland 2007; Hajiboland and Manafi 2007). In hydroponic experiments with high concentrations of nickel (Ni), Hajiboland (2007) found that *S. montanum* accumulated Ni in both roots and shoots, and its tolerance was associated with high endogenous cysteine and Ni-induced cysteine accumulation. High amounts of Ni accumulated in the LMW fraction of *S. montanum* indicated chelation with cysteine or other low molecular weight compounds. In susceptible species, accumulation occurred in the HMW fraction, indicating Ni association with proteins and structural macromolecules. Translocation of Ni from roots to shoots and from older leaves to younger leaves also differed between susceptible and tolerant plants. In *S. montanum*, mature leaves served as a sink for Ni with no retranslocation to younger leaves. Peroxidase and catalase activity were lower in *S. montanum*, whereas activity of polyphenol oxidase was induced by Ni, pointing to the possibility of lignification as a mechanism of tolerance. *S. montanum* also exhibited tolerance to the heavy metal, Zinc (Zn) (Hajiboland 2007).

S. montanum is considered a useful rangeland species in the semi-arid western United States where it germinates vigorously under a wide range of conditions (Buman and Abernethy 1988) and has been found to be a successful competitor against downy brome (*Bromus tectorum* L.) in southern Idaho and

with bromegrasses (*B. tectorum* and *B. japonicus* Thunb) that dominate old surface coal mine sites in southeastern Montana (Andersen et al. 1992). Bromegrasses are invasive, short-lived annual species that provide early spring pasturage in the western mountains of the US, but suppress more desirable perennial grasses. A variety of measures to control downy brome have proven unsuccessful. However, in plantings of mixed perennial species, *S. montanum* was found to successfully compete as a seedling with the invasive weeds. But after the second year, *S. montanum* died back enabling bromegrass to reemerge. Mountain rye was thought to have potential for use as a biocontrol for brome, provided that accessions with greater longevity and other favorable characteristics could be found (Buman et al. 1988) and was deemed useful for temporary weed control, soil enrichment, and erosion control.

Early efforts to transfer useful genes from rye to wheat involved the construction of addition and substitution lines, which were used as a bridge to induce translocations (Lukaszewski and Gustafson 1983; Lukaszewski 2000). Once the mechanism for pairing control of wheat chromosomes was described, more direct methods of chromosome engineering became available (Riley et al. 1968; Sears 1972, 1981; Miller et al. 1994). The use of wild *Secale* germplasm for gene transfer has been limited in spite of numerous favorable characteristics, such as resistance to biotic and abiotic stress (Lukaszewski and Gustafson 1987; Jouve and Soler 1996), primarily owing to linkage drag. In 1915, Jesenko reported on the first hexaploid triticale produced by crossing *Triticum dicoccoides* with *S. montanum* and a hybrid of *S. montanum* × *S. cereale*. The first reported hexaploid triticale in USSR by A.I. Derzhavin was a hybrid between *T. durum* and *S. montanum* (Jouve and Soler 1996). Intergeneric hybrids results mostly in sterile offspring but may be useful to produce CMS lines and for hybrid rye breeding (Lapinski 1972). Several methods to reduce the size of the alien chromatin segment introduced into wheat chromosomes have been devised including the use of ionizing radiation, tissue culture, and gametocidal genes (Sears 1956, 1981; Lapitan et al. 1984; Endo 1988; Tsujimoto and Noda 1988). Studies were carried out on *S. montanum* and substitution and translocation lines involving wheat and *S. montanum* (Miller 1973; Montero et al. 1986; Cuadrado and Jouve 1995). Intergeneric hybrids and amphiploids are useful for studying gene expression of useful traits in alien cytoplasm. In Taeb et al. (1993) genetic variation for waterlogging tolerance among several grass species, including intergeneric amphiploids with Chinese Spring (CS) wheat and various alien addition lines, the CS–*S. montumum* amphiploid produced significantly longer roots under anoxia than either CS or *S. cereale*.

The transfer of desirable agronomic traits from rye to wheat might presumably be accomplished more readily by crossing wheat and the wheat–rye amphiploid, triticale (× *Triticosecale* Wittmack). Several Russian hexaploid triticales with the rye genome from *S. strictum* (syn. *montanum*) exhibited strong resistance to Russian wheat aphid (RWA) (*Diuraphis noxia* Mordvilko) (Bennett et al. 1978; Nkongolo et al. 1996; Lukaszewski et al. 2001). Nkongolo et al. (1996) crossed two of these lines with wheat-producing sterile hybrids, which were backcrossed and then selfed and screened for RWA reaction. All resistant lines and a few susceptible contained rye chromosome 4R from *S. strictum*, which was determined to carry a dominant allele for the type of resistance carried by PI 386150. Lukaszewski et al. (2001) attempted to transfer RWA immunity from PI 386148 into wheat by induced homoeologous recombination to identify the chromosome conferring resistance. Further homoeologous recombination was induced by crossing a wheat–rye translocation showing resistance with a *ph1b* mutant line of Pavon. Recombinant lines identified by C-banding and resistance was determined to be on chromosome arm 4RL. Their conclusion was that infrequent recombination and poor homology between donor and recipient made the transfer of small segments of rye chromatin by induced homoeologous recombination impractical. Nkongolo et al. (2009) identified the rye chromosome conferring resistance as 1R using GISH and rye-specific microsatellites. The uncertainty about the number and location of the RWA resistance loci in Nkongolo et al. (2009) and earlier studies may derive from the reliance on GISH without C-banding to identify the chromosomes.

Numerous wheat cultivars carrying the 1R chromosome or 1RS arm from *S. cereale* have been released worldwide (Villareal et al. 1994). However, the germplasm base of the introgressed rye chromatin is very narrow. In an extensive review of the pedigrees of this introgressed material, Rabinovich (1998) found that the rye came primarily from four sources: two German wheats, one Japanese, and one from the USA.

According to Schlegel and Korzun (1997), the donor of rye chromatin in the majority of wheat cultivars with 1R or 1BL.1RS can be traced back to the single rye variety "Petkus." The stripe rust (*Puccinia striiformis* f. sp. *tritici*) gene *Yr9* on Petkus-derived 1R and 1BL.1RS in wheat cultivars was initially an important source of race-specific resistance to stripe rust, but is no longer effective in most wheat-growing areas in China and elsewhere (Mago et al. 2002; Li et al. 2006). Ko et al. (2002) selected a new 1BL.1RS translocation using a Korean rye variety as the source. The use of *S. africanum* as a new source of 1RS was investigated by Yang et al. (2009). Two wheat–*S. africanum* amphiploids were analyzed using GISH and FISH, and non-Robertsonian translocations between the wheat and *S. africanum* were detected. F_2 introgression lines were evaluated in the field for resistance with very virulent strains of stripe rust. Three highly resistant lines were selected, one of which was found to contain a 1BL.IRS translocation. In addition to adding a new source of resistance genes, the ease with which the translocation was found may be related to the reduced heterochromatin in the *S. africanum* genome (R^a) and to the low level of tandem repeats of pSc250, which were detected only on subtelomeric regions of *S. africanum* chromosome $6R^a$. This material offers a new source of 1RS resistance useful for breeding programs.

8.6.2 Somatic Hybridization and Genetic Transformation

Rye is known to be highly recalcitrant to tissue culture, making somatic cell regeneration difficult (Rybczyński and Zduńczyk 1986; Rybczyński 1990). De la Pena et al. (1987) reported a method for rye transformation without single cell culture and regeneration, by injecting DNA directly into immature floral tissues; however, this method was not substantiated. Castillo et al. (1994) used particle bombardment on embryonic callus tissue from immature embryos to create a few stable transformations. Rakocyz-Trojanowska and Malepszy (1995) determined that regeneration of rye in vitro is controlled by recessive genes. The recalcitrance of *Secale* spp. to somatic cell regeneration, which has restricted the development of transgenic rye using *Agrobacterium tumefaciens*, seems to have been surmounted. Recently, Herzfeld (2002) described a genetic transformation protocol for rye involving biolistic and *Agrobacterium*-mediated gene transfer. Popelka and Altpeter (2003) and Popelka et al. (2003) and Altpeter (2006), using a time-critical strategy for embryo culture and avoiding the stumbling-block of herbicide selection in the medium, succeeded in producing a high number of single-copy transgene inserts. No attempts to use the wild species for DNA transformation have been reported although *S. vavilovii* was reported to be less recalcitrant to androgenic regeneration than *S. cereale* (Fleinghaus et al. 1991).

8.6.3 Agronomically Useful Traits

Wild ryes are a potential source for value-added traits such as those for high protein content, disease resistance, and other morphological and biochemical traits, for wheat (*Triticum*) and triticale (× *Triticosecale* Wittmack) improvement (Chikmawati et al. 2005). *S. montanum* is tolerant of low temperatures and high levels of soil aluminum and manganese (Culvenor et al. 1985), and thus has potential as a gene source to improve other cereals (De Bustos and Jouve 2002). An interspecific hybrid (*S. cereale* × *S. montanum*) perennial pasture grass with good forge quality "Perenne" was registered in Hungary and found to have acceptable baking quality for bread-making (Füle et al. 2004, 2005). Many similar *S. montanum* derivative "PC rye" lines have been studied for drought tolerance and forage utility globally. In a test of abiotic stress response, Taeb et al. (1993), cited previously, found that among hybrids of *T. aestivum* "Chinese Spring" with a number of related genera, the amphoploid of CS × *S. montanum* produced the longest roots in waterlogged soils, showing good penetrance and little root inhibition under anoxia.

The quality of wheat bread dough is determined by glutenin proteins, which are multimeric aggregates of high molecular weight (HMW) and low molecular weight (LMW) subunits. The LMW allele *Glu* A3c was shown to have a larger effect than the HMW allele *Glu* A1b on dough properties (Gupta et al. 1989). Rye flours contain 9% secalins, consisting of HMW and gamma-75k secalin protein types (Gellrich et al. 2003), which cause rye dough to be less elastic. To

improve the quality of rye flour, wild *Secale* species may be used as a source of LMW alleles. Three LMW glutenin-like genes (designated as *Ssy1*, *Ssy2*, and *Ssy3*) were successfully isolated from and characterized for *S. sylvestre*. These three genes consist of a predicted highly conservative signal peptide with 20 amino acids, a short N-terminal region with 13 amino acids, a highly variable repetitive domain, and a less variable C-terminal domain. The LMW glutenin-like genes of *S. sylvestre* were orthologous with the LMW-GS genes of wheat and B hordein genes of barley, with functions similar to the *Glu*-3 locus in wheat and its related species (Shang et al. 2005).

Although many wheat–rye translocation lines carrying useful genes have been produced, such as insect resistance (Friebe et al. 1990, 1995a; Mukai et al. 1993; Lukaszewski et al. 2001; Mater et al. 2004), disease resistance (Lapitan et al. 1986; Friebe et al. 1995b, 1996; Mater et al. 2004), and abiotic stress resistance (Leach et al. 2006) to name a few, most have not produced successful wheat cultivars. Rye segments either do not compensate well for the loss of wheat chromatin or contain undesirable genes (Jiang et al. 1994). On the other hand, the 1BL.1RS and 1AL.1RS translocation lines are prevalent in global wheat breeding programs (Lukaszewski 1990; Schlegel 1997; Rabinovich 1998). The 1RS arm contains genes for resistance to powdery mildew (*Pm8* and *Pm17*), stem rust (*Sr31*), leaf rust (*Lr26*), and stripe rust (*Yr9*) (Jiang et al. 1994; Kim et al. 2004). In addition, 1RS arm also carries genes that positively affect agronomic traits including yield performance, yield stability, and wide adaptation (Schlegel and Meinel 1994; Moreno-Sevilla et al. 1995; McKendry et al. 1996; Kim et al. 2004). The distribution of the wheat–rye translocation 1BL.1RS was studied in 31 winter wheat varieties from Bulgaria and the presence of the translocation was verified in 17 varieties (Landjeva et al. 2006). Approximately 69.4% and 38% of the wheat cultivars in Southwest and North China, respectively, which were released in the 1980s and 1990s, were found to carry the 1BL.1RS chromosome originating from the cultivar "Aurora" and "Kavkaz" (Yang and Ren 1997; Zhou et al. 2004). Nevertheless, a very few sources of 1RS comprise the progenitors of hundreds of commercial wheat (Schlegel and Korzun 1997; Rabinovich 1998; Zhou et al. 2004; Landjeva et al. 2006). Bartos (1993) reported that the resistances provided by genes *Lr26*, *Yr9*, and *Pm8* carried on that rye chromosome are no longer completely effective. Virulence against *Yr9* and *Pm8* has been reported in Southwest China (Yang and Ren 1997; Yang et al. 2003). Villareal et al. (1998) reported that genetic vulnerability was the consequence of the narrow genetic base of the 1RS chromosome arm from cv. Petkus rye widespread in the 1BL.1RS cultivars. Although some new 1BL.1RS translocation lines have been developed (Ko et al. 2002; Tang et al. 2008b; Yang et al. 2009), the critical step is the development of successful wheat cultivars. Therefore, it is imperative that production of more sources of resistance becomes a high priority for wheat breeding programs.

Bread baking quality is reduced by the presence of secalins in 1RS.1BL or 1RS.1AL translocated wheat (Graybosch 2001). The methodology to reduce the length of alien segments thereby reducing the introduction of deleterious genes was described many years ago (Sears 1981). Lukaszewski (2000) used the *ph1b* mutation to induce further recombination with wheat group 1 chromosomes. Ribeiro-Carvalho et al. (2001) reported spontaneous introgression of very small rye segments into chromosome arm 2DL of the Portuguese wheat landrace "Barbela." Nejad et al. (2002) have successfully transferred small rye chromosome segments (1R satellite) into wheat chromosomes 2A, 2D, 3D, 5D, and 7D using a gametocidal system. Wheat–rye translocation resulting from genetic instability caused by monosomic addition of rye chromosome in wheat was described by Ren and Zhang (1997).

The genetic variability of 1RS is another important consideration in breeding programs. For example, the *Pm8* gene for resistance to powdery mildew derived from Petkus rye is different from *Pm17* derived from Insave, and the *SrR* gene for resistance to stem rust derived from Imperial rye differs from *Sr31* from Petkus. The resistance gene in Amigo wheat is derived from Insave. Thus, surveys of the potential variability in disease resistance genes in chromosome 1RS in different rye species and genotypes would be useful. Kim et al. (2004) indicated that the effect of source of 1RS on agronomic performance was greater than its position effect in wheat genome, and they reported that those with 1RS derived from "E12165" (CIMMYT) and "Amigo" induced higher mean grain yield and T1DL.1RS derived from "BH1146/Blanco rye" had the lowest grain yield. Yan et al. (2005) obtained a wheat line 843-1-1 carrying a pair of 1BL.1RS chromosomes lacking expression of the Sec-1 site, indicating wide genetic variability for

certain traits, which can be exploited by the wheat breeders. Rye chromosome 1RS is likely to have continued importance despite the loss of disease resistance.

For wheat breeding programs, technologies for screening wheat lines containing alien chromatin are very important. C-banding technique cannot detect small segments of translocated rye chromatin. The technique of ISH with total genomic DNA may be helpful in detecting small-segment translocations in some situations; however, the method has resolution limits (Lukaszewski et al. 2005). Katto et al. (2004) developed a PCR-based marker to detect various segments of rye chromosome incorporated into wheat, which can be utilized in a large-scale screening for wheat accessions carrying rye segments that are too small to detect cytologically. In addition, some PCR-based markers located on individual rye chromosomes or chromosome arms have already been obtained (de Froidmont 1998; Seo et al. 2001; Chai et al. 2006; Kofler et al. 2008; Liu et al. 2008; Tang et al. 2008a) and are useful for the detection of individual rye chromosome segment in wheat background.

8.7 Genomics Resources

8.7.1 Databases

The International Nucleotide Sequence Database Collaboration (INSDC) (*http://www.insdc.org/page.php?page=home*) serves as a portal for the cooperating partners EMBL-Bank/GenBank/DDBJ databases: The DNA Data Bank of Japan (DDBJ), Michima, Japan (*http://www.ddbj.nig.ac.jp/*); the European Molecular Biology Laboratory (EMBL) Nucleotide Sequence Database (EMBL-Bank), Cambridge, UK: (*http://www.ebi.ac.uk/embl/*); and the National Center for Biotechnology Information (NCBI) or GenBank, Bethesda, MD, USA (*http://www.ncbi.nlm.nih.gov/*).

As of May 2009, sequence data and references for 158 nucleotide accessions and 39 protein accessions of wild *Secale* spp. were to be found in the public database NCBI in addition to over 1,400 nucleotide accessions of cultivated and weedy ryes. The nucleotide accessions include 17 designated as *S. strictum*, 17 as *S. strictum* subsp. *kuprijanovii*, 15 as *S. strictum* subsp. *anatolicum*, four as *S. strictum* subsp. *strictum*, 26 as *S. strictum* subsp. *africanum,* 40 as *S. montanum*, two as *S. montanum* subsp. *montanum*, 32 as *S. sylvestre* and four intergeneric hybrids with *S. strictum* subsp. *africanum,* and one with *S. sylvestre* (see Appendix 2 for nucleotide sequences and related references). The protein accessions include 23 retrieved as *S. strictum*, seven as *S. montanum*, and nine as *S. sylvestre*. The most frequent use of these sequences has been for phylogenetic studies. In addition, a number of the rye sequences represent genome-specific markers useful to detect introgression and translocation involving small segments of rye chromatin.

Protein sequences and related data, which have been submitted by the authors to the EMBL-Bank/GenBank/DDBJ databases, are integrated into UniProtKB/TrEMBL. UnitprotKB Protein Knowledgebase (*http://www.uniprot.org/*) currently lists 35 wild *Secale* spp. protein sequence accessions.

GrainGenes (2008) (*http://wheat.pw.usda.gov/GG2/index.shtml*) provided by the Agricultural Research Service of the US Department of Agriculture is an integrated package of databases, curation services, documents, internet links, and news in support of Triticeae and Avena research. Taxonomic information, genetic maps and linkage data, sequence annotation, images, references, etc. are submitted by contributors and queried by users.

Gramene (2009) (*http://www.gramene.org/species/index.html*) provides a comparative mapping resource for grains. Gramene *Secale* lists genes and chromosome locations cross-referenced with GenBank accessions numbers, UniProt accession numbers, and GrainGenes sequence numbers (*http://www.gramene.org/species/secale/rye_stat.html*). A total of 132 markers are listed for wild and weedy species of rye.

International Triticeae EST Cooperative (ITEC) (*http://wheat.pw.usda.gov/genome/*) hosts a public database of expressed sequence tags (ESTs), which have been deposited with the NCBI GenBank dbEST (ITEC 2007).

8.7.2 ESTs, Transcripts, Metabolites, Gene Sequences, Mutants, BAC Sequences

Compared to wheat and barley, the rye EST database is small. NCBI/GenBank listed 9,298 ESTs of rye as of

May 2009 compared to more than one million for wheat and more than half a million for barley. The Dana-Farber Cancer Institute (DFCI) Gene Index Project, which integrates research data from international EST sequencing and gene research projects, includes a Rye Gene Index (RyeGI) for *S. cereale* but presumably would include all rye sequences submitted. The DFCI RyeGI attempts to amass non-redundant sequences of all Rye genes and data on their expression patterns, cellular roles, functions, and evolutionary relationships (*http://compbio.dfci.harvard.edu/tgi/cgi-bin/tgi/gimain.pl?gudb=s_cereale*). As of July 3,2008, the site lists 9,152 ESTs. The total number of unique sequences for rye including singleton EST and tentative consensus sequences was 5,587, which is the smallest number of sequences among all species included in the Gene Index Project (DFCI 2008).

Gene sequences of rye such as 5S ribosomal gene (Reddy and Appels 1989) and ω-secalins gene (Hull et al. 1991) are also available. In addition, 661 gene sequences of rye can be obtained from NCBI. Šimková et al. (2008) constructed a deep coverage BAC library specific for the short arm of chromosome 1R, which will facilitate 1RS – wheat breeding. A BAC library of *S. cereale* cv. Blanco has been created that represents a valuable resource for *Secale* molecular studies. The library is composed of a 6 × genome coverage of *S. cereale*, which has a 8.1 Gb genome. The BAC clones have an average insert size of 131 kb, and the library is composed of ~2% of empty or organelle derived clones. Shi et al. (2009) analyzed several BAC clones containing allelic forms of genes at or near the *Alt4* locus, which revealed heterozygosity within cv. Blanco.

8.8 Scope for Domestication and Commercialization

8.8.1 Therapeutic Compounds, Herbal Drugs, Dietary Supplements, etc.

Little is to be found in either the scientific or popular literature about therapeutic uses of wild *Secales*. However, the cultivated ryes are known to contains a high concentration of dietary fiber (16.1 g/100 g), the major components being arabinoxylan (60%), cellulose (15%), and β-glucan (9%) (Nilsson et al. 1997). The effect of *S. cereale* in a high fat diet on the development of colonic cancer was studied in F-344 rats treated with the carcinogen azoxymethane (Davies et al. 1999). Leinonen et al. (2000) indicated that *S. cereale* bread offered a practical dietary means of reducing serum cholesterol in men. Linko et al. (2005) analyzed alklresorcinols (Ars) in human plasma after a controlled, 8-week crossover intervention study with intake of whole-grain *S. cereale* and found that introducing whole-grain rye into the diet increased plasma AR levels. Ars have several biochemical properties such as antimicrobial and anticarcinogenic activities (Kozubek and Tyman 1999). Concentrations of Ars in whole grain *S. cereale* have been found to vary between 360 and 3,200 μg/g (Ross et al. 2001). The pollen extracts of rye have been found to contain high concentrations beta-sterols and sterolins. Commercial products containing these extracts have been widely used in Europe and Japan to alleviate prostate enlargement, prostate pain, and chronic prostatitis. Some studies have found that rye pollen extracts modestly improve urological symptoms associated with prostatitis, including nocturia (MacDonald et al. 2000). In addition, the content of foliates in rye is relatively high, provided these bioactive compounds are not discarded in the milling process. Germination or malting increases foliate synthesis, but the compounds are degraded by heat and other processes required for food preparation. Kariluoto et al. (2006) investigated the effects of temperature and germination on foliate content, concluding that the foliate profile of germinated rye more resembled that of vegetables than of cereal grains and that novel methods of food preparation could retain bioactivity. Nyström et al. (2008) studied a selection of ten European rye varieties, including modern high-yielding cultivars and older, non-commercial populations and found significant variation among the lines for dietary fiber (arabinoxylan and beta-glucan) and phytochemicals (folate, tocols, phenolic acids, alkylresorcinols, and sterols). This indicates a potential for improvement of rye for health benefits.

The fungus *Claviceps purpurea* has been identified as the causal agent of ergotism in rye and other grasses, for which there seems to be no resistance among the wild species of *Secale* (Bushuk 2001). Reduced fertility in interspecific and intergeneric

hybrids might increase susceptibility to infection and development of the fungal schlerota. The use of ergot as a specific remedy for migraine headaches evolved during the twentieth century (Eadie 2004). The drug ergomatine was widely used until triptan derivatives were introduced. Tfelt-Hansen et al. (2000) concluded that ergotmaine was no longer the drug of choice for the treatment of migranes and that its continued use as a pharmaceutical is unlikely. While little information is available about therapeutic uses of the wild *Secale* species, they may be viewed as a source of value-added traits to enhance the nutritional and medicinal benefits of commercial varieties for human and animal populations.

8.8.2 Industrial Uses

Rye, predominantly *S. cereale*, is grown for distilling into whiskey in the United States, Canada, and Europe (Bushuk 2001). *S. montanum* is sometimes malted for beer (Pomeranz et al. 1973). Rye and rye malt are used by distillers for whiskey in the US, for gin in Holland, and for beer in Germany and Russia. In the US, Rye whiskey, by law, may contain no less than 51% rye in the mash (US Government Printing Office 27CFR5). In Russia, Ukraine and eastern European countries, a traditional, fermented, non-alcoholic (or marginally alcoholic) soft drink called Kvass is popular. One of a very few cereal-based beverages, Kvass production uses either dried sourdough rye bread or rye mash as raw materials (Dlusskaya et al. 2008). Rye is also used for the production of non-potable alcohols ethanol and acetone-butyl-alcohol (Wickens 2004). The wild *Secale* species have not been widely explored for medical, industrial, or other value-added uses.

8.8.3 Introduction as Alternative Crops

Rye contains primary phytotoxic compounds including Cyclic hydroxamic acids (benzoxazinones), which have allelopathic effects on a number species including other grasses (Fomsgaard et al. 2004). The potential for exploiting the allelopathy of *S. strictum* (syn. *montanum*) for alternative weed management and cropping systems has been mentioned previously (see Sect. 8.6.1).

Abiotic and biotic stresses trigger some of the same defense mechanisms in plants (Zhang et al. 2006). The general tolerance of the wild *Secales* for marginal environmental conditions and resistance to pathogens raises interest in a variety of studies on the mechanisms of stress response. Antifreeze proteins that retard the formation of ice-crystals in plant tissues in subzero temperatures can be extracted from winter rye leaves (Hon et al. 1994, 1995). Again, most of the research in these areas has focused on *S. cereale*.

Cultivated rye (*S. cereale*) as a cover crop fits well into many erosion control programs and can protect agricultural land over winter. Rye has been used successfully as to fill gaps between other crops. Moreover, as a green manure crop, rye is particularly suitable because of its winter hardiness and frost tolerance, its high germinability, and rapid growth early in the spring. The potential for wild species to be used in self-regenerating, permanent pastures (Oram 1996) has not been fully explored.

8.9 Problems and Remedies

Troublesome characteristics of the wild species include small seed size, shattering and pre-harvest sprouting, divergent breeding systems, including both selfing and self-incompatible species, and a range of life cycle habit from annual to short lived perennial. The wild species exhibit varying degrees of susceptibility to typical cereal diseases including leaf rust (*Puccinia recondita*), stem rust (*P. graminis* f. sp. *secalis*), ergot (*Claviceps purpurea*), leaf blotch (*Rhynchosporium secalis*), Fusarium diseases, and soilborne viruses (Cox et al. 2002).

Linkage drag has limited the usefulness of the wild *Secale* spp. for wheat, rye, and triticale improvement using conventional breeding strategies. It has hampered efforts to increase the nutritive value of cultivated rye thru interspecific crosses. *Secale* spp. pose other challenges depending on the breeding objective. The plant cell walls contain soluble pentosans that can cause feeding problems when large amounts of rye are used in animal feed. For instance, rye pentosans are linked to depressed weight gain and nutritional and digestive developmental abnormalities in poultry (Lázaro et al. 2004). Pentosans also present some disadvantages for brewing (Wickens 2004).

Rye storage proteins, the secalins, have been implicated in human immunoreactive celiac (also: coeliac) disease. The wild species exhibit a siminar secalin profile as the cultivars (Yang et al. 2001b) and are presumed to be equally toxic to susceptible individuals. Shan et al. (2002) have found a 33-mer peptide that appears to be the primary inducer of the inflammatory response in celiac sprue patients. Protein sequence alignments (BLASTP searches) found significant homology only with gliadins, hordeins, and secalins, the toxic food-grain proteins.

Rye species are known to hybridize in the wild, and wheat–rye varieties are commercially available as triticales and perennial hybrids. Spontaneous wheat–rye introgression is of concern, with respect to erosion of genetic diversity and to the development of transgenic rye. In order to assess ecological risks and to formulate strategies to manage gene transfer to hybrids, Hedge and Waines (2004) studied introgression between wheat and its wild relatives. They reported that a few natural introgressive hybrids between herbicide-resistant wheat and rye have been created or recovered in North America. A greater understanding of reproductive ecology would seem to be warranted.

8.10 Conclusion and Recommendations for Future Action

8.10.1 Conclusion

While providing an important cereal staple and a source of value-added traits and for expanding the genetic base of other cereal species, the genus *Secale* is generally afforded a secondary status with respect to collections, preservation and conservation, and the research support necessary to carry out these vital activities. Considering its value as a crop and its considerable importance for wheat improvement, the *Secale* genus is a fertile arena for future work. Collections of species types are limited, old, and difficult to locate even with the enormous resources of the biodiversity initiatives, consortiums of museum herbaria, and global surveys. Available *Secale* passport information from the past is inconsistent and idiosyncratic. Germplasm collections are weak in breadth of species, are too few to adequately serve the entire producing community, and exhibit uneven record keeping. This is not a reflection on the dedication of hardworking scientists in the field but of the general perception of rye as a secondary crop. Resources applied to the conservation of the *Secale* genus, germplasm collections, taxonomic studies, and experimental research are most complete in the countries where rye has been a vital food and agricultural resource.

8.10.2 Recommendations for Future Action

As the global community develops greater collective appreciation of the need for action to protect and preserve biodiversity, it is hoped that the following areas of need, with respect to the *Secale* genus, will be addressed:

1. Advanced scientific training in basic botany and taxonomy of the genus, field botany and scientific collection of specimens and germplasm, ecology, and in situ conservation, cytological and molecular genetics, bioinformatics, and biotechnology.
2. Cooperative, multiple site collection, and curation efforts with small, in-country germplasm and herbaria specimens maintained to the specification of the museum and national germplasm banks.
3. Complete, standardized passport information (using the FAO/IPGRI multicrop passport descriptors), automatically digitized, in the field where possible, and entered into local and international searchable, interactive databases. Digital, cellular, and GPS technologies can be applied.
4. In situ and ex situ conservation of species in critical wild ecosystem, natural grassland preserves, botanical gardens, seed storage facilities and genomic.
5. Increase studies involving gene transfer from the wild species of the genus *Secale* to related crop species, especially wheat. For specific recommendations regarding breeding programs, see Sect. 8.6, particularly Sect. 8.6.3.

If the Millennium Development Goals (MDG) of the United Nation are to be achieved by the deadline of 2015, practical steps need to be taken in every area. One contribution would be the achievement of Goal #7 "Ensure Environmental Sustainability" Target 9 "Reverse Loss of Environmental Resources" with respect to all of the wild species of the genus *Secale* especially *S. africanum* (= *S. strictum* subsp. *africanum*).

Appendix 1

Rye Genetic Map References by Year of Publication

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Consensus Map by Börner and Korzun (1998) from 12 Individual Linkage Maps

Devos et al. (1993)
Plaschke et al. (1993)
Plaschke et al. (1995)
Wanous and Gustafson (1995)
Wanous et al. (1995)
Korzun et al. (1996)
Loarce et al. (1996)
Senft and Wricke (1996)
Korzun et al. (1997)
Börner et al. (1998)
Korzun et al. (1998)
Voylokov et al. (1998)

Appendix 2

Wild *Secale* Nucleotide Sequence References (From: *http://www.ncbi.nlm.nih.gov/*)

Secale strictum

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Chapter 9
Sorghum

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9.1 Basic Botany of the Species

The *Sorghum* genus is found in the family Poaceae, tribe Andropogoneae, with the cultivated *Sorghum bicolor* being the most well known species. Selection for superior characteristics, either for food or feed crops, has resulted in distinct classes of sorghum varieties. The genus consists of 25 species and has been split into five sections: *Eu-Sorghum*, *Chaetosorghum*, *Heterosorghum*, *Para-Sorghum*, and *Stiposorghum*. Cultivated *Sorghum* is divided into five distinct races, namely, *bicolor*, *caudatum*, *guinea*, *durra*, and *kafir*. The broad geographical distribution of the five sections of the *Sorghum* genus has been well described and summarized by Price et al. (2005). The *Eu-Sorghum* is distributed throughout Africa and southern Asia. Species within the *Para-Sorghum* are found in Asia, Australia, and Central America. *Chaetosorghum* and *Heterosorghum* are monotypic and native to Australia and Southeast Asia. In comparison, the *Stiposorghum* are comprised of ten species only found in northern Australia. Most of the Australian *Sorghums* are found across the tropical and subtropical northern belt of Australia, with only *S. leiocladum* being widespread across eastern Australia.

S. bicolor ($2n = 4x = 20$), cultivated sorghum, is an important cereal, pasture crop and is closely related to maize and sugarcane (Dillon et al. 2007; Paterson et al. 2009a). It is the world's fifth most widely grown cereal crop after wheat, rice, maize, and barley. Sorghum has been used for human consumption by ancient tribes, dating back to 8000 BC. Originating from Africa, Ethiopia is reported to be the center of genetic diversity for the species. The progenitor species of cultivated sorghum may include *S. arundinaceum*, *S.* × *drummondii*, *S. halepense* ($2n = 20$), and *S. propinquum* ($2n = 20$). Sorghum is one of the oldest cultivated crops and is currently grown in over a hundred countries. Annual production of cultivated sorghum is only about 60 million tons, which is a much lower level of production than the major cereal crops wheat and rice (Sasaki and Antonio 2009). This is partly because sorghum and its wild relatives have not been exploited to their true breeding potential.

The species within the *Sorghum* genus fall into a primary, secondary, and tertiary gene pool. The primary and secondary gene pools consist of *Eu-Sorghum* (*S. bicolor, S.* × *almum, S.* × *drummondii, S. halepense, S. propinquum, S. arundinaceum*), while the broad tertiary gene pool consists of *Chaetosorghum* (*S. macrospermum*), *Heterosorghum* (*S. laxiflorum*), *Para-Sorghum* (*S. grande, S. leiocladum, S. matarankense, S. nitidum, S. timorense*), and *Stiposorghum* (*S. amplum, S. angustum, S. brachypodum, S. bulbosum, S. ecarinatum, S. exstans, S. interjectum, S. intrans, S. plumosum, S. stipoideum*) (Dillon et al. 2004; Price et al. 2005), which are increasingly being exploited in biotechnology and breeding programs. *S. bicolor* is strictly a short-day plant and very sensitive to photoperiod. It is cultivated in harsh conditions, on marginal land with minimum resources requiring less water (high water use efficiency, WUE) and low dosage of fertilizers/nutrients.

Dillon et al. (2001, 2004, 2007) presented the broad phylogenetic relationships within the *Sorghum* genus. They include 25 species of *Sorghum* found worldwide, which are divided into five taxonomic groups. In a

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study conducted on wild sorghum genetic variability from Ethiopia and Africa, Ayana et al. (2004) found that genetic variability among races were found to be low to moderate both on a population and regional basis. Wild sorghum is resistant to various degrees of *Striga asiatica* and, therefore, may be used for introgression of valuable genes into the cultivated species (Rich et al. 2004). They also observed that wild sorghum slows down spore germination, growth, and haustorial initiation.

Plant breeders can exploit the sorghum gene pool through crosses between wild and cultivated species in order to improve agronomic traits (Dillon et al. 2001). A number of Australian native sorghum wild species (categorized into *Eu-sorghum*, *Chaetosorghum*, *Heterosorghum*, *Para-sorghum*, and *Stiposorghum*) are resistant to insects and pests, including sorghum midge. Overall, the wild *Sorghum* species can be exploited for their genes, which may impart resistance to biotic stress (Dillon et al. 2001). This is possible by interspecific crosses, which can be made between donor (wild type) and receptor (cultivated) *Sorghum* species. Many species have been reported to be very similar to *S. bicolor* and this includes the Australian native *Sorghum* species *S. laxiflorum* and *S. macrospermum* (Dillon et al. 2004) and a distinct group found in China (De Oliveira et al. 1996).

Growth and flowering data for 12 wild *sorghums* grown concurrently in a glasshouse in northern NSW (Fig. 9.1) have been compared to *S. bicolor*. Germination takes between 3 and 10 days for *S. bicolor*, while the spread of germination amongst the wild species was 3–8 days. Days to flowering was also contrasted, with *S. bicolor* reported as 55–70 days and the wild species taking between 61 and 136 days, although three species did not flower at all during the 160 days of observation. Wild species also had a significantly increased number of seed heads per plant with up to 235 seed heads recorded for *S. exstans* (Dillon et al. 2007).

9.2 Conservation Initiatives

9.2.1 In Situ *Conservation*

Sorghum species are not specifically protected in their natural environment. Some rare species could benefit from in situ conservation efforts.

9.2.2 Ex Situ *Conservation*

Sorghum landrace and wild species have been collected from Africa, Asia, the Americas, and India since the early 1900s. The first major targeted collection of sorghum germplasm was made in 1973 by Gebrekidan and Ejeta from northeastern Ethiopia (Shewayrga et al. 2008). Since then, there have been focused collection activities to conserve *S. bicolor* landraces and traditional village cultivars, and more recently, the wild species of sorghum. These valuable collections are now conserved as seed in Plant Genetic Resource Centers around the world. The seed is maintained under medium- or long-term storage conditions (humidity and temperature controlled) to ensure that this important genetic diversity of sorghum germplasm is freely available to the global research community.

Extensive collections of *S. bicolor* cultivars and landraces are held in these centers, with increasing numbers of the wild *Sorghum* species now being represented. Table 9.1 summarizes the collections of wild *Sorghum* species held in the major Plant Genetic Resource Centers globally. From here, it is clear that the primary and secondary gene pools of sorghum (*Eu-Sorghum* species) are well represented. These species have a few genetic incompatibilities with *S. bicolor*, and some of these species have been extensively used in genetic studies and mapping. The tertiary gene pool *Sorghum* species are conserved primarily in the Australian Tropical Crops and Forages Germplasm Centre (*www.dpi.qld.gov.au/auspgris*), which has been undertaking targeted wild sorghum collecting activities into northern Australia since 1980s. Some of these tertiary gene pool species are poorly represented as they are rare or located in extremely isolated areas, while other species are highly represented as they are widespread and easily accessible. Future collection activities will be targeted towards poorly represented species, and also in representing the geographic spread of these species.

9.3 Role in Elucidation of Origin and Evolution

It has been estimated that there are about 9,000 current species of grasses, which have evolved from a common ancestral species that lived 50 million years ago

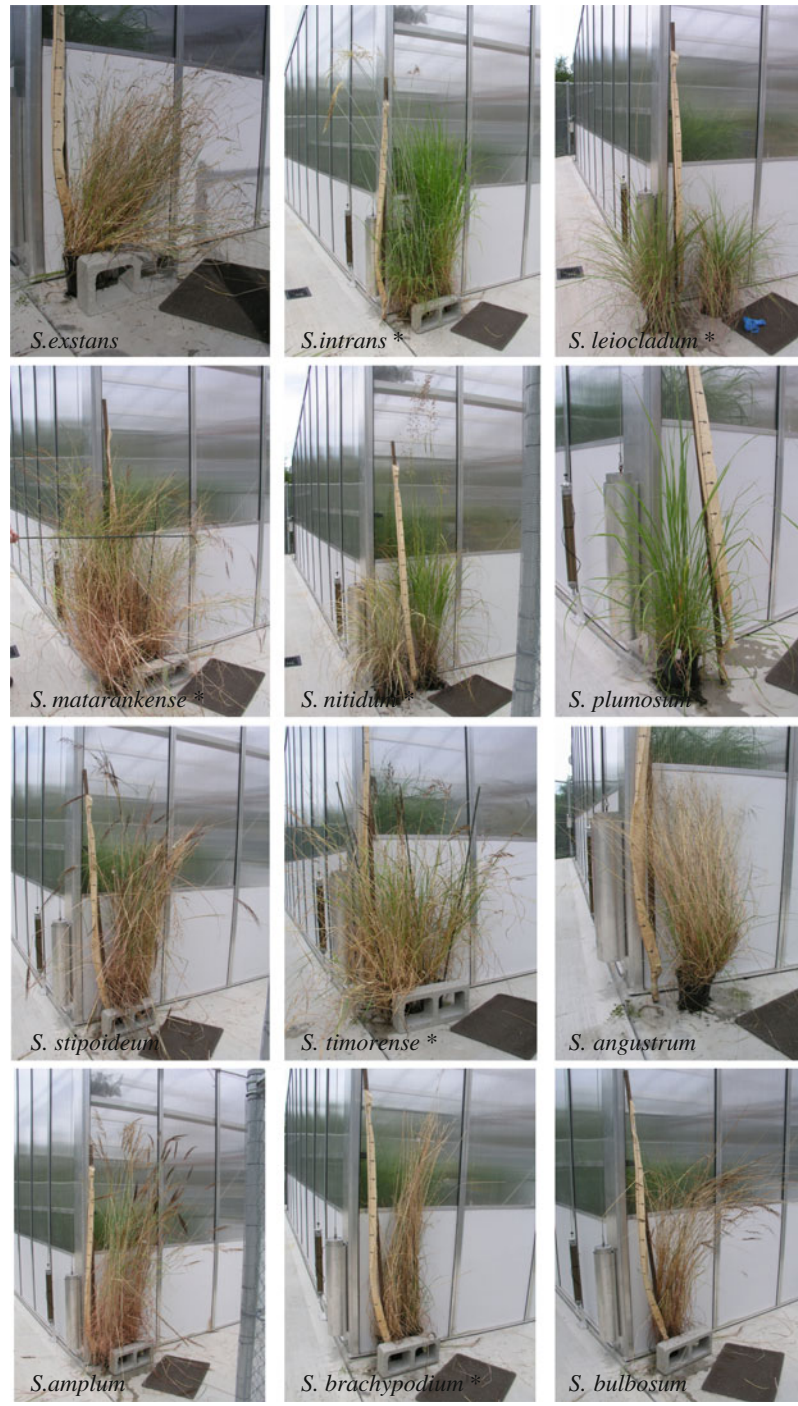

Fig. 9.1 Wild *Sorghum* species planted June 2004 and grown in a glasshouse in Northeast NSW. Species marked with an *asterisk* have two plants in the figure

(Mya) (Paterson et al. 2004; Bennetzen 2007). Evolution of the grass species has been so rapid that they have acquired widely different chromosome numbers and genome sizes. Further, Bennetzen (2007) suggested that polyploidy, segmental duplication, and subsequent gene loss are standard phenomena in grass species. He further pointed out that when any two grass species are compared, even the copy number of a given gene,

Table 9.1 Number of accessions for each wild *Sorghum* species held in the major ex situ plant genetic resources holdings globally: Australia – Australian Tropical Crops and Forages Collection, Biloela QLD Australia (AusPGRIS: *http://www.dpi.qld.gov.au/auspgris*); India – International Crops Research Institute for the Semi-Arid Tropics (ICRISAT: *http://singer.cgiar.org/index.jsp?page=taxatoz*); USA – National Genetic Resources Program (GRIN: *www.ars-grin.gov/npgs/search-grin.html*); Colombia – International Centre for Tropical Agriculture (CIAT: *http://www.ciat.cgiar.org/ http://singer.cgiar.org/index.jsp*); Africa – International Livestock Research Institute (ILRI: *http://singer.cgiar.org/index.jsp*)

Species	AusPGRIS	ICRISAT	GRIN	ILRI	CIAT
Eu-Sorghum					
S.× almum	5	–	30	11	3
S. arundinaceum	1	–	60	–	–
S. × drummondii	5	174	82	–	–
S. halepense	2	22	31	–	–
S. propinquum	1	–	1	–	–
Chaetosorghum					
S. macrospermum	3	1	–	–	–
Heterosorghum					
S. laxiflorum	24	1	2	–	–
Para-Sorghum					
S. grande	2	–	–	–	–
S. leiocladum	27	–	–	–	–
S. matarankense	9	–	–	–	–
S. nitidum	9	–	1	–	1
S. purpureo-sericeum	4	6	1	–	–
S. timorense	43	3	–	–	–
S. versicolor	7	3	4	–	3
Stiposorghum					
S. amplum	2	–	–	–	–
S. angustum	11	–	–	–	–
S. brachypodum	3	–	–	–	–
S. bulbosum	22	–	–	–	–
S. ecarinatum	12	–	–	–	–
S. exstans	4	–	–	–	–
S. interjectum	20	–	–	–	–
S. intrans	28	5	3	–	–
S. plumosum	56	1	4	–	–
S. stipoideum	42	5	2	–	–

its position, and expression may have diverged and each of the species may have an individual lineage. Most grasses are polyploids, and large expansion of genome size has taken place by gene duplication and the presence of multiple transposable elements (Class I mobile elements, retrotransposons, long sequence repeats, LSRs), which has led to divergence (Moore et al. 1995). These evolutionary processes within the Poaceae help explain the diversity of *Sorghum* species.

Bowers et al. (2005) found that in sorghum–rice–maize system, insertion, deletion, or rearrangement was common at euchromatic regions. The authors hypothesized that rearrangements are often not inherited and tend to accumulate in parts of chromosome where recombination events are rare (i.e., near centromeric regions though Ma and Bennetzen (2006) proved that is not always the case as in rice).

In a study conducted by Deu et al. (1995) on mitochondrial DNA diversity in wild and cultivated sorghum, they found that genetic diversity in cultivated sorghum showed marked geographic grouping. The study included samples of cultivated sorghum diverse landraces, and *Eu-Sorghum* species and encompassed the primary and secondary gene pools. Deu et al. (1995) were able to distinguish wild and cultivated sorghum based on DNA markers. This study showed that two of the cultivated *S. bicolor* races have origins from wild species (the *arundinaceam* race is homogeneous whereas the *verticilliflorum* race consists partly of *arundinaceam*). In another study, Aldrich and Doebley (1992) found that cultivated sorghum seemed to be derived from the wild species *S. arundinaceum*, and this wild gene pool is genetically similar to cultivars from central-north-eastern Africa. These observations may explain the principal area of origin and domestication of cultivated sorghum.

Sorghum and maize seem to have diverged from a common ancestor about 5 Mya (Thomasson 1987) and from rice about 50 Mya. Sequence information further reveals the similarity of sorghum genes with those of rice, thus revealing that grasses have conserved genes across families and species. The sorghum genome is larger than the rice genome (380 Mbp) and comparatively one fourth of maize (2,500 Mbp) and sugarcane (500–4,200 Mbp, variation due to ploidy level). Dillon et al. (2004) presented broad taxonomic classification of the entire *Sorghum* genus, divided into three groups (A, B, and C) and subdivided into twenty (A), six (B), and two (C) subgroups.

9.4 Role in Classical and Molecular Genetic Studies

A diverse range of wild sorghum germplasm is conserved in seedbanks (Table 9.1), with increasing use of the Australian native species occurring in the last 10 years. This germplasm is freely available to be used in any research program, with some species now being

used successfully in interbreeding with *S. bicolor*. Primary and secondary *Eu-Sorghum* species have little genetic barriers for crossing with *S. bicolor* and have been shown in the ancestral history of *S. bicolor*.

The use of tertiary gene pool species is very difficult due to genetic incompatibilities and barriers for crossing. However, successful introgression of genes from these species has been reported (Hodnett et al. 2005; Price et al. 2006; Kuhlman et al 2008).

An *S. bicolor* line was identified by Laurie and Bennett (1989) and used by Price et al. (2006) to create hybrids between *S. bicolor* and the tertiary gene pool species *S. angustum* and *S. nitidum*. The *S. bicolor* parent used was recessive for the inhibition of alien pollen (*iap*) gene. The presence of this gene enabled the pollen from the tertiary gene pool species to germinate and reach the ovary and create an F_1 hybrid embryo. If allowed to develop unhindered, the embryo aborts due to tissue breakdown. Recovering the embryos before death by embryo-rescue and tissue culture allows fertile F_1 hybrid plants to be recovered. To date, the only fertile F_1 hybrid plant to reach maturity is between *S. bicolor* and *S. macrospermum*, a rare Australian endemic species (Kuhlman et al 2008).

Molecular analysis has established relationships between species in the genus that is guiding breeders to use species that are closely related and more readily able to be used to produce fertile crosses. There have been very few published studies on the development of markers for the wild relatives of the *Sorghum* genus; however, markers and genomic information from *S. bicolor* can be utilized in these species. To aid in our understanding of sorghum genomics and crop improvement, detailed amplified fragment length polymorphism (AFLP) and restriction fragment length polymorphism (RFLP) markers were developed (Boivin et al. 1999). Also, other PCR-based markers such as simple sequence repeat (SSR) and cleaved amplified polymorphic sequence (CAPS) markers have been developed for sorghum improvement. SSR markers are mostly codominant and are usually very effective at detecting genotypic variation caused by a high degree of polymorphism. These markers will be useful in marker-assisted selection (MAS) and also in map-based cloning (MBC) of the traits of importance including drought resistance and tolerance to heavy metals like aluminum.

In addition, previous studies by Tuinstra et al. (1996) identified several quantitative trait loci (QTLs) linked to plant architecture. Identified genomic regions related to plant stature. These have not been confirmed in wild species.

9.5 Role in Crop Improvement Through Traditional and Advanced Tools

Wild *Sorghum* species have many characteristics of potential value in sorghum improvement. Limited genetic analysis of this potential has been undertaken. One wild species, *S. macrospermum* (Chaetosorghum), has some interesting features; it is (1) a non-host of sorghum midge (*Stenodiplosis sorghicola*); (2) resistant to sorghum downy mildew (*Peronosclerospora sorgh*) (Kamala et al. 2002); and (3) scarcely infested by shoot fly (*Atherigona soccata*) (Sharma et al. 2005). Therefore, it can act as a suitable candidate donor for gene transfer to cultivated sorghum to improve its genetic potential. Chromosome pairing revealed that *S. macrospermum* (AAB1B1YYZZ, with Y- and Z- being unknown genomes) has two genomes similar to the genomes of *S. bicolor*, and further in situ hybridization revealed conserved sequences between these two species (Kuhlman et al. 2008).

SSR markers derived have been used to evaluate the molecular genetic diversity of wild sorghum species including those from the secondary and tertiary gene pools (Dillon et al 2005). The *S. bicolor*-derived SSR markers were highly transferable to the tertiary gene pool species and showed that the wild species had high levels of diversity compared to *S. bicolor*. This would be expected as a majority of the tertiary gene pool species is outcrossing (Dillon et al 2005).

S. propinquum ($2n = 20$) is predominantly a noxious weed, perennial in nature with profuse tillering, narrow leaf, and distinct rhizomes, and predominantly Asian origin (Chittenden et al. 1994). The cross between *S. bicolor* and *S. propinquum* resulted in a third species *S. halepense* ($2n = 40$), commonly known as Johnson grass. This has helped researchers to develop a detailed genetic map, which has assisted in answering questions related to the domestication of *S. bicolor*. Comparative mapping between sorghum and maize revealed the evidence of gene duplication upon separation of these two groups 5 Mya (Bowers et al. 2003).

Sorghum is a very effective converter of solar energy among the members of the plant kingdom as they have a C_4 photosynthesis pathway. This C_4 photosynthetic pathway is an effective means to endure conditions of extremes of temperature, light intensity, low water availability, and high carbon dioxide fixing efficiency compared with the C_3 pathway found in most crop plants, e.g., wheat and rice (Wang et al. 2009).

It is expected that further genome sequencing of Poaceae will identify gene-rich regions, which have been conserved for many millions of years during the evolution of grasses. For example, the gene encoding granule-bound starch synthase (GBSS1) was recently sequenced in *S. leiocladum* and *S. nitidum*. Although complete sequence for *S. nitidum* was not determined, comparative analysis of the putative amino acid sequences of *S. bicolor* and *S. leiocladum* ascertained that *S. leiocadum's* protein was three amino acids shorter; however, it retained 97% homology with *S. bicolor*, with most of the variation occurring in the transit peptide sequence (Shapter et al. 2009a). Conversely, other genes present in the cereals are not present in *S. bicolor*. Grain texture is an important property, which influences usage. Recent studies by Charles et al. (2009) showed the origin and evolution of the *Ha* gene, for grain softness. Comparative genomic analysis revealed that *Ha*- or *Ha*-like genes are present in wheat and Brachypodium and non-existent in sorghum. It is now believed that the evolution of *Ha* or *Ha*-like genes took place some 50 Mya, after the cereal lineage diverged from Panicoideae, of which cultivated sorghum belongs.

As more complete grass genomes are sequenced and annotated, our understanding of the evolution and spread of cereal crops throughout the world will improve. For instance, this may include the evolution of sorghum in Africa and wet land rice in Asia (Sasaki and Antonio 2009) and help us to understand the impact of land mass separation, which occurred millions of years ago.

Seventeen of the twenty-five recognized *Sorghum* species are native to Australia (Dillon et al. 2005). Australia's relatively short agricultural history has led to these wild sorghums being an untapped and very unique genetic resource for sorghum breeding programs. There is potential for these species to be used in the genetic improvement of *S. bicolor* or in the domestication of new species as novel grain foods, feedstocks for biofuel production, and as native pastures. Although not specifically cultivated as pasture, wild sorghums provide an important component of Australia's natural grazing systems (Cook and Andrew 1991).

Recent evaluation of the potential of Australian Native *Sorghums* to produce grain for human or animal consumption has identified wide diversity in the grain morphology between the wild and cultivated *Sorghums*. While cultivated *S. bicolor* has a tightly packed panicle with awnless exposed grains, wild *Sorghums* have an open panicle with grains encased in glumes. All Australian wild *Sorghums* have awns, which can vary from ~10 to 90 mm in length. The naked caryopsis is ovoid in shape, as opposed to the spherical shape of cultivated *Sorghums*, and does not have the hard pericarp associated with cultivated *S. bicolor*. The appearance of the caryopsis can vary in color from off-white to reddish-brown. Caryopsis size also varies between species from ~2–4 mm wide by ~3–8 mm long (Spangler 2003).

The use of scanning electron microscopy (SEM) has identified novel starch granule formation and protein distributions in the wild *Sorghum* grain's starchy endosperm. Starch granules have variations in their shape, size, arrangement, and surface texture when compared to *S. bicolor*. Some of the species have starch granules with pores on the granule surface or channels, which have the potential to improve the digestibility of the starch. Protein distribution in the starchy endosperm was also variable with some of the wild species having far more, and some far less, protein bodies. The distribution of the protein bodies also varied when compared to *S. bicolor* (Shapter et al. 2008). *S. bicolor's* starchy endosperm is described as having a distinct vitreous (outer) layer with polygonal starch granules and an obvious protein matrix embedded with protein bodies, and a floury (inner) layer with large ovoid granules and far less protein (Duodo et al. 2002). Many of the native species had a single continuous morphology throughout the endosperm. It was hypothesized that these differences in morphology may have positive implications for human nutrition, if this wild germplasm was integrated into breeding programs with cultivated sorghums.

The pericarp and subaleurone layer of the starchy endosperm of Australian wild species have also been identified as having significant variation from *S. bicolor*. Use of SEM and histochemical analysis confirmed the presence of a discontinuous proteinacious subaleurone

morphology in all the wild species examined, which has not previously been reported in cereals (Shapter et al. 2009b). The pericarp of the wild species was noted as significantly thinner than *S. bicolor*. A thick pericarp in sorghum is known to be associated with increased levels of phenolic compounds, which have been reported as having both positive and negative nutritional ramifications for humans (Parr and Bolwell 2000). Increased protein in the starchy endosperm may have implications for digestibility for human and animal consumption and may also be a unique adaptation for supporting germination in low nitrogen soils (Shapter et al. 2009b).

The allelopathic properties of some *S. bicolor* subspecies may drastically reduce growth of other crop species grown in the vicinity or in rotation (Baerson et al 2008). Sudan grass (*Sorghum sudanense*) reduces the growth of weeds in the field in which it is grown without the use of chemical herbicides. These herbicides increase the cost of crop production drastically and have a negative environmental impact, which means identification of the gene(s) for allelopathy would have enormous benefits for crop improvement.

9.5.1 Biofuels

The growing need to find fossil fuel alternatives for the production of energy has led to the use of plant biomass as a carbon source as feedstock for conversion processes to produce alternatives to petroleum fuels. Until recently, the preferred feedstocks have been food crops, such as sugarcane, maize, and sorghum, which have led to concerns about human food commodities being diverted to a higher value market. Environmental concerns regarding the overall carbon neutrality of these systems have now led to further technological advances, which have led to the refinement of the first generation bioenergy technologies and development of second generation technologies such as large scale pyrolysis and enzyme-based systems. With these recent advances, the question of the most appropriate sources of biomass has now moved outside the sugarcane and cereals to find efficient, non-competitive, environmentally sustainable sources of biomass. For example, the United States has been working with switch grass for ethanol production and has calculated a 94% reduction in greenhouse gas emissions compared with conventional fuel from oil life-cycle analysis (Schmer et al. 2008).

Wild *Sorghums*, which have previously been overlooked as sources of feed or fodder may now have applications as biomass sources for these new generation bioenergy technologies. Many of the wild *Sorghums*, particularly those native to the northern parts of Australia, have intrinsic adaptations enabling them to grow in what are considered to be marginal areas of agricultural land, where little or no other intensive agriculture is viable. Furthermore, many of these species are perennial, which means less tillage, better water use efficiencies, and advantageous use of seasonal conditions once they are established. The biomass production potential of many of these wild species is unquantified through field observations, and taxonomic descriptions describe these plants growing as high as 2–3 m in a season. In conjunction with these qualitative observations, their close relationship to sorghum and sugarcane indicate that further investigation of these species may show them to be good candidates for domestication as biofuel crops. Alternatively, wild sorghums have already been used in sorghum breeding programs to confer pest and disease resistance to cultivated *S. bicolor* and may have other beneficial characteristics to develop new cultivars specifically targeted at biofuel production.

9.5.2 Breeding Tools for Improvement

Xin et al. (2008) advocated the use of 0.1–0.5% (v/v) EMS solution to induce mutagenesis in sorghum. Chemical mutation breeding programs are favored over genetic modification (GM) approaches, as it is not subjected to the same regulatory process. Development of hybrid sorghum varieties has contributed to a global increase in productivity. The keys to hybrid variety development include genetic male sterility and subsequent use of fertility restorer lines. Also, exploitation of heterosis is an essential component. Sarath et al. (2008) advocated that brown-midrib mutations in sorghum are responsible for alteration in cell wall composition, a reduction of lignin, and thus could be used for biofuel production and act as a model species for C_4-grasses.

Menkir et al. (1994) advocated the use of a broad based recurrent parent selection for introgressing suitable genotypes, thus providing robust opportunities for developing suitable lines resulting from the adapted × wild type (exotic) crosses.

9.6 Genomics Resources Developed

A few genomic resources are available for wild *Sorghum* species. The sorghum genome sequence (Paterson et al. 2009b) is a resource that will allow analysis of wild germplasm and generation of genomic resources in these species. Maqbool et al. (2001) identified tissue culture, molecular marker development, genetic manipulation, and genomics resources as key tools in the improvement of sorghum. In an extensive paper on sorghum expressed sequence tags (EST), Pratt et al. (2005) reported key genes for drought and pathogenesis from a large set of 16,801 transcripts. These ESTs and other cereal genomics data can be used as a platform to ascertain the sequence of their homologs in the wild species (Shapter et al. 2009a).

Databases for comparative genomics, *http://www.grassius.org*, GRASSIUS (Grass Regulatory Information Services), deal with transcriptional factors (TFs) and have up to date information on sorghum (*S. bicolor*), sugarcane (*Saccharum* spp.), maize (*Zea mays*), and rice (*Oryza sativa*). TFs influence gene regulation and therefore play an important role in crop development. Further, Yilmaz et al. (2009) indicated that classification of TFs will be helpful to uncover orthologous relationships between the members of the grass family. Jardim (2007) advocated the use of genome information to pinpoint genes responsible for tolerance to aluminum and soil toxicity in soil. This has potential interest for growing crops in marginal and degraded lands.

The genetic map for sorghum is a particularly useful resource available online at *http://www.SorghumGenome.tamu.edu*. New browsers such as TARGeT (Tree Analysis of Related Genes and Transposons), which compares DNA/amino acid seed query to genomic databases, will be an added advantage. Additionally, the TARGeT browser is also able to identify many gene families at a time. Han et al. (2009) evaluated TARGeT to identify a typical gene family of rice, sorghum, and many transposable element families.

9.7 Scope for Domestication and Commercialization

Wild species have much to contribute to domesticated sorghum as a genetic resource. Some species have potential for more direct domestication. Selection of new energy crops and food crops adapted to hotter or dryer environments might favor the use of other species in the genus.

9.8 Some Dark Sides and Their Addressing

The weedy nature of many *Sorghum* species needs to be considered. Analysis of weedy and non-weedy species may help us to define the essential nature of these species as weeds.

9.9 Recommendations for Future Actions

Sorghum is highly valued as a food, feed, and fodder crop and even for green manure production. It can be grown in areas of severe temperature and scarce moisture and nutrient regimes. The *Sorghum* genus has a reservoir of genes, particularly from wild species, which can be exploited for crop improvement. Recent completion of cultivated Sorghum Genome Initiative has made sequence information available for further gene annotation and discovery. This data can be compared with other cereals, for which sequence information is already available, and will help us to understand the ancestral cereal genome (Bolot et al. 2009). This will also facilitate target-induced local lesions in genome (TILLING) projects involving mutagenized populations for sorghum improvement. Further, chemical-induced mutation projects (like TILLING) remain more acceptable to general public than transgenic approaches in many countries.

With the recent advances in next generation sequencing technologies (including reduced costs per base), more genomic data will become available, extending the development of extensive genome surveys and physical maps. This will allow the components, which

are important for productivity, to be analyzed, leading to the development of better strategies for genetic manipulation and crop improvement.

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Chapter 10
Triticum

Eviatar Nevo

10.1 Domestication as a Gigantic Human Evolutionary Experiment

Domestication of plants and animals revolutionized human cultural evolution and is the major factor underlying human civilization. Domestication is a gigantic evolutionary experiment of adaptation and speciation, generating incipient species (Darwin 1905). It was performed by humans primarily during the last 10,000 years (Zohary and Hopf 2000; Feldman and Kislev 2007), mimicking speciation in nature (Wei et al. 2005). It leads to adaptive syndromes fitting human ecology (Harlan 1992). Domestication and the emergence of agricultural economies from pre-agricultural ones established human sedentism, urbanization, culture, and an unprecedented population explosion. The domesticated adaptive syndromes in cereals include *non-shattering spikes* and *higher yields* (see Table 1 in Harlan 1975; see details in Gill et al. 2007). Domestication makes the cultivars human-dependent, capable of surviving only under cultivation in human agricultural niches to meet human needs and culture.

10.2 Wheat: The Domesticated *Triticum* Species

Wheat is the universal cereal of Old World agriculture (Zohary and Hopf 2000) and the world's foremost crop plant (Feldman et al. 1995; Gustafson et al. 2009), followed by rice and maize. Wheat and barley constituted the principle grain stock founding Old World agriculture. They were among the earliest domesticated crop plants, 10,000 years ago in the pre-pottery Neolithic Near East Fertile Crescent (Harlan and Zohary 1966). Wheat is the most widely cultivated food crop and is the staple food, with an annual production of more than 620 MT produced in more than 40 countries and for over 35% of the global population (Williams 1993). The earliest present evidence for wheat utilization is from Ohalo, a site near the Lake of Galilee where a 19,000-year-old wild brittle tetraploid wheat *Triticum dicoccoides* was found, permitting sedentism and cereal agriculture (Kislev et al. 1992). However, wild emmer was first cultivated in the southern Levant in the Pre-Pottery Neolithic A (PPNA) 10300–9500 BP. Domesticated emmer (with a *non-brittle* spike) appeared several hundred years later in the late PPNB (9500–9000 BP), which was grown mixed with wild emmer in many Levantine sites. Types with naked, free threshing grains emerged in the late PPNB (9000–7500 BP) (Feldman and Kislev 2007). Mutations affecting spike traits including shattering, also called brittle rachis (controlled by genes *Br1* and *Br2*), tough glume (controlled by genes *Tg* and *Sog*), and speltoid spike (*q*, non-free threshing) were largely responsible for wheat domestication (Gill et al. 2007). Today, wheat ranks first in the world's grain production and accounts for more than 20% of the total human food calories. Wheat is now extensively grown in the temperate, Mediterranean-type, and subtropical parts of both world hemispheres from 67°N in Norway, Finland, and Russia to 45°S in Argentina. The world's main wheat-producing regions are in temperate and southern Russia, central plains of US, southern

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Canada, the Mediterranean basin, north-central China, India, Argentina, and southwestern Australia.

Wheat enabled large food production, expanding sedentism, population expansion, and the development of early human civilization. Wheat cultivars are superior to most other cereals in their nutritive value. They contain 60–80% starch and 8–15% protein, which rise in elite wild genotypes of *T. dicoccoides* up to 13.9–28.9% (Avivi 1978, 1979; Avivi et al. 1983; Grama et al. 1983; Nevo et al. 1986a; Levy 1987). Wheat, and particularly wild wheat, has unique bread baking qualities. Wheat is the staple food for billions of people. In the still exploding world population (approaching 10 billion in 2050), wheat will continue to serve as the major food ingredient through the production of bread.

10.2.1 Wheat Speciation

Modern wheat cultivars belong primarily to two species (1) hexaploid bread wheat, *Triticum aestivum* ($2n = 42$ chromosomes, AABBDD), and (2) tetraploid, hard or durum-type wheat, *T. turgidum* ($2n = 28$, AABB) used for macaroni and low-rising bread. Other species are relict (for a detailed account see Zohary and Hopf 2000; Gill et al. 2006, 2007; Feldman and Kislev 2007). Wheat is almost fully self-pollinated; hence, genetic diversity is represented in the wild by numerous clones and in cultivation by some 25,000 different cultivars. Cultivated primitive wheat forms have hulled grains, whereas advanced cultivated wheat are free-threshing. Likewise, wild wheat has brittle ears that disarticulate at maturity into individual spikelets. Each spikelet, with the wedge-shaped rachis internode at its base, constitutes an arrow-like device that inserts the seed into the ground (Zohary 1969). By contrast, cultivated wheat has non-brittle ears that stay intact after maturation, depending on humans, for reaping, threshing, and sowing (Figs. 1.1, 1.2 in Nevo et al. 2002; all hitherto cited figures and tables will refer to this book unless otherwise indicated). The non-brittleness and nakedness of cultivated wheat depends on the *Q* locus (Luo et al. 2000), located on chromosome 5 of genome-A, and it may have arisen from the *q* gene of the hulled varieties by a series of mutations (Feldman et al. 1995). Gill et al. (2007) cloned the *Q* gene, unraveling the structural and functional nature of the free-threshing trait and other early domestication events. The family Poaceae (grasses) originated 50–70 million years ago (Mya) and the subfamily Pooidae is 25 Mya.

10.2.2 Allopolyploidy as a Major Speciation Mode in Plants: Cytogenetic and Taxonomic Background

The tribe Triticeae is economically the most important group in the family Gramineae. It has given rise to cultivated wheat, barley, rye, oats, and a number of important range grasses. Polyploidy is a major speciation mode in plants (Stebbins 1950; Soltis and Soltis 1999). Hybridization among genera within the tribe has allowed the exchange of genetic material and given rise to polyploidy in the form of amphiploidy. Wheat (genus *Triticum*) comprises a series of amphiploids between *Triticum* species and diploid species of the genus *Aegilops* (Kihara 1944, 1954; Riley 1965; Sears 1969; Miller 1987; Kimber and Feldman 1987; Dvorak et al. 1993; van Slageren 1994; Feldman et al. 1995; Feldman and Levy 2005; Wendel 2000; Caligari and Brandham 2001; Gill et al. 2007; Gustafson et al. 2009; Feuillet and Muehlbauer 2009; Figs. 1.1 and 1.2 in Nevo et al. 2002).

The wild diploid species, some of which have contributed to the polyploidy of wheat, are presumably monophyletic in origin, though they have diverged considerably from each other. This divergence is particularly evident in the morphologically well defined seed-dispersal units of the species and their specific ecological requirements and geographic distributions. Cytogenetic data have corroborated the taxonomic classification by showing that each diploid species contains a distinct genome (Kihara 1954). The related chromosomes of the different genomes show little affinity with each other and do not pair regularly in interspecific hybrids, so the result is complete sterility and isolation of the diploid species from each other. Besides alloploidy, natural selection played a major role in orienting wheat evolution (Gustafson et al. 2009).

Wheat polyploid species are a classic example of evolution through amphiploidy (Levy and Feldman 2002, 2004; Feldman and Levy 2005; Feldman and

Kislev 2007; Kimber and Feldman 1987; Gill et al. 2007; Gustafson et al. 2009). They behave like typical genomic amphiploids; that is, their chromosomes pair in a diploid-like fashion and the mode of inheritance is disomic. The allopolyploid nature of the *Triticum* polyploids has been verified by cytogenetic analysis of hybrids between species of different ploidy levels. Each polyploid species can be identified as a product of hybridization followed by chromosome doubling.

Allopolyploidy is a major evolutionary driving force of the wheat genome, hybridizing *Triticum* and *Aegilops* species, thereby increasing genetic diversity and adaptive radiation (Levy and Feldman 2002, 2004; Feldman and Levy 2005; Ma and Gustafson 2005; Gustafson et al. 2009), first in the formation of new allotetraploid wheat species, *T. dicoccoides* (~0.5 million years ago) and second in the formation of hexaploid wheat species, *T. aestivum* (~10,000 years ago). Elimination of *non-coding* sequences from one of the two homologous pairs in tetraploids and hexaploids causes homologous chromosomes' divergence in the polyploid, advancing gene inactivation (silencing), and diploidization. Rapid activation of genes by demethylation, or retroelement adjacent gene activation, innovate expression patterns or silence others (Kashkush et al. 2002; Kashkush 2007). Gradual evolutionary changes involve a horizontal intergenomic transfer of chromosome segments, repeats and transposons (Belyayev et al. 2000), genome recombination, diversification, and mutations, all increasing genome structural and functional plasticity and adaptive radiation in wheat, cereal crops, and other grasses (Kellogg et al. 1996; Kellogg 1998, 2001).

The pivotal genome concept (Zohary and Feldman 1962; Gustafson et al. 2009) and cell cycle differences contributed substantially to wheat evolution (Gustafson et al. 2009). Modified genomes along with unmodified or pivotal (buffering) genomes widespread in Triticeae increase adaptability, contribute to rapid expansion of bread wheat in the world, and facilitate gene introduction from other species and genera in wheat breeding, hence, the importance of all wheat relatives for wheat improvement. Diploidization (*ph* mutant, reviewed in Sears 1977), preventing multivalent chromosome formation with deleterious intergenomic exchange, was critical for wheat polyploidy stabilization. Gene mutations complemented polyploidization in expanding wheat diversity and successful speciation. There also seems to be a relationship between DNA content, nuclear volume, and mitotic cell cycle, and ultimately plant growth affecting polyploidy evolution (Gustafson et al. 2009 and references therein).

At the diploid level, there are two species of einkorn wheat: *Triticum monococcum* L. and *Triticum urartu* Thum. The sterility of their hybrids (Johnson and Dhaliwal 1976) indicates that they are valid biological species. Diploid *T. monococcum*, or einkorn wheat (genome AA or Ab, Ab), comprises both wild and cultivated forms (Fig. 2 in Zohary and Hopf 2000). Cultivated einkorn, with its hulled grains, was an important grain crop in the past, surviving today only as a relic. Diploid *T. urartu* (genome-AA or AuAu-) presumably exists only in its wild form. Morphologically, *T. urartu* closely resembles the wild forms of *T. monococcum* (Zohary and Hopf 2000), and yet, *T. urartu* is reproductively isolated from both wild and cultivated einkorn wheat. Interspecific F_1 hybrids of this wheat are male sterile and do not produce seeds upon self-pollination. Allozymically, these two diploid wheats are distinct. *T. urartu* is almost totally confined to the Near East (Valkoun et al. 1998); it prefers basalt soils and often grows in mixed stands with wild einkorn wheat.

At the tetraploid level, there are two species, *T. turgidum* L., which include wild emmer *T. turgidum dicoccoides* (Korn.) Thell (designated hereafter as *T. dicoccoides*), the focus of this review, and cultivated emmer wheat, durum wheat, and several other cultivated tetraploid forms including rivet, polish, and Persian (Figs. 3 and 4 in Zohary and Hopf 2000). Molecular analysis (Dvorak et al. 1988, 1993; Jaaska 1997) has shown that genome-A of tetraploid *T. turgidum* is close to the diploid A-genome of *T. urartu*, whereas the genome-A of *T. monococcum* is more distant. Thus, tetraploid *T. dicoccoides* comprises the *T. urartu* A-genome.

10.2.3 *Einkorn Wheat:* T. monococcum

Today, einkorn is a relic crop, although it is still sporadically grown in western Turkey, Balkan, Germany, Switzerland, Spain, and Caucasia (Nesbitt and Samuel 1996). Prominent in Neolithic agriculture, its importance declined gradually since the Bronze

Age, competitively replaced by free-threshing wheat. Einkorn is a small plant with a relatively small yield; yet it survives on poor soils where other wheats fail (Figs. 2, 6, 7 in Zohary and Hopf 2000). While nutritious, einkorn produces bread with poor rising qualities. It is primarily used as porridge and also as animal feed.

Wild einkorn (*T. boeticum* included as a subspecies of *T. monococcum*, AA, Ab, Ab) is fully fertile with cultivated *T. monococcum* and they are morphologically similar except for the brittle ears of wild einkorn (see Fig. 2 in Zohary and Hopf 2000). *T. boeticum* is widespread in western Asia and southern Balkans (Harlan and Zohary 1966). It ranges primarily in the northern, cooler, and rainier regions of the Near East Fertile Crescent (map 1 in Zohary and Hopf 2000), in oak and steppic widespread ecologies, and secondarily in edges of cultivations and roadsides. It prefers cooler climates and does not penetrate drier and warmer Israel, but prevails in Turkey in the Karakadağ Mountains where cultivated einkorn may have evolved (Heun et al. 1997).

10.2.4 *Emmer and Durum Wheat:* T. turgidum

The varied aggregates of cultivated tetraploid wheat share the AABB genome, and all are fully interfertile (Zohary and Hopf 2000; Feldman and Kislev 2007). As a result, two groups are classified according to their threshing properties:

1. Hulled emmer wheat, *T. turgidum* L. subspecies *dicoccum* (Schrank). Thell. (traditionally designated as *T. dicoccum* Schubl), in which the threshing products are individual spikelets. Hulled emmer represents the primitive situation in cultivated *T. turgidum* wheat.
2. Durum wheat, *T. turgidum* conv. *durum* (Desf.) MacKey (synonym *T. durum* Desf), and other rivet wheats, represent the more advanced free-threshing tetraploids that evolved under domestication from hulled emmer (Fig. 4 in Zohary and Hopf (2000)). Less common types of rivet wheat include Polish wheat with thin glumes. Threshing frees the naked grain in these cultivated tetraploids.

10.2.5 *Bread Wheat:* T. aestivum

This is the most variable aggregate of cultivated wheat and, economically, the most important wheat species (Zohary and Hopf 2000; Tsunewaki 2005; Feldman and Kislev 2007; Gill et al. 2007; Gustafson et al. 2009). Bread wheat accounts for about 90% of total world wheat production today and includes thousands of diverse types. All types are hexaploid ($2n = 42$) and interfertile; all share the AABBDD genomic constitution. For information on hybrid wheat, see Aimin and Tiecheng (1998). For Genetics and Genomics of the Triticeae, see Feuillet and Muehlbauer (2009). For a special issue on Triticeae see Breeding Science vol. 59:455–686, December 2009; on line at *http://www.nacos.com/jsb*.

Hexaploid *T. aestivum* is a new wheat species that evolved 10,000 years ago under cultivation and from the already domesticated tetraploid *T. turgidum* stock. In contrast to diploid and tetraploid wheat, it does not have a wild hexaploid counterpart. Bread wheat is a classic example of evolution by polyploidy. Genome analysis studies have shown (Kihara 1954; McFadden and Sears 1946) that *T. aestivum* is a hybridization product between a tetraploid *turgidum* wheat (genomic constitution AABB) and a diploid wild grass *Aegilops tauschii* Coss. (genomic constitution DD) (see HMW glutenins of *Ae. tauschi* in Gianibelli et al. 2001). Thus, hexaploid bread wheat originated by hybridization, adding a third genome to the two genomes already present in *T. turgidum*. Bread wheat has been synthesized in the laboratory by crossing the two putative parents and doubling the chromosomes in the hybrids. Since no AABBDD hexaploid wheat occurs in the wild, this development could have occurred only under cultivation, i.e., by the hybridization of the already domesticated tetraploid wheat with the wild *Ae. tauschii*. Hexaploid wheat fall into two groups according to their response to threshing: hulled (mostly relic crops represented by spelta wheat) and free-threshing bread wheat, which is predominant today as bread wheat *T. aestivum* (Figs. 5a–c and 6c in Zohary and Hopf 2000).

Experimental evidence indicates that the first hexaploid wheat was spelta-like. Artificial synthesis of *T. aestivum* (by crossing and fusing tetraploid AABB *T. turgidum* with diploid DD *Ae. tauschii*) always

results in hulled products (Kerber and Rowland 1974), irrespective of whether the *turgidum* AABB parent is hulled or naked. It also suggests that the free-threshing condition in hexaploid bread wheat was brought about by two events: the appearance of the free-threshing gene, *q*, located on chromosome 5A, and the mutation from *Tg* to *tg* in the gene responsible for the tenacious glumes trait on chromosome 2D. All present-day 6 naked wheat examined carry the *tg*/*tg q*/*q* genotype (Gill et al. 2007).

Domestication of tetraploid wheat apparently occurred independently in several sites across the Levant (Feldman and Kislev 2007) rather than in a small core area in southeastern Turkey (Lev-Yadun et al. 2000). Presumably, non-brittle (non-shattering) genes, tough glumes, and threshing controlled by multiple genetic pathways and mutations in different loci may have led to domestication-driven convergent evolution (Gill et al. 2007). These spike traits were transferred polycentrically to numerous wild emmer genotypes through many spontaneous hybridizations followed by human selection, generating highly polymorphic populations resistant to biotic and abiotic stresses superior under cultivation. The data indicate that populations of *Ae. tauschii,* native to Armenia and the southwest part of the Caspian Sea belt, are closest to the genome-D found in hexaploid wheat (Dvorak et al. 1998).

Finally, it is attractive to speculate that the addition of the D-genome greatly extended the range of adaptation of wheat (Zohary 1969). Cultivated tetraploid wheat derived from a Near East progenitor was adapted to grow in Mediterranean-type environments with mild winters and warm rainless summers. The incorporation of the *Ae. tauschii* genome enabled the hexaploid plant to withstand continental winters and humid summers. This, no doubt, facilitated the spread of hexaploid bread wheat.

Hulled emmer *T. turgidum* subsp. *dicoccum* was the principle wheat of Old World agriculture in the Neolithic and early Bronze ages and was used for food and beer (Zohary and Hopf 2000). It was later replaced by more advanced free-threshing tetraploid and hexaploid types. Emmer is still sporadically grown in the Balkans, Anatolia, western Asia, and India and is massively grown in Ethiopia. Durum wheat is the main representative of the free-threshing AABB tetraploid wheat. They were domesticated in Neolithic times as the major wheat crop in the summer-dry, warm Mediterranean region. In temperate areas with summer rains and in more continental climates, traditional bread wheat production depended heavily on other free-threshing wheat, e.g., hexaploid *T. aestivum*, the major current bread wheat across the planet, with many thousands of land-races and modern lines.

10.3 Comparative Genomics of Cereals

Cereals comprising wheat, barley, maize, sorghum, millet, and rice belonging to the grass family Poaceae comprise some of the most important crops for human and animal nutrition. The genomes of grasses are different in size, ploidy, and chromosome numbers (Gill et al. 2007). Nevertheless, comparative mapping revealed that the linear order (colinearity) of genetic markers, genes, and quantitative trait loci (QTLs) for agronomic traits is conserved between different grass genomes (Gale and Devos 1998; Kellogg 1998; Bennetzen 2000; Feuillet and Keller 2002; Paterson 2006; Salse and Feuillet 2007; Tang et al. 2008; Bolot et al. 2009; Feuillet and Muehlbauer 2009). Syntenic blocks are retained in spite of a 40-fold variation in chromosome size (Gill et al. 2007). The development of large insert libraries elucidated their genome organization. Remarkably, genes are non-randomly clustered in high gene density in species with large genomes.

Colinearity is largely retained at the molecular microlinear level. This elevated the small genome size of rice as a grass evolutionary model, and for sequencing, followed later by the small genome of 355 Mb of *Brachypodium distachyon*. Large expressed sequence tag (EST) collections and bacterial artificial chromosome (BAC) sequences from maize, sorghum, rice, wheat, and barley revealed rearrangements disrupting colinearity during grass evolution in the past 50–70 million years (Salse and Feuillet 2007; Feuillet and Muehlbauer 2009).

10.3.1 International Wheat Genome Sequencing Consortium

The International Wheat Genome Sequencing Consortium (IWGSC) was established by a group of plant scientists, breeders, and growers dedicated to sequencing the wheat genome to enhance our knowledge on the

structure and function of the wheat genome. By gaining increased understanding of the biology of agronomically important traits and deploying state-of-the-art molecular tools, plant scientists and breeders will be able to accelerate wheat improvement to meet the challenges of the twenty-first century. The Consortium is committed to ensuring that the sequence of the wheat genome and the resulting DNA-based tools are available for all to use without restriction. To achieve the vision of a sequenced wheat genome, the IWGSC established strategic plans with short- and mid-term goals, defined areas of coordination, facilitates and coordinates research projects, and funds efforts at the national and international levels. Recently, increased marker density and genome sequencing of several cereal genomes revealed detailed intragenomic colinearity enabling the identification of paleo-duplications and propose a model of grass genome from a common ancestor. On the basis of five ancestral chromosomes, the "inner circle" was defined as providing new insights into the origin of evolution of grasses (Bolot et al. 2009). The physical mapping of the wheat genome, conducted by the International Wheat Genome Sequencing Consortium (IWGSC, *http://www.wheatgenome.org*) since 2005, will provide excellent tools for optimizing breeding (Feuillet and Eversole 2007; Feuillet and Muehlbauer 2009).

10.4 Chromosomal Speciation

Chromosomal repatterning is a source of new adaptive character combination and speciation, particularly in plants (Grant 1981). Structural chromosomal rearrangements including deletions, duplications, translocations, and inversions are often associated with heterochromatic repetitive DNA regions bordering with euchromatin (Badaeva et al. 2007). The diploid/polyploid *Triticum*/*Aegilops* complex exemplifies abundant C-banding polymorphism based on chromosomal rearrangements (Raskina et al. 2008 and references therein). The distal/terminal chromosomal regions enriched by transposable elements (TE) appear as a faster evolving genomic region (Belyayev and Raskina 1998). The repetitive DNA fraction is also involved in polyploidization changes (Feldman and Levy 2005; Ma and Gustafson 2005). In allotetraploid *T. dicoccoides* (AABB), the distribution pattern of highly repetitive DNA clusters and Ty1 Copeia retroelements differ from that of its diploid progenitors, *T. urartu*, A-genome, and *Aegilops speltoides*, B-genome (Raskina et al. 2002). In *T. dicoccoides*, part of the A-genome heterochromatin was substituted by satellite DNA of the B-genome (Belyayev et al. 2000). Enrichment of these clusters with mobile Ty1-copeia retroelements may have contributed to stabilizing *T. dicoccoides* as a new species. Furthermore, insertions of transposable elements (TEs) may create a new crossing over "hot spot," which provokes TEs rapid chromosome reorganization during speciation and allopolyploid stabilization. Bennetzen (2002) described the mechanisms and rates of genome expansion and contraction in flowering plants. Significant temporal fluctuations in the copy number of TEs provide new insights into genome evolution of the wild diploid wheat, *Aegilops speltoides*, in its marginal population in Israel. The revealed temporal dynamics of TEs could promote and intensify morphological and karyotypic changes affecting microevolution and the evolution of new species under stressful and rapid climatic change (Belyayev et al. 2010).

10.5 Mobility of rDNA Clusters, Robertsonian Rearrangements, and Speciation

The dynamics of rDNA clusters strongly reflect intragenomic processes (Raskina et al. 2004a and references therein). Chromosomal sites may move chromosomally without translocations (Dubcovsky and Dvorak 1995). These chromosomal dynamics are caused by TE and may create mutations, alter gene expressions, and promote chromosomal aberrations and meiotic abnormalities that may cause reproductive isolation (Belyayev et al. 2007). These studies call for further investigations, following Baum et al. (2004), who showed different chromosomal positions for rDNA in diploid *T. urartu, Ae. speltoides,* and *Ae. tauschii* than those found in polyploids (*T. dicoccoides* and *T. aestivum*). McClintock (1946, 1984) suggested that TEs could cause chromosomal breakages, which become highly unstable and can fuse with other broken ends. They could also stimulate de novo rapid

Fig. 10.1 (**a**) Wild emmer wheat, *Triticum dicoccoides*, ripe natural populations from Qazrin, Golan Heights, with some black morphs (photo by E. Nevo). (**b**) Wild emmer wheat, *T. dicoccoides*, spike color polymorphism: *black*, *green*, and *yellow* morphs (photo by E. Nevo). (**c**) Wild emmer wheat, *T. dicoccoides*, spike color polymorphism: *black*, *green*, and *yellow* morphs (photo by E. Nevo)

synthesis of telomeres, increase recombination and satellite loss, and promote Robertsonian translocations causing post-zygotic reproductive barriers and speciation (Raskina et al. 2008 and references therein). The combination of chromosomal and molecular integrative future research programs may highlight both speciation and adaptation processes in the wheat genome and generally in grasses (Fig. 10.1a–c).

10.6 Variability of Chromosomal Distribution of Ty3 Gypsy Retrotransposons in *Ae. speltoides* and *H. spontaneum* (Belyayev et al. 2005)

Fish cytogenetic experiments revealed mini-cluster organization of Ty3-gypsy element chromosomal distribution in wild Triticeae species (Belyayev et al. 2005). They include (1) stable species-specific clusters associated with rRNA genes; (2) variable clusters that represent 68% of clusters in the genome of *Ae. speltoides* and 20% in *H. spontaneum*, and (3) population-specific clusters primarily inserted into a centromeric domain in populations from regionally hot and dry environments across Israel in both *speltoides* and *spontaneum* (Fig. 4 in Belyayev et al. 2005). Remarkably, this insertion of specific clusters into the centromeric domain is also observed in *Hordeum spontaneum* populations at the "Evolution Canyon" microsite (Nevo 2006, 2009) on the southern-facing hot and dry "African" slope as compared with the cool and humid "European" north-facing slope, separated by a distance of only 200 m (Kalendar et al. 2000; Belyayev et al. 2005). Retrotransposon-mediated changes in centromeric regions can lead to meiotic abnormalities, which can (though rarely) generate reproductive isolation in adaptive evolution (Grant 1981). Partial post-zygotic isolation was shown by comparing intra- and interspecific crosses in wild barley at "Evolution Canyon" (Nevo 2006; Parnas 2006). High transposon dynamics of *Ae. speltoides* may have led to the speciation of *Ae. sharonensis* in Haifa, Israel (Raskina et al. 2004b).

10.6.1 Transposon Activity

10.6.1.1 En/Spm-like Transposon Activity in Meiosis in Small, Isolated Peripheral Populations of *Aegilops speltoides*

The involvement of enhancer/suppressor mutator (En/Spm) transposons leading to the rise of new fertile

genomic forms was demonstrated in a small, isolated peripheral population of *Ae. speltoides* (Raskina et al. 2004b). A wide spectrum for chromosomal abnormalities was recorded including extra chromosomes, chromosomal rearrangements, and variability in chromosomal position/number of 45S and 5S rDNA sites in *Ae. speltoides*. This transposon is active during male gametogenesis, forming separately or in combination with rDNA, clusters of "hot spots," provoking large chromosomal rearrangements. The appearance of at least part of the mobile rDNA sites in the genome of *Ae. speltoides* is connected with meiotic activity of En/Spm transposons, leading to karyotype remodeling.

10.6.2 Evolutionary Dynamics of Repetitive DNA in T. dicoccoides and Ae. speltoides

Genomic in situ hybridization (GISH) of *T. dicoccoides* chromosomes probed with DNA from the A/B diploid genome ancestors, *T. urartu* (AA) and *Ae. speltoides* (BB), displays evidence of enrichment of the A-genome with the B-genome (Fig. 10.2). In both *T. dicoccoides* and *Ae. speltoides*, at least part of the heterochromatin blocks are formed by Ty1-copeia retrotransposons and their derivatives. The peculiarity of plant genomes and particularly the large genomes of cereals is the high percentage of retroelements and their panchromosomal distribution (Fig. 2.4 in Belyayev et al. 2003). Retroelements constitute about 50–80% of the maize nuclear genome and up to 90% in wheat (Kashkush 2007). Hypothetically, the relatively weak correlation among different systems facilitates movement, insertion, and accumulation of retroelements and their derivatives in the plant genome. In both species, there are "hot spots" of Ty1-copeia clusters, active in the evolution of both alloploidization and speciation. Detection of alien chromosomes from S-genome species in the addition/substitution lines of bread wheat *T. aestivum* and visualization of A-, B-, and D-genomes by GISH appear in Belyayev et al. (2001, Fig. 10.2).

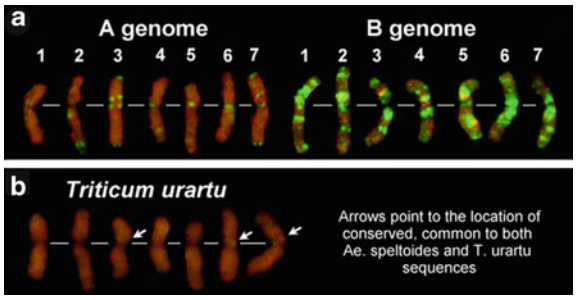

Fig. 10.2 (**a**) GISH-banding karyotype of *Triticum dicoccoides*. Probe: DNA of *Aegilops speltoides* (detected in *green*) plus DNA of *T. urartu* (detected in *red*). (**b**) Karyotype of *T. urartu*. The same probe was used (Belyayev et al. 2000)

10.6.3 Repetitive DNAs of Wild Emmer Wheat, T. dicoccoides, and Their Relation to Sitopsis Genome Species: Molecular Cytogenetic Analysis

We explored the B-genome origin of *T. dicoccoides* by GISH analysis in three populations of wild allopolyploid wheat *T. dicoccoides* in Israel: Amirim, Tabigha, and Gilboa (Raskina et al. 2002; Belyayev et al. 2003, Fig. 2.3). *Ae. speltoides*, the assumed B-genome of wild emmer (see Gustafson et al. 2009 and references therein), showed almost complete affinity of its repetitive DNA to C-heterochromatin of *T. dicoccoides*. By contrast, other S-genome species (*Ae. searsi, Ae. longissima, Ae. sharonensis*, and *Ae. bicornis*) demonstrated relatedness only to distal heterochromatin of *T. dicoccoides*. This further substantiates the priority of *Ae. speltoides* as the most similar to the wheat B-genome donor in comparison with other *Sitopsis* species. Molecular banding analysis reveals polymorphism between the aforementioned three wild emmer wheat populations. Salina (2007) described the modern concepts of the structural organization and evolution of the main macrosatellite families in *Triticum* and *Aegilops* species and their rearrangements in amphiploids.

10.7 Wild Emmer, *T. dicoccoides*, Wheat Progenitor: Origin and Evolution

10.7.1 Origin

Genetic and morphological evidence clearly indicates that cultivated tetraploid *turgidum* wheat (both hulled

dicoccum forms and free-threshing *durum* varieties) is closely related to the wild wheat that is native to the Near East and traditionally called *T. dicoccoides* (Korn) Aaronsohn (wild emmer wheat) (for review see Zohary 1969; Chapman et al. 1976; Miller 1987, 1992; Harlan 1992; Feldman et al. 1995; Zohary and Hopf 2000; Nevo et al. 2002; Feldman and Kislev 2007; Gill et al. 2007). *T. dicoccoides* (Fig. 10.1a–c) includes the A- and B-genomes of *T. aestivum*, with which it makes fertile hybrids. *T. dicoccoides* is the origin of all cultivated bread wheat, *T. aestivum,* which constitutes the most important wheat, involving several primitive hulled types (spelta wheat) and numerous modern free-threshing forms (see Table 3 and Figs. 2–5 of diploid, tetraploid, and hexaploid wheat in Zohary and Hopf 2000). Timopheev's wheat, *T. timopheevi*, comprise both cultivated and wild forms that are genomically different (AAGG) and, reproductively, effectively isolated by hybrid sterility from the more common tetraploid *turgidum* group (see details on *T. timopheevi* and *T. araraticum* in Zohary and Hopf 2000, pp 58–59).

Wild emmer wheat is predominantly self-pollinating, annual tetraploid wheat, with large and brittle ears and big elongated grains (Fig. 10.1a–c) similar to cultivated emmer and durum wheat. It is the only wild ancestor in the genus *Triticum* that is cross-compatible and fully interfertile with cultivated *T. turgidum* wheat. Hybrids between wild *T. dicoccoides* and all cultivated members of the *T. turgidum* complex show normal chromosome pairing in meiosis, indicating that all tetraploid wheat contains homologous chromosomes of the AABB genomic constitution. Hybrids do form occasionally on the plot borders between cultivated tetraploid wheat *T. durum* and *T. dicoccoides*. Therefore, *T. dicoccoides* has been regarded as the wild race of cultivated tetraploid and durum wheat, and it is sometimes ranked as the wild subspecies of the *T. turgidum* complex. Clearly, *T. dicoccoides* occupies a central place in wheat evolution and deserves to be regarded as a good biological species, separated ecologically and genetically from other tetraploids (Nevo et al. 2002).

10.7.2 Classification

The nomenclature and classification of wheat are varied and confusing (Miller 1987, 1992). In this review, I adhere to the traditional view of *T. dicoccoides* as a valid biological species, as Miller (1992) did, for the following reasons. We now know that speciation can occur with very little genomic and morphological changes (Ayala 1975; Avise 1976; Nevo and Cleve 1978; Nevo 1991; Orr 1991; Coyne 1992; Thibert-Plante and Hendry 2009; Magalhaes et al. 2009). *T. dicoccoides* has a unique ecological niche in nature. Likewise, a conspicuous diagnostic difference exists between the wild type and the cultivars that are safeguarded by humans, i.e., in the seed dispersal mechanism (Zohary 1969). *T. dicoccoides* has brittle ears that shatter upon maturity into individual spikelets. Each spikelet operates as an arrow-like device disseminating the seeds by inserting them into the ground. "Wild type" rachis disarticulation and spikelet morphology reflect adaptive-specialized traits in seed dissemination that ensure survival in nature. Under the man-made system of reaping, threshing, and sowing, this adaptation broke down, and non-brittle types were selected. The shift from a brittle spike in *T. dicoccoides* to a non-brittle spike in *T. dicoccum* wheat is described in Gill et al. (2007). Wild and cultivated forms also differ in kernel morphology (van Zeist 1976). In the cultivars, the grain is wider, thicker, and rounder in cross-section than in *T. dicoccoides* (Fig. 3 in Zohary and Hopf 2000). This trait can distinguish the wild type from the cultivars and, at archeological sites, helps to determine the advent of domestication (Zohary and Hopf 2000; Feldman and Kislev 2007). Likewise, important unique chromosomal translocations (Kawahara et al. 1993; Nishikawa et al. 1994; Joppa et al. 1995; Kawahara and Nevo 1996) and genetic polymorphisms (Nevo 2007; Nevo et al. 1982; Nevo and Beiles 1989; Fahima et al. 1998, 1999, 2002; Nevo 1998a, 2001a; Li et al. 1999, 2000a, b, c, d, 2001, 2002, 2003) characterize *T. dicoccoides*. This combined evidence justifies its traditional ranking as a biological species, which was the *progenitor – not the derivative* of *T. turgidum*, as is implied in the name *T. turgidum* subsp. *dicoccoides*. What is the unique ecological niche of *T. dicoccoides* in nature? (Fig. 10.3).

10.7.3 Ecology

Triticum dicoccoides (Fig. 10.1a, b) is more restricted in distribution and ecology than wild einkorn, *T. boeticum*, and wild barley, *H. spontaneum*. It is found in Israel and Syria, which are its centers of

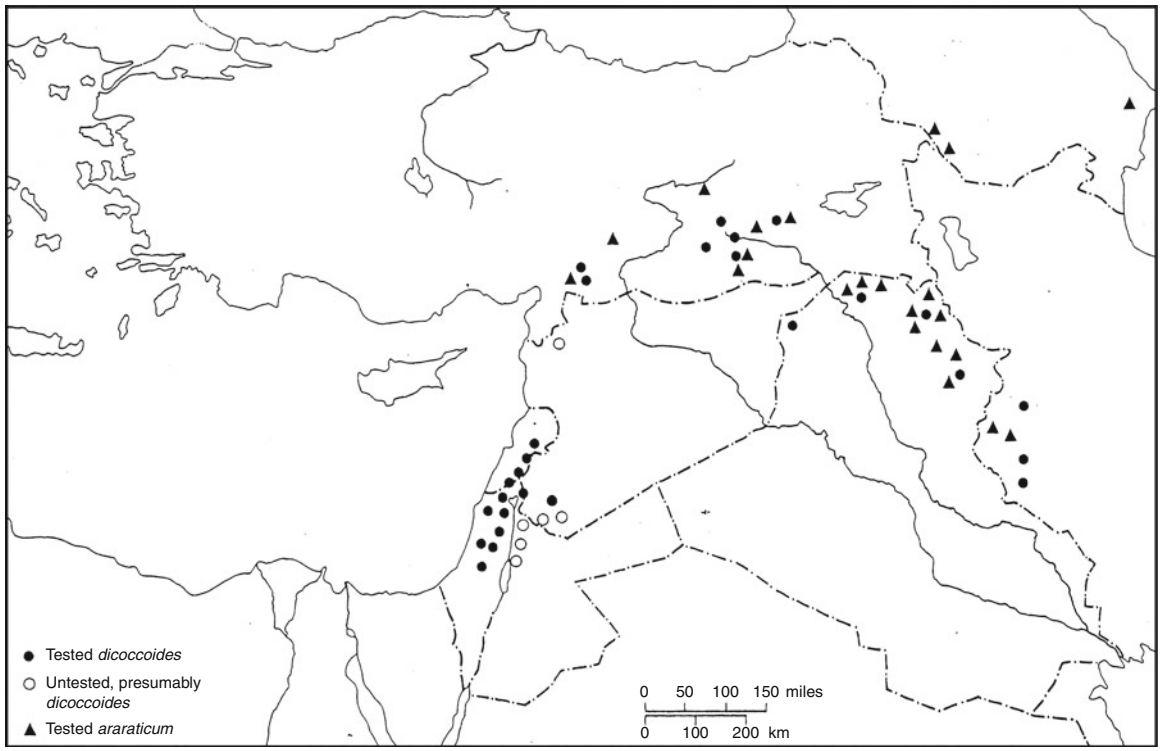

Fig. 10.3 Distribution of the wild tetraploid wheat (filled circle, open circle) (i) wild emmer wheat, *Triticum turgidum* subsp. *dicoccoides* (*T. dicoccoides*) (filled circle) (ii) wild Timopheev's wheat, *T. timopheevi* subsp. *araraticum* (*T. araraticum*), (filled triangles); Collections represent sites that were tested cytogenetically. (Zohary and Hopf 2000, and references therein)

distribution based on genetic diversity (Nevo and Beiles 1989; Nevo 1998a), Jordan, Lebanon, southern Turkey, northern Iraq, and western Iran (Fig. 10.3). It was rediscovered in 1906 in eastern Galilee and on the slopes of Mt. Hermon by Aaronsohn, who had already recognized its potential importance for wheat improvement (Aaronsohn and Schweinfurth 1906; Aaronsohn 1910, 1913; Schiemann 1956; Feldman 1977; Nevo 1983, 1989, 1995, 2001a; Nevo et al. 2002). The discovered genetic resources of wild emmer, *T. dicoccoides*, for wheat improvement far exceeded Aaronsohn's vision (Nevo 2001a; Nevo et al. 2002; Peng et al. 1999, 2000a, b, c, 2003; Israel Journal of Plant Sciences 2001, 2007).

T. dicoccoides grows as a common annual component in the herbaceous habitat of the Tabor oak (*Quercus ithaburensis* Decaisene), open park forest belt, together with related steppe-like herbaceous plant formations such as wild barley, *H. spontaneum* (Nevo 1992), and wild oats, *Avena sterilis* L. in peripheral western populations. *T. dicoccoides* may grow in openings in the maquis of the live oak *Quercus calliprinos* Webb or in the open park forest of *Ceratonia siliqua* L. Wild emmer is an ecological specialist primarily confined to basaltic and hard limestone bedrocks, weathering into basalt and terra rossa soils, respectively. It grows infrequently on hard chalks and is absent from marls and sandstones. Its central area of distribution is the catchment area of the Upper Jordan Valley (eastern upper Galilee and Golan Heights), where it grows in large continuous stands, becoming sporadic, semi-isolated, and isolated in central Israel as well as in Turkey, Iraq, and Iran (Nevo and Beiles 1989). In the sympatric areas with *T. araraticum* in the northeastern distribution of *T. dicoccoides*, the two species are separated by strong sterility barriers (Maan 1973), though they are similar and practically indistinguishable morphologically without cytogenetic analysis (Fig. 10.3).

T. dicoccoides is genetically highly polymorphic in its center of distribution in northern Israel and southern Syria. Morphologically, it is polymorphic for glume hairiness and spike color (Fig. 10.1b, c), and genetically, it is polymorphic allozymically (Nevo et al. 1982; Nevo and Beiles 1989; Li et al. 2000d, 2001)

and for various DNA diversities: rDNA diversity (Flavell et al. 1986), RAPDs (Fahima et al. 1999; Li et al. 1999), and microsatellites (Fahima et al. 1998, 2002; Li et al. 2000a, b, c, d; 2002, 2003; Wang et al. 2008, 2009), resistance gene analog polymorphisms, RGAPs (Dong et al. 2009), sequences-related amplified polymorphism, SARP (Dong et al. 2010), and single nucleotide polymorphism, SNP (Wang et al. 2010a,b). Diverse ecotypes relate to climatic and altitudinal variations. *T. dicoccoides* ranges from 200 m below sea level in the Jordan Valley up to 1,600 m on Mt. Hermon, only 80 km apart from each other, and up to 1,600 m on the Zagros Mountains of western Iran. The mean annual temperature ranges from 11 to 24°C; mean rainfall ranges from 167 to 1,400 mm, and mean annual number of rainy days from 39 to 75. For the range of other climatic soil and biotic parameters, see Table 1 in Nevo and Beiles (1989) and Peleg et al. (2008a, b). Robust early-maturing ecotypes occupy the winter-warm basin north of the Sea of Galilee. Slender ecotypes occur either as a late-blooming form in the steppic high-altitude habitats of Mt. Hermon or as an early-blooming form in low-altitude habitats in the eastern Samarian steppes.

10.7.4 Domestication

T. dicoccoides, like *T. boeticum* and *H. spontaneum*, was probably collected from the wild, long before its domestication (Kislev et al. 1992; Zohary and Hopf 2000; Lev-Yadun et al. 2000; Nesbitt, In: Caligari and Brandham 2001; Feldman and Kislev 2007). Brittle *T. dicoccoides*-like remains with relatively narrow grains appear in several early Neolithic and Natufian Near Eastern settlements. However, nine to ten millennia ago, they coexisted with non-brittle seeds in Jarmo, Iraq, Cayonu, Turkey, Tel Aswad, and Tel Abu Hureira, northern Syria (see archeological evidence and citations in Zohary and Hopf 2000 and their Map 3; Nesbitt and Samuel 1996; Lev-Yadun et al. 2000; Feldman and Kislev 2007). Domesticated forms of einkorn wheat, emmer wheat, and barley appeared in core area Neolithic sites at Tel Abu Hureira 2A and Cafer Huyuk about 7500 BC, and soon thereafter at Cayonu and Nevali Cori (Kislev et al. 1992; Nesbitt and Samuel 1996), Jericho, Gilgal, and Netiv Hagdud (see details in Table 2 of Feldman and Kislev 2007). From the early beginning of agriculture in the Near East (approximately 10,000 years ago) and through the Chalcolithic and Bronze times, emmer was the principle wheat of the newly established farming settlement, subsequently spreading to Egypt, the Indian subcontinent, and Europe approximately 7,000 years ago from its Near Eastern area of origin. *T. dicoccoides* apparently speciated in the Golan Heights and its vicinity, including northern Syria, based on the high polymorphism found there (Nevo and Beiles 1989), through allopolyploidy of genome-A, *T. urartu,* and genome-B, *Ae. speltoides,* 300,000–500,000 years ago based on molecular data (Huang et al. 2002; Dvorak and Akhunov 2005).

The evolution of hexaploid bread wheat, *T. aestivum,* originated outside the Fertile Crescent area where emmer reached the range of *Ae. tauschii* (van Zeist 1976; van Zeist and Bakker-Heeres 1985). This probably happened in the Caspian Sea region 7,000–8,000 years ago. Notably, *T. durum* wheat is adapted to Mediterranean environments, whereas the hexaploid *T. aestivum* grows in cooler and more continental parts of Europe and western Asia. Thus, *T. dicoccoides,* the progenitor of all wheats, deserves a particular in-depth study, correctly suggested by Aaronsohn (1910, 1913), as a potential genetic resource for improving all wheats, both tetraploid and hexaploid. Here, I will review theoretical and applied studies on *T. dicoccoides* that are important for wheat evolution and improvement. These studies, initiated in 1980, include genetic structure across its range, based primarily on the wild cereal research program of wild cereals at the Institute of Evolution, Israel (Fig. 3.1 in Nevo et al. 2002) (see Nevo list of wild cereals at *http://www.evolution.haifa.ac.il*). Special emphasis was on the genetic resources of wild emmer (Nevo 1983, 1989, 1995, 2001a; Nevo et al. 2002). These topics are critically important to overcome the dangerous process of genetic erosion and consequent homogeneity of cultivated wheat (Haudry et al. 2007; Fu and Somers 2009). Likewise, I discuss genome organization, mapping, and evolution of *T. dicoccoides*, and the current efforts of wheat genome sequencing (*http://www.wheatgenome.org*).

10.8 Macrogeographic Population Genetic Studies of *T. dicoccoides* in the Fertile Crescent, Israel and Turkey: Allozyme and DNA Polymorphisms

10.8.1 Allozymic Diversity: General Overview of Molecular Evolution

Genetic diversity within and among species is central to evolutionary biology and its two major processes: speciation and adaptation. Molecular biology permitted the characterization of genetic diversity among individuals, populations, and species. It did so first by revealing the relationship between genes and proteins (Lewontin 1974), and second, by elucidating, at the nuclear and extranuclear *coding* and *non-coding* DNA regions, the structure, expression, function, and evolution of genes, intergenic spacers, and multigene families, and the vast regions of *non-coding* genomic regions. The genetic structure and differentiation of natural populations of plants and animals are now understandable at the molecular level, primarily in a dynamic, ecological context over space and time.

The evidence of genetic diversity found in natural populations of *T. dicoccoides*, the wild progenitor of all cultivated wheats, is important for two major reasons; first, *theoretically*, for understanding the evolutionary history and dynamics of this unique progenitor of wheat, and second, *practically*, for evaluating the potential genetic resources for conservation and utilization of crop improvement. Modern genetic techniques permit the transference of genetic material between ancestors and their derived domesticates (Lupton 1987; Feuillet and Eversole 2007). Very few ancestors have been studied across their geographic range at the interface of population genetics and ecology. This interface is important for displaying the actual genetic differentiation of the progenitor and its ecological determinants, thereby yielding clues about the evolutionary scenario and the potential genetic resources it harbors for breeding (Nevo 1983, 1987, 1989, 1995, 2001a; Tanksley and Orton 1983; Nevo et al. 2002; Peng 2000; Peng et al. 1999, 2000a, b, c, 2001, 2003). Current efforts focus on sequencing the hexaploid wheat genome (*http://www.wheatgenome.org*).

10.8.2 Patterns of Allozyme Diversity of Wild Emmer

T. dicoccoides was studied for allozyme diversity of proteins encoded by 42 gene loci in 1,815 plants (1,658 and 157 from Israel and Turkey, respectively), representing 37 populations: 33 from Israel and 4 from Turkey, sampled in 33 localities from Israel (Nevo and Beiles 1989; Fig. 4.1a, b; Table 4.1; *all figures and tables are from Nevo et al. 2002 unless otherwise specified*) and studied allozymically from 1979 to 1988 (Nevo and Beiles 1989). The results indicated that (1) six loci (14%) were monomorphic in all populations, 15 loci (36%) were locally polymorphic, and 21 loci (50%) were regionally polymorphic, similar to the first studies of 12 populations in Israel (Nevo et al. 1982) and in Turkey (Nevo et al. 1988a). All polymorphic loci (except four) displayed high local levels of polymorphism ($\geq$10%). (2) The number of alleles per locus, *A*, was 1.252 (range: 1.050–1.634). The proportion of polymorphic loci per population, *P*, averaged 0.220 (range: 0.050–0.415), and genic diversity, *He*, averaged 0.059 (range: 0.002–0.119). (3) Altogether, there were 119 alleles at the 42 putative loci tested, 114 of them in Israel. (4) Genetic differentiation was primarily *regional* and *local*, not clinal; 70% of the variant alleles were common ($\geq$10%) and not widespread, but *localized* or *sporadic*, displaying an "*archipelago*" population genetics and ecology structure (see later).

The coefficients of genetic distance between populations were high and averaged $D = 0.134$, range 0.018–0.297, indicating sharp genetic differentiation over short distances. (5) Discriminant analyses differentiated the Israeli from the Turkish populations and within Israel, between central and three marginal regions as well as different soil-type populations (Fig. 4.2; Table 4.2). (6) Allozymic diversity comprised 40% within and 60% between populations (Table 4.3). (7) Linkage disequilibria were abundant, and their number positively correlated ($r_s = 0.60$, $p < 0.01$) with the humidity (Table 4.4). (8) Multilocus organization (Brown et al. 1980) was substantial and also positively correlated with humidity. (9) Allozyme diversity, overall and at single and multilocus structures, was significantly correlated with and partly predictable by climatic and edaphic factors (Table 4.5). (10) The distribution of the significant positive and

negative autocorrelations, and their absence in the correlogram, revealed no similar geographic patterns across loci, eliminating migration as a prime factor of population genetic differentiation.

These results suggest (1) during the evolutionary history of wild emmer, diversifying and balancing natural selections, through climatic, edaphic, and biotic factors, were the major agents of genetic structure and differentiation at both the single and multilocus levels; (2) wild emmer harbors large amounts of genetic diversity exploitable as genetic markers in sampling and abundant with adaptive genetic resources utilizable for wheat improvement (Xie and Nevo 2008).

10.8.3 Population Genetic Structure of Wild Emmer Wheat

10.8.3.1 "Archipelago" Population Genetic Structure of Wild Emmer Wheat

Wild emmer grows in lush and extensive stands in the catchment area of the Upper Jordan Valley (in northern Israel, in the eastern Upper Galilee Mountains, and the Golan Heights). However, elsewhere in the Fertile Crescent (Fig. 4.1a,b in Nevo et al. 2002), populations of wild emmer are semi-isolated and isolated and mostly display a patch structure. At least in Israel, but possibly also elsewhere across the range of wild emmer in the Fertile Crescent, populations are subdivided into demes or clumps of varying sizes including large, medium, and small patches. This "archipelago" structure (Table 4.4 in Nevo et al. 2002) was originally described for a smaller sample of wild emmer in Israel (Nevo et al. 1982) and Turkey (Nevo et al. 1988a), and for wild barley, *H. spontaneum*, in the Near East (Nevo 1992).

The highly subdivided "archipelago"-type ecological population structure of wild emmer is even more distinct than that of wild barley and is matched by its genetic population structure. We found substantially more gene differentiation within and between populations that were sometimes geographically very close in Israel than between wild emmer in Israel and Turkey (Table 3 in Nevo and Beiles 1989). Of the total genetic diversity of *T. dicoccoides*, 40% was found between Israel and Turkey as metapopulations (see *Gst* analysis, Table 4.3 in Nevo et al. 2002) Nevo et al. 2002. This conclusion is reinforced by the microgeographic allozyme analysis based on local edaphic, topographic, and temporal differentiation (Nevo et al. 1988b), and in the extreme case of local differentiation on the Golan Heights (Nevo et al. 1982; Golenberg and Nevo 1987). Similar patterns to those of the allozyme analysis were obtained with DNA analyses at macro- (Fahima et al. 1999, 2001) and microscales (Li et al. 1999, 2000a, b, c, d; 2001; 2002; 2003). The high genetic differentiation within and between populations of *T. dicoccoides* is also reflected by the analysis of allele distribution. The latter revealed sharp, local, and regional differentiation over all genetic indices, such as *P* and *He* (Table 4.1 in Nevo et al. 2002), as well as in individual allele frequencies (Appendix in Nevo and Beiles 1989). Out of the 119 alleles, 61 occurred in Turkey and, remarkably, 114 were found in Israel. Of the 61 alleles at 42 shared loci across Israel and Turkey, five alleles (8.2%) were *unique* for Turkey. Likewise, allele *uniqueness* was also found for the ecogeographic subdivisions in Israel as follows (1) out of 93 alleles occurring in *central* populations, 17 alleles (18.3%) were unique. In marginal populations (2) the southeastern margins (Samaria and Judea steppes) had 84 alleles; six were unique (7.1%). The western margins (western Galilee and Carmel Mountains) had 79 alleles; six were unique (7.6%). (4) The northeast (Mt. Hermon) margins had 53 alleles; one were unique (1.9%). (5) Based on soil types: basalt had 89 alleles; 11 were unique (12.4%); and finally, (6) regarding population size: "large" populations had 93 alleles; 17 were unique (18.3%); "medium": populations had 78 alleles; 11 were unique (14.1%); and "small" populations had 82 alleles; 10 were unique (12.2%) (see discriminant analysis in Table 4.D and Figs 2a-f in Nevo and Beiles 1989).

Moreover, 70% of all variant alleles were not widespread but revealed *localized* and *sporadic* distribution. Likewise, the analysis of genetic distances between populations supports the conclusion that, based on genetic differentiation and allele distribution, sharp local differentiation over short geographic distances is the rule, and the frequency of some common alleles (>10%) is *localized* and high. The population genetic structure of wild emmer is mosaic. This genetic mosaic appears to reflect the underlying ecological heterogeneity that derives from

local and regional geological, edaphic, climatic, and biotic differentiations. The resulting structure, as in wild barley, is an ecological, genetic "archipelago," where the genetic structures are in accordance with the ecological heterogeneity. The extreme case is that of wild barley, *H. spontaneum*, at "Evolution Canyon," Mount Carmel, where the Nei genetic distance between the opposite slopes, separated by 200 m, was $D = 0.48$, which was as large as that between the populations of Jerusalem and Sede Boqer, separated by 100,000 m (×500 apart)!

10.8.4 Spatial Autocorrelation of Allozymes

Spatial autocorrelation analysis predicts that migration will cause high positive correlations (similarity) in the low-order distant groups (i.e., between neighbors), starting from the first distant group in the transect. *Migration is expected to cause similarity between loci and alleles*. This prediction was not realized in our results. By contrast, loci and alleles of wild emmer differ drastically in their autocorrelation pattern between loci. In addition, positive autocorrelations emerge at intermediate and distant groups, thus negating migration as an important evolutionary factor (Table 6 in Nevo and Beiles 1989).

The autocorrelation analysis also predicts that *genetic drift will not create any autocorrelative pattern*. Our data are structured, thus *negating randomness*. However, despite the between loci variation, a general tendency of more positive correlations on the left-hand side of the table and more negative correlations on the right-hand side are evident, suggesting that neither migration nor genetic drift can generate the observed patterns, and selection remains a vital explanatory model.

Environmental selection is also partly autocorrelated and affects loci differentially but differently from migration. This is supported by our data for the following three reasons (1) variation among loci; (2) positive correlation in different distant groups, and not necessarily the first one, as would be expected if migration determined the interpopulation genetic structure; and (3) the predominance of negative correlations in the larger distant groups is expected due to decreasing ecological similarity, often with increasing distance (see Table 6 for details and discussion in Nevo and Beiles 1989).

10.8.5 Multilocus Structure of Allozymes

The multilocus organization estimates expressed by X (2) in Table 4.1 need elucidation. The measure was first suggested by Brown et al. (1980) as an estimate of multilocus associations for summing up multiple-linkage disequilibria. The behavior of this statistic, including its strengths and drawbacks, was discussed by Brown et al. (1980). The merit of this estimate, compared to a set of two-locus linkage disequilibria, is that it effectively reduces multilocus association to a few parameters for comparative studies of populations and species. This measure is an inverse function of two-locus gametic frequencies in inverse analogy with the single-locus Simpson diversity measure. The drawbacks include, first, a severe loss of information and ignorance of the behavior of particular allelic combinations. Second, it is assumed that all loci are of equal interest, which is biologically unjustified. Third, in some cases, the summation combines positive and negative contributions, and thus existing multilocus organizations may be obscured. Finally, this measure is partially dependent on single-loci diversity and/or the number of loci scored, which is important in comparing estimates that are based on radically different single-locus databases.

We acknowledge all of the four abovementioned drawbacks. In our present case, there was no one locus that was polymorphic across all 37 populations (appendix in Nevo and Beiles 1989). Therefore, we based our analysis on 29 loci that were polymorphic to different degrees in different populations. Our results indicated a significant negative Spearman rank correlation of X(2) with evaporation across the range ($r_s = -0.20$, $p < 0.05$), reinforced in the large, highly polymorphic, central populations. We speculate that the intensity of X(2) as a summary statistic may vary in content, though it may be similar in level because of different linkage disequilibria, LDs, in diverse ecologies. Thus, the same level of intensity of genome organization may be caused by different ecological stresses.

10.8.6 Adaptive Nature of Allozyme Polymorphisms

Much evidence for wild emmer supports the hypothesis that allozyme polymorphisms are at least partly adaptive and determined by natural diversifying and balancing selection. The evidence includes (1) the association of single loci and multilocus structures with climatic and soil parameters (Table 4.5). Genetic differentiation appears to match, to a large extent, the ecologically heterogeneous background. (2) Genetic differentiation is not correlated with geographic distance but with local ecological conditions of soil and climate (Tables 1, 3, and 6 of Nevo and Beiles 1989). (3) Macrogeographic genetic differentiation is partially paralleled by microgeographic soil fluctuations as well as topographical and yearly climatic fluctuations (compare Nevo et al. 1991a). (4) The genetic differentiation of wild emmer is far from random at the level of single-, two-, and multilocus organization. Significant linkage disequilibria abound, displaying allele association at the two-locus level (Table 4.4). (5) Strong allele association, above those expected by chance, occur at the multilocus level (Table 4.1). (6) Spatial autocorrelation analysis reveals different significant spatial patterns for different alleles and loci (Table 6 in Nevo and Beiles 1989), ruling out migration as a differentiating factor. Likewise, the general order of negative and positive correlations rules out random drift as a major agent of genetic differentiation in wild emmer.

Our overall results indicate that genetic diversity among localities and over time (see Appendix in Nevo and Beiles 1989, and Table 4.1 for microsite spatial and temporal variation at Tabigha, and Table 5 for autocorrelation analysis in Nevo and Beiles 1989) display weak correlations with geography and stronger ones with ecology for single loci and for multilocus organization. This evidence permits us to eliminate genetic drift and migration as basic factors of genetic differentiation in wild emmer. By contrast, the structures described suggest that climatic and edaphic diversifying and balancing selection regimes at single- and multilocus levels operate on different spatial scales from the *micro-* to the *macrogeographic*. These results appear to match those found in wild barley locally (Nevo et al. 1981, 1983, 1986c) and regionally (Nevo et al. 1979, 1989; Nevo 1992). Remarkably, similar patterns described in wild emmer are demonstrated by DNA diversity, as shown with random amplified polymorphic DNA (RAPD) and short sequence repeats (SSRs) at both regional (Fahima et al. 1999, 2002) and local (Li et al. 1999, 2000a, b, c, d, 2001, 2002a, 2003) scales.

Stochastic factors certainly interact with natural selection in varying environments over space and time. Some of the very small populations of wild emmer described in Nevo and Beiles 1989 (e.g., now 28, 29, 31, and 33 in Table 4.1 of Nevo et al. 2002) may have derived their low levels of *P* and *He* from either founder effects and/or genetic drift. The latter can be a potent force for removing variation, particularly in small populations and when the random environment tends to occasionally push alleles down to low frequencies (Nei 1987; but see Nevo et al. 1997). However, most of the populations described here are medium and large, and they appear to derive and maintain their genetic polymorphisms and structure from climatically and edaphically varying environments.

The maintenance of polymorphisms in wild emmer may be explicable by both spatial and temporal variations in selection. Theory indicates that selection, acting differentially in space, coupled with limited migration (which is typical of wild emmer, mean dispersal of 1.25 m per generation at Yehudiyya, Israel; Golenberg 1987), will maintain a substantial amount of polymorphism (Karlin and McGregor 1972; Hedrick 1986; Nevo et al. 2000). Two-niche models (Levene 1953) can explain genetic differentiation in wild emmer. Different homozygotes are favored in different climatic and edaphic niches. These operate on all levels, from regional to local and on a minuscule level at a locality (see later).

In the case of an additive two-locus panmictic model, we found that, in both haploids and diploids, a stabilizing selection with a cyclically moving optimum may be an efficient factor in protecting polymorphisms for linked loci additively affecting the selected trait (Korol et al. 1994, 1996; Kirzhner et al. 1995, 1999). We found that, within the same class of fitness function, non-equal gene action and/or a dominance effect for one or both loci may lead to local polymorphic stability with substantial polymorphism-attracting domains. These important mechanisms may be relevant also to situations with different violations of panmixia (e.g., Ryndin et al. 2001), including inbreeders such as wild emmer, but they need additional critical, theoretical studies.

The limited migration of seeds between niches over many generations and the accumulated seed banks in the soil provide rich reservoirs of genetic diversity for natural selection to operate on. Such accumulated seed pools provide a memory of past selection regimes. These seed pools can greatly reduce the fitness uncertainty generated by cyclical or random environments and thus free the plant population from having to respond genetically to the fitness conditions occurring from year to year (Templeton and Levin 1979). The temporal differentiation in allele frequency, presumably realized by the richness of seed pools, is exemplified in our microsite emmer wheat study in Tabigha (Appendix in Nevo and Beiles 1989, and details in Nevo et al. 1981). We found higher correlations of spatiotemporal allele frequency changes within, rather than between, allozymic groups, a strong indicator of ecological selection (Li et al. 2000a, b, c, d, 2001, 2002, 2003).

10.9 RAPD Genetic Diversity

Random amplified polymorphic DNA (RAPD) markers represent amplification products from a polymerase chain reaction (PCR) utilizing arbitrary primers and genomic DNA (Williams et al. 1990). Most variation among individuals for RAPDs probably arises from base pair substitutions or insertions/deletions that modify (or eliminate) the primer site or insertions in the genomic sequence that separate the primer sites to a distance that will not permit amplification (Williams et al. 1990). RAPD markers have been useful in studies of taxonomic identities, systematic relationships, population genetic structures, species hybridizations, and parentage identifications (for review see McClelland and Welsh 1994; Penner 1996). RAPDs have contributed to the estimation of genetic diversity among diploid (Vierling and Nguyen 1992), tetraploid (Joshi and Nguyen 1993a; Fahima et al. 1999), and hexaploid (Devos and Gale 1992; He et al. 1992; Joshi and Nguyen 1993b) wheat.

10.9.1 Patterns of RAPD Variation and Genetic Diversity

Genetic diversity of RAPDs was studied at the Institute of Evolution in 110 genotypes of *T. dicoccoides* from 11 populations sampled in Israel and Turkey (Fahima et al. 1999) that were tested allozymically earlier (Nevo et al. 1982; Nevo and Beiles 1989). Our results showed high levels of diversity of RAPD markers in wild wheat populations in Israel. Latitude, temperature, and water availability factors, singly or in combination, explained a significant proportion of the variation in polymorphism of RAPDs. The best two variable-predictors of *P* and *He* were Lt and Tdd (latitude and day–night temperature difference), which significantly explain the 0.70–0.80 of their variance. A three-variable combination involving both geographic (latitude) and climatic factors (day–night temperature difference and mean annual temperature, Lt, Tdd, and Tm) accounted significantly ($p = 0.001$) for 0.94 of the variance in *P*, while the combination LtTddRv (latitude, day–night temperature difference, and mean relative variability of rainfall) accounted significantly ($p = 0.01$) for 0.88 of the variance in *He*.

RAPD loci in wild emmer can cautiously be classified into several categories in terms of their prime ecological predictor (a) water factors (Rn, Hu, Ev, and Rd); (b) soil type (Ba, Tr, and Ren); (c) temperature (Ta, Td, Tm, and Trd); (d) water + temperature (Hu, Rr, Trd, and Tm); (e) soil + temperature (Ba, Ren, Tr, Tj, Tdd, Trd, and Ev); (f) soil + water (Ba, Ren, Rd, and Dw) (Fahima et al. 1999). Our results showed agreement between RAPD and allozyme genetic diversity profiles at regional (Fig. 4.5) and local (Li et al. 1999, 2000a, b, c, d, 2001, 2002, 2003) scales. However, RAPDs yielded much higher values of diversities than allozymes, both regionally and locally. The average regional RAPD values for *P* and *He* were 0.523 (range: 0.407–0.627) and 0.188 (range: 0.138–0.213), as compared with allozyme values of 0.200 (range: 0.100–0.308) and 0.068 (range: 0.026–0.116), respectively. These results showed that RAPD loci were more polymorphic than allozyme loci among wild wheat populations from Israel and Turkey, both *regionally* and *locally* (Li et al. 2000d).

There was a positive, albeit small, Spearman rank correlation ($r_s = 0.41$) between RAPD gene diversity (*He*) values and allozyme *He* values (Fig. 4.5). All the populations showing high allozyme *He* values also showed high RAPD *He* values. However, in many cases, low allozyme *He* values were accompanied by high RAPD values, suggesting that these two genetic

systems are selected independently and do not display a clonal genotypic "hitchhiking" due to the high inbreeding level of *T. dicoccoides*. There were no opposite cases in which high allozyme *He* values were accompanied by low RAPD *He* values. It is noteworthy that the allozyme analysis (Nevo and Beiles 1989) included 423 *T. dicoccoides* individuals (an average of 38 genotypes per population) belonging to the same 11 populations tested for RAPD, while the RAPD analysis included only 110 genotypes (10 genotypes per population) (Fahima et al. 1999). We would expect a higher correlation level between RAPDs and allozymes if the analysis was based on identical plant material with the same sample size. Other studies have also compared the patterns of variation detected at allozyme vs. RAPD loci in several species (Puterka et al. 1993; Isabel et al. 1995; Peakall et al. 1995; Lanner-Herrera et al. 1996). As in our case, the overall patterns of allozymes and RAPDs were generally in good agreement, although the levels of genetic variation and fine-scale genetic structures sometimes differed with the two kinds of markers. The parallels between RAPD and allozyme patterns indicate that similar, primarily deterministic (selection) evolutionary forces, are involved in shaping the genomic structure of both RAPDs and allozymes, i.e., *non-coding* and *coding* genomic regions.

10.10 Microsatellite Diversity

Microsatellites are a relatively new class of molecular markers based on tandem repeats of DNA sequences called simple sequence repeats (SSRs) (Litt and Luty 1989). These 2- to 6-bp repeats are highly polymorphic, even among closely related cultivars, due to mutations causing variation in the number of repeating units. This kind of polymorphism at specific loci is easily detected by the use of specific primers in the flanking regions of such loci and subsequent amplification by PCR (Litt and Luty 1989; Weber and May 1989). The high level of polymorphism combined with the high interspersion rates make microsatellites an abundant source of genetic markers. Extremely high-density molecular maps based on microsatellite DNA markers have been constructed in both human (Dib et al. 1996) and mouse (Dietrich et al. 1996) genomes. Microsatellites appear to serve as a major source of "tuning knobs" for molecular adaptive evolution, including the evolutionary control of the mutation process itself (reviewed and critically analyzed in Kashi et al. 1997; King et al. 1997; Li et al. 2002b, 2004; Kashi and King 2006).

The usefulness of microsatellites as genetic markers in plants was demonstrated for many species including soybean (Akkaya et al. 1995), rice (Wu and Tanksley 1993), maize (Senior and Heun 1993), *Arabidopsis* (Bell and Ecker 1994), and tropical trees (e.g., Aldriche et al. 1998). These studies indicated that microsatellites in plants can be up to tenfold more variable than other marker systems, such as restriction fragment length polymorphism (RFLP). Furthermore, the efficiency of microsatellite markers was demonstrated also for self-pollinating species with relatively low levels of intraspecific polymorphism, such as hexaploid wheat, *T. aestivum* (Plaschke et al. 1995; Röder et al. 1995), and cultivated barley, *Hordeum vulgare* (Saghai-Maroof et al. 1994; Liu et al. 1996; and wild barley Turpeinen et al. 2001; Nevo et al. 2005). The studies conducted at the Institute of Evolution, University of Haifa, demonstrate the application of wheat microsatellite (WMS) markers for the differentiation and estimation of genetic diversity among different accessions of populations and subpopulations of *T. dicoccoides* (Fahima et al. 1998, 2001; Li et al. 2000a, b, c, d, 2001, 2002a) and for tagging of agronomically important genes (Chague et al. 1999; Peng et al. 1999, 2000a, b, c, 2003).

10.10.1 SSR Genetic Diversity

We examined the diversity of wild emmer wheat *T. dicoccoides* based on 20 microsatellite loci (SSRs) in 15 populations (135 genotypes), representing a wide range of ecological conditions of soil, temperature, and water availability, in Israel and Turkey (Fahima et al. 2001). A large amount of diversity at microsatellite loci was observed despite the predominantly selfing nature of this plant species. The 20 Gatersleben wheat microsatellites (GWM), representing 13 chromosomes of genomes-A and -B of wheat, revealed a total of 363 alleles, with an average of 18 alleles per GWM marker (range: 5–26). The proportion of polymorphic loci per population averaged 0.90 (range: 0.45–1.00); genic diversity, *He*, averaged 0.50 (range

0.094–0.736); Shannon's information index averaged 0.84 (range 0.166–1.307). The coefficients of genetic distance between populations were high and averaged $D = 1.903$ (range: 0.923–3.348), an indication of sharp genetic divergence over short distances. Inter-population genetic distances showed no association with geographic distance between the population sites of origin, negating a simple isolation-by-distance model.

Genetic dissimilarity values between genotypes, calculated from the GWM-derived data, were used to produce a dendrogram of the relationships among wild wheat populations using the UPGMA unweighted pairgroup method with arithmetic averages (Fig. 4.6). The results showed that all of the wild emmer wheat populations could be distinguished. Microsatellite analysis was found to be highly effective in distinguishing genotypes of *T. dicoccoides* that originated from diverse ecogeographic sites in Israel and Turkey, with 88% of the 135 genotypes correctly classified into sites of origin by discriminant analysis. Our present microsatellite results were *non-random* and in agreement with the previously obtained allozyme (Nevo and Beiles 1989) and RAPD (Fahima et al. 1999) patterns, although the genetic diversity values obtained with microsatellites were much higher.

Significant correlates of microsatellite markers with various climatic and soil factors suggest that, as in allozymes and RAPDs, natural selection causes adaptive microsatellite ecogeographic differentiation not only in *coding* but also, most importantly, in *non-coding* genomic regions. Hence, the concept of "junk DNA" needs to be replaced by one, which at least partly takes into account a DNA regulatory function (reviewed in Li et al. 2002b, 2004). The results obtained suggest that microsatellite markers are not only adaptive markers but are also useful for the estimation of genetic diversity in natural populations of *T. dicoccoides* and for tagging agronomically important traits derived from wild emmer wheat (see Chap. 8 in Nevo et al. 2002).

10.10.2 Summary of SSR Genetic Diversity of T. dicoccoides

A summary of the genetic data for each of the 15 populations of *T. dicoccoides* is given in Table 10.2. Mean levels of the proportion of polymorphic loci, *P* (5%), genetic diversity, *He* (Nei 1987), and Shannon information index of the 15 populations of wild emmer wheat were 0.90, 0.50, and 0.84, respectively. The range of diversity of *He* between the wild wheat populations was large (0.094–0.736). The highest value of *He* (0.736) was obtained for the high-altitude and arid-cold steppic population of Mt. Hermon, which represents one of the most stressful xeric-cold habitats in Israel. In contrast, low levels of diversity, with *He* values of 0.094 and 0.249, characterized the small isolated populations from Bet-Oren on Mt. Carmel and Givat-Koach near Tel Aviv on the coastal plain, respectively.

10.10.3 SSR Versus RAPD and Allozyme Genetic Diversity Profiles

The genetic diversity profiles obtained in the microsatellite study were compared with those obtained previously with allozyme (Nevo et al. 1982; Nevo and Beiles 1989) and RAPD (Fahima et al. 1999) markers (Fig. 4.7 in Nevo et al. 2002). All populations studied here showed a higher proportion of polymorphic loci (*P*) and higher genetic diversity (*He*) values for microsatellite loci than for allozyme and RAPD loci. The average microsatellite values for *P* and *He* were 0.90 (range: 0.445–1.00) and 0.50 (range: 0.094–0.736), compared with allozyme (Nevo and Beiles 1989) values of 0.200 (range: 0.100–0.308) and 0.068 (range: 0.026–9.116), and RAPD (Fahima et al. 1999) values of 0.524 (range: 0.407–0.627) and 0.188 (range: 0.138–0.213), respectively. These results showed that microsatellite loci were far more polymorphic than allozyme and RAPD loci among wild wheat populations from Israel and Turkey. Similar results were obtained in the microscale analysis at Ammiad (Li et al. 2000d).

10.10.4 Genetic Structure of Wild Emmer Wheat Populations Based on SSR

Wild emmer wheat grows in lush extensive stands in the catchment area of the Upper Jordan Valley, in the

eastern Upper Galilee Mts., and the Golan Heights in Israel. However, as previously stated, elsewhere in the Fertile Crescent, populations of wild emmer are semi-isolated and isolated and largely display a patchy structure (Nevo et al. 1982, 1995; Nevo and Beiles 1989). Genotypes chosen for this study spanned most of the ecological range of emmer wheat in Israel. Central populations (Yehudiyya, Gamla, Rosh-Pinna, and Tabigha) were collected in warm, humid environments of the Golan Plateau and near the Sea of Galilee. Marginal steppic populations were collected across a wide geographic area in the northern (Mt. Hermon), southeastern (Mt. Gilboa, Gitit, Kokhav-Hashahar), and southern (Jaba) borders of Israeli wild emmer distribution, ranging from xeric-cold to xeric-warm regions. Marginal mesic (Mediterranean) populations (Amirim, Bet-Oren, Bat-Shlomo, and Givat-Koach) were collected from the western border of the wild emmer range (Nevo et al. 1982; Nevo and Beiles 1989). The dendrogram (Fig. 4.6) and the discriminant analyses (Fig. 4.8) show a clustering together of the central populations and a clear separation of most of the isolated marginal populations (e.g., Hermon, Givat Koach, Gitit, Bet-Oren, and Turkey) from the other populations. These results demonstrate population divergence based on the *non-coding* genomic regions. Interestingly, the *non-coding* DNA genome also outlines an archipelago-type genetic structure of wild emmer, as demonstrated earlier by the *coding* DNA genome expressed by allozymes (Nevo and Beiles 1989).

10.10.5 Ecological SSR Correlates of SSR Polymorphism

A test of the best predictors of *P*, *He*, Shannon's information index, allele number per locus, and representative allele frequencies (alleles that were present in at least four populations) was conducted by stepwise multiple regression (MR) analysis (Fahima et al. 2001). These characteristics were used as dependent variables, and the geographic, climatic, and edaphic factors were used as independent variables. The results are given in Table 4.10. Altitude, temperature, and water availability factors, singly or in combination, explained a significant proportion of the diversity in the polymorphism of the microsatellites. The best two-variable predictors of *He* and Shannon's Information index were Al and Ta (altitude and mean temperature in August), explaining the significance of the 0.38–0.40 of their variance. A three-variable combination, involving both geographic (altitude) and climatic (mean temperature in August and mean annual rainfall) variables, AlTaRn, accounted significantly ($p = 0.05$) for 0.49 of the variance in *P* and Shannon's information index.

Based on the number of microsatellite alleles per locus, microsatellite loci in wild emmer can be classified into several categories in terms of their prime ecogeographic predictors (a) *water factors* (Rn, Hu, Rv), (b) *soil type* (So), (c) *temperature* (Td), (d) *geographic factors* (Al), (e) *water + temperature* (Rn, Hu, Trd), (f) *soil + temperature* (So, Trd, Td, Ta), (g) *geographic factors + temperature* (So, Trd, Td, Ta), and (h) *geographic factors + water* (Al, Ln, Dw, Rn, Hu, Rv, Rd). Clearly, ecogeographic factors affect the differentiation pattern of primarily *non-coding genomic* SSRs, the same as they affect *coding* genomic regions (allozymes). The idea that *non-coding genomic* regions are "junk DNA" again seems unrealistic (Li et al. 2002b, 2004). As indicated for RAPD diversity, although SSR and allozymes are correlated, cases of low SSR and high allozymes exist, possibly suggesting that the SSR ecological correlates may be partly independent from allozymes; so the SSR pattern is not only a result of the inbreeding system but is also due to direct selection on the SSRs.

10.10.6 SSR Diversity Associated with Aridity Gradient in Wild Emmer Wheat Populations

The association between 54 SSR loci diversity and ecogeographical variables was studied in 145 genotypes from 25 populations (*T. dicoccoides*) sampled across an aridity gradient in Israel (Peleg et al. 2008). The results revealed that 56% of the genetic variation resided among accessions within populations, while only 44% of the variation resided between populations. The UPGMA tree, based on the microsatellite allelic diversity, divided the 25 populations into six major groups. Several groups were comprised of populations that were collected in ecologically similar, but geographically remote, habitats. Furthermore, genetic differentiation

between populations was independent of the geographical distances. A unimodal relationship was found between allelic diversity and annual rainfall ($r = 0.74$, $P < 0.0002$), indicating higher allelic diversity in populations from intermediate environmental stress (e.g., rainfall 350–550 mm per year). SSR diversity appears to be adaptively selected by drought stress and could be a guideline in wheat breeding.

10.10.7 Genetic Diversity of Wild Emmer Versus Cultivated Wheat

In the regional wild wheat study (Fahima et al. 2001) using 20 Gatersleben wheat microsatellite markers (GWM), 363 alleles were revealed among 135 wild wheat genotypes, an average of 18 alleles per GWM. In a previous study, also using GWM markers, Plaschke et al. (1995) reported a total of 142 alleles among 40 cultivated wheat lines, an average of 6.2 alleles per GWM. The results obtained in the wild emmer study demonstrated the high diversity in microsatellite sequences among *T. dicoccoides* genotypes compared with the cultivated germplasm, as was already demonstrated by RAPDs (Fahima et al. 1999) and allozyme markers (Nevo and Beiles 1989). Clearly, genetic diversity was eroded across the *coding* and *non-coding* genome during the domestication of major cereal crops (Nevo 1986; Fahima et al. 1998, 1999, 2001; Haudry et al. 2007; Fu and Somers 2009). This contributed to the susceptibility and vulnerability of the wheat cultivars to abiotic and biotic stresses.

The dendrogram presented in Fig. 4.6 clearly demonstrates the ability of microsatellites, developed from *T. aestivum* sequences, to detect a large amount of genetic diversity in wild emmer wheat and to identify intergroup differences. Our results demonstrated that the SSR DNA polymorphism of wild emmer wheat was correlated with *macro-* and *micro*scale ecogeographic factors. In particular, the Israeli populations exhibited high interpopulation and interregional polymorphism. SSR results demonstrate geographic and genomic congruence and continuity from *macro-* to *microscales* and from *coding* (protein) to *non-coding* (presumably partly regulatory) DNA (Nevo et al. 1982; Nevo and Beiles 1989; Li et al. 1999, 2000a, b, c, d, 2001, 2002a, b).

10.10.8 Single Nucleotide Polymorphism

A single nucleotide polymorphism (SNP) is a DNA sequence variation occurring when a single nucleotide – A-, T-, C-, or G – in the genome differs between members of a species (or between paired chromosomes in an individual). SNPs are the ultimate genomic variation. We showed (Wang et al. 2008, 2010) in α-amylase inhibitors that SNPs, like other genetic markers, are variable, partly and adaptively, and are subjected to natural selection (see later).

10.11 Genome-Wide Reduction of Genetic Diversity in Wheat Breeding

Public concerns about crop uniformity introduced by modern plant breeding and genetic vulnerability to biotic and abiotic stresses have been one of the major forces driving long-term efforts in plant germplasm conservation for future food security. However, such concerns have gained little empirical support, as recent molecular diversity analyses of improved crop gene pools did not reveal much reduction from early to recent breeding efforts. Fu and Somers (2009) conducted a genome-wide examination of 75 Canadian hard red spring wheat (*T. aestivum* L.) cultivars released from 1845 to 2004 using 370 simple sequence repeat (or SSR) markers that were widely distributed over all 21 wheat chromosomes. A total of 2,280 SSR alleles were detected. Allelic reduction occurred in every part of the wheat genome, and a majority of the reduced alleles resided in only a few early cultivars.

Significant allelic reduction started in the 1930s. Considering 2,010 SSR alleles detected in the 20 earliest cultivars, 38% of them were retained, 18% were new, and 44% were lost in the 20 most recent cultivars. The net reduction of the total SSR variation in 20 recent cultivars was 17%. This clear-cut evidence supports the contention that modern plant breeding reduced the genetic diversity of Canadian wheat and underlies the need for conserving wheat germplasm and introducing genetic diversity into wheat breeding from its relatives. A massive loss of nucleotide diversity in wheat during domestication was described by Haudry et al. (2007).

T. dicoccoides is one of the important genetic resources to genetically enrich and diversify the impoverished bread wheat *T. aestivum* (Nevo 1983, 1986, 1988, 1995, 2001a; Nevo et al. 1985, 2002; Cakmak et al. 2004; Xie 2006; Xie and Nevo 2008), together with other wheat relatives.

10.12 Ribosomal DNA Diversity

Ribosomal RNAs are involved in translation and interact directly with translation initiation, hence their cardinal importance in protein synthesis. The rRNA genes are separated by non-transcribed intergenic spacers. What could be the function of these spacers? We explored the variation in rRNA gene structures and its relationship to allozymic diversity in wild emmer (Flavell et al. 1986). The rRNA genes, the infrastructure of protein synthesis, lie in tandem arrays at the nucleolus organizers (NOR) on chromosomes 1B and 6B of *T. dicoccoides*. In hexaploid wheat and in *T. dicoccoides,* there is variation in the amount of DNA residing between the transcribed regions specifying the rRNAs. This variation often involves the number of 135-bp repeats that are clustered in this intergenic region (Appels and Dvorak 1982). A map of a typical rDNA repeat unit from chromosomes 1B or 6B from hexaploid wheat is shown in Fig. 1 in Flavell et al. (1986). The variable segment containing the 135-bp repeats is marked. To understand the incidence and possible significance of the spacer-DNA variation in hexaploid wheat, it was considered useful to study the equivalent variation in the Israeli populations of *T. dicoccoides* that had already been genetically and ecologically studied.

Variation in the intergenic spacer of ribosomal DNA (rDNA) of tetraploid wild emmer wheat, *T. dicoccoides*, in Israel was examined in 112 individual plants representing 12 populations (Flavell et al. 1986) that had been tested earlier for allozymic diversity encoded by 50 gene loci (Nevo et al. 1982). The variation, detected by means of restriction endonucleases, results in part from variation in the number of 135-bp repeats in tandem array in the intergenic DNA. Intergenic spacers of genes within a single locus, such as in the rDNA locus, are relatively homogeneous, but populations of *T. dicoccoides* display a wide spectrum of rDNA-spacer-length variation. Some populations are very homogeneous, whereas others are heterogeneous. The most homogeneous population (Bet Meir, Table 1 in Flavell et al. 1986) consists of plants with spacer-rDNA lengths that are the most common in *T. dicoccoides* in Israel.

We showed that (1) natural populations of tetraploid *T. dicoccoides* in Israel display a wide spectrum of rDNA diversity. (2) The allozymic and rDNA diversities are highly and significantly intercorrelated. (3) Both allozymic and rDNA diversities are significantly correlated with climatic variables. It thus appears that both allozyme and rDNA genic markers may be molded, at least partly, by diversifying natural selection in accordance with extreme xeric ecological stresses, such as drought in the Samaria eastern-xeric marginal-biotic environment.

The rDNA phenotypes of wild barley *H. spontaneum* were also more diverse than those of cultivated barley and correlated with different climatic, edaphic, and ecogeographical microsites and niches at "Evolution Canyon" (EC) and Tabigha (Gupta et al. 2002). The sharp microsite ecogeographic variation, either climatic at EC or edaphic in Tabigha, appear adaptive and channeled by climatic and edaphic natural selection, either directly on rDNA loci or indirectly on linked loci.

Of prime importance for evolutionary theory of protein and DNA diversities is the fact that the genetic landscape is *non-random* between loci, populations, and habitats, displaying possible adaptive patterns since allozyme and DNA variation are predictable on the basis of climatic factors. Allozymic and rDNA diversities are not only positively intercorrelated (Table 3, Flavell et al. 1986), but, most importantly, both polymorphic systems are significantly correlated with, and predictable by, climatic factors. The extent of fragment length polymorphism was found to be high in the steppic Samaria populations (Taiyiba and Kokhav Hashahar). These populations occupy a climatically highly stressful and fluctuating region situated between the Mediterranean and Samaria steppe climatic regimes. Could this spacer polymorphism represent a regulatory adaptation to a climatically fluctuating environment characterized by interchanging dry-hot and wet-cool years and, complementarily, an adaptation to shorten the life cycle to avoid the early summer drought (Nevo et al. 2002)?

In summary, the population genetics of allozymes and DNA markers (RAPD and SSR) of wild emmer

wheat, *T. dicoccoides*, the progenitor of most cultivated wheats, have been reviewed across its regional geographic range in the Near East Fertile Crescent. We found massive *non-randomness* and *heavy structure*. Diversity in both the *coding* (allozymes) and *non-coding* (primarily RAPD and SSR DNA) genomes was largely correlated with, and predictable by, abiotic and biotic factors. Natural selection, both diversifying and balancing selection, seems to be the major evolutionary driving force responsible in wild emmer for the pattern of primarily adaptive structures to environmental stress and heterogeneity. Are the macroscale genetic patterns also reflected at the microscale evolutionary theaters? We tested genetic polymorphisms at four microsites in northern Israel, in the Golan Heights (Yehudiyya, Qazrin), and eastern Upper Galilee (Tabigha, Ammiad), displaying microclimatic, edaphic, and topographic ecological contrasts at a *microsite*.

10.13 Microgeographic Studies of Allozyme, DNA, and Candidate Gene Polymorphisms in *T. dicoccoides*

Microgeographic studies of allozymes, DNA markers and sequences, and candidate genes elucidate the nature and dynamics of genetic diversity and the evolutionary driving forces shaping evolution. During the last three decades, we conducted extensive studies at the Institute of Evolution, University of Haifa, on allozymes and DNA differentiation in many species of bacteria, fungi, plants, animals, and humans (see full and subdivided lists of publications of Nevo at *http://evolution.haifa.ac.il* and Nevo 2001b). The microgeographic studies on genetic diversity in *T. dicoccoides* have been overviewed by Nevo et al. (2002, Chap. 5) and in Nevo's list of Genetic Evolution of Wild Cereals and Crop Improvement at *http://evolution.haifa.ac.il*, particularly in Nevo et al. (1991a) and Li et al. (1999, 2000a, b, c, d, 2001, 2002a, 2003). These studies suggest that both allozyme and DNA polymorphisms are at least partly adaptive across the *coding* and *non-coding* genomic regions. Our microsite studies, incorporating microclimate, rock, soil, and topographical variation, indicate that microniche ecological selection is the primary cause of genetic differentiation in wild emmer and other model organisms studied, rather than stochastic processes and the neutrality of allozymic and DNA variants. Here, I will provide a very brief overview of the four different microgeographic studies in wild emmer wheat related to microclimate (Yehudiyya), soil (Tabigha), and topography (Qazrin-Yehudiyya, Ammiad).

10.14 Yehudiyya: Microclimatic Sun/Shade Genetic Divergence

10.14.1 Allozymes

Localized in an open park forest of *Quercus ithaburensis* in the basaltic lower foothills of the Golan Heights, we compared wild emmer (in an area habitated by thousands of plants) in 12 repeated experiments in Yehudiyya, subdivided in two microniches (shady under a tree canopy and sunny in immediate tree circumference); each tree with its two microniches was considered an experiment. Trees with a canopy diameter up to 20 m were separated by only 10–20 m. The comparison between the two microniches, shady and sunny, separated by 2–4 m (see microclimate divergence in Table 5.1a in Nevo et al. 2002), near each tree, included five plants from each niche (altogether 120 plants). Wild emmer was sparse in the shade (more stressed for light) and abundant in the sun. Allozymes encoded by 48 loci diverged significantly at single, two, and multilocus structures (Figs. 5.3, 5.4 and Tables 5.2-5.5 in Nevo et al. 2002 and Fig. 10a,b, here). These strongly divergent results indicate that the sun-shade differences are adaptive and selected by temperature, water, and light variables, singly or in combination, rather than by migration or chance. The differential stresses involved high solar radiation, temperature, and drought in the sunny niche and light deprivation in the shady niche. The level of polymorphism, *P*, number of alleles per locus, gene diversity partitioning, discriminant analysis, linkage disequilibria, and multilocus organization diverge between sun and shade. These *micro* results corroborate *macro* results in wild emmer and other organisms (Nevo et al. 2002) (Fig. 10.4a, b here).

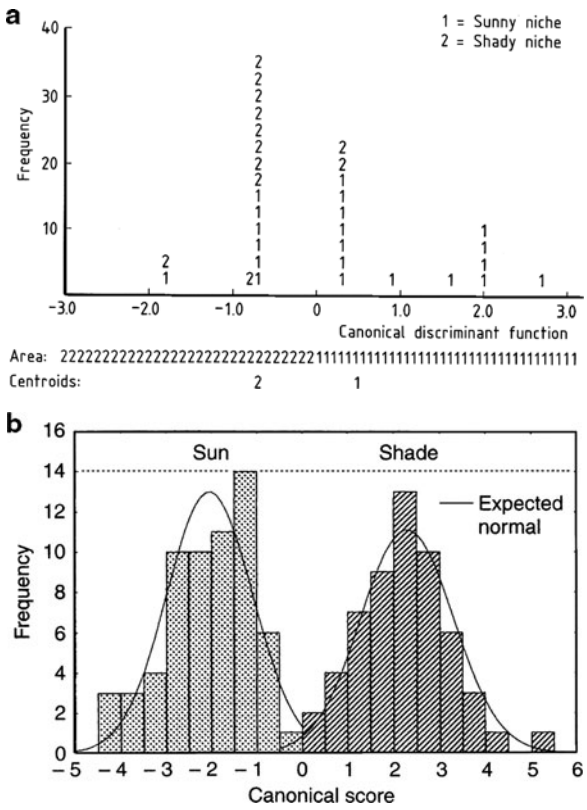

Fig. 10.4 (**a**) Discriminant analysis of wild emmer wheat in Yehudiyya according to two climatic microniches, sunny and shady, based on seven polymorphic isozyme loci (Nevo et al. 1988b). (**b**) The histogram of frequencies of canonical scores for wild emmer wheat at the shady and sunny niches in Yehudiyya according to 25 polymorphic RAPD loci (Li et al. 1999)

10.14.2 RAPD Analysis of Wild Emmer at Yehudiyya (Li et al. 1999, 2001b)

RAPD discrimination, based only on two of the 12 selected loci, succeed in correctly and significantly classifying genotypes into the sunny and shady microclimatic niches (see details in Nevo et al. 2002, Sect. 5.2.2) (Fig. 10.4b here).

Thus, largely *non-coding* RAPD divergence significantly identifies plant niche origin similar to allozymes. Two allele linkage disequilibria were extensive in the entire population (52), shade (56), and sun (40) (Li et al. 1999). These DNA polymorphisms, like allozymes, appear to be significantly associated with *unique* microclimatic stresses, suggesting that they are the result of adaptive natural selection rather than chance due to population subdivision or founder effects (Li et al. 1999).

10.14.3 SSR Analysis of Wild Emmer in Yehudiyya

SSR polymorphism of wild emmer from Yehudiyya, in the same layout as for allozymes and RAPD (Li et al. 2002a), was high (92.9%). Significant microclimatic divergence of many loci displayed asymmetric and *non-random* distribution of repeat numbers. Niche-specific and unique alleles and linkage disequilibria abound in the two niches, shady and sunny, separated by a few meters. SSR diversity is *non-random* and uniquely affected by microclimatic stress. Genetic factors (genome, chromosome, motif, locus, and LD) significantly affected SSR diversity. Genetic niche divergence across a few meters and correct allele niche classification at the largely *non-coding* SSR loci is substantial, as was true for largely *non-coding* RAPD loci and *coding* DNA for allozymes. The evidence displays interaction between ecological stresses and genetic factors oriented by natural selection (see Nevo et al. 2002, Sect. 5.2.3.1). Genome-B showed a larger average repeat number (ARN) than genome-A, whereas genome-A showed a larger *variance* in repeat number than genome-B.

10.14.4 The Tabigha Microsite

We have also shown that edaphic, topographical, and temporal factors at the Tabigha microscale in northern Israel affect *non-random* qualitative and quantitative allozymes, and DNA divergence of wild emmer (Nevo et al. 1988b; Li et al. 2000a, b) significant allozyme, SSR divergence in allele distribution, repeat length, genetic diversity, and LDs were found between the two soil types, basalt and terra rossa, across the 100 m transect subdivided into 50 m basalt and 50 m terra rossa.

10.14.5 Golan Heights

This study was conducted on a dipping basalt slope between Qazrin and Yehudiyya (Golenberg 1986a, b, 1987; Golenberg and Nevo 1987, Fig. 5.1 in Nevo et al. 2002). Two allozyme multilocus genotypes (MGPs) defined by eight loci (Mdh-1, Ipol, B Glu, Pept-1, Pept-3, 6 Pgdh-2, Hk and Rc) were distributed in a step cline along a 10-km transect. Linkage

relationships and chromosomal locations of the eight loci were determined. Linkage disequilibria between loci increased over four generations, and probable epistatic selection was established. Significant differences were found between Yehudiyya and Qazrin in morphology, germination, and phenology. Reciprocal translocation did not reveal local adaptations, which was possibly due to the short and interrupted experiment. Outcrossing was below 1%. Gene flow per generation was low, about 1.25 m. Our results suggested that sporadic selection pressures, high selfing rates, and limited gene flow are substantial in multilocus evolution of wild emmer.

10.14.6 The Ammiad Microsite

The fourth microsite in the multidisciplinary research program (Anikster et al. 1991; Israel Journal of Botany 1991) is a national effort to unravel the ecological, genetic, and morphological aspects *over time* of *T. dicoccoides* (at a microsite) differentiated lithologically and topographically in an attempt to optimize in situ conservation (Anikster and Noy-Meir 1991). Ecologically, the site could be divided into four major habitats and 11 subhabitats. Environmental variation was also caused by wide differences in annual totals and distribution of the Mediterranean winter rainfall. In the years 1984 to 1989, plants were monitored at closely spaced permanent sampling points along four topographically diverse transects (Anikster and Noy-Meir 1991). The dominant ecological factor causing heterogeneity within the site and vegetation and, presumably, the wild wheat population is the rock microrelief, operating through soil moisture and possibly also through local modification of grazing pressure and competition (Noy-Meir et al. 1991). Rainfall, rockiness, and grazing are the main primary factors affecting the demography of wild emmer; hence, they are also the major selective forces affecting genetic structure.

10.14.7 Allozymes

Allozymic variation in proteins encoded by 43 loci was analyzed electrophoretically from 1983–1984 to 1986–1987, during four consecutive growing seasons, in 812 individual plants of wild emmer wheat, *Triticum dicoccoides*, from microsites at Ammiad, north of the Sea of Galilee (Nevo et al. 1991a). Significant genetic differentiation, according to vegetationally and topographically defined habitats and subhabitats, was found primarily in *space* over very short distances and secondarily over *time*. The highest gene diversity occurred in the Karst formation where soil moisture was most variable, i.e., in the habitat displaying the broadest niche. Our results suggest that allozyme polymorphisms in wild emmer wheat are partially adaptive. Genetic differentiation appears to be primarily affected by environmental factors related to topography and temporal climatic changes, probably through drought, e.g., aridity stress.

10.14.8 Spatiotemporal Polymorphism of High-Molecular-Weight Glutenins in Wild Emmer at Ammiad

Variation in the seed electrophoretic pattern of the high-molecular-weight (HMW) glutenin subunits was studied in *T. dicoccoides* at Ammiad during a 5-year period (1983–1984 to 1987–1988) (Felsenburg et al. 1991). The population was found to be highly polymorphic: the HMW glutenin loci of genome-A, *Glu-AI-1* and *Glu-AI-2*, had four and two alleles, respectively, and those of genome-B, *Glu-BI-1* and *Glu-BI-2*, had five and seven alleles, respectively. The A-genome alleles appeared in four combinations, and the B-genome alleles appeared in 12 combinations. There were 18 intergenomic combinations (A- and B- genotypes), some of which were very rare, while others were abundant and distributed along transects in clusters. The spatial distribution of these genotypes was *non-random*, with each of the 11 habitats characterized by different genotype frequencies. A high affinity between specific HMW glutenin genotypes and certain habitats was found, suggesting natural selection as the major evolutionary driving force.

10.15 Genetic Resources of Wild Emmer for Wheat Improvement

Domestication, evolution, and particularly modern agriculture practices have drastically reduced the genetic diversity of cultivars, making them vulnerable

and susceptible to biotic (diseases and pests) and abiotic (salinity, drought, soil, and mineral poverty) environmental stresses. The current threat of the extremely virulent new race of stem rust Ug99 from East Africa to genetically, relatively uniform, wheat *T. aestivum* is evident (Borlaug 2007). A recent extensive study in Canadian *T. aestivum* (Fu and Somers 2009) demonstrated that the net reduction of the total SSR variation in 20 recent cultivars was 17%. This is clear-cut evidence of wheat genetic impoverishment, highlighting the need for conserving wheat germplasm and introducing genetic diversity into wheat breeding (see also Haudry et al. 2007).

Wheat wild relatives are the best hope for replenishing the impoverished genomes of the cultivars (Feldman and Sears 1981; Nevo 2001a, 2004; Nevo et al. 2002). This is especially important in a world where populations are still expanding, food requirements are constantly increasing, yet world food production is threatened (*http://www.ftp.fao.org/docrep/fao/010/i0112e/i0112e03.pdf/*). We have recently reviewed the importance of wild emmer, *T. dicoccoides*, in the future of wheat improvement by elucidating its genetic resources, gene mapping, and potentials for wheat improvement. An extensive review of the genetic resources of wild emmer appears in Nevo et al. (2002), Part III; likewise, see also Israel Journal of Botany (1991); Israel Journal of Plant Sciences (2001, 2007); Nevo (1983, 1986, 1989, 1995, 2001a); Peng et al. (2000a, b, c, 2001, 2003); Distelfeld and Fahima (2007); Gladysz et al. (2007); Fu et al. (2009), and Xie and Nevo (2008).

10.16 Genetic Variation in Phenotypic and Genotypic Agronomic Traits

10.16.1 Genetic Variation in Agronomic Traits

Wild emmer wheat in Israel varies not only in genetic polymorphisms of allozymes and DNA markers, but also in quantitative traits of agronomic importance (Nevo et al. 1984). These include agronomic traits, disease resistance, amino acids, protein content, and ecological tolerances to drought, salt, herbicides, and photosynthetic yield. These traits, briefly described below, are economically significant and should be conserved and utilized in wheat crop improvement. Our results substantiated earlier claims that wild emmer in Israel displays remarkable morphological and physiological variation both within and between populations in each of the agronomic characteristics studied (Table 2 and Fig. 1 in Nevo et al. 1984; Fig. 6.1). These traits are derived by natural selection as adaptive traits at both *macro-* and *microgeographic* scales. A detailed quantitative trait loci (QTL) mapping of 11 agronomic traits appears in Peng et al. (2003).

We compared and contrasted agronomic traits (germination, earliness, biomass, and yield variables) of different Israeli populations of wild emmer in common garden experiments in the Negev desert and Mount Carmel Mediterranean environment (Nevo et al. 1984). The results indicated that the characteristics studied are partly genetically determined. Striking genotypic and phenotypic variation was found between and, at least for some characters, also within populations in each site, whereas remarkable environmental variation was found between the mesic (Haifa) and xeric (Negev) sites (Fig. 6.1, Nevo et al. 1984).

10.16.2 Heat Production in Wild Cereals

We calorimetrically compared and contrasted heat production in seedlings incubated at 5°C and 24°C, testing genotypes from cold and warm Israeli populations of the wild progenitors of barley (*H. spontaneum*) and wheat (*T. dicoccoides*) (Nevo et al. 1992c, Fig. 6.2a, b). Our results indicated that (1) in both wild cereals, heat production was significantly higher at 5°C than at 24°C; (2) interspecifically, wild barley generated significantly more heat than wild wheat at both 5°C and 24°C; (3) intraspecifically, at 24°C, wild barley from warm environments generated significantly more heat than wild barley from cold environments. We hypothesized that both *inter-* and *intraspecific* differences in heat production evolved adaptively by natural selection in accordance with the genetic niche width variation hypothesis (van Valen 1965). These differences presumably enhance biochemical processes, and hence growth, thereby leading to the shorter annual cycle of barley than that of wheat. They may also explain the wider range in wild and cultivated gene pools of barley as compared with those of wheat. We proposed that shortening the growth period through the utilization of heat

production gene(s) is feasible in breeding by classical methods and/or modern biotechnology.

10.16.3 Abiotic Stress Tolerance and Variation in Physiological Performance: Salt and Drought Tolerance

A recent review on salinity and water stress improvement of crop efficiency appears in Ashraf et al. (2009) and a review on drought and salt tolerance in wild relatives for wheat and barley improvement appears in Nevo and Chen (2010). Natural populations of *T. dicoccoides* are characterized by abundant genetic variation in resistance to physiological stresses, such as salinity and drought. This is highlighted by the tremendous variation both between and within populations observed in our extreme desert station in the Negev at Avedat (Table 2 and Fig. 2 in Nevo et al. 1984). In our Avedat experiments, under a water regime of 130 mm and highly saline soil conditions, some populations and superior genotypes have proved outstanding in both growth and yield despite these extremely dry ecological conditions. Thus, the genetic resources of the progenitor display striking morphological and physiological traits that are of potential economic importance for wheat improvement. We conducted critical salt tolerance tests on these populations as described below.

10.16.3.1 Salinity Tolerance

Salt tolerance was tested under 250 and 350 mM NaCl in the progenitors of the cultivated cereals, wild barley (*H. spontaneum*), and wild emmer wheat (*T. dicoccoides*), from Israel (Nevo et al. 1993c; Table 6.1). We found superior genotypes of *T. dicoccoides* ripening at 250 mM (=40% seawater) and of *H. spontaneum* ripening at 350 mM NaCl (=60% seawater).

10.16.3.2 Genetic Polymorphisms in ^{22}Na Uptake

We also found variation in ^{22}Na uptake in wild emmer, which may provide salt tolerance resources for wheat improvement (Nevo et al. 1992a). Polymorphisms in ^{22}Na uptake relative to the uptake into *T. durum* cv. Langdon (relative uptake Langdon, RUL) were found among 230 genotypes of tetraploid *T. dicoccoides* in Israel. The accessions were collected from 15 xeric margins of the Israeli range (Table 6.2). The results indicated that (1) there is widespread genetic variation both within and primarily between populations in ^{22}Na uptake. (2) Remarkably, the xeric Samaria eastern and southern populations, characterized by a drier and hotter climate, displayed significantly lower values of relative ^{22}Na uptake as compared with the mesic western populations (0.38 vs. 0.61 RUL). (3) Ecological factors and allozyme markers, either singly or in combination, explained up to 81% of the variance in RUL and, therefore, appear to be good guidelines for predicting the ecogeographic location and allozymic constitution of specific genotypes that have low ^{22}Na uptake and, hence, are presumably associated with high salt tolerance. We concluded that *T. dicoccoides* harbors genetic resources for salt tolerance that can be utilized in wheat improvement by both classical plant breeding and by modern biotechnology. Notably, *Ae. tauchii* (D-genome of bread wheat) also carries salt tolerance genes as well as other distant wheat relatives (Nevo et al. 2002). Salt tolerance in wild emmer is an ongoing research project conducted by researchers at the Institute of Evolution. Wide genetic diversity of salinity tolerance, sodium exclusion, and growth in wild emmer are described in Shavrukov et al. (2010).

10.16.3.3 Drought Tolerance

Drought is the main environmental stress that limits plant and crop success. This is particularly true under global warming and increasing drought, including worldwide desertification and salinization. Our preliminary experiments in growing wild emmer from different Israeli populations under a common desert environment in Avedat (northern Negev desert, 130 mm annual rainfall!) showed differential tolerance of wild emmer to drought resistance (Nevo et al. 1984). Further comprehensive studies of wild emmer across a southward Israeli aridity gradient (Peleg et al. 2005, 2007, 2008) established our preliminary observations that wild emmer, *T. dicoccoides,* is superior in drought resistance to *T. durum* cultivars. Wild emmer displays better water-use efficiency than the cultivars

and could be used efficiently in reinforcing drought resistance of cultivated wheat as envisioned by Aaronsohn and Schweinfurth (1906) more than 100 years ago. It displays high allelic diversity associated with high drought resistance capacity. The distinct drought resistance of xeric wild emmer populations from the steppic regions of Israel harbor important genetic diversity for molecular, biochemical, morphological, and physiological drought-resistance resources for wheat improvement. Numerous QTLs and genes underlying drought resistance in wild emmer (Peleg et al. 2007, 2008) could advance drought resistance wheat marker-assisted breeding in both tetraploid (*durum*) and hexaploid (*aestivum*) cultivated wheat.

We have identified genes for drought resistance by comparative genome-wide transcriptome analysis in two wild emmer genotypes, *susceptible* and *resistant* (Krugman et al. 2010). Among 221 drought-induced selected transcripts in the resistant genotype, 26% are involved in multilevel regulatory processes, transcriptional regulation, alternative splicing, protein phosphorylation, and abscisic acid signaling implicated in stomatal closure. The identified proteins are involved in drought adaptations, such as cell wall adjustment, cuticular wax deposition, lignification, osmoregulation, redox homeostasis, dehydration protection and drought-induced senescence. The drought-resistant genes described in this study could be used in breeding drought-resistant wheat.

10.16.3.4 Herbicide Response

Crop species can show differential responses to successful, widely used herbicides. Some varieties are unaffected by application, whilst others show symptoms of damage, ranging from a slight reduction in vigor to complete plant death (Snape et al. 1991a). The elucidation of the genetic control of such responses is important for developing strategies in breeding for herbicide resistance within crop species and also in understanding the modes of action of the herbicides and in the evolution of resistance in weed species.

The responses of wild populations of *T. dicoccoides* from different ecogeographic areas of Israel to three herbicides (difenzoquat, chlorotoluron, and metoxuron), commonly used on cultivated wheat, were studied (Snape et al. 1991b). Although cultivated wheat was polymorphic in their response to difenzoquat, all families of all populations of the wild species were resistant. However, these wild species were polymorphic in their response to both chlorotoluron and metoxuron. In addition, there appeared to be differences between populations in the frequencies of resistant and susceptible morphs for these herbicides (for chlorotoluron, see Table 6.4 in Nevo et al. 2002). The implications of these findings for understanding the evolution of herbicide resistance and for developing strategies in breeding for resistance of cultivated species were thus highlighted.

We demonstrated (Krugman et al. 1997) that the scores and frequencies of chlorotoluron (CT) and metoxuron (MX) resistant and susceptible phenotypes of *T. dicoccoides* are correlated with ecological factors and allozyme markers (Nevo et al. 1992b). Some isozymic markers located on chromosome 6B (e.g., *Adh*, *Est-4*, and *Got*), which harbor the CT and MX resistance genes, are good genetic markers for herbicide-resistance breeding. Significant correlations between herbicide and photosynthetic characteristics suggest that the evolution of herbicide-resistance polymorphisms may be related to the process of photosynthesis in nature and pre-dated domestication of cultivated wheat. The genetic mapping of CT-resistance in cultivated and wild emmer appears in Krugman et al. (1997).

10.16.3.5 *T. dicoccoides*: An Important Genetic Resource for Increasing Zinc and Iron Concentration in Modern Cultivated Wheat

Genotypic variation for Zn and Fe concentration in seeds among wheat cultivars is relatively narrow and limits the options to breed wheat genotypes with high concentration and bioavailability of Zn and Fe in seeds. Among the collections of wild emmer wheat, *T. dicoccoides* (825 accessions) showed impressive variation and the highest concentrations of micronutrients, significantly exceeding those of cultivated wheat (Cakmak et al. 2004). The concentrations of Zn and Fe among the *dicoccoides* accessions varied from 14 to 190 mg kg^{-1} DW (dry weight) for Zn and from 15 to 109 mg kg^{-1} DW for Fe. Also, for total amounts of Zn and Fe per seed, *dicoccoides* accessions contained very high amounts of Zn (up to 7 μg per seed) and Fe (up to

3.7 μg per seed). Seed concentrations of Zn and Fe were lower and less variable in modern cultivated wheat. Screening different series of *dicoccoides* substitution lines revealed that chromosome 6A, 6B, and 5B of *dicoccoides* resulted in an increase in Zn and Fe concentrations when compared with their recipient parent and other chromosome substitution lines. The results indicate that *T. dicoccoides* is an important genetic resource for increasing concentration and content of Zn and Fe in modern cultivated wheat (Cakmak et al. 2004). Recently, Uauy et al. (2006) reported the positional cloning of *Gpd-B1*, a wheat QTL associated with increased grain protein and Zn and Fe content. The ancestral wild emmer allele, *non-functional in modern wheat varieties*, encodes a NAC transcription factor (*NAM-B1*) that regulates senescence and improves grain protein and zinc and iron content (Distelfeld and Fahima 2007).

10.17 Grain Protein Quality and Quantity

World food assessments indicate shortages in, and the biased distribution of, high-quality food. The primary deficiency is protein, and more than half of the people in the world do not consume enough high-quality protein for a nutritionally balanced diet (Nevo et al. 2002). The ongoing population explosion, primarily in less-developed countries, and the current billion people starving in developing countries, which is predicted to reach ten billion people in 2050, highlights the seriousness of the problem. Protein deficiency is most serious for the young and may cause permanent brain damage. The current serious protein shortage threatens to become a real nutritional disaster in the near future, primarily because Asia and Africa have about 80% of the protein of human diets supplied by plants and only about 20% is from animal sources. This problem dictates an active search for additional, genetically-based, high-quality protein sources in nature. The urgent need to increase high-quality protein sources is exacerbated by the major problems affecting cultivated crops with respect to the drastic reduction in genetic diversity, which includes the decline in grain protein content in cultivars (Plucknett et al. 1983, 1987; Nevo 2004; Haudry et al. 2007; Fu and Somers 2009). As indicated earlier, the amount of storage grain proteins in wheat cultivars is 7–22%, but 13.9–28.9% (Grama et al. 1983) in *T. dicoccoides* in nature and 24–43% under greenhouse conditions (Levy and Feldman 1987). Furthermore, in wild emmer the high-protein content is under distinctly strict genetic control (Grama et al. 1983). We have shown that molecular markers and ecological factors (Nevo et al. 1986a) can predict high-quality and high-quantity protein parameters both in highly competitive dense populations in northern Israel and the Golan, and in marginal xeric *Samaria* populations (Nevo et al. 1986a). Transformation to elite wheat cultivars of rust resistance, coupled with high-protein content, started in the Volcani Center in Israel (Gerechter-Amitai and Grama 1974, 1977; Grama et al. 1983). This process excluded negative traits of the progenitors, thus combining elite cultivar and positive progenitor traits.

In our test (Nevo et al. 1986a), the results indicate that protein percentage and kernel and protein weight (the product of the former two values) vary both within but *particularly between* populations (Table 6.5b, Fig. 6.3). Notably, the three marginal populations (Fig. 6.3) exhibited high-protein content but low-kernel weight. The central populations displayed a lower protein percentage but high kernel weight, and hence higher protein weight. Three-variable combinations of climatic factors explained $R^2 = 0.70$ of the variance in kernel weight and $R^2 = 0.60$ of the variance in protein weight. Likewise, three-variable combinations of allozyme genotypes significantly explained the spatial variances in protein percentage and kernel and protein weight (R^2 0.60, 0.69, and 0.54, respectively) (Tables 6.5a, 6.6).

We concluded, together with other researchers (Gerechter-Amitai and Grama 1977; Avivi 1978, 1979; Avivi et al. 1983; Grama et al. 1983; Levy and Feldman 1987), that natural populations of wild emmer in Israel contain a large number of yet untapped genes for elite protein and high-seed weight. These could be effectively screened and utilized for producing high-quality and high-quantity protein wheat cultivars by means of effectively following ecological factors and molecular markers as predictive guidelines in screening natural populations of wild emmer wheat. Importantly, in wild emmer grain, protein potential is under distinctly strict genetic control.

10.17.1 A NAC Gene Regulating Senescence Improves Grain Protein, and Zinc and Iron Content in Wheat

Indeed, Uauy et al. (2006) reported on the positional cloning of *Gpc-B1*, a wheat QTL associated with pleiotropic effects that increase grain protein and zinc and iron content. The ancestral wild emmer wheat allele encodes functional NAC transcription factor (*TtNAM-B1*) that accelerates senescence and increases nutrient remobilization from leaves to developing grains, whereas modern wheat varieties carry a *non-functional NAM-B1* allele. Reduction in RNA levels of the multiple NAM homologs by RNA interference delayed senescence by more than 3 weeks and reduced wheat grain protein and zinc and iron content by more than 30%. This NAC discovery from wild emmer may contribute to the more efficient manipulation in crops and increase the nutritive value of cultivated wheat (Distelfeld and Fahima 2007).

10.17.2 Wheat Storage Proteins and Glutenin DNA Diversity *in* T. dicoccoides *in Israel*

Glutenin and gliadin prolamin storage proteins comprise most of the wheat grain endosperm. Glutenin consists of approximately 20% HMW glutenin subunits and 80% low-molecular-weight (LMW) subunits. The biochemistry and genetics of endosperm proteins in bread wheat have been extensively studied (e.g., Kasarda et al. 1976; Konzak 1977; Payne et al. 1980, 1981a, b, 1984; Payne and Rhodes 1982; Galili and Feldman 1983, 1985; Nevo and Payne 1987; Levy and Feldman 1987, 1988; Gepts 1990; Felsenburg et al. 1991) due to their importance in bread baking quality (Wall 1979) (see Gianibelli et al. 2001 for *Triticum tauschi* and elaboration and references in Nevo et al. (2002:195)).

10.17.3 Utilization of Glutenin Diversity in Bread-Making Quality

Glutenins, like other wheat grain proteins, play a key role in food processing such as in the manufacture of bread, biscuits, breakfast cereals, and pasta products (Payne and Rhodes 1982). A major effort of plant breeders is to develop elite bread wheat with improved bread-making quality. Significant associations were found for certain HMW subunits of glutenin and bread-making quality (Payne et al. 1981b). These results have been confirmed (Moonen et al. 1982) and expanded (Payne et al. 1984). Dough strength is the limiting factor in the bread-making process in western European wheat. This characteristic is controlled by the elasticity of glutenin, which, in turn, is determined by its specific composition of LMW and HMW subunits.

One approach to improving the quality of wheat cultivars for bread-making is by utilizing genes from (1) landraces of primitive agriculture (Payne et al. 1984) and (2) wild relatives of bread wheat (Law and Payne 1983). Wild emmer could provide a host of genetic variants of the endosperm storage proteins for improving bread-making quality (Nevo and Payne 1987; Felsenburg et al. 1991; Pagnotta et al. 1995), as it does for other agronomic traits (Nevo 1983, 1989, 1995, 2001a; Feldman 1979). The range of allelic variation in the glutenin loci of wild emmer is remarkable, and this variation could be readily transferred to bread wheat for testing by plant breeders in an attempt to improve baking quality (Fig. 10.5).

10.17.4 Genetic Differentiation of High-Molecular-Weight Glutenin Subunits

The diversity of HMW glutenin subunits in *T. dicoccoides* was studied electrophoretically in 231 individuals representing 11 populations of wild emmer from Israel (Nevo and Payne 1987 and Fig. 10.5a–e). The results showed that (1) the two HMW glutenin loci, *Glu-A1* and *Glu-B1*, were rich in variation, having 11 and 15 alleles, respectively (Fig. 10.5). (2) Genetic variation in HMW glutenin subunits was often severely restricted in individual populations, supporting an island population genetic model (Tables 6.7a, b and 6.8). (3) Significant correlations were found between glutenin diversity and the frequencies of specific glutenin alleles and physical (climate and soil) and biotic (vegetation) variables (Table 6.8). Our results suggest that at least part of the glutenin polymorphisms in wild emmer can be accounted for by

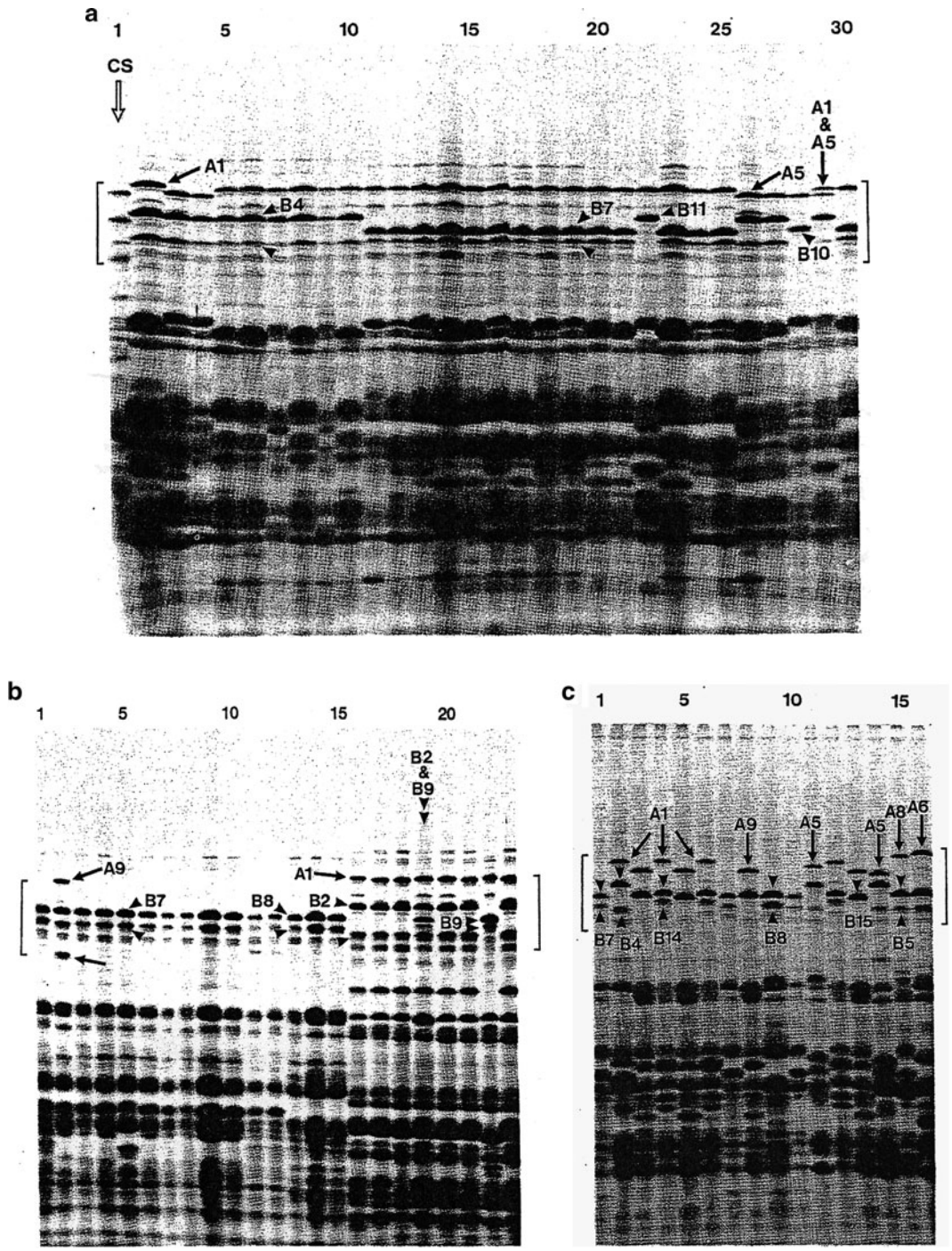

Fig. 10.5 (**a**–**c**) SDS-PAGE of grain proteins found in populations of wild emmer from Kokhav Hashahar, near Jerusalem. The region of the gel containing the HMW glutenin subunits is marked by *brackets*. The proteins thought to be coded by the *Glu-A1* and *Glu-B1* loci are prefixed with letters A and B, respectively. The HMW glutenin subunits of bread wheat cultivar Chinese Spring are separated in Slot 1. They are, with increasing mobility, 2 (chromosome 1D), 7 + 8 (1B), and 12 (1D). (From Nevo and Payne 1987). (**b**) SDS-PAGE of grain proteins in two wild emmer populations: *lanes* 1–15 Qazrin, *lanes* 16–23 Yehudiyya (from Nevo and Payne 1987). (**c**) SDS-PAGE of a range of wild emmers to show the extent of allelic variation in HMW glutenin subunits. Grains were taken from populations Qazrin (*lane 1*). Yehudiyya (*lanes 2, 7, 8,* and *10*). Tabigha (*lane 12*). Bet-Meir (*lane 14*). Rosh Pinna (*lane 11*). Bat Shelomo (*lane 9*), Mt. Hermon (*lane 15*), Sanhedriyya (*lane 3–6,* and *13*), and Taiyiba (*lane 16*). (Nevo

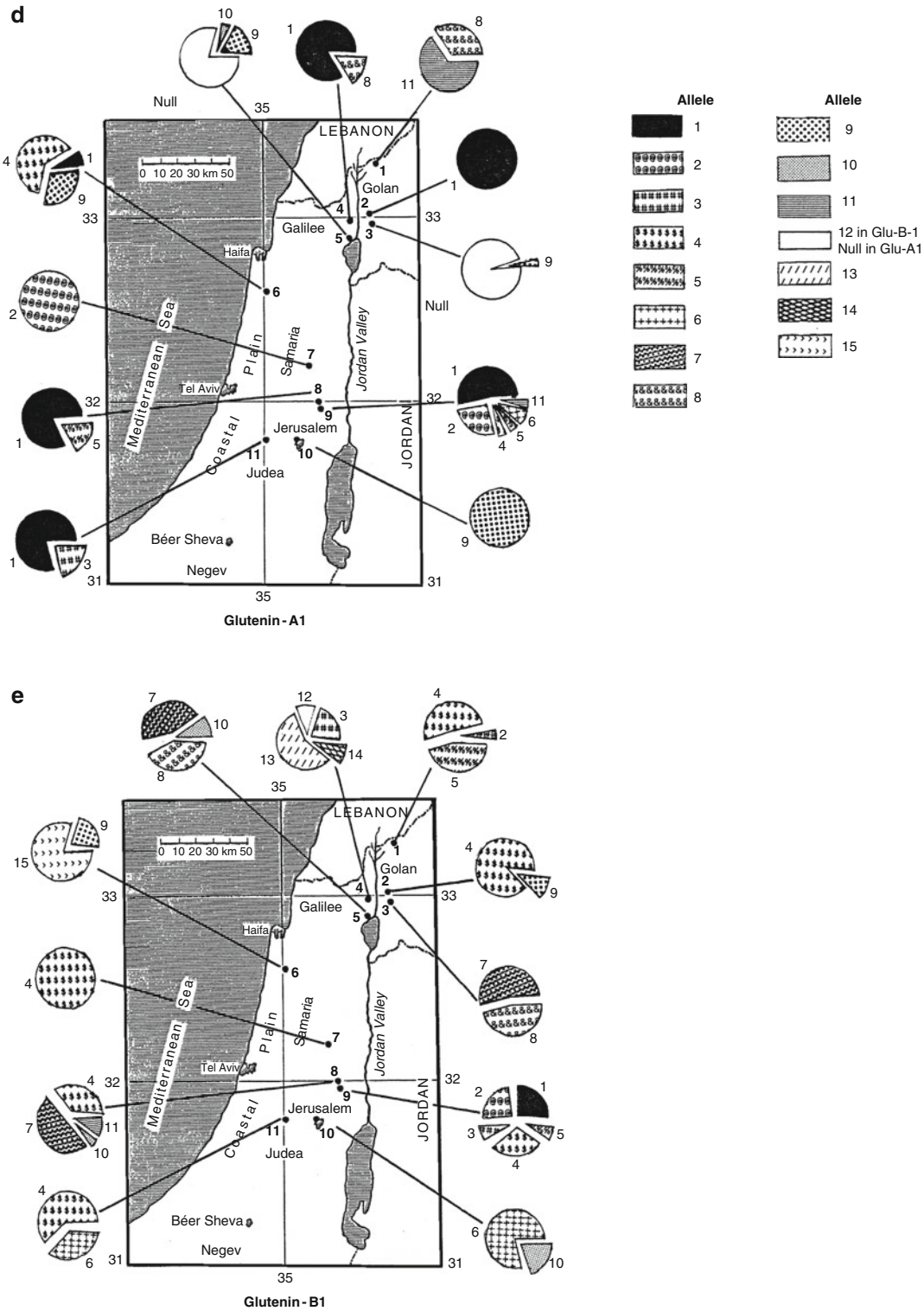

Fig. 10.5 (continued) and Payne 1987). (**d**, **e**) Pie diagrams displaying the percentage of the 11 alleles of F *Glu-A1* and 15 alleles of *Glu-B1* in 11 populations of wild emmer wheat, *Triticum turgidum dicoccoides*, and their geographical location in Israel. Populations are numbered as in Table 1. A *Glu-A1*; B *Glu-B1*. C Key to diagrams; alleles are numbered as in Table 2. (Nevo and Payne 1987)

environmental factors. Furthermore, the endosperm of wild emmer contains many allelic variants of glutenin storage proteins that are not present in bread wheat and could be utilized in breeding varieties with improved bread-making qualities.

Three main features characterize glutenin differentiation in natural populations of wild emmer in Israel (Nevo and Payne 1987) (1) extensive variability, (2) extreme population differentiation, and (3) correlation with ecological factors. First, allele diversity is remarkable in glutenin subunits and is similar to that of hordein storage proteins in wild barley, *H. spontaneum* (Nevo et al. 1983), in which 15 hordein (Hor)-1 phenotypes and 16 hordein (Hor)-2 phenotypes have been described in Israel. In line with this extremely high hordein diversity, we found 11 Glu-A1 and 15 Glu-B1 alleles in wild emmer (Fig. 10.5a–e). Similarly, our unpublished studies also revealed extensive allelic variation in the gliadin proteins of wild emmer. The high levels of allele diversities of hordein and glutenin storage proteins contrast sharply with the relatively lower levels of allozyme allele diversity per locus in each population of wild barley and wild emmer in Israel.

We have also showed extensive glutenin HMW and LMW DNA diversity in wild emmer correlated with the environment (Nevo et al. 1995).

10.18 Amino Acid Resources in *T. dicoccoides*: Polymorphisms and Predictability by Ecology and Isozymes

Amino acid content was measured in 109 genotypes from 22 populations of wild emmer wheat, *T. dicoccoides*, across its ecological range in Israel. The results are given in Table 6.1 in Nevo and Beiles (1992). *T. dicoccoides* was high in lysine and isoleucine content, as compared to six other species of wild wheat, but low in threonine and proline. Significant correlations were found among lysine and the other essential amino acids. Significant differences between Israeli populations of *T. dicoccoides* were found for isoleucine, methionine, leucine, and threonine but not for lysine, arginine, proline, and glutamic acid. Significant differences between regions were only found for methionine. Ecological factors and allozyme markers appear to be good guidelines for predicting the eco-geographic location of specific elite amino acid genotypes, either singly or in combination. *T. dicoccoides* may be used in the future as a promising genetic resource for genetically improving and optimizing the nutritional value of cultivated wheat.

10.19 Amylases

Amylases are of fundamental importance in wheat germination and growth. α- and β-amylase isozyme diversity was studied electrophoretically in *T. dicoccoides* (Nevo et al. 1993a). We analyzed 225 plants from 23 populations encompassing the ecological spectrum of *T. dicoccoides* in Israel. The results were as follows (a) band and MGP polymorphisms abounded and varied within and between the four amylase components, malt, green (α-amylases), dry, and germinating seed (β-amylases) (Fig. 6.6, Table 6.12). (b) The number of bands of malt, green, germinating and dry seeds were 20, 6, 11, and 13, generating 40, 6, 51, and 51 patterns, or MGPs, respectively. The MGPs varied widely within and between populations, from monomorphic in some populations with a single pattern to highly polymorphic ones (Table 6.13). (c) Mean 11 values for malt, green, germinating, and dry seed amylases were 0.053, 0.055, 0.088, and 0.077, respectively; the mean number of bands per individual was 11.8, 4.4, 7.6, and 4.0, respectively (Table 6.13). (d) The percentages of 50 bands and 148 MGPs (in parentheses) (Table 6.13) were classified into widespread, sporadic, and localized groups: 84.4 (10.8), 8.9 (12.2), and 6.7 (77.0), respectively. Notably, 89.2% of the patterns were *not widespread*, but rather *sporadic* and *localized*, as was shown earlier for other allozymes (Nevo and Beiles 1989). (e) The mean value of genetic distances among populations (Nei's *D*) for the four amylase groups was $D = 0.136, 0.175, 0.288$, and 0.307, respectively, not displaying geographic correlates. (f) Most of the α- and β-amylase diversity is between populations ($G_{st} = 68–75\%$). (g) Significant environmental correlates occurred between either bands or patterns and climatic diversity (water and primarily temperature

factors). (h) There were significant associations of multilocus amylase bands across Israel (Table 6.14). Likewise, significant linkage disequilibria, LD, occurred within populations and positively correlated with climatic variables, primarily with temperature (Table 6.14). (i) Discriminant analyses correctly classified (95–100%) the 23 wild emmer populations into their ecogeographic region and soil type (Fig. 6.7). (j) Autocorrelation analysis showed no correlation between bands and geographic distance, thereby excluding migration as a major factor of amylase differentiation. These results suggested that diversifying climatic, edaphic, and biotic natural selection rather than stochastic factors or migration is the major evolutionary force driving amylase differentiation at both the single and multilocus levels. Furthermore, wild emmer harbors high levels of α- and β-amylase diversity, detectable both as single bands and multilocus adaptive genetic patterns. These are exploitable both as genetic markers for QTLs and as direct adaptive genetic resources to improve wheat germination and growth through classical breeding and/or biotechnology.

10.19.1 The Amylase Multigene Family (and the "Archipelago" Structure): Evolutionary Considerations

The amylase multigene family displays an "archipelago" pattern of population–genetic structure similar to that of allozymes in wild emmer wheat and wild barley (Nevo and Beiles 1989). The diversity patterns of α- and β-amylases in natural populations of *T. dicoccoides* suggest that natural selection appears to be the predominant differentiating and orienting evolutionary force.

The "archipelago" pattern of amylase population genetics is *non-random*, structured, and correlated with ecology across the range of *T. dicoccoides* in Israel, negating genetic drift as an explanatory model. The predominance of ecogeographically structured polymorphisms (displaying significant climatic and soil correlates) *excludes randomness* as a major evolutionary factor and strongly suggests the operation of ecologically *diversifying natural selection*. The numerous genes and rich polymorphisms of wild emmer α- and β-amylases may provide unique genetic resources of agricultural importance for improving wheat germination and growth in adverse and stressful environments.

10.19.2 Molecular Evolution of Dimmer α-amylase Inhibitor Genes in Wild Emmer Wheat and Its Ecological Association

Wheat α-amylase inhibitors (WMAI) are candidates for the control of seed weevils, as these insects are highly dependent on starch as an energy source. We described the structure and diversity of dimeric α-amylase inhibitor genes in wild emmer wheat from Israel and elucidated the relationship between the emmer wheat genes and ecological factors using SNP markers. Likewise, we correlated the relationship between SNPs in functional protein-coding genes and the environment (Wang et al. 2008).

A total of 348 dimeric α-amylase inhibitor genes were obtained from 13 accessions in 10 wild emmer populations. Seventy-five polymorphic positions and 74 haplotypes were defined by sequence analysis. Sixteen out of the 75 SNP markers were designed to detect SNP variations in wild emmer wheat accessions from different populations in Israel. The proportion of polymorphic loci, *P* (5%), the expected heterozygosity, *He*, and Shannon's information index in the 16 populations were 0.887, 0.404, and 0.589, respectively. The populations of wild emmer wheat showed great diversity in gene loci both between and within populations. Based on the SNP marker data, the genetic distance of pairwise comparisons of the 16 populations displayed a sharp genetic differentiation, largely geographically independent but ecologically dependent. The values of *P, He*, and Shannon's information index were negatively correlated with three climatic moisture factors, whereas the same values were positively correlated by Spearman rank correlation coefficients' analysis with some of the other ecological factors.

In conclusion, the populations of wild emmer wheat showed a wide range of diversity in dimeric α-amylase inhibitors, both between and within populations. SNP markers are useful for the estimation of genetic

diversity of functional genes in wild emmer wheat. SNPs in the α-amylase inhibitor genes are significantly correlated with ecological factors affecting diversity. Ecological factors, singly or in combination, explained a significant proportion of the variations in the SNPs, and the SNPs could be classified into several categories as ecogeographical predictors. It was suggested that the SNPs in the α-amylase inhibitor genes have been subjected to natural selection, and ecological factors had an important evolutionary influence on gene differentiation at specific loci.

In a follow-up study (Wang et al. 2009), we revealed that AMY1 (amy 32) in wild barley was under natural selection across populations by the expected ratio of dN/dS. The results of purifying and the positive selection hypothesis ($P < 0.05$) also showed that the sequences of AMY1 (amy 32) were selected at different domains. Most amino acid changes occurred at the C-terminal (positive selection domain), and most of the amino acids in the middle domain were conserved thereby ensuring the stability of AMY1 (amy 32) to keep their correct 3D structure to combine with insect α-amylase. Ecological factors, singly or in combination, explained a significant proportion of the variations in the SNPs, and the SNPs could be classified into several categories as ecogeographical predictors such as water, temperature, and geographic factors. A sharp genetic divergence (large *D*) over very short geographic distances against small genetic divergence (small *D*) between large geographical distances also suggested that the SNPs in the monomeric α-amylase inhibitor genes of wild emmer were subjected to natural selection, and ecological factors had an important evolutionary role in gene differentiation at these loci, insects being the main selective factors.

10.20 Disease Resistance Polymorphisms in *T. dicoccoides* (Host–Pathogen Coevolution in the Center of Diversity)

Crop diseases cause loss of yield and prevent a rapid increase in food production. Crop improvement should therefore aim to minimize the risks of epidemic and endemic diseases in an attempt to increase production efficiency without affecting the agronomic properties of existing varieties. In general, natural plant ecosystems are not devastated by disease since, over the course of host–pathogen coevolution, they have developed efficient defense mechanisms. This evolutionary interaction has resulted in a balanced genetic polymorphism, with the highest genetic diversity for virulence in the pathogen and for resistance in the host (Anikster and Wahl 1979; Anikster 2001).

Israel is one of the epicenters where active coevolution of several crop ancestors and their pathogens occurred, including wild barley, wild wheat, and their rust and powdery mildew pathogens. In keeping with the theory and genetics of host–parasite coevolution, the highest and broadest genetic immunity against devastation in the progenitors of some crops would be expected in the Fertile Crescent, in general, and in Israel, in particular. Israel is ideal for the exploitation of resistance genes, which could provide useful material for crop improvement for both the short-term and, more importantly, the long-term solution to disease problems in agriculture (Segal et al. 1980, 1982).

10.20.1 *Powdery Mildew:* Erysiphe graminis tritici

Powdery mildew of the wheat *T. aestivum* L., incited by the pathogen *Blumeria graminis* f. sp. *tritici,* is one of the most damaging diseases of wheat worldwide. It causes significant losses in wheat production in the United States, United Kingdom, New Zealand, Hungary, China, and elsewhere (Nevo et al. 2002; Xie and Nevo 2008). The reactions of 233 *T. dicoccoides* accessions to infection with cultures of *Blumeria* (*Erysiphe*) *graminis tritici* (collected at ten sites in Israel and elsewhere) were determined (Moseman et al. 1984). The results indicated that the number of sources of resistance to *B. graminis tritici* obtained from *T. dicoccoides* plants, growing wild in Israel and elsewhere, are almost unlimited. Of the accessions, 114 (49%) were resistant, and 137 (59%) were resistant or moderately resistant to infection with four cultures of *B. graminis tritici*, which possess the virulence genes corresponding to most of the identified resistance genes in wheat. Accessions collected at sites with marginal habitats, where *T. dicoccoides* grows poorly and has a lower grain weight, were more susceptible than accessions collected at central sites with an optimal habitat for growth of *T. dicoccoides*. Powdery

mildew resistance genes from *T. dicoccoides* have been extensively used in wheat improvement in Europe and North America.

Plant disease resistance genes (*R*-genes) are a major component of the plant response to pathogen attacks. The majority of *R*-genes encode proteins with a nucleotide binding site (NBS) and a leucine-rich repeat (LRR) domain associated at the N-terminus, either with a Toll interleukin receptor-like (TIR) domain or a coiled-coil (CC) domain. R-proteins directly or indirectly perceive pathogen-emitted effecter molecules and trigger a resistance reaction mainly based on a hypersensitive response leading to cell death (Jones and Dangl 2006). Yahiaoui et al. (2009) have identified 61 *Pm3* allelic sequences from wild and domesticated tetraploid wheat subspecies, corresponding to 24 different haplotypes. A new functional gene was identified in a wild emmer wheat accession from Syria. This gene, *Pm3k*, conferred intermediate race-specific resistance to powdery mildew and consists of a mosaic of gene segments derived from non-functional alleles. The *Pm3* sequence variability and geographic distribution indicated that diversity was higher in wild emmer wheat from the Levant area compared with the accessions from Turkey (Yahiaoui et al. 2009). Recombination is diversifying force of the *R*-gene, *Lr10* in *T. dicoccoides* (Sela et al. 2010). High genetic diversity at resistance gene analog polymorphism (RGAPs) were recorded in *T. dicoccoides* associated with ecology (Dong et al. 2009).

Several resistance genes (*Pm16, Pm26, Pm36*, and *MlZec*1) derived from wild emmer were identified and mapped using molecular markers. *Pm16,* originally mapped on chromosome 4A by monosomic analysis (Reader and Miller 1991), was recently mapped on 5BS using SSR markers (Chen et al. 2005). *Pm31*, previously reported as derived from wild emmer (Xie et al. 2003), was subsequently identified as *Pm21* because of a pedigree error (Xie 2006; Zy Liu personal communication). Recently, two novel powdery mildew resistance genes (temporarily designated as *PmG3M* and *PmG16*) were mapped on chromosomes 6BL and 7AL, respectively (Table 1 in Xie 2006). *PmG3M* from accession G-305-3M conferred resistance to 41 powdery mildew isolates collected from different wheat species and from various locations in Israel. *PmG16* from accession 18-16, which originated from a dry environment and also contained drought-resistant QTLs (Peleg et al. 2008), conferred resistance to 29 of 42 Israeli isolates. Since *T. dicoccoides*-derived *Pm*30 was resistant to all 20 tested isolates in China (Chen et al. 2005), it is expected that *PmG16* might provide good resistance to powdery mildew outside of Israel, and its usefulness needs to be further investigated (Xie 2006). Accession 18-16 might provide both drought and powdery mildew resistance for wheat improvement. Recently, Li et al. (2009) described a new powdery mildew resistance gene, *Pm41*, by marker-assisted selection. Clearly, *T. dicoccoides* is an excellent resource of powdery mildew-resistant genes from Mt. Hermon mapped on chromosome 3 BL of *T. dicoccoides*. The resistance gene was introgressed into common wheat by backcrossing.

10.20.2 Rust Resistances

The three rusts caused by *Puccinia triticina* (leaf rust), *Puccinia graminis* f. sp. tritici (stem rust), and *Puccinia striiformis* f. sp. *tritici* (yellow rust or stripe rust) affect wheat production worldwide, attracting much attention and interest in research and application, especially after the devastating effect of Ug-99 in Asia. Moseman et al. (1985) assessed the seedling responses of 687 *T. dicoccoides* accessions to culture PRTUS6 of *P. triticina*. Ninety-eight accessions (14%) were at least moderately resistant. Evaluations of seedling and adult plant responses of 742 accessions by Anikster et al. (2005) led to the identification of some highly to moderately resistant accessions; adult resistance was more common than seedling resistance. However, only one leaf rust resistance gene derived from *T. dicoccoides* has been chromosomally mapped; *Lr53* was located on 6BS using monosomic, telosomic, C-banding, and RFLP analyses (Marais et al. 2005; Table 1 in Xie and Nevo 2008). Some polymorphism in response to leaf rust and stem rust was detected in populations of *T. dicoccoides* (The et al. 1993; Nevo et al. 1985; Anikster et al. 2005), but the stem rust resistance gene was not identified or mapped.

Wild emmer is a valuable source of stripe rust (or yellow) resistance (Gerechter-Amitai and Stubbs 1970; Nevo et al. 1986b; The et al. 1993). Gerechter-Amitai and Stubbs (1970) reported that accession G-25 from Rosh Pinna, Israel, was resistant to many races of *P. striiformis* from different geographical origins, and the gene responsible was later identified

as *Yr15* (Gerechter-Amitai et al. 1989). *Yr15* was mapped on 1BS using cytogenetic (McIntosh et al. 1996) and molecular analyses (Sun et al. 1997; Chague et al. 1999; Zakari et al. 2003). Peng (2000) and Peng et al. (1999, 2000c) identified and mapped another *T. dicoccoides*-derived stripe rust resistance gene, *YrH52*, in accession H52 from Mount Hermon, Israel, identified by Nevo et al. (1986b). High density molecular mapping of the chromosome region harboring *YrH52* and *Yr15* suggested close linkage, rather than allelism between these two genes (Peng 2000; Peng et al. 2000c). However, further ongoing investigation is required to decide if they are truly different. The isolate possessing *Yr15* virulence, recently identified in Denmark (Hovmoller 2007), could help to discriminate between them. Marais et al. (2005) identified *Yr35* on chromosome 6BS. It was completely linked with Lr53. A high temperature adult-plant (HTAP) resistance gene, *Yr36*, was also mapped on chromosome 6BS, and this gene was closely linked to the grain protein content locus *Gpc-B1* (Uauy et al. 2006; Table 10.1). Fu et al. (2009) described the map-based cloning of the *Yr36* (WKS1) gene, which confers resistance to a broad spectrum of wheat stripe rust races at high temperatures (25–35°C). Importantly, *Yr36* is present in *T. dicoccoides* emmer but is absent in *T. durum* and *T. aestivum*; it could introduce rust resistance to these cultivars.

10.20.3 Fusarium *Head Blight Resistance*

Fusarium head blight (FHB), caused mainly by *Fusarium graminearum* in North America and *F. culmorum* in many parts of western Europe, leads to mycotoxin-contaminated cereal products and is another destructive disease of wheat. Host resistance is the most effective method of controlling FHB in wheat, and resistance to the various *Fusarium* species is assumed to have the same genetic basis. Modern *durum* wheat cultivars are mostly highly susceptible to FHB. A search for resistance in wild emmer revealed moderate FHB resistant lines (Miller et al. 1998; Stack et al. 2002; Buerstmayr et al. 2002; Gladysz et al. 2007; Oliver et al. 2007). The evaluation of a set of Langdon durum-*T. dicoccoides* chromosome substitution lines identified the line LDN(DIC-3A), having a *T. dicoccoides* chromosome 3A replacing its Langdon homolog, as being consistently less susceptible than the other substitution lines (Stack et al. 2002). The QTL *Qfhs.ndsu-3AS* linked with microsatellite locus *Xgwm2* explained 37% of the phenotypic and 55% of the genotypic variances in FHB resistance response (Otto et al. 2002). Another QTL, *Qfhs.fcu-7AL* present in accession PI478742, was identified and mapped on chromosome 7AL (Kumar et al. 2007). These sources of FHB resistance could also be transferred to hexaploid wheat using marker-assisted selection.

10.20.4 Resistance to Wheat Soil-Borne Mosaic Virus

A fungal vector, *Polymyxa graminis* Ledingham, carries the wheat soil-borne mosaic virus (WSBMV). The virus particles are carried on or in the fungal zoospores (swimming spores). The fungus invades root hairs of the young wheat plants within affected areas. Losses caused by WSBMV are variable, depending on weather conditions, varying from 10 to 50% (see details in *http://www.ext.nodak.edu/extpubs/plantsci/smgrains/pp646w.htm*).

The reaction to WSBMV of 318 plants representing 90 genotypes in 25 populations of *T. dicoccoides* collected in Turkey and Israel was determined by symptomatology and enzyme-linked immunosorbent assay (ELISA) (Hunger et al. 1992). A range of reactions to WSBM existed in the wild emmer collection, although the virus has not been reported as a severe disease in Israel or Turkey. Nine populations contained only WSBM-resistant plants. The other 16 populations contained different ratios of resistant and susceptible plants that ranged from nearly all susceptible to nearly all resistant. Thus, wild emmer may represent a source of resistance to WSBMV. This could, in turn, help diversify the genetic base for bread-wheat resistance to WSBMV.

10.21 Photosynthetic Characteristics in *T. dicoccoides* and Their Predictability by Ecological and Genetic Factors

Crop productivity, in general, and specifically grain yield of wheat, is genetically determined by a hierarchy of physiological factors. Central to this hierarchy is the

photosynthetic process. We tested *T. dicoccoides* for genetic variation (*Vc*) in photosynthetic characteristics residing within and between native populations sampled from three ecogeographic regions of Israel. Our aim was to identify potential sources of high photosynthetic efficiency for future wheat improvements (Carver and Nevo 1990). Accessions sampled in the center of wild emmer distribution (catchment area of upper Jordan Valley) in a relatively narrow geographic range showed the greatest diversity in CO_2 assimilation rate per unit leaf area (A) or per unit chlorophyll (A/Chl). Genetic variation was absent for internal CO_2 concentration (*Ci*) and water-use efficiency (WUE) and was generally lacking for stomatal conductance (*gs*). Leaf area, although quite variable, was not a significant cofactor for assessing genetic potential for photosynthesis. Some genotypes within a given population showed ten times more variation in A and A/Chl than populations sampled from different locations in a region. Genotypes with the highest photosynthetic efficiency derived from upland steppic populations located in marginal habitats extending in Samarian eastern steppes facing the Jordan rift valley. Some accessions having high photosynthetic capacity ($A = 32$ ml m^2 s^{-1}) with no significant reduction in leaf size constitute a potentially valuable genetic resource, as yet untapped, for genetic improvement of hexaploid (*T. aestivum* L.) wheat. We, therefore, crossed these accessions into hexaploid wheat.

In a twin study (Nevo et al. 1991b), we analyzed, using univariate and multivariate methods, the differentiation of variation in photosynthetic characteristics of the same 107 genotypes from 27 populations of wild emmer in Israel, as analyzed by Carver and Nevo (1990). These populations were distributed in three ecogeographic regions including central, xeric-marginal (northern cold and eastern warm), and mesic-marginal (western) populations. The highest photosynthetic efficiency was displayed by populations of the xeric-marginal region, but most variation for photosynthetic capacity occurred between accessions within ecogeographic regions and populations. Genotypes and populations of *T. dicoccoides* having high photosynthetic capacity can be identified by climatic factors and isozyme markers. Out of 107 genotypes tested in discriminant analysis, 97 (91%) were correctly classified into their respective sets. *The best photosynthetic performance was that of the xeric-warm-marginal populations*. These xeric-marginal populations were selected for high photosynthetic yield together with other traits, e.g., high levels of storage proteins (Nevo et al. 1986a) and high rDNA diversity (Flavell et al. 1986), to shorten the life cycle in the steppic environment and avoid the early hot summer. Identification by genetic mapping can facilitate the maximization of conservation, in situ or ex situ (Nevo 1998a), as well as the utilization of these photosynthetic genetic resources for improvement of hexaploid wheat, *T. aestivum*.

10.22 Diurnal Rhythms of mRNAs for the Chlorophyll a/b Binding Protein in Wild Emmer Wheat and Wild Barley in the Fertile Crescent

Biological rhythms in nature are entrained to a 24-h (circadian) period by environmental cues (*Zeitgeber*). Circadian clocks are abundant in both plants and animals. In plants, morphological, cellular, biochemical, physiological, and photosynthetic oscillations have been observed (Britz et al. 1987); however, the molecular basis of these rhythms is still largely unknown. The mRNAs encoding the chlorophyll-a/b binding (CAB) proteins of the light-harvesting system were monitored in wild emmer wheat, *T. dicoccoides*, and wild barley, *H. spontaneum*, the progenitors, respectively, of all cultivated wheat and barley (Nevo et al. 1993b). Significantly different mRNA levels were detected at different time points during the 24-h cycle, with generally low levels around sunrise, sunset, midnight, and maximum levels around noon (Fig. 6.12 in Nevo et al. 2002). These results indicate that there is diurnal control of CAB gene expression in these ancient species (Fig. 10.6).

10.23 Domestication Quantitative Trait Loci in *T. dicoccoides*

We studied the domestication–related QTL in *T. dicoccoides* (Peng et al. 2003). The studied traits included brittle rachis, heading date, plant height, grain size, yield, and yield components. Our mapping population

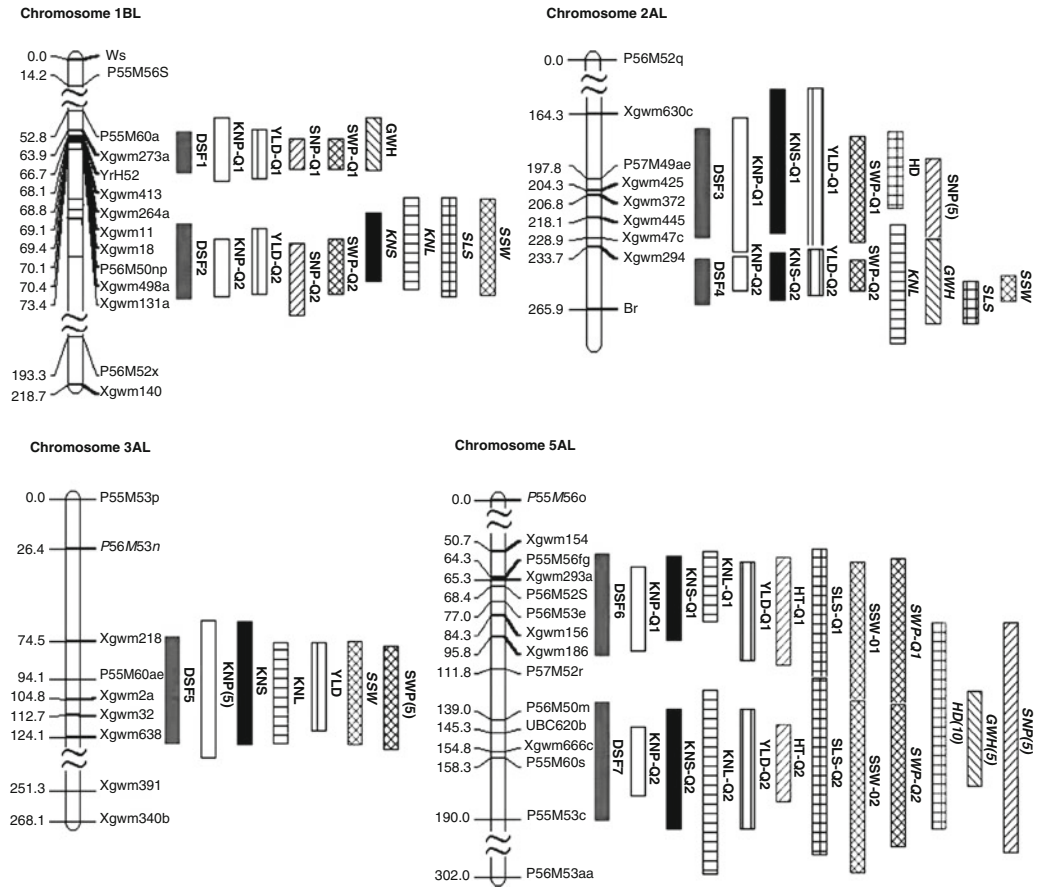

Fig. 10.6 Map locations of DSFs and their involved QTLs in L version maps of wild emmer wheat, *T. dicoccoides*. (*Upper*) Short arms of chromosomes. (*Right*) DSFs and corresponding QTLs: *gray-filled bar*, DSF; *dark-filled bar*, KNS; *bar with horizontal fill*, kernel number/spikelet (KNL); *bar with vertical fill*, YLD; *bar with upper right to lower left thin fill*, HT; *bar with plus*, spikelet number/spike (SLS); *bar with diagonal thin crosshatch fill*, single spike weight (SSW); *bar with diagonal bold crosshatch fill*, spike weight/plant (SWP); *empty bar*, kernel weight/plant (KNP); *bar with edged plus*, HD; *bar with upper left to lower right fill*, GWH; *bar with upper right to lower left thick fill*, spike number/plant (SNP). The regular trait name represents a single QTL; the italic trait name represents a single QTL (Q2) detected by linked-QTL analysis; the regular trait name tailed with Q1 means the first QTL and tailed with Q2, the second QTL in a pair of linked QTLs. A tailed trait name (5) means that the QTL effect is not significant at the level of 5% of FDR but is significant at FDR 10%; (10) means that the effect is not significant at FDR 10% (Peng et al. 2003)

was derived from a cross between *T. dicoccoides* and *T. durum*. Approximately 70 domestication QTL effects were detected, *non-randomly* distributed among and along chromosomes (Fig. 10.6). Seven domestication syndrome factors were proposed, each affecting 5–11 traits (Fig. 10.6). We showed (1) clustering and strong effects of some QTLs; (2) remarkable genomic association of strong domestication-related QTLs with gene-rich regions, and (3) unexpected predominance of QTL effects in the A-genome. The A-genome of wheat (*T. urartu*) may have played a more important role than the B-genome during domestication evolution. The cryptic beneficial alleles at specific QTLs derived from *T. dicoccoides* may contribute to wheat and cereal improvement.

10.24 Potential and Actual Genetic Resources of *T. dicoccoides* and Future Wheat Breeding

Diverse studies in Israel and the Near East Fertile Crescent of wild emmer, *T. dicoccoides*, the progenitor of most tetraploid and hexaploid wheats (briefly reviewed above), have revealed rich genetic resources

applicable in wheat improvement due to their diverse single- and multilocus adaptations to stressful abiotic (climatic, soil, and minerals) and biotic (pathogens, parasites) environments (Nevo et al. 2002; Xie and Nevo 2008; Israel Journal of Botany 1991; Israel Journal of Plant Sciences 2001, 2007). Most of the discovered resources are *yet untapped*, and it takes many years to explore, evaluate, and especially to utilize these genetic resources in improving wheat. The genetic reinforcement of wheat cultivars, which suffered great genetic erosion during domestication, could enormously benefit from the rich adaptive genetic resources of wild emmer and other wheat relatives, primarily the *Aegilops* species (e.g., Millet 2007).

The Near East, in general, and Israel and Syria in particular (Nevo 1986), are the centers of origin and diversity of wild emmer, where it developed wide genetic adaptations against multiple pathogens and diverse ecological stresses contributing to its fitness in heterogeneous and stressful ecologies (Aaronsohn 1910). Genetic diversity is transferable from the wild to the cultivated gene pool, so genes and their biochemical pathways and regulatory systems of wild emmer are of primary importance for future wheat improvement (e.g., Uauy et al. 2006; Fu et al. 2009). Genetic resources and diversity of wild emmer, described here and previously, are geographically structured (Nevo et al. 1982; Nevo and Beiles 1989) and are predictable by ecology and molecular (allozyme and DNA) markers (Nevo 1987, 2001a). Extensive genome mapping and QTL mapping (Peng et al. 2000a, b, c, 2003) provide rich genetic resources for wheat improvement. Consequently, exploration by optimizing sampling strategies, of in situ and ex situ conservation (Nevo 1998a), along with utilization programs (involving classical and modern technologies), should maximize the contribution of wild emmer to wheat improvement (Xie and Nevo 2008). For earlier references, see Nevo et al. (2002).

10.25 Conclusion: Wild Emmer Wheat as a Model Organism for Elucidating Wheat Evolution and Improvement

Wild emmer, the origin of most cultivated bread wheat, is an excellent model organism for advancing evolutionary theory, wheat evolution, and wheat improvement (Feldman and Millet 2001; Nevo et al. 2002). It is a rich, mostly untapped, genetic resource for improving cultivated wheat (together with other wheat relatives). Regional and local genetic patterns reveal significant adaptive spatial and temporal genic protein and DNA diversities, even across several meters at local niches. Genetic patterns across *coding* and *non-coding* genomic regions are correlated with, and predictable by, environmental stress (climatic, edaphic, and biotic) and niche heterogeneity as was also shown in other species across life, locally, regionally, and globally (Nevo 1998b). The genomic organization of wild emmer is *non-random*, heavily *structured*, and largely *adaptive*. Spatiotemporal natural selection, and species interaction (Kirzhner et al. 1999) primarily diversifying, balancing, and cyclical selection, appears to be the major genomic architects to adapt populations to multiple ecological niches and stresses. Wild emmer has a unique "archipelago" ecological genetic structure across its range.

The center of wild emmer origin and diversity are in the massive and lush populations of the catchment area of the Upper Jordan Valley (Golan and eastern Upper Galilee Mountains). Southward in Israel and northward in Turkey, it becomes fragmented into semi-isolated and isolated populations. Across the Near East Fertile Crescent, but particularly in Israel, with its extraordinary physical and biotic diversity, wild emmer developed a wide range of spatiotemporal *non-random* biochemical, morphological, and immunological complex adaptive diversity to multiple diseases and climatic and edaphic stresses, both within and between populations. These genetic resources (mostly untapped) represent the best hope for wheat improvement for abiotic (e.g., drought, salt, heat, cold, and Fe and Zn deprivation) and biotic (viral, bacterial, fungal, and herbicide) stresses as well as for amino acids storage proteins, protein genes, amylases, dimeric α-amylase inhibitors, and photosynthetic yield.

The current genetic map of *T. dicoccoides* with 549 molecular markers and 70 QTLs for 11 traits of agronomic importance (Peng et al. 2003) makes it possible to unravel and identify beneficial alleles of candidate genes that are otherwise hidden. The *Gpc-B1* QTL locus originated from wild emmer with pleiotropic effects on plant senescence, grain protein, and Zn and Fe content. Wild emmer, unlike cultivated wheat, carries a functional *TtNAM-B1* allele of the

NAC transcription factor controlling senescence, causing higher levels of proteins and minerals (Zn and Fe) in mature grains, and was then transgressed into bread and durum wheat available for distribution worldwide from Dubcovsky's lab in Davis, CA, USA (Uauy et al. 2006). Likewise, a kinase-START gene, absent in modern wheat, confers extensive temperature-dependent resistance to stripe rust (Fu et al. 2009). Other genes have already been identified and mapped and are potentially ready for wheat improvement (Xie and Nevo 2008). Clearly, the origin and evolution of wild emmer makes it an ideal species for wheat improvement.

10.26 Prospects

Wild emmer wheat could be probed in-depth to evaluate its structural, functional, and regulatory polymorphisms adapting it to environmental stresses (Parsons 2005). Next-generation sequencing transforms today's biology (Schuster 2008), dramatically advancing both theory and application. In particular, the relationship with genomic and epigenomic diversities (Kashkush et al. 2002; Levy and Feldman 2004; Kashkush 2007) could be highlighted by deciphering the regulatory function of *non-coding* genomes on genic components. *Regulation* might be the key in the future. It might decipher both speciation and adaptation processes to stressful, heterogeneous, and changing environments. The *non-random* adaptive processes and complexes in wild emmer and other wheat relatives could provide the basis for wheat improvement as single genes, QTLS, and interacting biochemical networks.

Theoretically, to decipher genomic and epigenomic interactions, function and regulation at *macro* and *microgeographical* scales of natural populations will bridge structure, function, and fitness over the entire life-cycle. Transplant experiments and genome-wide mapping, over space and time, could identify fitness components. Molecular cytogenetics could probe the nuclear, chloroplast, and mitochondrial genomes (e.g., Raskina et al. 2008). Exploration of *non-random* mutations, splice variants, lateral transfers, recombination, stress genes, regulatory elements, and their association with ecological heterogeneity, environmental stress, and adaptive systems could be advanced. Identifying the interactions of the driving evolutionary processes of allopolyploidization and domestication processes (hybridization, selection, drift, and migration) could link theory and application, and epigenetics and evo–devo processes could highlight growth and development, evolution, and applications.

In application, unique genes and QTLs of agricultural importance should be probed to unravel much more than the currently known resources of wild emmer and other wheat relatives, and introduce them into elite cultivars via marker-assisted selection (Lemaux and Qualset 2001). Molecular cloning of adaptation genes, expressed sequence tags (ESTs), transcription factors, and genetic transformation of defined target genes/alleles. Exploring novel genes and regulatory elements in small genome grasses, such as rice and *Brachyopodium,* could facilitate the mapping and cloning of wild emmer genes due to colinearity. Last but not least, it is essential to follow domestication processes and unravel many functional and regulatory genes that were eliminated from the cultivars during domestication, primarily by modern breeding.

Identifying the polycentric sites of wild emmer domestication in the southern Levant versus monocentric ideas is feasible by tracking non-brittle rachis remains, during initial phases of the "agricultural revolution," which may have been a gradual rather than a revolutionary process. This future research could identify lost adaptive genes during domestication and their active introgression from wild emmer back to cultivated wheat for genetic reinforcement.

The current efforts of physical mapping and sequencing of the wheat genome (Feuillet and Eversole 2007; Feuillet and Muehlbauer 2009), conducted by the International Genome Sequencing Consortium (IWGSC) since 2005, develop the needed background for sequencing the hexaploid wheat genome and provide theoretical evolutionary perspectives and application tools for improving wheat.

This is a long-term, milestone-based strategy that delivers products and tools while working towards the ultimate goal of enabling profitability throughout the industry. It involves the following perspectives:

- Physical map of bread wheat genome
- Genome sequencing launching pad

- Robust bioinformatics platform
- Whole genome enabled functional genomics

Twenty countries and more than 200 members participate in this heroic effort to sequence the ~17,000 Mb bread wheat genome with its ~85% repeat sequences, which is 120-fold *A. thaliana* genome and 45-fold rice and *Brachyopodium* genomes. Clearly, wheat represents a challenge for genomic studies and sequencing. An update of diversity, agronomical traits and phenotypes, and integrative perspectives of wheat genome evolution, both theoretically and practically, appears in the abstracts of the 19th International Triticeae Mapping Initiative, 3rd Cost Tritigen (Clermont-Ferrand, France, August 31–September 4, 2009), and in Feuillet and Muehlbauer 2009.

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Chapter 11
Zea

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11.1 Some Important Characteristics of Wild *Zea* Species

11.1.1 Geographical Distribution and Genetic Diversity

The teosintes are annual and perennial grasses native to Mexico and Central America. Most of these wild *Zea* species and subspecies are distributed across narrow ranges and can only be found in some tropical and subtropical areas of Mexico, Guatemala, Nicaragua, and Honduras. The geographic distributions of the teosintes are listed in Table 11.1. A point worth highlighting is that wild *Zea* species do not have uniform distributions and are enormously sensitive to even the slightest environmental changes in day length and moisture (Sánchez-González and Ruiz-Corral 1997). Hence, we also list in Table 11.1 some specific climate, elevation, and soil conditions under which teosintes are found.

Two subspecies of *Z. mays* (*parviglumis* and *mexicana*) harbor high levels of molecular genetic variation and show genetic substructuring along geographic lines (Doebley et al. 1984). Phylogeographic and diversity analysis demonstrated that *parviglumis* originated from the eastern part of Central Balsas, Mexico, and spread from east to west, while *mexicana* originated from the Central Plateau of Mexico and spread along multiple paths to the north and east (Fukunaga et al. 2005). Other species of wild *Zea* (*Z. luxurians*, *Z. diploperennis*, *Z. perennis, Z. mays* ssp. *mexicana*, *and Z. nicaraguensis*) show less diversity, as expected from their very narrow geographic distributions.

11.1.2 Morphology

As the wild ancestor of modern maize, the plant architecture and general growth forms of teosinte are similar to maize. A typical teosinte plant usually has a main stalk that typically contains a series of nodes and elongated lateral branches at most nodes. The internodes can reach up to 20–30 cm in length. The ears occur in clusters of 1–5 (or more) at each node along the branch. The essential morphological features of the teosintes are given in Table 11.2.

The main morphological differences between teosinte and maize are their branches and inflorescences. Teosinte plants contain more branches and smaller female inflorescences than maize. For wild *Zea* species, the inflorescences can only form 5–10 triangular or trapezoidal black or brown seeds with a hard fruitcase. By comparison, maize usually has 100 or more naked seeds (Fig. 11.1). Recently, it was reported that pollen, starch grain, and phytoliths can also be used for differentiating between teosintes and maize (Holst et al. 2007).

11.1.3 Cytogenetics

Zea species have been one of the most favored models for extensive cytogenetic studies since the 1950s.

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Table 11.1 Geographic distribution of the wild *Zea* species (teosinte)

Populations	Distribution	Growing environment
Z. mays ssp. *parviglumis*	Southwestern Mexico	Middle altitude (400–1,800 m), subhumid temperate
Z. mays ssp. *huehuetenangensis*	Northwestern Guatemala	Low elevation (900–1,650 m)
Z. mays ssp. *mexicana*	Central and northern Mexico	High elevation (1,700–2,600 m), dry environment
Z. diploperennis	Sierra de Manantlán biosphere reserve region, Mexico	Highlands (1,900–2,100 m), average annual temperature about 16°C
Z. perennis	Western Mexico	Highlands (1,500–2,000 m), moderate climate (average annual temperature of 16–27°C)
Z. luxurians	Southeastern Guatemala; Honduras and Nicaragua	Altitudes between sea level and 1,100 m
Z. nicaraguensis	Southwestern Nicaragua (Pacific coast)	Coastal plain and very mesic environment (some grow in water)

Data are from Sánchez-González and Ruiz-Corral (1997); Fukunaga et al. (2005)

Here, we briefly summarize the cytology of *Zea* chromosomes. Additional descriptions of cytogenetic studies on wild *Zea* species are presented in Sect. 11.4.

11.1.3.1 Basic Chromosome Number and Size

All the annual wild *Zea* species are diploid with $2n = 20$ chromosomes. One perennial species, *Z. diploperennis*, is also diploid and has $2n = 20$. The other perennial species Z. *perennis* (also called *Z. tetraperennis*) is a tetraploid with 40 chromosomes (Molina and Naranjo 1987). Generally, the teosintes have larger chromosomes than those found in cultivated maize where they have an average length of 11.2 μm. Among the wild species, Z. *nicaraguensis* has the largest chromosomes, with an average length of 19.6 μm (Ellneskog-Staam et al. 2007). *Z. perennis* has the smallest average chromosome size and the smallest heterochromatic blocks (Tito et al. 1991).

11.1.3.2 Chromosome Knobs

Chromosome knobs are enlarged structures consisting of condensed heterochromatin on mitotic and meiotic chromosomes. They can be used for taxonomic studies in the genus *Zea* because they exhibit fixed numbers and positions on chromosomes within a specific accession, but vary between accessions (Kato 1976). In most cases, the main chromosome knobs in maize are internal or subterminal. In contrast, the chromosomes of *Z. nicaraguensis*, *Z. luxurians*, *Z. diploperennis*, and *Z. perennis* (Section *Luxuriantes*) have many terminal chromosome knobs and lack internal knobs. The main cytological difference between the *Z. nicaraguensis* and *Z. luxurians* genomes is that chromosome 10 of *Z. nicaraguensis* is knobless (Ellneskog-Staam et al. 2007). On the other hand, all of the subspecies of *Z. mays* (Section *Zea*), in general, have many internal knobs and few or no terminal knobs except *Z. mays* ssp. *huehuetenangensis*, which has many terminal chromosome knobs (Wilkes 1967). These chromosomal knobs contain thousands to millions of tandem 180 bp and 350 bp repetitive DNAs that have high frequencies of preferential segregation as a result of meiotic drive (Buckler et al. 1999).

11.1.4 Agricultural Status

The wild species within the genus *Zea* are considered by many maize farmers as harmful weeds in some parts of Mexico, while in other areas, they are regarded as beneficial companion plants that can hybridize with maize. Currently, a good deal of interest is focused on delivering beneficial teosinte traits, such as insect resistance, perennialism, and flooding tolerance, to cultivated maize. However, this is difficult due to linked deleterious traits in the teosinte genomes. Some wild *Zea* species are also used locally for food and/or forage in Guatemala and Honduras. Although the wild *Zea* species may provide an important germplasm resource for the improvement of cultivated maize, several populations of teosinte are either threatened or endangered. *Z. diploperennis* exists in a very

Table 11.2 Morphological features of the wild *Zea* species

	Z. mays ssp. *parviglumis*	*Z. mays* ssp. *huehuetenangensis*	*Z. mays* ssp. *mexicana*	*Z. diploperennis*	*Z. perennis*	*Z. luxurians*	*Z. nicaraguensis*
Habit	Annual	Annual	Annual	Perennial	Perennial	Annual	Annual
Height	2–5 m	up to 5 m	1.5–4 m	2–2.5 m	1.5–2 m	3–4 m	2–5 m
Tassel branches	Slender, 20–100 branches	Slender, 20 or more branches	Slender, 10–20 branches	Thicker, lax, 2–10 branches	Thicker, erect, 2–8 branches	Thicker, erect, 4–20 branches	Slender, lax, 27–38 branches
Male spikelet	Paired, 4.6–7.2 mm long, smaller size pedicels (4.6–7 mm)	Paired, longer pedicels (5–7 mm)	Paired, longer pedicels (6–10 mm)	Paired, 10 mm long, shorter pedicels (1.5–3.5 mm)	Shorter pedicels	Shorter pedicels (1.5–3.8 mm)	Paired, shorter pedicels (1.5–3.5 mm)
Female spikelet	Slender and distichous	Slender and distichous	Slender and distichous	Slender and distichous	Slender and distichous	Slender and distichous	Slender and distichous
Blooming date	September through October	Late November to January	September through October	September through October	September through October	September through October	Mid-October to early November
Fruit cases	Small, blunt, triangular (30–80 mg)	Small, triangular (30–60 mg)	Large, acute triangular (60–95 mg)	Trapezoidal (68–75 mg)	Trapezoidal (70–83 mg)	Trapezoidal (76–99 mg)	Trapezoidal (76–99 mg)
Rhizomes	Absent	Absent	Absent	Slender, both cord-like and tuber-like with short internodes (2–6 mm)	Slender, cord-like with long internodes (1–6 cm)	Absent	Absent

Data are from Iltis et al. (1979); Iltis and Doebley (1980); Doebley (1983); Iltis and Benz (2000)

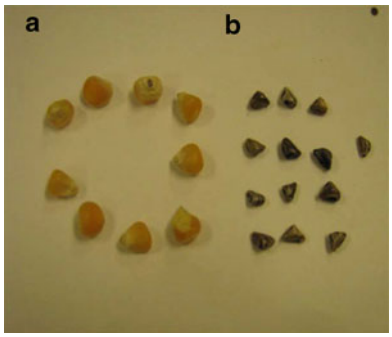

Fig. 11.1 Maize and teosinte seeds. (**a**) maize; (**b**) *Z. mays* ssp. *parviglumis*

narrow area in Mexico and could go extinct in the wild if not protected (Sanchez-Velasquez et al. 2002). *Z. nicaraguensis* appears to be restricted to an area of 200 × 150 m in Chinandega, Nicaragua (Iltis and Benz 2000).

11.1.5 Summary

In summary, the teosintes are naturally distributed in Mexico, Guatemala, Honduras, and Nicaragua. The morphology and cytogenetics of the wild *Zea* species are quite similar to maize but have significant differences in branches, inflorescences, and ears, and in chromosome length and knob distributions. The distributions of wild *Zea* species are very limited. Fortunately, the Mexican and Guatemalan governments have taken action in recent years to protect wild *Zea* populations, using both in situ and ex situ conservation methods.

11.2 Conservation Initiatives

Teosintes have been generally accepted as the closest relatives of domesticated maize and thus are expected to exhibit many of the wild traits that were present in the ancestor(s) of maize. The natural distributions of teosintes covered more than a thousand square kilometers in Mexico and Guatemala in the 1960s (Wilkes 1967). However, in the last 40 years, teosinte populations have declined dramatically. Once an abundant element of rural field margins and fallow plots, two of the wild teosintes in Mexico (*Z. mays* ssp. *mexicana* and *Z. mays* ssp. *parviglumis*) have now become fragmented and scattered. Today, the current distributions of teosintes in Mexico have shrunk to 50% of their size in 1900 (Wilkes 1997). Teosinte in Guatemala (*Z. luxurians* and *Z. mays* ssp. *huehuetenangensis*) is near extinction and is expected to be eliminated within the next decade if no immediate conservation efforts are undertaken (Wilkes 2007). Three of the annual teosinte populations (*Z. nicaraguensis*, *Z. luxurians* and *Z. mays* ssp. *huehuetenangensis*) are considered "rare," occurring at single locations, while others are considered "vulnerable" according to the terms of the Species Survival Commission of the International Union for Conservation of Nature and Natural Resources (IUCN) in Switzerland (National Research Council US 1993).

Several factors appear to have hastened the decline of the teosintes (1) *Genetic erosion* has greatly affected the genetic diversity of wild *Zea* species. Teosintes often hybridize with cultivated maize, especially on roadsides and in the populations growing on the borders of maize fields (FAO 2002). The hybrids may lose some unique characteristics of their teosinte parents and lead to the extinction of some rare species. (2) *Increasing human population* is an important factor for the decline of wild teosintes. The rate of teosinte disappearance will accelerate with an increase in the number and size of roads and houses. (3) *Overgrazing* is another principal threat to teosinte. Teosintes are a good source of forage for livestock. Livestock relish the sweet, green leaves and stems of teosinte that appear after summer rains (Tuxill and Nabhan 2001). (4) *Replacement of maize* with coffee or other crops that are not compatible with the teosinte growth environment can also lead to the eradication of wild *Zea* germplasm (Wilkes 2007).

11.2.1 Evaluation of Genetic Erosion in Teosinte Germplasm

Genetic erosion is the loss of genetic diversity, both the loss of individual genes and the loss of particular combinations of genes. Crop plants and their wild relatives can interbreed because of their close evolutionary relationship. Gene flow from the wild *Zea*

species is predicted to often be beneficial for maize improvement and hybrid development. However, gene flow from the vast populations of highly inbred maize into the wild *Zea* species inevitably causes genetic erosion and thereby restricts the genetic diversity of teosinte. The frequency of gene flow is quite different between maize and different species and subspecies of teosinte. A 3-year field experiment showed that maize and *Z. mays* ssp. *parviglumis* hybridized at a high rate, while *Z. mays* ssp. *mexicana* and *Z. luxurians* show cross-incompatibility with maize (Evans and Kermicle 2001; Ellstrand et al. 2007). In addition, allozyme comparison of *Z. luxurians* and *Z. diploperennis* revealed that there is an extremely low level of introgression from maize to these wild teosinte species (Doebley 1990b). Maize has coexisted and coevolved with its wild relatives over thousands of years. It is likely that maize and *Z. mays* ssp. *parviglumis* have been exchanging genes for the last several thousand years. It is reported that crossing between maize and *Z. mays* ssp. *parviglumis* usually occurs in September to October when the flowering period of teosinte plants overlaps the end of the flowering period of maize (Baltazar and Schoper 2002). But further attempts to exactly predict the extent of genetic erosion is not possible because ancestral contributions cannot be demarcated clearly (Smith and Goodman 1981).

The isolated teosinte populations that survive in or near maize fields may be genetically "swamped" and replaced by maize. In the major areas of teosinte distribution in Mexico, where maize also predominates, 10% or more of the teosinte may actually be hybrids (Doebley 1984). Teosintes have a dispersal advantage because they possess articulate seed cases. However, they usually lose this ability after hybridization with maize, thus leading to the possible extinction of small teosinte populations.

11.2.2 *Attempts at* In Situ *and* Ex Situ *Conservation*

Conservation of plant genetic resources can be achieved by protection of populations in nature (in situ) or by preservation of samples in germplasm banks and botanical gardens (ex situ). As early as the 1960s, the principal zones of teosinte distribution in Mexico and Guatemala were surveyed (Wilkes 1985). In 1985, the government of Guatemala prohibited the collection of wild *Zea* species and their removal from the country. Over the last 40 years, with financial support from the Rockefeller Foundation, the United States Department of Agriculture (USDA), and the governments of Mexico and Guatemala, in situ and ex situ conservation efforts have been expanded for the wild *Zea* species.

11.2.2.1 In Situ Conservation

In situ (on site) conservation is to protect, monitor, and manage the selected species in their natural habitats, including where they have evolved. The benefits of in situ conservation of crop wild relatives (CWR) have been well acknowledged (Prescott and Prescott 1981). In the mid-1980s, Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT), Instituto Nacional de Investigaciones Forestales y Agropecuarias (INIFAP), and Instituto de Ciencia y Tecnología Agrícolas (ICTA) came together to start a periodic and long-term in situ monitoring program on each teosinte population with the participation of local farmers. The intention was to check the status of each teosinte population and take preservation actions whenever a population appeared to be in danger of extinction (Eltringham 1984). However, besides the in situ monitoring program, the establishment of in situ protected areas has been slow to emerge due to various social and economic reasons (FAO 1997). So far, the only successful in situ conservation project is that of *Z. diploperennis* in Mexico's Sierra de Manantlan Biosphere Reserve (Fig. 11.2). This 1,396 km^2 reserve area was established in 1987 and encompasses 12.5 km^2 of the *Z. diploperennis* germplasm distribution (Benz 1988). In Guatemala, attempts to establish large in situ conservation areas were unsuccessful and there was only some small village-level protected areas established by ICTA. Researchers in Nicaragua are planning to start an in situ conservation program funded by USDA (Meilleur and Hodgkin 2004).

11.2.2.2 Ex Situ Conservation

Ex situ (off site) conservation is to remove the genetic materials from their native habitats and maintain them in managed, easily accessible locations such as

botanical gardens, nurseries, or germplasm banks. Ex situ is a complementary method to in situ conservation with a relatively low cost, facile establishment, and easy access for users (Hawkes et al. 2000). About 80% of the extant genetic diversity in the teosintes is believed to have been sampled and conserved ex situ through various methods (Smith et al. 2004). A few teosintes are cultivated in some important botanical gardens such as the Brooklyn Botanic Garden and the Aztec Botanical Garden in the USA and Mexico, respectively. Most wild *Zea* species are conserved through germplasm banks.

The major teosinte collections in germplasm banks are those of CIMMYT and INIFAP in Mexico. The other two germplasm banks with large numbers of teosinte accessions are the North Central Regional Plant Introduction Station (NCRPIS) of the USDA at Iowa State University and at the University of Guadalajara, Mexico. Antonio Narro, Autonomous Agrarian University in Mexico, also preserves a small number of teosinte accessions. In 2003, ICTA constructed an active germplasm storage facility for teosinte and maize in Guatemala. Teosinte accessions collected in the main germplasm banks are listed in Table 11.3.

In addition to seed storage, an international cooperative project to regenerate the endangered wild *Zea* species has been in progress since 1993 (Taba 2003). Several teosinte accessions are grown annually at CIMMYT and planted in their field plots located in El Batan and Tlaltizapan, Mexico. ICTA (Guatemala) is planning to replenish seed stocks of all the new accessions of *Z. mays* ssp. *huehuetenangensis* as well as some of the accessions at CIMMYT in the next several years.

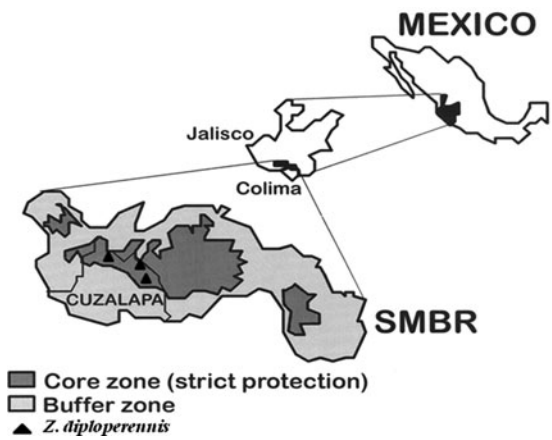

Fig. 11.2 Map of Sierra de Manantlan Biosphere Reserve (SMBR). Figure is adapted from Louette et al. (1997)

11.2.3 Modes of Preservation and Maintenance

Different germplasm banks often preserve seeds in different quantities and under varied conditions. For short storage (less than 10 years), the materials are usually stored in glass jars or plastic boxes at 4–15°C and 10–30% humidity. This is the so-called "active bank" where the seeds are frequently used by researchers and farmers. For medium-term storage (up to 30 years), the temperature is maintained at 0–4°C. Of the teosinte germplasm banks, only CIMMYT and USDA-ARS NCRPIS have adequate and specialized long-term storage (more than 30 years) facilities for

Table 11.3 Number of teosinte accessions held in germplasm banks

Species/number	Germplasm banks			
	INIFAP	CIMMYT	USDA (GRIN)	University of Guadalajara
Zea mays ssp. *parviglumis*	135	50	216	96
Zea mays ssp. *mexicana*	103	65	74	57
Zea mays ssp. *huehuetenangensis*	0	1	7	0
Zea perennis	4	2	10	1
Zea luxurians	0	1	20	0
Zea diploperennis	7	2	13	2
Zea nicaraguensis	0	1	1	0

Data are from the USDA Germplasm Resources Information Network (GRIN) online database, *http://www.ars-grin.gov/* (Cited 12 Oct; Global Crop Diversity Trust 2007)

teosinte. A small part of the maize collection (including some teosinte seed) is preserved in INIFAP's long-term storage facilities.

Cryopreservation is also used for long term storage of teosinte seed at CIMMYT. CIMMYT's long-term storage facility has 240 m^3 of storage space for maize and teosinte seed, and it was constructed in 1996. The teosinte seed are packaged in aluminum-laminated bags containing 1–1.5 kg of seed per bag on movable shelves to optimize use of the available space (Pardey et al. 2001). This storage area is maintained at a temperature of −18°C and at a relative humidity of 6–8%. Germplasm bank employees monitor the teosinte seed viability periodically to ensure that the viability remains between 85 and 100%.

11.2.4 Summary

Because of their small and isolated populations, most of the teosintes are threatened with extinction, and their germplasm is continually being eroded, largely by gene flow from domesticated maize. Specific efforts to conserve teosinte alleles of *Zea* genes are needed. Both in situ and ex situ conservation methods are important. They have distinct advantages and disadvantages and therefore are complementary approaches for teosinte protection. In situ conservation of teosinte germplasm has only recently been initiated, and this only in two regions of Central America, so additional populations need to be protected. Ex situ conservation is more advanced, with germplasm banks established for short, medium, and long-term storage at a number of facilities around the world.

11.3 Role in Elucidation of the Origin and Evolution of Maize

Most, perhaps all, crop plants have wild relatives that currently exist in natural populations. Since the end of the nineteenth and the beginning of the twentieth century, the relationships between the teosintes and other plant species have been controversial. Initially, some taxonomists proposed that teosintes were closely related to rice (reviewed in Doebley 2004). Subsequently, fertile hybrids crossed between maize and some types of teosinte were noted, leading some scientists to consider one or more teosintes to be plausible candidates as the ancestor(s) of maize (Schuman 1904; Collins 1919). In 1939, Beadle proposed the "teosinte hypothesis," which stated that modern maize was derived directly from a teosinte (Beadle 1939). The idea itself was not new; however, Beadle was the first person who successfully used experimental results to verify this hypothesis and pointed out five key mutations from teosinte to maize (Beadle 1939, 1972, 1980). A recent commentary has described the accumulated and overwhelming evidence in support of wild *Zea* species being the sole progenitors of maize (Bennetzen et al. 2001). To help understand the important role of the teosintes in the origin of maize, we outline the morphotaxonomy and chemotaxonomy of teosinte and maize. Additionally, the outcomes of molecular marker analyses used to elucidate the issues on the origin and evolution of maize are summarized. At the end of this part, we briefly describe some useful statistical techniques and computer software.

11.3.1 Morphotaxonomy of Teosinte and Maize

The latest taxonomic relationships of teosinte and maize were discussed in the first part of this chapter. Here, we discuss the classification outcomes for the *Zea* species based on morphological traits.

Although maize has held the interest of botanists and taxonomists for several hundred years, little attention was paid to teosinte taxonomy and phylogeny. The first formal taxonomic study on the teosintes was attempted by Gilly in the 1940s (Table 11.4). However, in his taxonomy, teosintes were only classified by their geographic distributions (Iltis and Doebley 1980). Subsequently, Wilkes presented a landmark monograph on a system of classification for the teosintes (Wilkes 1967). Unlike Gilly, Wilkes carried out long-lasting field explorations of teosinte species in Mexico, Guatemala, and Honduras several times. He described the geographic distribution of the teosintes clearly and divided *Z. mexicana* into six allopatric races (Table 11.4). However, Wilkes relied heavily on geographical distribution for classification and

Table 11.4 Taxonomic studies of the teosintes and maize and their proposed classification systems

Gilly (unpublished specimen annotations, 1948)	Wilkes (1967)	Iltis and Doebley (1980); Doebley (1983)
Zea	Section Zea	Section Zea
Zea mays L	*Zea mays* L	*Zea mays* L.
Euchlanea	Section Euchlanea	ssp. *mexicana*
Euchlanea mexicana	*Zea mexicana*	Race Chalco
Phase Durango	Race Nobogame	Race Central Plateau
Phase Bajio	Race Central Plateau	Race Nobogame
Phase Chalco	Race Chalco	ssp. *parviglumis*
Phase Barranca	Race Balsas	ssp. *huehuetenangensis*
Phase Guerrero	Race Guatemala	ssp. *mays*
Phase Huehuetenango	Race Huehuetenango	Section Luxuriantes
	Zea perennis	*Zea diplorennis*
		Zea perennis
		Zea luxurians
		Zea nicaraguensis

Adapted from Doebley et al. (1984); Doebley (1990a)

paid little attention to floral morphology (Doebley 1983). Thus, some of his races are clearly distinct, while others are differentiated narrowly. Moreover, he did not accept Beadle's teosinte hypothesis for the origin of maize (Wilkes 1985). Further taxonomic study of the genus *Zea* was published by Iltis and Doebley, establishing a new hierarchical system of classification based on the morphology of the male inflorescence (tassel) and spikes (Doebley and Iltis 1980; Iltis and Doebley 1980). Furthermore, Doebley stated that morphological distinctions made it clear that the genus *Zea* should be divided into two sections: Luxuriantes and Zea (Doebley 1983). In section *Zea*, cultivated maize (*Z. mays* ssp. *mays*) was grouped together with several teosintes (*Z. mays* ssp. *mexicana*; *Z. mays* ssp. *parviglumis*; and *Z. mays* ssp. *huehuetenangensis*). Section Luxuriantes includes *Z. diploperennis*, *Z. perennis*, and *Z. luxurians*. This taxonomic system placed the taxa in a sequence that is believed to reflect their phylogenetic origins. More importantly, the morphological data provided strong evidence that *Z. mays* ssp. *parviglumis* and/or ssp. *mexicana* was a direct ancestor of maize.

Because of the striking differences in the female inflorescence (ear) between maize and teosinte (Fig. 11.3), there is still a lot of debate about the evolution of maize. Iltis carefully evaluated the ear morphology of teosinte and maize and used these observations to construct his catastrophic sexual transmutation theory (CSTT; Iltis 1983). In the CSTT model, Iltis proposed that teosinte is the ancestor of maize and that the maize ear evolved from the teosinte ear through a one-step feminization of the tassel. Iltis has now replaced his CSTT model with his sexual translocation theory (STLT) to explain the ear evolution from teosinte to maize. STLT theory proposed that maize ear has evolved from teosinte tassels through one or two homeotic sexual translocations, which replace the primary and secondary branch tassels with a female structure (Iltis 2000). The female inflorescence development of teosinte was also investigated in other published studies (Benz and Iltis 1992;

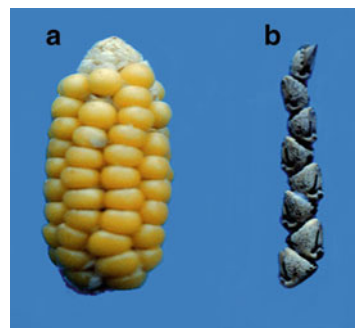

Fig. 11.3 Extreme differences between maize and teosinte ears. (**a**) A short maize ear; (**b**) Ear of pure teosinte (*Z. mays* ssp. *parviglumis*). Figure is adapted from Doebley (2004). Reprinted, with permission from the *Annual Review of Genetics*

Orr and Sundberg 1994; Orr et al. 2002). Their results further supported Iltis's STLT model and confirmed the important role that teosinte played in the origin of maize.

11.3.2 Chemotaxonomy Based on Biochemical Markers

Chemotaxonomy or chemosystematics classifies organisms by chemical or biochemical methods. Because of the importance of the taxonomy of *Zea*, a considerable amount of work using biochemical markers (enzymes, other proteins, and amino acids) has been undertaken. The electrophoretic patterns of zeins and reduced alcohol-soluble glutelins in teosintes were found to be quite similar to those in maize, but those of the sister genus *Tripsacum* were found to exhibit marked differences (Paulis and Wall 1977). Similar banding patterns were found on denaturing acrylamide gels for water-soluble seed proteins in comparisons between maize and Mexican teosinte. These results further suggested that a Mexican teosinte was an ancestor of maize (Smith and Lester 1980). Subsequently, influential work on the chemotaxonomy of the genus *Zea* was done by Doebley and his colleagues through the analysis of isozymes (Doebley et al. 1984, 1985, 1987a). In their work, 13 enzyme systems were investigated in 56 populations of teosinte and 99 populations of maize from Mexico and Guatemala, and a phylogenetic tree was constructed (Fig. 11.4). The classification on the basis of isozyme data agreed well with previous studies based on morphological traits.

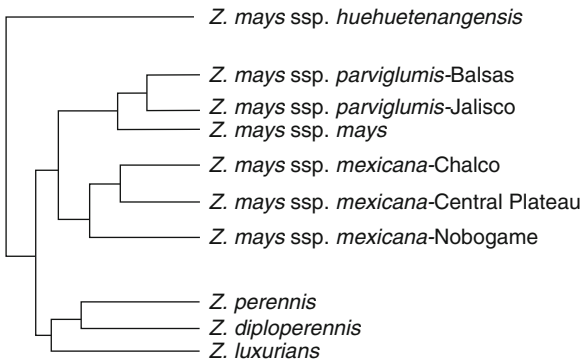

Fig. 11.4 The phylogenetic tree of teosinte and maize based on isozyme data. Figure is adapted from Doebley et al. (1984)

Several morphologically similar species (*Z. mays* ssp. *mexicana* and ssp. *parviglumis*, *Z. perennis*, and *Z. diploperennis*) were well-differentiated based on these data. This study also indicated that one of the Mexican annual teosintes, *Z. mays* ssp. *parviglumis,* was the closest relative of maize because it had indistinguishable isozyme allele frequencies from those of maize.

11.3.3 Molecular Markers and Their Application for the Study of Maize Origins and Evolution

Because of their abundance, ease of use, high level of polymorphism, robustness, and constantly decreasing cost, the development of DNA-based markers has provided an excellent opportunity to evaluate the relationship between the teosintes and modern maize. Molecular markers have been used to examine the mitochondrial, chloroplast, and nuclear DNA of teosinte and maize.

In early molecular analyses, chloroplast and mitochondrial DNA fragments produced by restriction enzyme digestion were used to characterize relatedness in the genus *Zea*. Using this method, maize, a perennial teosinte, and six races of annual teosinte (Guatemala, Huehuetenango, Balsas, Central Plateau, Chalco, and Nobogame) were characterized (Timothy et al. 1979). The results showed that the annual teosinte Guatemala and the perennial teosinte differed markedly from maize, while the other five races of annual teosinte were similar to maize, suggesting a common ancestry. Phylogenetic analysis based on chloroplast DNA digestion patterns split the genus *Zea* into two sections that were fully congruent with those revealed by isozyme analysis and morphology (Doebley et al. 1987b). These results supported the hypothesis that maize is a domesticated form of either *Z. mays* ssp. *mexicana* or *Z. mays* ssp. *parviglumis*. However, distinguishing between these two teosinte subspecies as an ancestor was not possible at this low level of resolution.

Two common types of molecular markers that have been used in maize and teosinte research are restriction fragment length polymorphism (RFLP) and simple sequence repeat (SSR) or microsatellite markers.

(1) *RFLPs*: Due to their inherent properties (numerous and codominant), RFLP markers have been used with great success in many genomic characterizations, including identification and mapping of quantitative trait loci (QTL) controlling traits (Phillips and Vasil 1994). A total of 52 genes that might be related to the major morphological differences between maize and *Z. mays* ssp. *mexicana* were investigated. The results demonstrated that these differences are controlled by five chromosomal regions, each with major effects on one or more of these traits (Doebley et al. 1990; Doebley and Stec 1991). These studies provided new evidence on maize evolution that were further supported by Szabo and Burr's experiments investigating maize and *Z. mays* ssp. *parviglumis* (Szabo and Burr 1996). Multiple QTL controlling morphological differences between *Z. diploperennis* and *Z. mays* ssp. *parviglumis,* and between *Z. mays* ssp. *parviglumis* and *Z. mays* ssp. *mexicana,* were also analyzed by RFLP markers (Westerbergh and Doebley 2002, 2004; Lauter et al. 2004). Based on QTL results, two genes were identified as strong candidates to be responsible for the major differences in plant morphology between maize and teosinte. These two genes, *teosinte branched1* (*tb1*) and *teosinte glume architecture1* (*tga1*), were cloned and functionally characterized by the Doebley laboratory (Doebley et al. 1997; Wang et al. 2005). More details about the function of these genes will be discussed in Sect. 5. (2) *SSRs*: Microsatellite or SSR markers are a series of tandemly repeated units (1–6 bp each) commonly found in eukaryotic genomes. SSRs have been exploited as tools to assess the genetic relatedness and diversity of crop plants because they are highly polymorphic, codominant, easy to assay, and relatively inexpensive (Bruford and Wayne 1993). More than 2,000 SSR markers have been developed for maize (*www.maizegdb.org/ssr.php*) and some of them can be applied to different teosinte species (Lubberstedt et al. 1998). Using SSR markers, the phylogenetic tree of the genus *Zea* was constructed with good resolution and the results revealed that *Z. mays* ssp. *parviglumis* is most closely related to the progenitor of maize (Matsuoka et al. 2002a). Moreover, the microsatellite-based study also indicated that maize originated from a single domestication event in southwestern Mexico about 8,700 years ago, which is consistent with the archeological and paleoecological records such as phytoliths, starch grains, and pollen (Piperno and Flannery 2001; Matsuoka et al. 2002b; Piperno et al. 2009; Ranere et al. 2009). Recently, phylogeny and population structure were investigated in a comprehensive study that genotyped numerous maize and teosinte accessions from the American continent using 93 microsatellite markers (Fukunaga et al. 2005). These results provide a solid framework for further studies of the genetic origins of maize domestication and diversification.

11.3.4 Relevant Statistical Techniques and Computer Software

Research on the origin and evolution of maize increasingly produce a large quantity of information that includes morphological data, molecular marker data, gene expression data, and protein-level data. A series of classical and modern statistical techniques have been widely used to allow more power and flexibility in handling these impressive sets of data. Here, we briefly describe some important statistical tools and computer programs used for the classification of the genus *Zea* and for elucidating the contributions of the teosintes to maize evolution. The software and their platforms, uses, and online links are summarized in Table 11.5.

11.3.4.1 Statistical Tools for Classification Within the Genus *Zea*

A clear taxonomy of the genus *Zea* is the backbone for the elucidation of the role played by teosintes in origin and evolution of modern maize. Principal component analysis (PCA), canonical analysis, and cluster analysis have been the three most widely used statistical methods. (1) *PCA*: PCA is an ordination statistical technique invented by Karl Pearson in 1901. In taxa classification, it is utilized to generate a 2- or 3-dimensional scatter plot on which the geometrical distances among the dots reveal the genetic distance and the aggregations of dots reflect the genetic similarity among the individual samples (Warburton and Crossa 2000; Mohammadi and Prasanna 2003). For example, on a PCA plot, *Z. perennis*, *Z. diploperennis*, and *Z. mays* ssp. *huehuetenangensis* were separated from each other and from the remaining annual Mexican

Table 11.5 Some widely used computer software for maize origin and evolution research

Name	Recent version	Platforms	Brief description	Useful online link
SAS/SAT	9.2	Windows (2003, xp, Vista); Linux; Solaris	PCA and canonical analysis; clustering analysis	*http://www.sas.com*
VisuMap	2.6	Windows (2000/xp)	PCA, especially for 3D maps	*http://www.visumap.net*
XLSTAT	2008	Windows (9x~vista); Mac, based on Excel	PCA and canonical analysis	*http://www.xlstat.com*
CANOCO	4.5	Windows; DOS	Canonical analysis	*http://www.microcomputerpower.com*
Microsat	1.5	Dos; Mac	Genetic distance measurement	*http://hpgl.stanford.edu/projects/microsat/*
LCDMV	–	Windows or Linux based on SAS (6.10 and above version)	Genetic distance measurement	*http://www.cimmyt.org/english/docs/manual/lcdmv/contents.htm*
STRUCTURE	2.2	Windows (9x/xp/2000/NT); Linux; Mac	Model-based method clustering; population structure	*http://pritch.bsd.uchicago.edu/structure.html*
PHYLIP	3.68	Linux; Mac; Windows; (9x/NT/me/2000/xp/vista)	Phylogenetic tree construction	*http://evolution.genetics.washington.edu/phylip.html*
PAUP*	4.0	DOS; Linux; Mac ; Windows (9x/me/2000/NT/xp)	Phylogenetic tree construction	*http://paup.csit.fsu.edu/*
MacClade	4.08	Windows; Mac	Phylogenetic tree construction	*http://macclade.org/macclade.html*
Mapmaker	3.0	Windows; Web-based	Linkage map	*http://www.broad.mit.edu/science/software/software*
QTL Cartographer	1.17	Windows; Mac; Linux	QTL mapping	*ftp://statgen.ncsu.edu/pub/qtlcart/*
Windows QTL Cartographer	2.5	Windows (9x, NT/ME/2000/xP)	QTL mapping	*http://statgen.ncsu.edu/qtlcart/WQTLCart.htm*

teosintes. *Z. mays* ssp. *mays* completely overlapped with *Z. mays* ssp. *parviglumis* (Doebley et al. 1984; Smith et al. 1984). As a 100-year-old method, it is not surprising that PCA can be performed with different softwares such as SAS, VisuMap, and XLSTAT. (2) *Canonical analysis*: Canonical analysis, also called multiple discriminant analysis, is an elegant multivariate statistical model for studying the relationships between two or more variables in a data set. This method was used to assess relationships within the genus *Zea* (Doebley and Iltis 1980). The function and the output graph of canonical analysis are both quite similar to PCA. However, canonical analysis is more suitable than PCA for taxonomic analysis because PCA is based on the variability among individuals and does not efficiently separate taxa that show few intrataxa differences (Doebley 1983). The software of choice for this type of analysis is CANOCO. Mathematically, this software is excellent. However, its ease of use and the graphics are not the best. Additionally, XLSTAT and SAS can also do the canonical analysis. (3) *Cluster analysis*: Cluster analysis or typological analysis refers to a common statistical technique whose purpose is to classify objects into groups based on the characteristics they possess so that individuals in the same group are more similar than objects in a different group. There are two types of cluster analysis methods: the distance-based method and the model-based method. Distance-based phylogenetic tree construction has predominated in maize origin and evolution studies. The most commonly used algorithms for the construction of phylogenetic trees are neighbor joining (NJ) and Fitch-Margoliash. An important step in producing a distance-based phylogenetic tree is to measure the genetic distance. Different genetic distance methods may produce different phylogenetic trees. The commonly used approaches for measuring genetic distance are Nei's standard distance (D_s) and the modified Rogers' distance (D_r). Trees constructed using four different genetic distance methods (D_s; D_r; Cavalli-Sforza and Edwards's chord distance

and Nei's chord distance) were compared (Matsuoka et al. 2002a). The results showed that the tree based on D_s is congruent with the expected tree based on Doebley and Iltis's classical taxonomy of *Zea* (Doebley and Iltis 1980), while the other three trees failed to show a monophyletic cluster for *Z. mays* ssp. *mexicana*. The genetic distance is usually calculated by MICROSAT, but this software is no longer updated. LCDMV, a software developed by CIMMYT, is also used to estimate genetic distance. To use this software, a recent version of SAS (later than version 6.10) is required. PHYLIP, PAUP*, and MacClade are the three most frequently cited computer programs for phylogenetic analysis.

11.3.4.2 Statistical Tools for QTL Mapping

QTL mapping is a powerful method for determining the number of genomic regions that control specific traits (Kao et al. 1999). A number of QTL that determine the morphological differences between teosinte and maize have been identified (Doebley and Stec 1993; Doebley et al. 1995; Lauter et al. 2004). There are two steps to make a QTL map: one is to construct the linkage map with the molecular markers and the other is to use quantitative phenotyping to map the QTL. The linkage map is usually made by a computer program called MAPMAKER, based on the maximum likelihood method (Table 11.5). In maize evolution research, interval mapping (IM) and composite interval mapping (CIM) are two widely used statistical techniques for mapping QTL. Theoretically, CIM should be more precise because CIM combines IM with multiple regressions so that the effects of QTL outside the test interval are included in the model (Zeng 1993). QTL Cartographer is one of the most commonly used QTL mapping programs. However, updated packages have not been released since 2004. Recently, a new computer program called Windows QTL Cartographer was developed on the basis of the original QTL Cartographer (Wang et al. 2006).

11.3.5 Summary

A wide set of morphological, biochemical, diversity, and mapping analyses have been employed to study the origin and evolution of modern maize and its teosinte relatives. The results of these studies support the idea that a specific teosinte (*Zea mays* ssp. *parviglumis*) was the ancestor of modern maize, and that a small number of genetic events gave rise to the major morphological differences that distinguish these species. A wealth of software is available to assist these analyses, but many of these tools are not easy to use and/or have not been recently updated.

11.4 Role in the Development of Cytogenetic Stocks and Their Utility

The basic cytogenetics of the wild *Zea* species was discussed in the first part of this chapter. In the genus *Zea*, all species are diploid with a basic set of ten chromosomes ($2n = 20$), except *Z. perennis*, a tetraploid with $2n = 40$. Due to their unique karyotypic and morphological features, the teosintes have played an important role in the development of cytogenetic stocks of maize. These stocks include substitution lines, haploids, polyploids, and aneuploids. These cytogenetic materials have been widely used in genetics research.

11.4.1 Substitution and Introgression Lines

A chromosome substitution line is generated by replacement of a single chromosome in a host plant by a chromosome from a donor parent. Introgression lines contain genomic fragments from a similar species. The teosintes are the closest wild relatives of maize, and they have a similar ($n = 10$) chromosomal composition. Thus, the teosintes have been used to produce maize–teosinte substitution and introgression lines by wide crosses followed by backcrossing to the maize inbred parents. These substitution and introgression lines have mainly been used for trait mapping and introgression of desirable traits from teosinte into the maize germplasm. (1) *Linkage map construction and QTL identification*. QTL have been identified by phenotypic and statistical analyses in F_2 populations or backcross populations derived from maize–teosinte crosses (Doebley and Stec 1991; Doebley et al. 1995,

1997). Compared to F_2 populations, maize–teosinte backcross populations (introgression lines) have been more informative because of their larger population size and resultant higher number of recombination events (Briggs et al. 2007). (2) *Maize improvement*. To transfer desirable characters from teosinte to maize, maize–teosinte substitution and introgression lines were created. For example, several maize × annual teosinte (*Z. mays* ssp. *mexicana* and *Z. luxurians*) substitution lines with high grain yield were generated (Cohen and Galinat 1984). Lines with superior disease resistance were developed by hybridization and backcrossing of maize in projects with two different perennial teosintes (Wei et al. 2003; Tang et al. 2005a).

11.4.2 Haploids

Haploids containing only one half of the chromosome number present in somatic cells provide a shortcut to develop inbred lines. The generation of maize haploids through in vitro (anther and microspore culture) and in vivo (genetic induction) approaches has been routine for quite some time (Coe and Sarkar 1964; Chase 1969; Kindiger and Hamann 1993; Tang et al. 2005b; Zhang et al. 2008). However, there are no reports of teosinte haploid production, probably due to the unclear cytogenetic and genetic background. However, several teosintes have been used as pollen sources to recover wheat haploids. High frequencies of haploid production in wheat were obtained through intergeneric hybridization with teosinte, following the elimination of teosinte chromosome during the development of the embryo (Ushiyama et al. 1991; Ushiyama and Yoshida 2008). The relative efficiencies of nine teosinte lines and several elite maize lines for the production of wheat haploids were evaluated by Suenaga et al. (1998). The results showed that *Z. mays* L. ssp. *mexicana* had a significantly higher efficiency of haploid production compared to Z. *mays* L. ssp. *mays* and to other teosinte species.

11.4.3 Polyploidy and Aneuploidy

With the exception of the tetraploid *Z. perennis*, crosses between maize and different teosintes will produce fertile F_1 hybrids at the diploid level ($2n = 20$). In contrast, *Z. perennis* is an excellent parent for the production of teosinte–maize polyploids. Triploid stocks were obtained by crossing Z. *perennis* ($2n = 40$) with *Z. mays* L. ssp. *mays* ($2n = 20$) or *Z. perennis* with other teosintes. The triploids are completely sterile due to the high number of univalents and trivalents produced in gametes (Poggio et al. 1990). One can produce tetraploid hybrids ($2n = 40$) from *Z. perennis* × *Z. mays* L. *mays* hybrids by doubling the chromosome number of maize with colchicine treatment before crossing (Molina and Garcia 1999). Additionally, a fertile tetraploid hybrid from the combination of an unreduced gamete of *Z. diploperennis* ($2n = 20$) with a normal gamete of *Z. perennis* ($2n = 20$) was generated by Molina (1983). Polyploid stocks of maize and teosinte have been used mainly for cytogenetic research to investigate ancestral relationships and taxonomic classification in the genus *Zea*. Different meiotic behavior was found between a triploid hybrid (*Z. perennis* × *Z. luxurians*) and a diploid hybrid (*Z. diploperennis* × *Z. luxurians*) that can be used for differentiating *Z. luxurians* from other teosintes (Poggio et al. 1999). The meiotic configuration of several triploid and tetraploid stocks provided important evidence regarding the ancestral allotetraploid nature of maize (Molina and Naranjo 1987; Poggio et al. 1999; Molina et al. 2004).

Aneuploids have extra or missing chromosomes, including monosomics ($2n - 1$) and trisomics ($2n + 1$). Maize monosomic and trisomic lines are usually produced by the r-X1 deficiency system and are extremely useful in mapping genes to linkage groups and specific chromosomes (Helentjaris et al. 1986; Weber 1986; Zhao and Weber 1988). At this date, there is no record of teosinte aneuploids.

11.4.4 Summary

A series of maize–teosinte substitution and introgression lines were created and used for maize improvement. Teosintes have been used to generate wheat haploids. Teosinte polyploids were used to investigate ancestral relationships in genus *Zea*. To date, no teosinte aneuploids have been reported.

11.5 Role in Classical and Molecular Genetic Studies

Maize and its wild relatives provide a tractable model system for both classical and molecular genetic research because maize and the teosintes are fully interfertile. The first maize–teosinte hybrid experiments can be traced back to the year 1896 (Harshberger 1896). In the past 100 years, through hybridization of maize with teosinte or teosinte with teosinte, a series of genetic linkage maps was constructed. These populations were also employed in the identification of important genes and polygenic clusters. The effects of ploidy level, the details of physiological pathways, and the properties of various host–parasite interactions were also investigated. These classical and molecular genetic studies of the teosintes and maize have made a major contribution to our understanding of plant biology.

11.5.1 Use in Classical Genetic Studies

Classical genetic studies on the teosintes were mainly focused on the inheritance of morphological traits (dominant, recessive, or intermediate) in maize–teosinte or teosinte–teosinte hybrids. These investigations helped to explain the origin of maize before any molecular studies were undertaken. Mexican agronomist José Segura first pointed out that the teosintes were more closely related to maize than previously thought based on his investigations of maize–teosinte hybrids (Harshberger 1896). Pioneering work on inheritance in maize and the teosintes was carried out in the early twentieth century (Collins and Kempton 1920; Beadle 1939; Mangelsdorf and Reeves 1939). As mentioned above, Beadle's classical genetic experiments (Beadle 1939) led to his hypothesis of a teosinte origin for maize that has been widely accepted by modern maize geneticists (Bennetzen et al. 2001). In his experiment, Beadle crossed one maize line with a teosinte and observed that the teosinte ear reappeared in the F_2 populations at a frequency of about one in 500. From these data, he concluded that a teosinte was the sole progenitor of maize and that only four or five loci controlled the major morphological differences between maize and teosinte (Beadle 1939). Subsequently, the inheritance patterns of other traits (tassel branch, male spikelets, bloom date, prolificity, etc.) were also investigated. The genetic behaviors of these traits in F_2 populations are summarized in Table 11.6. Some of these traits are inherited in a similar manner and are closely linked, as seen in the cases of TBN (tassel branch number) and TBAL (tassel branching axis length) and of VBL (number of veins between primary lateral veins) and TV (total vein number).

Teosintes were also used in classical cytogenetic research that investigated genome origins within the genes *Zea*. DeWet and Harlan's cytogenetic studies provide strong evidence that maize and teosinte (*Z. mays* ssp. *mexicana*) are conspecific and that teosinte could not be derived from a hybrid of maize and *Tripsacum,* as has been hypothesized by Mangelsdorf (Mangelsdorf and Reeves 1939; Dewet and Harlan 1976).

11.5.2 Use of Teosintes as Parents in Interspecific/Intergeneric Crosses for Construction of Classical and Molecular Genetic Linkage Maps

Genetic linkage maps were constructed using the progenies of teosinte–maize or teosinte–teosinte crosses (F_1, F_2 or backcross). The development of these linkage maps has been useful for mapping QTL responsible for the morphological, developmental, and stress tolerance differences between maize and teosinte or between different teosinte species. It has also been helpful for marker-assisted selection (MAS) for maize improvement. (1) *Genetic linkage maps and QTL related to morphological evolution in the genus Zea*. In 1947, Mangelsdorf showed that the inflorescence differences between maize and Nobogame teosinte (*Z. mays* ssp. *mexicana*) were linked with markers on chromosomes 1, 3, 4, 8, 9, and 10 (Mangelsdorf 1947). However, these early linkage map results on the chromosomal regions controlling the morphological differences between maize and teosinte were contradictory. For instance, the single pistillate spikelet of *Z. mays* ssp. *parviglumis* was associated with different chromosomes by different research groups, as summarized by Szabo and Burr

Table 11.6 Genetic behavior of some traits in F_2 population from a cross between maize and teosinte

Trait	Description	Cross	Genetic behavior of F_2 population	Reference
TBN	Tassel branch number	*Zea perennis* × *Zea mays* L. ssp. *mays* (Gaspe)	Transgressive inheritance and high TBN is dominant (traits from maize)	Palacios and Magoja (1984)
TBAL	Tassel branching axis length	*Zea perennis* × *Zea mays* L. ssp. *mays* (Gaspe)	Transgressive inheritance and long TBAL is dominant (traits from maize)	Palacios and Magoja (1984)
LTBIL	Lateral tassel branch internode length	*Zea perennis* × *Zea mays* L. ssp. *mays* (Gaspe)	No significant difference from mean parental value	Palacios and Magoja (1984)
PGZ	Pollen grain size	*Zea perennis* × *Zea mays* L. ssp. *mays* (Gaspe)	No significant difference from mean parental value	Magoja and Palacios (1984)
EUN	Number of ears in the uppermost node	*Zea mays* ssp. *parviglumis* × *Zea mays* L. *ssp. mays*	Transgressive inheritance and high number is dominant (from teosinte)	Corcuera (1991)
ET	Number of ears per tiller	*Zea mays* ssp. *parviglumis* × *Zea mays* L. ssp. *mays*	Transgressive inheritance and high number is dominant (from teosinte)	Corcuera (1991)
PN	Number of productive nodes per tiller	*Zea mays* ssp. *parviglumis* × *Zea mays* L. ssp. *mays*	Transgressive inheritance and high number is dominant (from teosinte)	Corcuera (1991)
BD	Bloom date	*Zea perennis* × *Zea mays* L. ssp. *mays*	No significant difference from mean parental value	Magoja and Benito (1981)
NL	Number of leaves	*Zea perennis* × *Zea mays* L. ssp. *mays*	No significant difference from mean parental value	Magoja and Benito (1983)
VBL	Number of veins between primary lateral veins	*Zea diploperennis* × *Zea mays* L. ssp. *mays*	Quantitative inheritance and low VBL is partially dominant (from maize)	Corcuera and Magoja (1991)
TV	Total vein number	*Zea diploperennis* × *Zea mays* L. ssp. *mays*	Quantitative inheritance and low TV is partially dominant (from maize)	Corcuera and Magoja (1991)

(1996). This disparity apparently was caused by the relatively small population sizes investigated and the absence of any statistical analysis. The application of molecular markers combined with statistical analysis made the genetic linkage maps more accurate. Based on maize–teosinte or teosinte–teosinte molecular genetic linkage maps, (a) the chromosome loci and QTL (major and minor) involved in maize morphological traits are distributed across all ten chromosomes (Doebley 2004). (b) Most morphological differences between maize and teosinte are controlled by relatively few chromosomes with large effects (five chromosome regions and about 50 QTL) plus several other regions with small effects. The morphological differences between different teosinte species are mainly controlled by several QTLs with small effect and no single QTL of strikingly large effect (Westerbergh and Doebley 2002, 2004). (c) The major differences in morphological traits between maize and the teosintes are located in a few chromosomal regions. A region on chromosome 3 is strongly associated with differences in kernel weight (Doebley et al. 1994). The cupulate fruitcase and the degree of glume induration are mainly controlled by a region on chromosome arm 4S. The differences in ear rank (the number of grain-bearing cupules) are determined primarily by chromosome 2S. A region on chromosome 1L accounts for the major differences in plant architecture such as primary lateral branches and female inflorescences (Doebley et al. 1990, 1995; Doebley and Stec 1991, 1993; Bomblies and Doebley 2006). (2) *Genetic linkage maps and QTL for maize improvement*. Some teosintes have additional important characteristics (flood tolerance, pest resistance, etc.) that could be of use in maize improvement. On the basis of current genetic linkage maps, it should be possible to efficiently introgress these unique characters into maize by a marker-assisted selection (MAS) approach. Recently, a genetic linkage map was constructed using an F_2 population generated from a cross of maize inbred B64 with the teosinte *Z. nicaraguensis*. This map employed 88 SSR markers and identified four QTL related to root aerenchyma (tissue with large airy compartments for gas exchange and storage) formation (Mano et al. 2007). These results could provide the genetic basis for the development of flooding-tolerant maize lines.

11.5.3 Mapping of Genes and Polygenic Clusters

Although genetic linkage map and QTL studies provided some evidence about the genetic foundations of the differences between maize and specific teosintes, they do not provide information about the genes underlying the QTL. For example, mapping data do not indicate whether a QTL for a trait represents a single major gene or a block of linked genes. To definitively resolve this issue, it is necessary to clone the genes responsible for the quantitative traits. In maize and teosinte research, several methods such as genetic complementation, association mapping, and human selection have been applied to identify the genes responsible for specific QTL (Mackay 2001; Yamasaki et al. 2005). By these approaches, a number of genes related to maize evolution and domestication have been identified, though most of their functions are not well characterized. These results demonstrated multifactorial inheritance of the key morphological traits associated with maize domestication from teosinte. However, these traits were not necessarily highly polygenic, except kernel weight, which exhibits oligogenic inheritance (Doebley et al. 1990, 1994; Barton and Keightley 2002). Here, we place these genes into three classes (some overlapping) according to their functions. The known genes and their roles in maize evolution and domestication or natural variation within the teosintes are listed in Table 11.7, and the details of their discovery and functional characterization are discussed below.

(1) *Genes controlling key morphological differences (threshold traits) between maize and teosinte*. As discussed in the first part of Sect. 11.1.2 on Morphology, the most obvious phenotypic differences between maize and the teosintes are that the wild *Zea* species have many lateral branches and the ears have cupulate fruitcases. Several genes controlling these morphological differences have been identified. The first such gene is *teosinte branched 1* (*tb1*), responsible for the QTL on chromosome 1L (Doebley et al. 1995). The *tb1* gene encodes a predicted helix–loop–helix DNA-binding protein. This protein, a probable transcriptional regulator, controls lateral branch growth and acts as a repressor of apical growth (Doebley et al. 1997). Maize with a *tb1* null mutation exhibits an outgrowth of axillary buds and loses apical dominance, causing it to resemble a teosinte in vegetative growth pattern (Doebley et al. 1995). Furthermore, insertional mutants of *tb1* indicate that this gene reduces the growth of axillary organs and aids in the development of female inflorescences (Doebley et al. 1997). Two other genes that control key morphological differences between maize and the teosintes are *teosinte glume architecture1* (*tga1*) and *barren stalk1* (*ba1*). The *tga1* gene controls the development of the cupulate fruitcase and the degree of glume induration (Dorweiler et al. 1993). Developmental analysis has shown that the allele of *tga1* in teosinte is responsible for increasing the length of inflorescence internodes and increasing the length, thickness, and curvature of the glume (Dorweiler and Doebley 1997). The function of *ba1* is similar to that of *tb1*. It also encodes a predicted helix–loop–helix protein that regulates

Table 11.7 Genes that contributed to maize domestication and/or natural variation within the teosintes

Gene	Chromosomal location[a]	Trait(s) influenced	References
teosinte branched1 (tb1)	1L	Lateral branches and female inflorescences	Doebley et al. (1995, 1997)
teosinte glume architecture 1 (tga1)	4S	Development of cupulate fruitcase	Dorweiler and Doebley (1997)
barren stalk1 (ba1)	3L	Lateral meristems	Gallavotti et al. (2004)
zea floricaula/leafy2 (zfl2)	2S	Ear rank and inflorescence structure; plant height in the teosintes	Bomblies et al. (2003); Weber et al. (2007)
teosinte crossing barrier1 (tcb1)	4	Crossing ability between maize and teosinte	Evans and Kermicle (2001)
terminal ear1 (te1)	3L	Internodes number in the teosintes	Weber et al. (2007)
tassel-replaces-upper-ear1 (tru1)	3L	Growth of lateral branches	Doebley et al. (1995)
ramosa1 and 2 (ra1 and ra1 2)	–	Ear structure variation in the teosintes	Weber et al. (2008)
zea agamous-like1 (zagl1)	–	Ear shattering variation in the teosintes	Weber et al. (2008)

L long arm; *S* short arm

the development of the vegetative lateral meristem (Gallavotti et al. 2004).

(2) *Genes with agronomic importance as signatures of selection during maize domestication*. Maize has experienced strong selective pressure directed at genes controlling traits of agronomic importance during its domestication from an ancestral teosinte. As a result, these agronomically important genes bear strong signatures of selection, where the nucleotide diversity of these genes is low compared to genes that are not under equally powerful selection. Using various gene mapping approaches, several candidate genes were identified and their functions were confirmed by comparing the nucleotide sequences of the target genes in maize to those in teosintes. The "domesticated alleles" of two genes discussed above that control key morphological differences, *tb1* and *ba1*, have undergone selective sweeps during maize domestication (Wang et al. 1999; Gallavotti et al. 2004). A MADS box gene of unknown function also displayed a selective sweep based on genomic scanning of 501 maize genes for signatures of selection (Vigouroux et al. 2002). Furthermore, eight genes with agronomic importance related to nutritional quality, maturity, and productivity were also identified by sequencing 1,095 maize genes and comparing sequence diversity with *Z. mays* ssp. *parviglumis* (Yamasaki et al. 2005). These genes encode for protein products, which are likely to be involved in auxin response, circadian clock, signal transduction, amino acid transport, and mediating protein–protein interactions.

(3) *Genes controlling natural genetic variation between and within the teosintes*. Natural variation, the genetic variation within wild species, has been considered as the main resource for evolutionary change of a species in response to its environment. Identification of genes associated with natural variation is one of the principal goals in evolutionary genetics (Shindo et al. 2007). Some key genes controlling the growth and development of maize are also involved in natural variation within teosinte species. For instance, three maize regulatory genes (*zfl2*, *zap1* and *tb1*) play important roles in the observed phenotypic variation within the *Z. mays* ssp. *parviglumis* germplasm (Weber et al. 2007). In addition, *Terminal ear1* (*te1*), a gene responsible for key morphological differences between maize and teosinte, was found also to be involved in natural variation within *Z. mays* ssp. *parviglumis* (Doebley et al. 1995; White and Doebley 1999; Weber et al. 2007). Studies on *Zea floricaula/leafy2* (*zfl2*) showed that this gene determines both ranks of cupules on the teosinte ear (a key difference between maize and teosinte) and the variation of plant height within teosinte species (Bomblies et al. 2003; Weber et al. 2007). Recently, 15 genes responsible for natural genetic variation within the teosintes were identified (Weber et al. 2008). Among them, a MADS-box gene, *zagl1*, was associated with teosinte ear shattering. Furthermore, variation in teosinte ear structure was found to be linked with two *ramosa* genes (*ra1* and *ra2*) that are transcription factors associated with inflorescence architecture in maize (Vollbrecht et al. 2005; Bortiri et al. 2006).

11.5.4 Assessment of Gene Action

Genetic studies usually involve two topics: one is the identification of genes that perform specific functions and the other is the determination of the means by which genes accomplish their tasks. Here, we will discuss assessment of gene action. Additive, dominant (recessive), partially dominant, overdominant, and epistatic gene actions are the five models for general gene function in any species, including maize and teosinte. (1) *Additive, dominant, partially dominant and overdominant gene action*: Genetic analysis of F_2 populations derived from maize crosses to teosintes has shown that most of the QTL involved in the domestication of maize exhibit additive gene action because F_2 values of the traits controlled by these QTL are usually close to the mid-parent value (Doebley et al. 1990, 1994; Doebley and Stec 1993; Table 11.6). A few QTLs, which control leaf length, leaf width, kernel weight, and lateral branch internode number, behave in a fully dominant/recessive fashion. Interestingly, teosinte alleles tended to be dominant in F_2 populations from the cross between *Z. mays* L. ssp. *mays* and *Z. mays* ssp. *mexicana* or *Z. mays* ssp. *parviglumis*, while these same traits were more likely to be maize-like in F_2 populations from a cross between *Z. mays* L. ssp. *mays* and *Z. diploperennis* (Doebley and Stec 1993; Doebley et al. 1994; Srinivasan and Brewbaker 1999). Besides, in maize–*Z. diploperennis* F_2 populations, maize alleles are partially dominant

for traits of total vein number and number of veins between lateral veins of male spikelet outer glume, which are two important characteristics for the taxonomy of genus *Zea* (Doebley and Iltis 1980; Corcuera and Magoja 1991). However, the modes of gene interaction for QTL controlling the differences among different teosinte species did not show the same relationships. For example, of the 38 QTL found to be responsible for differences in growth habit between *Z. diploperennis* and *Z. mays* ssp. *parviglumis*, 10 exhibited dominant gene action, 11 were inherited in an additive manner, and the remaining 17 exhibited overdominance (Westerbergh and Doebley 2004). (2) *Epistatic gene action*: Epsistasis is a term that connotes a phenotypic interaction between different genes. Studies on the action of *tb1* showed that it involves a complex network of gene interactions with at least one QTL that may be an upstream regulator (Lukens and Doebley 1999). Epistatic effects were also detected between the large-effect QTL and other QTL controlling the inheritance of two leaf sheath characters used to distinguish *Z. mays* ssp. *mexicana* and *Z. mays* ssp. *parviglumis* (Lauter et al. 2004).

11.5.5 Physiological Pathways

Dissecting the physiological pathways that lie between genes and traits is the fundamental challenge for developmental and evolutionary genetics (Nadeau and Dunn 1998). Here, the contributions of teosinte research to the understanding of two well characterized pathways, those for anthocyanin and starch biosynthesis, will be described.

(1) *Anthocyanin biosynthesis*. Anthocyanins are flavonoids found as water-soluble pigments within the vacuole. Anthocyanins are found in floral and vegetative tissues of both maize and teosinte, and they range in color from red to blue (Fig. 11.5). Some maize landraces and varieties also have anthocyanin-pigmented kernels while teosinte kernels are colorless and enclosed by hard fruit cases with unidentified brown pigments on their external surface. The anthocyanin biosynthetic pathway in maize has been well characterized, with the identification of eight structural genes (*a1*, *a2*, *bz1*, *bz2*, *c2*, *chi*, *pr* and *whp*) and six regulatory genes (*b*, *c1*, *pl*, *r*, *a3* and *vp1*) (Coe et al. 1988; Holtont and Cornish 1995; Robinett et al. 1995) plus a number of less well characterized modifier genes. The role of both structural and regulatory genes in the anthocyanin pathway was investigated in *Z. mays* ssp. *parviglumis* (Hanson et al 1996). The results showed that the *Z. mays* ssp. *parviglumis* genome possesses competent versions of all of the structural genes required for the synthesis of anthocyanins. Therefore, the colorless kernels in *Z. mays* ssp. *parviglumis* are probably the result of non-functional regulatory genes. Subsequently, in order to confirm this conclusion, the regulatory gene *c1* was investigated and differences in *cis*-regulatory elements of this gene were found between maize and *Z. mays* ssp. *parviglumis*. This finding was proposed to explain why *Z. mays* ssp. *parviglumis* has the C1 functional protein in aleurone but only during germination and not during kernel maturation. A recessive (non-functional) allele of another maize anthocyanin pathway regulatory gene, *r*, is also presumed to be present in *Z. mays* ssp. *parviglumis* because the red anthocyanin color that was lacking from seed (regulated by *R*) was found to be present in vegetative tissues (where *R* is not required and *B* fills the same role) (Hanson et al. 1996). Lauter et al. (2004) investigated anthocyanin biosynthesis in two teosintes, *Z. mays* ssp. *Mexicana*, which has red leaf sheaths and *Z. mays* ssp. *Parviglumis*, which has green leaf sheaths. The results indicated that two regulatory genes, *b* and *a3*, were not functional in the leaf sheaths of the *Z. mays* ssp. *parviglumis* line analyzed (Lauter et al. 2004).

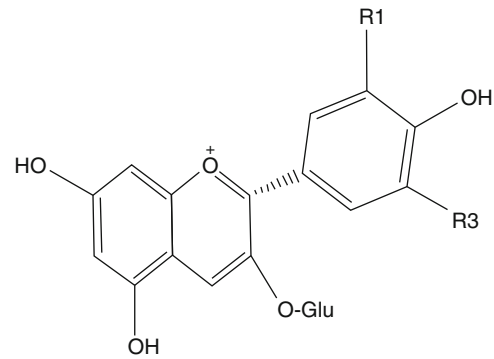

Fig. 11.5 Chemical structures of anthocyanins. Pigmentation depends on the presence of specific side groups. *Red color*: R1 = H, R3 = H; *Purple color*: R1 = OH, R3 = H; *Blue color*: R1 = OH, R3 = OH

(2) *Starch synthesis pathway*. The properties of two enzymes (soluble starch synthases and starch branching enzyme) related to starch synthesis in

Z. diploperennis were examined and found to be similar to those in maize by a chromatographic method (Boyer 1982). The study of nucleotide diversity of six key genes of maize starch biosynthesis in 30 maize inbred lines and 10 accessions of *Z. mays* ssp. *parviglumis* found that the diversity in these teosintes is much higher than in maize, especially for *su1*, *bt2*, and *ae1* (Whitt et al. 2002). These results suggest that these genes underwent extreme human (artificial) selection during the domestication and improvement of maize from its teosinte ancestor.

11.5.6 Host–Parasite Interactions

The coevolution of host–parasite interactions is arguably the single most important source of biodiversity on earth and thus has a dramatic impact on ecological and agricultural fitness (Rausher 2001). Plants have evolved multiple defense strategies in response to the invasion of pathogens. Some traditional genetic studies in plant pathology have aimed to elucidate the genetic mechanisms and the major genes involved in both the host (resistance genes, *R*) and the parasite (avirulence genes, *Avr*). Many but not all genetic relationships between the host and the parasite can be explained by the gene-for-gene model, in which the specific *R* gene expressed by the host has a corresponding *Avr* gene that is expressed by the pathogens (Flor 1955; Thompson and Burdon 1992). In addition, a plant immunity system also has a series of defense genes encoding some proteins, enzymes, and secondary metabolites, which directly or indirectly inhibit the growth, reproduction, or infection of parasite (Nimchuk et al. 2003; Moeller and Tiffin 2005). Gene-for-gene *R* loci have not been extensively studied in the teosintes, but the evolution and selection patterns of several defense genes have been characterized by examining their nucleotide diversity in maize and the teosintes. Geneticists chose teosinte because it was suitable to investigate the long-term evolutionary dynamics and the genetic effect of domestication on these genes (Zhang et al. 2002). These studies showed that the defense genes *wp1, wip1*, *mpi, chiA*, *chiB*, and *chiI* all had a neutral evolutionary history and that their diversity was maintained by either balancing selection or episodic selection (Tiffin and Gaut 2001; Tiffin 2004; Tiffin et al. 2004). In contrast, the sequence diversity distribution of two *R* genes that are not of the gene-for-gene class, *hm1* and *hm2*, was inconsistent with neutral evolution (Zhang et al. 2002). The results on the molecular evolution of 16 plant innate immunity genes (such as *chiA* and *chiB*) indicated that geographically variable selection had little influence on the nucleotide variation of these genes (Moeller and Tiffin 2008).

11.5.7 Detection of Duplicated Genomic Regions and Precise Ploidy Level

Cytogenetic studies on maize and the teosintes, such as those investigating the structure and meiotic behavior of chromosomes, have been a hot topic in both classical and molecular genetics since the beginning of the last century. In previous sections of this chapter, we have already discussed the basic chromosome number (Sect. 11.1.3) and various cytogenetic stocks (Sect. 11.4). Here, we will focus on the detection of duplicated genomic regions in the chromosomes of wild *Zea* species because they play important roles in confirming the precise ploidy level, in designing chromosome markers, in clarifying the taxonomy of the genus *Zea*, and in elucidating the evolution of maize.

In an early molecular study, a family of moderately repetitive sequences isolated from *Z. diploperennis* showed the same genomic organization patterns and similar copy numbers in all other *Zea* species investigated (Raz et al. 1991). These results further supported the long held idea that maize and the teosintes were derived from the same ancestral *Zea* population. An 180-bp maize-knob-repeat sequence was found to be present in all teosinte species except *Z. perennis*, and further studies showed that *Z. luxurians* contains not only this 180-bp repeat but also other species-specific repetitive sequences (Dennis and Peacock 1984; Poggio et al. 1999, 2000). This characteristic in *Z. luxurians* has been used as a species-specific marker to differentiate chromosomes from other species by genomic in situ hybridization (GISH) (Poggio et al. 2000).

The ancestral ploidy level and chromosomal origins within the genus *Zea* has been a subject of some controversy. Early mapping studies suggested that maize contained extensive regions of colinear genes (Rhoades 1951), suggesting a tetraploid origin, and

this hypothesis has been confirmed by numerous molecular mapping and segmental sequencing studies (Helentjaris et al. 1988; Gaut and Doebley 1997; Ilic et al. 2003; Lai et al. 2004; Swigonová et al. 2004). Although they currently show normal diploid pairing and inheritance, it is believed that maize and the other wild *Zea* species except *Z. perennis* are derived from a common ancestral allotetraploid ($n = 5$) and that *Z. perennis* originated from an ancient allooctoploid (Molina and Naranjo 1987; Poggio et al. 1990). Comparative sequence analysis of the retained homoeologous regions from this *Zea* allotetraploid indicated that it originated in the last few million years from the fusion of two ancestral genomes that themselves diverged about 12 million years ago (Swigonová et al. 2004). Since that time, more than 50% of the genes duplicated in the polyploid event have been lost by a process of accumulated small deletions and point mutations (Ilic et al. 2003; Lai et al. 2004). The cytogenetic detection of duplicated genomic regions in teosinte genomes has also supported this hypothesis (Poggio et al. 1999; Takahashi et al. 1999; Gonzalez et al. 2006).

11.5.8 Identification and Use of Potentially Useful Teosinte Alleles for Maize Improvement

As discussed in Sect. 11.1.4, teosinte populations with unique characteristics can serve as reservoirs of favorable alleles for the improvement of maize (Galinat 1985). Genetic studies of wild *Zea* species have already identified a series of potentially useful alleles for yield, flooding tolerance, and perennialism. Using advanced backcross QTL (AB-QTL) analysis, valuable alleles for higher yield were identified and transferred from *Z. perennis* to maize (Harjes et al. 1999). Perennial plants might help address many environmental and agricultural problems including soil erosion, nutrient loss, and abiotic stress (Cox et al. 2006). *Z. perennis* and *Z. diploperennis,* two perennial species in the genus *Zea*, have the potential to contribute to the generation of new, more environmentally friendly, maize lines (Moffat 1996). Cluster of QTL controlling the perennialism of teosinte were identified on chromosomes 2 and 6 (Westerbergh and Doebley 2004). Furthermore, four QTL from *Z. nicaraguensis* were identified for increased flooding tolerance in maize (Mano et al. 2007). More details about the application of these favorable alleles on maize breeding will be discussed in Sect. 11.6 below.

11.5.9 Summary

Maize–teosinte hybrids have been used to construct genetic maps, assess gene actions, and identify potentially useful alleles for maize improvement. Teosinte studies have also played an important role in elucidating physiological pathways and host–parasite interactions.

11.6 Role in Crop Improvement Through Traditional and Advanced Tools

In previous sections, we discussed the place of teosinte in the evolution of maize and the role of teosinte studies in understanding maize genetics. Teosinte has been proposed to be of practical use for maize breeding (Reeves 1950). Various teosinte species have been used as germplasm sources for breeding maize with traits such as higher nutritional quality, higher yield, and increased stress resistance (Taba 1995). Here, we introduce some traditional and molecular marker-assisted breeding efforts for maize improvement using wild *Zea* species.

11.6.1 Introgression of Wild Germplasm into Maize

Introgression is the incorporation of genes or chromosomal regions from one species or accession into another species or accession. The natural introgression between maize and teosinte that may lead to genetic erosion has been discussed in Sect. 11.2.1. Here, we describe the introgression practiced by maize breeders to transfer valuable traits from wild *Zea* species to maize. This "artificial" introgression is usually conducted by recurrent backcrossing of the elite maize line with the initial interspecific hybrid (Fig. 11.6) to select genetically superior varieties. At the end of this process, the presence of a whole chromosome or a

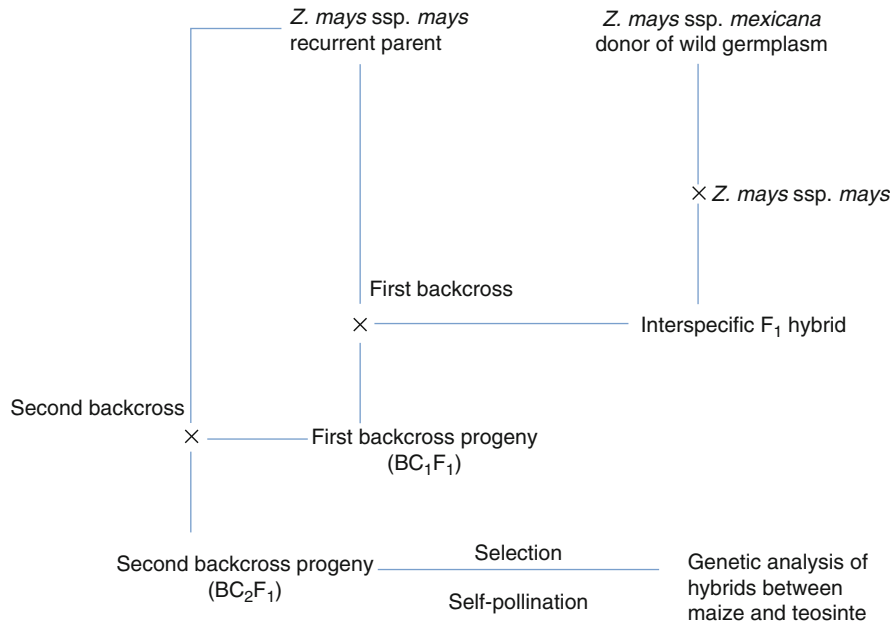

Fig. 11.6 Schematic diagram showing introgression of genes or chromosomal regions from wild germplasm into maize. Figure is adapted from Wang et al. (2008b)

segment from an introgressed teosinte chromosome is confirmed by fluorescence in situ hybridization (FISH), GISH, or molecular markers (Kato and Sanchez 2002; Tang et al. 2005a). Several introgression lines were created using various teosinte parents including *Z. perennis*, *Z. diploperennis*, *Z. mays* ssp. *mexicana*, and *Z. luxurians* (Pischedda and Magoja 1985; Ray et al. 1999; Wei et al. 2003; Wang et al. 2008b). The interspecific cross between maize ($2n = 20$) and *Z. perennis* ($2n = 40$) mostly produced infertile F_1 hybrids (<5% fertile) with $2n = 30$ due to a strong incompatibility barrier (Tang et al. 2005a). F_2 seeds were obtained from F_1 hybrids by using short day length and treatment with gibberellic acid (Tang et al. 2005a). An intergeneric cross introduced a disease resistance gene allele ($Rp1^{TD}$) from *Tripsacum dactyloides* into maize, conferring resistance to strains of *Puccinia sorghi* that were virulent on maize with only the $Rp1^{D}$ allele (Bergquist 1981).

11.6.2 Breeding for Heterosis

Heterosis (or hybrid vigor) is the phenomenon where the performance of an F_1 hybrid is genetically superior to either parent. In the decades since Shull's original description of heterosis, agronomists have been widely utilizing heterosis as a means for improving crop productivity (Shull 1948; Kallo et al. 2006). A basic requirement for heterosis breeding is to find suitable parents that produce hybrids with a sufficient level of heterosis. In maize breeding, much of the heterotic vigor is attributed to introgressive hybridization from its closest relative, teosinte (Wilkes 1972). Heterotic hybrids with higher yield were obtained through crossing *Z. mays* ssp. *mays* and *Z. mays* ssp. *mexicana* (Cohen and Galinat 1984). Other hybrids such as maize–*Z. perennis*, maize–*Z. diploperennis*, maize–*Z. mays* ssp. *parviglumis*, and maize–*Z. luxurians* also show such heterotic traits as vigorous development and high prolificity (Magoja and Benito 1982; Magoja and Palacios 1987; Corcuera 1991). Different maize–teosinte heterotic hybrids were compared and hybrids using teosinte belonging to Section *Luxuriantes* were found to have much higher prolificity and vigorous plant traits than those obtained using accessions belonging to Section *Zea*, with maize–*Z. perennis* hybrids being the most heterotic (Aulicino and Magoja 1991).

11.6.3 Somatic Hybridization and Genetic Transformation

Although some teosinte accessions have been used to enrich domesticated maize germplasms for the

purposes of maize breeding, most of these valuable genetic resources are sitting unused in the germplasm banks. The major reason is the difficulty in identifying useful characters in these materials and transferring the desired genes to maize (Hoisington et al. 1999). Fortunately, somatic hybridization techniques can overcome the constraints related to hybridization incompatibility between maize and some teosinte species (Garcia and Molina 2001). Somatic hybridization operates through the regeneration of somatic hybrids that were generated by the fusion of protoplasts from two different species. Through the fusion process, the genomes from both parents are mixed in the hybrid cells and the desired genes or traits will be transferred (Carlson et al. 1972; Liu et al. 2005). For instance, using this technique, hybrids of maize and *Z. perennis* were obtained, which otherwise are cross-incompatible (Molina and Garcia 1999). The chromosomal composition of somatic hybrids and the gene transfer are usually confirmed by FISH with molecular probes such as labeled repetitive elements and large genomic clones (Kato et al. 2006; Lamb et al. 2007).

11.6.4 Alleles for High Yield and Resistance to Biotic and Abiotic Stresses

Based on the development of traditional and modern breeding techniques, several maize hybrids were created with various desirable characters transferred from teosinte including high yield and various stress resistances. (1) *High quality*. One of the major objectives of maize breeders is to develop high yielding maize germplasm with improved nutritional quality. The hybridization of perennial teosinte and maize was observed to produce some progeny with high protein content in the kernel (Perini et al. 1991). The kernel protein content in some maize populations with introgression from *Z. diploperennis* was up to 50% higher than that observed in standard commercial maize (Perini and Magoja 1988). Recently, a new introgression line was obtained through the intraspecific cross between *Z. mays* ssp. *mexicana* and maize (Wang et al. 2008a). Amino acid analysis of endosperm from progeny of this cross exhibited a relatively high concentration of lysine. Low lysine content is a significant nutritional problem when using maize as a food or feed. (2) *Biotic and abiotic stress resistances*. Biotic and abiotic stresses cause significant decreases in maize production and quality. Many teosinte species are resistant to some important diseases and pests of maize and some can survive in a number of harsh environments (Wilkes 1977). These beneficial traits are available to improve modern maize. The infection frequency of maize stalk rot, rough dwarf disease, and brown spot in *Z. mays* ssp. *mexicana* introgression populations of maize was much lower than standard maize inbred lines (Wang et al. 2008b). Some of the alloplasmic lines with resistance to *Helminthosporium turcicum* and *Helminthosporium maydis*, two important fungal pathogens of maize, were created from *Z. mays* ssp. *mays* × *Z. diploperennis*, in which a part of the cytoplasmic genomes of maize was replaced by cytoplasmic segments from *Z. diploperennis* (Wei et al. 2003). *Z. nicaraguensis* grows in frequently flooded lowlands and has special root aerenchyma adapted to flooding (Iltis and Benz 2000). This character was transferred to maize by advanced backcross-QTL mapping and could be useful in developing flooding-tolerant hybrids (Mano and Omori 2008).

11.6.5 Summary

Compared to the huge potential value of teosinte genes for maize improvement, it is still a largely "untapped" genetic resource. The first efforts to introduce teosinte alleles for traits that influence heterotic potential, disease resistance, seed protein content, and flooding tolerance have been quite promising. Further introgression of teosinte alleles into maize germplasm is highly warranted.

11.7 Genomics Resources Developed

There are large differences in some morphological traits and physiological characters (yield, biotic and abiotic resistance, etc.) between modern maize and teosinte. Genomic research on maize and teosinte is needed to determine the genetic foundations of these

differences. To date, genome sequence data, expressed sequence tags (ESTs), full length cDNA sequence, protein sequences, and metabolomic and other data have been generated primarily for maize. Here, we summarize genomic resources for teosinte.

11.7.1 Genomic and Protein Sequences

Raw genomic and protein sequences for teosinte have been stored at NCBI (*http://www.ncbi.nlm.nih.gov*). Several species-specific or data-type-specific databases also provide access to teosinte genome data such as the Maize Genetics and Genomics Database (MaizeGDB) (*http://www.maizegdb.org*) and the Plant Genome Database (PlantGDB) (*http://www.plantgdb.org*). Various teosinte protein and genomic sequence data are listed in Table 11.8, including genome survey sequences (GSSs), sequence tagged sites (STSs), and ESTs. Compared to maize genomic sequence data, the information on teosinte is very limited. Among the wild *Zea* species, most of the sequence data are from *Z. mays* ssp. *parviglumis,* the proposed ancestor of maize. There is still no sequence information on *Z. nicaraguensis,* a new teosinte named in 2000 that has few samples available for research (Iltis and Benz 2000).

11.7.2 Transcripts

PlantGDB regularly assembles unique transcripts using ESTs plus full-length and partial cDNA sequences collected from NCBI GenBank (Dong et al. 2004). Figure 11.7 shows the total number of assembled transcripts in different teosinte species. These teosinte transcripts, when compared to comparable transcripts from maize, can provide some idea of differences in the transcribed component of the genome.

11.7.3 Mutants

The information about mutations affecting maize and teosinte phenotypes that was originally stored in ZmDB has now been transferred to Maize GDB (Lawrence et al. 2004). At this point in time, there are only three records related to teosinte in the mutation and phenotype database of Maize GDB. These pertinent entries are for teosinte branched, teosinte glume architecture, and teosinte glumes.

11.7.4 Summary

There is very little information on the structure, evolution, or genetic composition of teosinte genomes. Additional sequences, mutational characterization, and expression tools are needed for this wild germplasm in order to enhance its usefulness for maize improvement. With the ever-decreasing costs of the so-called "next generation" sequencing (Mardis 2008), it will be appropriate to "resequence" the genomes of several *Zea* species in the next few years and use the impending full genome sequence of maize inbred B73 (Tian et al. 2009) as the framework to assemble these teosinte sequences.

Table 11.8 Various types of sequence data available for teosintes

Species	Total number of each sequence type				
	Protein	GSSs	STSs	ESTs	Other DNA sequences
Z. mays ssp. *parviglumis*	873	1,593	17,972	3	2,884
Z. mays ssp. *mexicana*	74	0	102	0	431
Z. luxurians	83	0	0	0	143
Z. mays ssp. *huehuetenangensis*	12	0	1	0	33
Z. perennis	69	0	0	1	122
Z. diploperennis	126	0	43	7	296
Z. nicaraguensis	0	0	0	0	0

EST data were obtained from NCBI's GenBank; others were from the PlantGDB database, the latest access: June 27, 2009

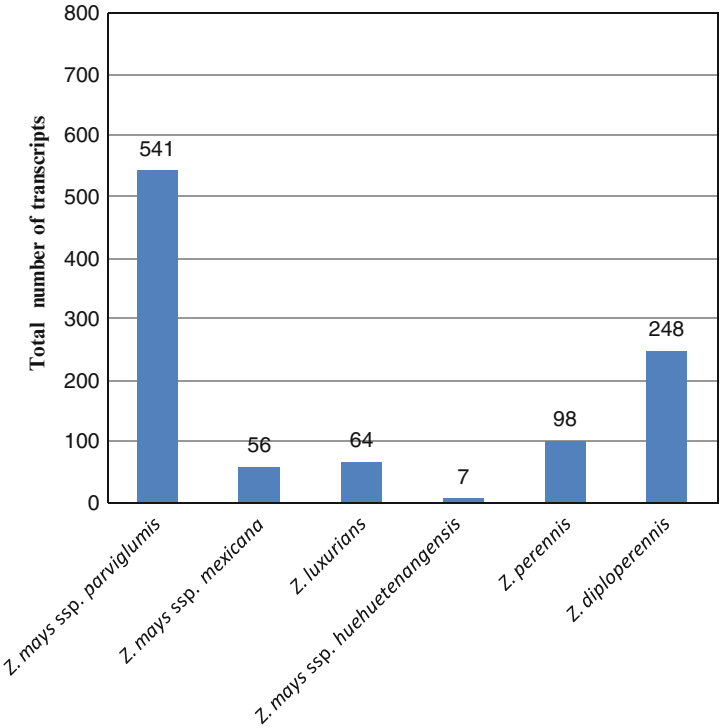

Fig. 11.7 PlantGDB-assembled Unique Transcripts (PUTs) of teosinte species. Latest access: June 27, 2009

11.8 History and Scope for Domestication and Commercialization

Some teosintes have proven to be a good quality and reliable forage source. In the nineteenth century, *Z. luxurians* was cultivated specifically for forage in some southern states of the United States (Schmidt and Colville 1963). Due to the natural pest-resistance of *Z. diploperennis*, the aqueous extract of its leaves has been proposed as a control agent for fall armyworm (Farias-Rivera et al. 2002). We are not aware of any reports regarding the use of teosinte in the herbal drug or perfume industries. Today, people seldom eat teosinte except as an ingredient of some salads (Coe 1994). Teosinte is not considered as an alternative crop because of the very low yield and bad taste of its grain. These observations make one wonder why and how maize was domesticated by pre-Columbian Amerindians from this almost useless and unattractive teosinte species. Anyone familiar with teosinte will appreciate this vexing question, especially due to the hard cupulate fruitcase covering the teosinte grains.

There are two competing hypotheses regarding the impetus for teosinte domestication into maize. One is George Beadle's "popcorn" hypothesis (Beadle 1939). Beadle proposed that popping attracted the pre-Columbian Amerindians when they accidentally threw teosinte seed into cooking or camp fires, and thereby produced a processed grain form that was suitable for consumption. Subsequently, when the hunter-gatherers settled down to fixed dwellings, they probably began to plant the teosinte seeds to help provide a dependable food source. If this hypothesis is true, there should be some phytoliths of teosinte fruitcases, but none have been found (Piperno 2006). An alternative hypothesis is "first for sugar and then for grain" (Iltis 2000). Iltis proposed that, initially, teosinte was harvested for its sugary stalks. His idea came from the observation that, even today, children in some areas of Mexico chew the raw pith of teosinte stalks (like chewing sugar cane). Iltis's hypothesis appears to be supported by some archeological evidences found in Honduras (Webster et al. 2005). However, recent archeological activities did not find any stalk phytoliths in Xihuatoxtla shelter, located in Central Balsas Valley of Mexico, the most likely region of

maize domestication. In contrast, some new maize cob phytoliths and starch grains from this area indicate that teosinte was not consumed for its stalks (Piperno et al. 2009). More evidence is needed to fully resolve the origin of maize domestication and the involvement of other factors such as the abundance of teosinte, the availability of other alternative resources, and the involvement of local culture (Piperno and Pearsall 1999; Hastorf 2009).

We are not aware of any redomestication of teosintes other than a proposed project from Doebley (reviewed in Doebley 2004) to see if he can recreate the "catastrophic transformation" of *Z. mays* ssp. *parvaglumis* into something resembling domesticated maize. It would be interesting, however, to see if biomass production for stalk sugars and/or lignocellulose could be further improved in one or more teosintes so that an additional *Zea* other than maize could be used as a crop for biofuel production. This approach might be most appropriate with one or more of the perennial species.

11.8.1 Summary

Today, the wild *Zea* species are mainly used for forage by small landholders in Central America. Teosinte (*Z. mays* ssp. *parvaglumis*) may have first attracted human attention because of their sugary stalks. Teosintes have played an important role in maize domestication. Some of the teosintes produce a good deal of biomass that might be improved for commercial development as a bioenergy fuel source.

11.9 Teosinte as a Possible Problem Weed

In previous sections, we discussed the roles played by teosinte in maize evolution and improvement, in genetic and genomic studies, and in the development of cytogenetic stocks. In this section, we will discuss some potentially harmful characteristics of the teosintes; after all, famers usually regard them as weeds. It is unlikely that any current teosinte will become an invasive species because teosinte populations are limited and some of them are endangered, as discussed earlier. However, attention has to be paid to the potential consequences of gene flow between transgenic maize and teosinte species. One possible problem that we have discussed in Sect. 11.2.1 is that gene flow may result in the extinction of teosinte species. The other concern is that gene flow may increase weediness and create "super weeds" if some resistance genes in transgenic maize enter into teosinte (Ellstrand 2003). Here, we outline recent progress on the conceptualization and analysis of possible enhanced weediness in the teosintes.

The risk evaluation of a potential enhanced weediness of teosinte usually involves three questions. (1) The first issue is whether the natural gene flow between transgenic maize and teosinte can occur. The spontaneous hybridization between such teosintes as *Z. mays* ssp. *parviglumis* and maize has been confirmed by several methods, including comparison of morphological traits (Wilkes 1977), use of molecular markers (Doebley 1990b; Blancas et al. 2002), and examination of pollen movement (Aylor et al. 2005). The gene flow rate was also measured by a 3-year field experiment (Ellstrand et al. 2007). These results showed that the natural hybridization rate between maize and *Z. mays* ssp. *mexicana* is low due to an incompatibility barrier, while maize and *Z. mays* ssp. *parviglumis* hybridized at a high rate. Hence, any transgene present in fertile maize will eventually find its way into some teosinte germplasm. (2) The second question is whether the transgene will persist. According to population genetics theory, and confirmed by many studies, an allele will stay in a population for a reasonable period of time even if the allele is neutral or moderately disadvantageous (Wright 1951; Varvio et al. 1986). Studies on the relative fitness of transgenic maize × *Z. mays* ssp. *mexicana* hybrids indicated that the hybrids have higher vigor than their teosinte parents (Guadagnuolo et al. 2006). (3) The third consideration is whether the transgene will enhance weediness. In theory, most transgenes that are beneficial for domesticated maize (e.g., those controlling herbicide tolerance or insect resistance) would be expected to benefit a teosinte for the same (if less intense) reasons, and would thus enhance weediness. However, some field fitness experiments did not support this idea (Guadagnuolo et al. 2006). One possible explanation is that these transgenes may have some unknown negative effect(s) on teosinte fitness (Ellstrand 1997).

From what is currently known, it is reasonable to expect that gene flow from transgenics may somewhat increase the weediness of some teosintes. Still, the likelihood of any wild *Zea* species becoming a problematic invasive seems relatively low. First, the genetic resource bases of teosintes are limited and scattered (Wilkes 2007). Moreover, *Z. mays* ssp. *mexicana* and *Z. luxurians* have significant sexual incompatibility with maize (Evans and Kermicle 2001). In the case of *Z. mays* ssp. *parviglumis*, it is separated from maize by temporal (different flowering date) and spatial factors (few sympatric regions) (Doebley 1990b). Second, teosinte has coexisted and coevolved with maize over thousands of years and has still maintained its distinct genetic constitution (Fukunaga et al. 2005).

11.9.1 Summary

The outcomes of gene flow between maize and teosinte seem to be quite different from that observed for some other crops and their wild relatives (Boudry et al 1993). Additional specific studies are needed regarding the flow and persistence of transgenes between maize and the teosintes, especially with respect to their possible contributions to weediness and to the possible in situ extinction of teosinte germplasm. These studies should also include analysis of seed dispersal from maize–teosinte hybrids. In combination, these proposed future experiments would provide a much more complete analysis of any possible threat associated with teosinte as a potential invasive weed.

11.10 Recommendations for Future Action

Although tremendous progress has been made in the last several decades on the protection, study, and utilization of wild *Zea* species, more research is needed. Also, more effort is needed to increase public awareness of the value of the teosintes as a part of the natural environment and a source of genes for future maize improvement.

CWRs play a role in maintaining ecosystem health as components of natural and agricultural systems. About 30 years ago, scientists identified wild *Zea* species as a target for enhanced conservation efforts (Frankel 1970). The current status and some achievements in teosinte conservation have already been discussed in this chapter. Here, we offer recommendations for additional teosinte conservation and use. (1) *Creating public awareness of the importance of teosinte*. Teosinte is a part of the natural heritage of humankind. However, little is known by the general public about the wild *Zea* species. Even some farmers in current regions of teosinte distribution regard it as a useless weed. Because efficient and comprehensive conservation requires efforts across the broad growth range of the teosintes, dealing with complex international policy and administrative elements will be necessary. Hence, it would be appropriate to establish a special non-profit organization that could educate, negotiate, and monitor. Staff of this non-profit would be responsible for training programs (producing and disseminating public awareness materials about teosinte) and coordination in Mexico, Guatemala Nicaragua, and Honduras (as a kind of "Wildlife Trust, US") (Meilleur and Hodgkin 2004), with the target audience being local populations, as well as government officials in these regions. (2) *Establishing more* in situ *conservation sites*. Until now, the only established in situ conservation site for teosintes is in the Sierra de Manantlan Biosphere Reserve, Mexico. The absence of other designated preserves may be due primarily to their high cost (natural capital, human capital, and social capital). Wilkes proposed an alternative low cost idea to separate teosinte populations into small plots (home garden size or bigger) and hire local farmers to maintain these plots. They would be allowed to harvest the maize introgression lines in the sympatric regions of maize and teosinte to help reward their service (Wilkes 2007). Equally important, farmers that protected teosinte could receive some prestige from their communities, if the heritage and importance of teosinte were common knowledge (Castineiras et al. 2000). Of course, this sympatric growth model has the potential to increase the rate of decay of teosinte germplasm novelty by encouraging gene flow from adjacent maize, but it might not be a dramatic effect because teosinte has a long history of growth alongside domesticated maize.

Regardless of the in situ preservation strategy employed, immediate efforts are needed to negotiate their creation, and this would be an appropriate task for

the aforementioned non-profit organization. This organization would also be needed to serve as an independent monitor of the status of preservation efforts. The sites that merit immediate in situ conservation are in southern Guatemala and Balas, Mexico (Wilkes 1997, 2007). However, in the long run, all current sites of teosinte growth need some level of protection, and planting of the rarer teosintes at new locations would be justified as an insurance against local extinction.

11.10.1 Summary

Public awareness of the significance and tenuous survival of teosinte is needed to help justify its preservation. This conservation will not happen by chance, but it needs the dedicated efforts of a small cohort of interested individuals. Billions of people worldwide now benefit from maize as a food, feed, forage, or industrial commodity. It is fully appropriate for the world to take an interest in teosinte conservation, to guarantee that it will continue to play an important role in maize research and improvement.

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