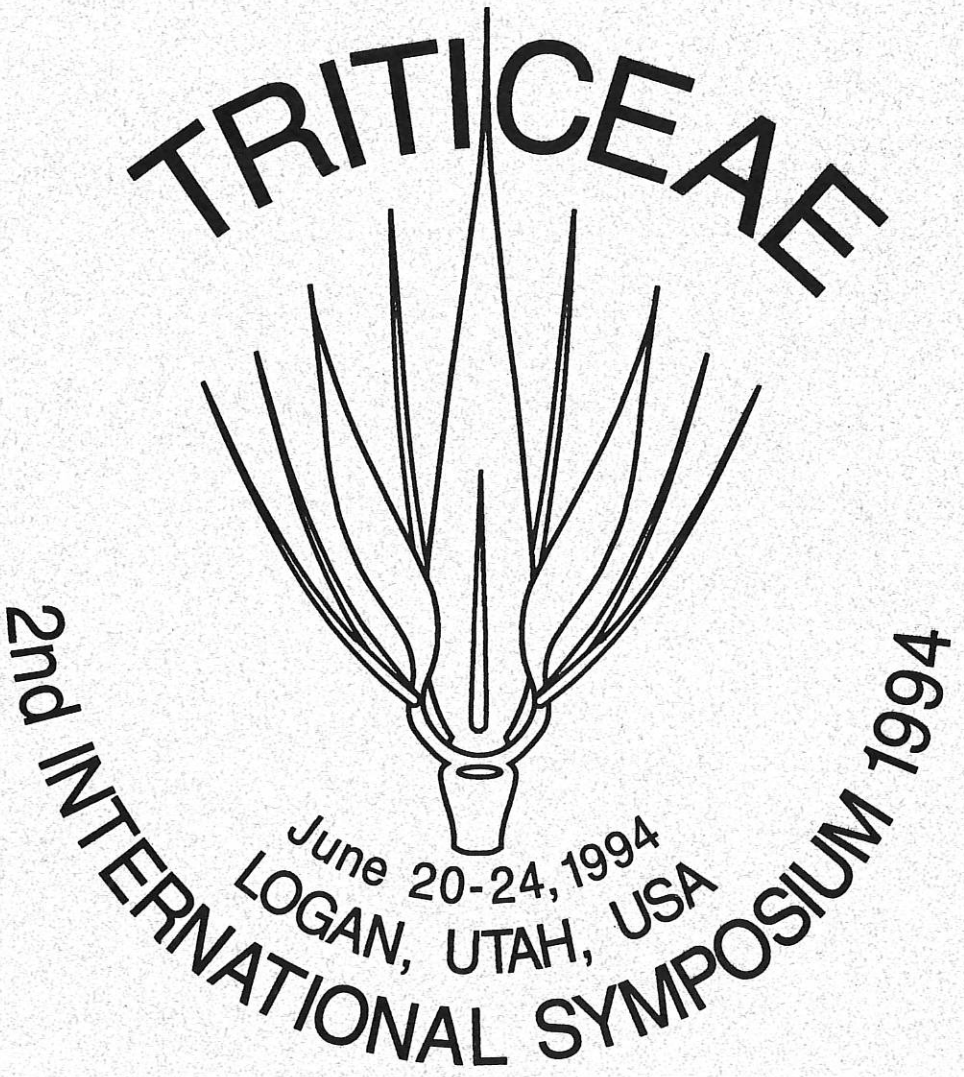
Proceedings of the 2nd International Triticeae Symposium



Logan, Utah U.S.A.,
June 20-24, 1994

Editors:
R. R.-C. Wang, K. B. Jensen, and C. Jaussi

Preface

At the First International Triticeae Symposium, Helsingborg, Sweden, July 29-August 2, 1991, the participants decided that subsequent meetings will be held every three years. Thus, the Second International Triticeae Symposium was held in Logan, Utah, U.S.A., June 20-24, 1994, with USDA Agricultural Research Service's Forage and Range Research Laboratory and Utah State University as hosts.

The purposes of the second symposium were: (1) to exchange the latest scientific information and advancements related to annual and perennial Triticeae species; (2) to promote the exchange of ideas for developing coordinated collaborative research; and (3) to provide an opportunity to see the biodiversity in the Triticeae by visiting the USDA Living Collection of Perennial Triticeae near Logan.

In addition to the above scientific goals, the participants also paid tributes to the late Dr. Douglas Dewey at the symposium banquet by presenting a plaque to Mrs. Lois Dewey and a slide show featuring Doug's activities and his many colleaques and associates.

Due to various reasons, some contributors of papers were unable to attend the symposium. Nevertheless, their manuscripts are included in the proceedings to benefit all Triticeae workers.

Conference support and travel grants from the USDA-CSRS Competitive Grant Program and the International Science Foundation enabled several speakers from China, former Soviet Union countries, and Estonia to attend the symposium.

Cooperation and assistance from our colleagues at the USDA-ARS Forage and Range Research Laboratory and the Location Administrative Office (Logan, Utah), the USDA Small Grain Collection (Aberdeen, Idaho), Utah State University and Utah Agricultural Experiment Station , and the Conference and Institute Division and the Student Services of Utah State University (Logan, Utah) made the symposium successful. We also thank many participants for reviewing manuscripts for the symposium proceedings. Most important are the contributions of the conference participants who presented the scientific information and ideas during and after the Second International Triticeae Symposium.

For the production of this publication, we thank all authors' patience, peer reviewers' effort, and the Publication Design & Production of Utah State University's excellent job.

The Editors

Richard R.-C. Wang
Kevin B. Jensen
Carolyn Jaussi

ACKNOWLEDGEMENT

We greatly appreciate receipt of conference support and travel grants from the USDA-CSRS Competitive Grant Program and the International Science Foundation.

Success of the symposium is dependent upon cooperation and assistance from our colleagues at the USDA-ARS Forage and Range Research Laboratory and the Location Administrative Office (Logan, Utah), the USDA Small Grain Collection (Aberdeen, Idaho), and Utah State University and Utah Agricultural Experiment Station (Logan, Utah). We thank them for all their support and efforts. Most important are the contributions of the conference participants who provided the scientific merits for the second International Triticeae Symposium.

We also thank the Conference and Institute Division and the Student Services of Utah State University, who provided critical administrative and logistical support.

ORGANIZING COMMITTEE

Programs of the 2nd International Triticeae Symposium

Sunday, June 19

9:00 a.m. - 4:00 p.m.	Visit Tony Grove Lake and surrounding area
3:00 p.m. - 6:00 p.m.	Visit U.S. Living Collection of Perennial Triticeae Grasses (Van leaving from the University Inn on the hour 3:00, 4:00, and 5:00 p.m.)
7:00 p.m. - 9:00 p.m.	Reception at the University Inn, Suite #302

Monday, June 20

7:30 a.m. - 9:00 a.m.	Registration at the Eccles Conference Center

SESSION I CHAIR: PROF. MARY BARKWORTH

9:00 a.m. - 9:15 a.m.	Welcome Address - Dr. H. Paul Rasmussen, Director of Utah Agricultural Experiment Station
9:15 a.m. -10:00 a.m.	Keynote Address "Triticeae: Past, Present and Future" - Dr. Roland von Bothmer, The Swedish University of Agricultural Sciences
10:00 a.m. -10:30 a.m.	Break
10:30 a.m. -11:15 a.m.	Plenary Lecture "Systematics of the Triticeae: progress and problems" (A66) - Dr. Elizabeth A. Kellogg, Harvard University Herbaria
11:15 a.m. -11:30 a.m.	"A Cladistic Analysis of the Monogenomic Genera of the Triticeae (Poaceae)" (A36) - S. Frederiksen* and O. Seberg
11:30 a.m. -11:45 a.m.	"The *Elymus trachycaulus* complex in North America: many questions, few answers" (A55) - Mary E. Barkworth
12:00 p.m. - 1:30 p.m.	Lunch (on your own)

Monday, June 20

SESSION 2 CHAIR: PROF. CLAUS BADEN

1:30 p.m. - 2:15 p.m.	Plenary lecture "The Genus *Elymus* L. in Asia. - Taxonomy and Biosystematics" (A45) - Dr. B. R. Lu, Institute of Botany, Chinese Academy of Sciences, Beijing, P.R. China
2:15 p.m. - 2:30 p.m.	"The presence of a repeated DNA sequence from *Triticum aestivum* in *Hordeum* species" (A21) - V. Schubert*, K. Hammer, F. Baldauf
2:30 p.m. - 2:45 p.m.	"A Family of Tandemly Repeated DNA Sequences Wide-Spreaded in *Triticeae*" (A62) - A.V. Vershinin*, S.K. Svitashev, P.O. Gummesson, B. Salomon, R. von Bothmer, T. Bryngelsson
2:45 p.m. - 3:00 p.m.	"RFLP Variation and phylogeny of the Genus *Hordeum*" (A6) - S. Svitashev*, T. Bryngelsson, A. Vershinin, T. Sall, and R. von Bothmer
3:00 p.m. - 3:15 p.m.	"Molecular Genome Organization in Regions Containing Tandemly Organized DNA Sequences in *Triticeae*" (A61) - A.V. Vershinin*, G. Harrison, J.S. Heslop-Harrison
3:15 p.m. - 4:00 p.m.	Break and Viewing of Posters

SESSION 3 CHAIR: PROF. CHI YEN

4:00 p.m. - 4:15 p.m.	"Isoenzyme Data on the Diploid Progenitors of Allotetraploid *Elymus* species" (A49) - V. Jaaska
4:15 p.m. - 4:30 p.m.	"The Phylogeny of *Psathyrostachys* - Are we able to see the wood for the trees?" (A1) - O. Seberg*, G. Peterson, and C. Baden
4:30 p.m. - 4:45 p.m.	"Are there three levels of endosperm protein electrophoretic specificity in *Elymus* Species?" (A30) - O. V. Agafonova*, A. V. Agafonov and E. V. Kostina

Monday, June 20

4:45 p.m. - 5:00 p.m.	"The Principle of Recombination Gene Pools (RGP) and Introgression Gene Pools (IGP) in the Biosystematic Treatment of *Elymus* Species" (A31) - A. V. Agafonov* and O. V. Agafonova
5:00 p.m. - 5:15 p.m.	"Phylogenetic Relationships and Genetic Diversity within the *Agropyron cristatum* Complex" (A53) - J. A. Matos, K. B. Jensen and M. Curto
5:15 p.m. - 5:30 p.m.	"Chemotaxonomy of Triticeae Grasses: Chatacterization of Truebreeding Lines and Hybrid Crosses" (A57) - J. H. Bennett, A. V. Agafonov, O. V. Aagafonova and K. B. Jensen
5:30 p.m. -	Dinner (on your own)
5:45 p.m. - 9:30 p.m.	Field Trip to Living Collection (Buses and vans will run leaving from the University Inn every 15 min.)

Tuesday, June 21

SESSION 4 CHAIR: DR. A. MUJEEB-KAZI

8:30 a.m. - 9:00 a.m.	"Use of C-banding and GISH for Genome Analysis in the Triticeae" (A7) - E. D. Badaeva*, N. S. Badaeva, J. Jiang, and B. S. Gill
9:00 a.m. - 9:15 a.m.	"Genomes, Chromosomes, and genes and the concept of Homology" (A2) - G. Peterson* and O. Seberg
9:15 a.m. - 9:30 a.m.	"The Study on N Genome of *Leymus* Species" (A15) - G. L. Sun*, C. Yen and J. L. Yang
9:30 a.m. - 9:45 a.m.	"Experimental Hybridization and Genome Analysis in *Elymus* L. Sect. *Caespitosae* and Sect. *Elytigia* (Poaceae: Triticeae)" (A20) - M. Assadi
9:45 a.m. -10:00 a.m.	"The Mechanism of the Origination of Auto- allopolyploid and Aneuploid in Higher Plants Based on the Cases of *Iris* and Triticeae" (A13) - C. Yen, G. L. Sun and J. L. Yang.
10:00 a.m. -10:30 a.m.	Break

Tuesday, June 21

SESSION 5 CHAIR: DR. V. SCHUBERT

10:30 a.m. -10:45 a.m.	"The Polyploidy Project - A study of the Evolution of Polyploid *Hordeum* species" (A5) - B. Salomon* and R. von Bothmer
10:45 a.m. -11:15 a.m.	"Genome Symbols in the Triticeae" (A22) - R. R-C. Wang*, R. von Bothmer, J. Dvorak, G. Fedak, I. Linde-Laursen, M. Muratmatsu
11:15 a.m. -11:30 a.m.	"Comparative Morphology of Dispersal Mechanisms in the Wheat Complex (*Triticum* L. and *Aegilops* L.) With Implications for Genetic Interpretations" (A24) - L. Morrison
11:30 a.m. -12:00 p.m.	"Recommendations For A Monographic Revision of *Triticum*" (A25) - L. Morrison*, A. B. Damania and T. E. Miller
12:00 p.m. - 1:30 p.m.	Lunch (on your own)

SESSION 6 CHAIR: DR. PATRICK MCGUIRE

1:30 p.m. - 2:15 p.m.	Plenary lecture "Plant Germplasm Resources" (A56) - S. A. Eberhart* and H. E. Bockelman, USDA-ARS
2:15 p.m. - 2:30 p.m.	"Triticeae Germplasm Collections: an analysis of current structure and identification of gaps" (A18) - W. G. Ayad
2:30 p.m. - 2:45 p.m.	"Ecogeographic Region and Related Triticeae Distribution in China" (A14) - J. L. Yang* and C. Yen
2:45 p.m. - 3:00 p.m.	"Geographic Distribution of Alleles for Esterase-5, Gliadin, α- and β-Amylase in *Triticum tauschii*" (A23) - Xueyong Zhang*, Yushen Dong, and Richard R.-C. Wang
3:00 p.m. - 3:15 p.m.	"Caryopsis Somatic Dimorphism in Relation to Floret Position, Germination, Growth, and Population Structure of *Dasypyrum villosum*" (A26) - C. De Pace* and C. O. Qualset
3:15 p.m. - 4:00 p.m.	Break and Viewing of Posters

Tuesday, June 21

SESSION 7 CHAIR: DR. VELLO JAASKA

4:00 p.m. - 4:15 p.m.	"α-Amylase Isozymes of *Aegilops cylindrica* Introduced into North America: Comparison with the Accessions from Ancestral Regions" (A44) - N. Watanabe*, K. Matsui and Y. Furuta
4:15 p.m. - 4:30 p.m.	"Germplasm Resources and its Utilization of Triticeae in Xinjiang" (A37) - Zhuomeng Yang* and Dafang Chui
4:30 p.m. - 4:45 p.m.	"Geographical Distribution, Ecology and Diversity of *Triticum urartu* Populations in Jordan, Lebanon and Syria" (A38) - J. Valkoun, A. B. Damania* and M. van Slageren
4:45 p.m. - 5:00 p.m.	"Forage Species in Xinjiang Northern Natural Grasslands: Grasses" (A40) - Bao-jun Li
5:00 p.m. - 5:15 p.m.	"Evaluation and Utilization of Genetic Resources of Triticeae for Crop Improvement" (A39) - A. B. Damania* and J. Valkoun
5:15 p.m. - 5:30 p.m.	"Chromatin characterization in *Dasypyrum*" (A59) - D. Pignone*, R. Mezzanotte, and R. Cremonini
5:30 p.m. - 7:00 p.m.	Dinner (on your own)
7:00 p.m. - 9:00 p.m.	Workshop on Taxonomy and Systematics - Mary Barkworth and Elizabeth A. Kellogg

Wednesday, June 22

SESSION 8 CHAIR: DR. KAY ASAY

8:30 a.m. - 9:15 a.m.	Plenary lecture "Procedures for the Transfer of Agronomic Traits from Alien Species to Crop Plants" (A68) - George Fedak*, K. C. Armstrong, L. O. Donoughe, and J. Simmonds. Agriculture Canada, Ottawa
9:15 a.m. - 9:30 a.m.	"Use of Annual and Perennial Triticeae Species for Wheat Improvement" (A4) - A. Mujeeb-Kazi

Wednesday, June 22

9:30 a.m. - 9:45 a.m.	"The Evaluation On Crossabilities of Chinese Wheat Landraces" (A12) - M. C. Luo*, C. Yen, J. L. Yang & Z. L. Yang
9:45 a.m. -10:00 a.m.	"Breeding Potential of Durum Wheat Landraces from Jordan III. Rate and Duration of Grain Fill" (A8) - A. A. Jaradat* and M. M. Ajlouni
10:00 a.m. -10:30 a.m.	Break

SESSION 9 CHAIR: DR. DOMENICO PIGNONE

10:30 a.m. -10:45 a.m.	"Breeding Potential of Durum Wheat Landraces from Jordan IV. High Molecular Weight Glutenin Subunit Variation" (A11) - A. A. Jaradat* and M. M. Ajlouni
10:45 a.m. -11:00 a.m.	"Physical Mapping of Micronutritional Genes in Wheat-Rye Translocations" (A10) - R. G. Kynast*, M. Röder and V. Römheld
11:00 a.m. -11:15 a.m.	"Progress in Polyhaploid Production Techniques of Hexploid Wheat through Wide Crosses" (A19) - M. N. Inagaki* and A. Mujeeb-Kazi
11:15 a.m. -11:30 a.m.	"Characterization of Wheat-*Aegilops* recombinant lines by *in situ* hybridization" (A41) - A. Castilho* and J. S. Heslop-Harrison
11:30 a.m. -11:45 a.m.	"Prospects for Gene Introgression from *Hordeum bulbosum* L. into Barley (H. vulgare L.)" (A42) - R. A. Pickering*, A. M. Hill, G. M. Timmerman-Vaughan, E. M. Forbes, M. G. Cromey, M. J. Gilpin, M. Michel and M. Scholz
11:45 a.m. -12:00 p.m.	"Variability of Exotic Barley Germplasm and its Effects on Agronomic Traits in Complex Crosses" (A43) - M. Vetelainen
12:00 p.m. - 1:30 p.m.	Lunch (on your own)

SESSION 10 CHAIR: DR. RICHARD PICKERING

1:30 p.m. - 2:15 p.m.	Plenary lecture "Biochemistry and Physiology of fructans (non-structural carbohydrates) in cool temperate grasses" (A67) - Dr. N. Jerry Chatterton, USDA-ARS

Wednesday, June 22

2:15 p.m. - 2:30 p.m.	"Genetic Effects of Alien Cytoplasms on Heat Tolerance in Wheat" (A46) - Q. X. Sun*, L. F. Gao and R. X. Xu
2:30 p.m. - 2:45 p.m.	"Influence of Climatic Factors on Distribution of Hordein Alleles in Barley" (A52) - A. A. Pomortsev*, B. A. Kalabushkin, and M. L. Blank
2:45 p.m. - 3:00 p.m.	"Attempts to Produce alien addition Lines in *Triticum durum*" (A60) - Domenico Pignone
3:00 p.m. - 3:15 p.m.	"Using Carbon Isotope Discrimination to Screen for Improved Water-Use Efficiency in Crested Wheatgrass" (A63) - D. A. Johnson*, K. H. Asay
3:15 p.m. - 4:00 p.m.	Break and Viewing of Posters
4:00 p.m. - 4:15 p.m.	"Wide hybridization for simultaneous improvement of wheat and *Leymus*" (A69) - Kesara Amanthawat-Jonsson* and R. Koebner
4:15 p.m. - 4:30 p.m.	"Variations in Structure Granule-Bound Starch Synthase (Wx protien) in Diploid, Polyploid Wheats and *Aegilops*" (A29) - N. Fujita*, K. Takaoka, M. Uematsu, A. Wadano, S. Okabe and T. Taira
4:30 p.m. - 4:45 p.m.	"Pathological Relationship Between Plant Parasitic Nematodes and Rangeland Grasses" (A35) - G. D. Griffin
6:00 p.m. - 9:00 p.m.	Symposium Dinner (Walnut Room, USU Student Union)

Thursday, June 23

8:00 a.m. - 9:00 p.m.	Field trip to Aberdeen, Idaho, to visit the USDA Small Grain Collection with stops for local grasslands and historical sites (Lunch provided)

Friday, June 24

8:30 a.m. - 8:45 a.m.	"Progress in the Development of GrainGenes, a Comprehensive Genome Database for Wheat and other Small Grains" (A65) - Susan B. Altenbach*, Olin D. Anderson, and David E. Matthews

Friday, June 24

8:45 a.m. -10:00 a.m.	Workshop on GRAINGENES computer database - Susan B. Altenbach
10:00 a.m. -10:30 a.m.	Break
10:30 a.m. -12:00 p.m.	Business Meeting and Closing of Symposium - Richard R-C. Wang and Kevin B. Jensen
12:00 p.m. - 1:30 p.m.	Lunch (on your own)
1:30 p.m. - 2:30 p.m.	Meeting of Committee on Genome Designations

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Table of Contents

TRITICEAE: a tribe for food, feed and fun

Roland von Bothmer and Björn Salomon
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INTRODUCTION

Triticeae is an important tribe in the grass family, Poaceae. It contains the cereals wheat, rye, triticale, and barley as well as a large number of wild species, some of which are utilized as forage grasses. The tribe combines all kind of biological mechanisms and genetic systems: diploids and polyploids; annuals and perennials, inbreeders and outbreeders, and even apomicts. Due to this large variation Triticeae is an excellent model group for research in genetics, plant breeding, genetic diversity, taxonomy, and speciation in plants.

Triticeae is distributed in almost all temperate areas of the world and consists of some 350-450 species (Dewey 1984, West *et al.* 1988, Tzvelev 1989). Most genera as defined today are exclusively either annuals or perennials, except the genera *Hordeum, Dasypyrum* and *Secale* that include annual as well as perennial species. Of the perennial genera, some are very large like *Elymus* with ca. 150 species down to the monotypic genera *Hordelymus*, *Peridictyon*, and *Pascopyrum* (Fig. 1). Apart from the *Triticum/Aegilops* group, which contains around 30 species, the other annual genera are small with 1-4 species.

There have been important contributions by many great scientists for research in Triticeae. Three persons should be mentioned who have had a great impact on the research in the tribe, but in different areas.

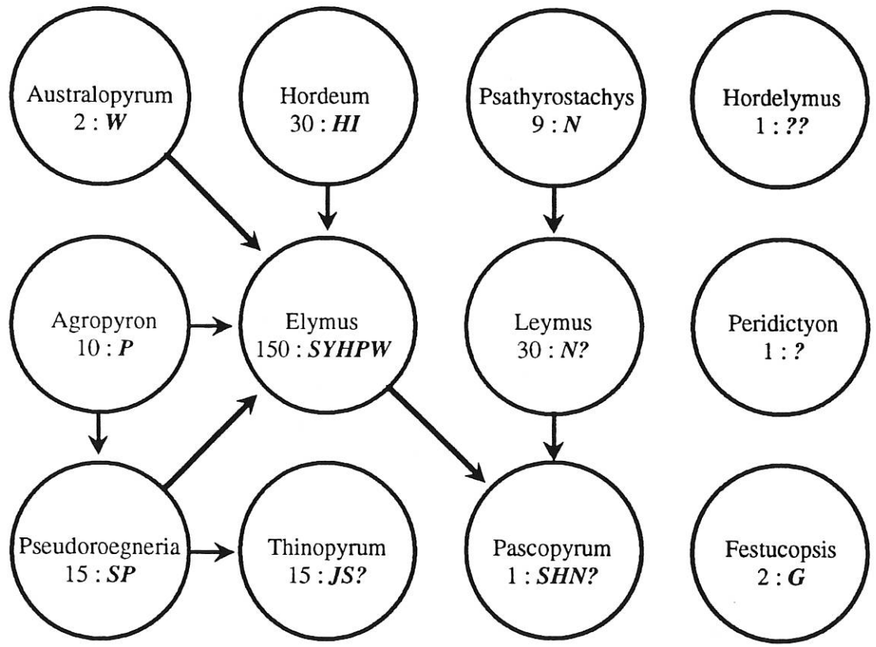

Figure 1 Perennial genera in the Triticeae with approximate number of species and genomes occurring within each genus.

S. A. Nevski (Fig. 2).

The excellent Russian botanist S.A. Nevski died in his early 30s after some outstanding achievements. He had a remarkably good eye for biological variation and good judgements in taxonomic delimitations. Nevski made a comprehensive monograph of *Hordeum*, he treated the whole tribe for the Russian flora and made several taxonomic contributions (Nevski 1934, 1941).

D. R. Dewey (1929-1993, Fig. 3)

A pioneer contribution to the broadened investigation on Triticeae was made in Logan by Doug Dewey. Patiently he collected material, produced interspecific and intergeneric hybrids and studied their meiosis over a period of more than 25 years. Thanks to his work there is now a basic knowledge on the genome content in most perennial groups of the tribe (Dewey 1982, 1984).

Á. Löve (1919 - 1994, Fig. 4)

The third person is more controversial, namely Áskell Löve. His very consequential treatment of the genomes (haplomes) as a basis for generic delimitation caused a very intense debate and his work encouraged people to work in Triticeae (cf. Löve 1982, 1984).

In this presentation four major areas of research and development and the current problems will be reviewed: (i) germplasm; (ii) taxonomy; (iii) phylogeny and relationships; and (iv) breeding aspects.

GERMPLASM

Collecting

Collecting of Triticeae germplasm has over the last decade been rather intense and a major undertaking for several national and international organizations and research groups. The target areas for collecting have primarily been the centers of diversity. For the crop species and their closest wild relatives this center is defined as the area with maximal genetic diversity, which, for the Triticeae, occurs in SW Asia (Fig. 5). For the other Triticeae species diversity centers are defined as areas where the highest number of species are distributed, namely in southern South America, western North America and particularly in Central Asia (Fig. 5).

The most intense collecting efforts have concerned wheat and barley, especially in the Fertile Crescent in SW Asia- The major part of these collections are landraces, weedy and primitive material, introgressional forms and wild taxa of the primary genepools. Large holdings of *Hordeum vulgare* ssp. *spontaneum* (C. Koch) Thell. and *Triticum*/*Aegilops* spp. have successively been built up at several genebanks, e.g. USDA, ICARDA, CIMMYT, John Innes Center, the Ethiopian Genebank and others. There are, however, still underrepresented areas, where further collecting should be encouraged. For the genus *Aegilops* areas to consider include parts of northern Iraq, eastern Iran and the Caucasus.

The genus *Secale*, including wild and primitive material of rye, is quite underrepresented in the world holdings. There have been no large scale missions for collecting of landraces, wild and weedy forms of *Secale*. The target areas for the rye group would be central Anatolia, the Caucasus area, and the region north of the Black Sea.

For the species more distantly related to the crops, the situation is somewhat different. Central Asia, parts of southwest Asia, South and North America are the most well collected areas, but there are still many regions from which living material is lacking. The more species rich areas where collecting should be made include parts of China, Afghanistan, Mongolia, Central Siberia, the Caucasus area, SW Asia, and parts of South America (Fig. 5). Some marginal areas like parts of SE Europe, New Zealand, North Africa, South Africa, and Erithrea need to be further explored. Some groups are underrepresented in the collections, like species of *Pseudoroegneria*, *Leymus*, and many of the annual genera.

The problems for earlier collectors were mainly of political nature due to wars and conflicts. The difficulty to

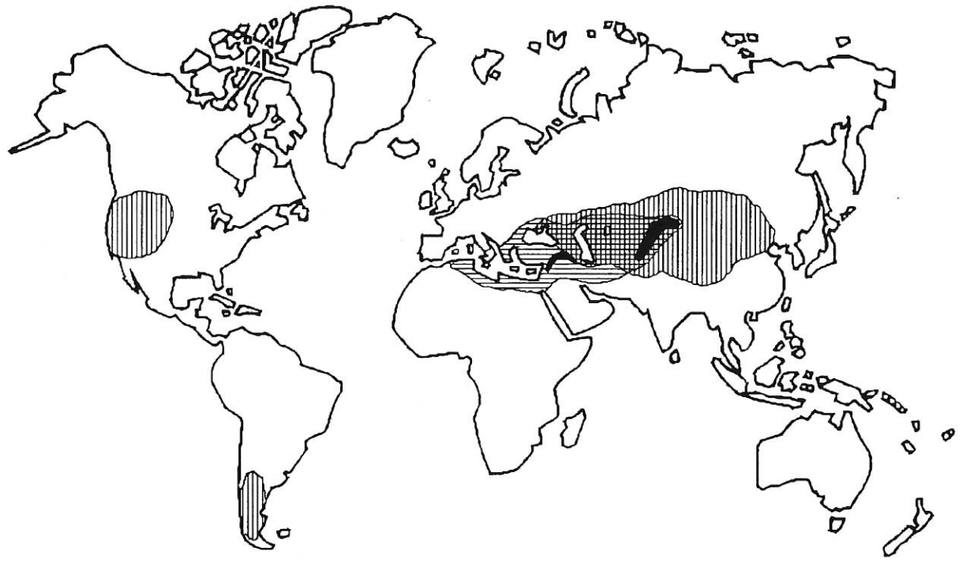

Figure 5 Diversity centers for the Triticeae; for the cereal crops and their closest allies, the center of diversity lies in the Middle East, and for the perennial genera, in Central Asia.

get access to material is now added with the possible problems arising with restrictions of collecting and free distribution of material. This is a result of the Rio convention which decided on the national ownership of genetic resources. Negotiations at FAO between member countries are going on and may hopefully lead to multilateral agreements about collecting and access to genetic resources. If this is not the case it will severely affect the possibilities to organize collecting missions and the access to germ plasm in the future.

Conservation and Genebank Problems

Material of wild species is invaluable for basic research and hopefully also for prebreeding programs and the need for collecting is obvious. It is, however, not self-evident that all material should be included in the gene banks. The importance of preservation of gene resources of primary and secondary gene pools for breeding purposes is well documented, but the value for preservation of other species of no immediate importance for breeding is not simple. Beside the general question of the value for preservation of the secondary and tertiary gene pools there are also several practical aspects which must be solved for the preservation of wild material.

Contamination. During multiplication and rejuvenation contamination through seed and pollen is common and difficult to avoid. It is a general problem for everyone dealing with wild species. The measure would be to develop effective isolation between plots either spatially or mechanically for keeping each accession as clean as possible. For each cycle of multiplication or rejuvenation the identity of each accession must also be carefully checked.

Loss of viability. The knowledge about the longevity of seeds is still fragmentary concerning wild species. Some species can survive in room temperature for decades, while others may loose their viability despite that careful precautions have been taken. The measure here is that more studies on seed storage conditions and seed physiology must be undertaken in a systematic way.

Labor intensive work. The keeping of seeds of many wild species means that most seed handling must be done by hand, which is time consuming, ineffective and costly. Development of new technology is highly desired. Due to the above mentioned practical problems it is out of question trying to preserve all wild material that has been collected. One fundamental problem concerning the wild species is the strategy about which material is prioritized for preservation. Unfortunately, no real strategy has been developed. As it is now the gene banks simply include whatever comes in. The aim for the preservation must be that the material in gene banks should optimally represent the entire variation amplitude of each species. Two major parameters can be used concerning what material to preserve, namely ecogeographic data on genetic diversity.

Ecogeographic data starts to be available for the primary gene pool of the cereals. In *Aegilops* information about geographical origin, altitude, soil conditions etc. for some of the about 30,000 accessions is available in databases (Hodgkin *et al*. 1992), but for other genera this information is fragmentary. When better facilities are obtained studies of *genetic diversity* with biochemical, molecular or adaptive characters must be applied.

If little material of wild species has been included in the gene bank the situation for the crops and the primary genepool is quite the reverse. Of wheat, the global holding constitute 570 000 accessions and of barley 320 000 accessions (Hintum 1994). These are very high numbers, but what the accessions represent in terms of genetic diversity is not known. There are also many types of duplicates among the total number of gene bank accessions (Hintum and Knüpffer 1994). The high number of accessions together with the unknown number of duplicates among them makes the access to the gene bank material rather difficult. One solution out of this problem is the set up of *core collections* (Brown 1989). The core collection is *"a selected and limited set of accessions optimally representing the genetic diversity of a crop and its wild relatives"*. That is where the ordinary germ plasm collection in a gene bank has an uneven distribution of accessions regarding ecogeographic or genetic diversity parameters the core collection should have an even distribution (Fig. 6). The Core Collection will not replace the regular gene bank holdings but, on the contrary, make an easier access to them. The first core collection to be realized concerns barley (the BCC). The number of accessions is decided to be about 2 000 representing about 1 % of the available gene bank accessions. The BCC is now under multiplication and the objective is that it will be completed and fully operating until the next Barley Genetics Symposium (1996 in Canada). Based on the BCC a number of investigations will be set up to actually test how much of genetic diversity the chosen set represents.

The creation of a "Triticeae *Core Collection*" (TCC) with a fixed set of accessions for each species which could serve as standards in basic investigations and for preliminary pre-breeding efforts should be discussed and decided upon. Two accessions from each taxon or cytotype could be included, which results in a TCC consisting of 700 to 800 accessions, which is fully feasible.

Utilization

The collected and preserved material should naturally be widely utilized in research and breeding. The more an accession is used the more information will be available and the more will it be justified to preserve. There is a gap between the collection and preservation on one side and utilization on the other. The major problem, especially in

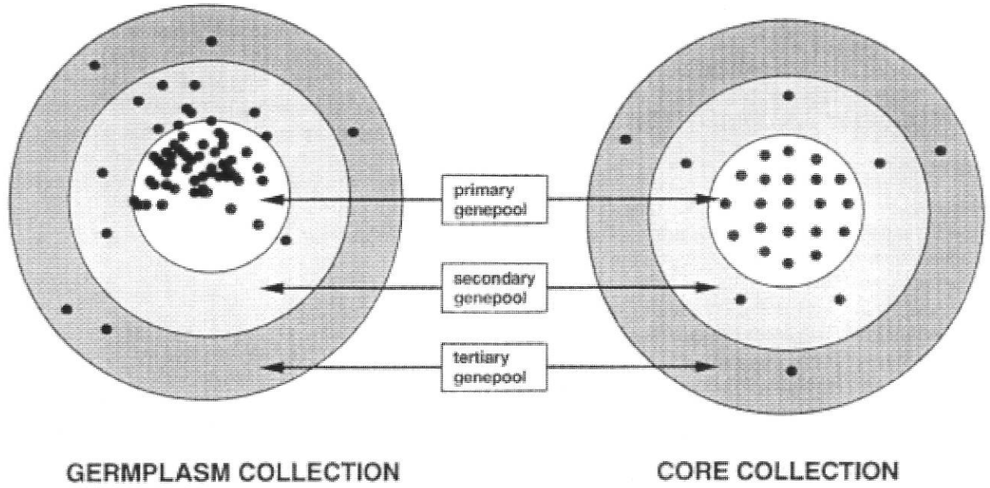

Figure 6 The core collection concept. A regular germ plasm collection has an uneven distribution of accessions whereas the core collection has an even distribution. (Modified from Hintum 1994).

research, is that the accuracy and the source of the material used often is neglected. If scientists were as careful about their material as they are about their methodology our knowledge about the Triticeae species would be far better. The use of unidentified or not verified material should not be allowed in publications. Accurate citations of the seed source with passport data or at least a number referring to a particular genebank accession should be obligatory, but it is sadly far from common. Documentation by voucher specimens for later verification of the identity is also desirable.

Taxonomy

The basis for our understanding of relationships and phylogeny is the species. If we know how the individual species look, how they vary and how they are distributed, there are better possibilities to choose material for phylogenetic studies and breeding. Classical taxonomic studies based on herbarium specimens are urgent and should have a high priority. Efforts should also be invested to gather data on habitat requirements which are lacking for many species. The taxonomic data at the species level will also throw light on the delicate and controversial discussion on generic delimitations.

Over the years many taxonomic studies of genera or groups of species have been made. One could thus get an impression that further basic taxonomic work is superfluous. Nothing could be more wrong! During the last two decades there have been surprisingly few taxonomic studies and there are still several complicated groups which have not been thoroughly investigated.

Some groups have been the subject for recent taxonomic treatments, for example, the annuals: *Dasypyrum, Eremopyrum, Henrardia, Amblyopyrum, Heteranthelium*, and *Taeniatherum* (Frederiksen 1986, 1990, 1991, 1993). The genera *Aegilops* and *Triticum* are at present under revision, where *Aegilops* and some of the *Triticum* species are ready for publication (Van Slageren, ICARDA, personal comm.). Among the perennials, which have been treated recently, are *Psathyrostachys* (Baden 1991), *Hordeum* (Bothmer *et al*. 1991), *Leymus* in North America (Barkworth & Atkins 1984), and some groups in *Elymus* (Salomon 1994, Lu 1995) and *Thinopyrum* (Jarvie 1992, Assadi 1994). Cladistic and numerical approaches based on morphological characters have also been carried out in the tribe (cf. Baum 1982, Kellogg 1989, (Frederiksen & Seberg 1992).

There are still several groups which are poorly known, for example *Leymus* and *Pseudoroegneria* in Asia, and the major parts of *Elymus* and *Thinopyrum*. Joint international efforts could solve some of these taxonomic problems. One such proposal is a Scandinavian initiative for an *Elymus* network with the aim to study the genus from different

angles and hopefully ultimately lead to a monographic treatment of this huge genus. National and regional initiatives for taxonomic treatments, especially in the diversity centers, should be greatly encouraged and financially supported.

PHYLOGENY AND RELATIONSHIPS

Cytogenetic Methods

Based on chromosomal pairing in the meiosis of interspecific and intergeneric hybrids classical cytogenetics has gradually built up the knowledge on genome relationships in the Triticeae (cf. Dewey 1984, Love 1984). There is now a framework done, and the genomic content is partly known for some genera with some genomes occurring in more than one genus (Fig. 1). The **H** genome occurs in the genera *Hordeum, Elymus* and Pascopyrum. The **S** genome occurs in species of *Elymus*, *Pseudoroegneria, Thinopyrum* and Pascopyrum. The **N** genome occurs in *Psathyrostachys, Leymus*, and Pascopyrum. The **J** genome occurs only in *Thinopyrum* in a broad sense and not in *Leymus* as formerly postulated (Zhang & Dvorak 1991, Wang & Jensen 1994).

There are still several species which are not studied at all. Including in particular species of *Elymus* and *Leymus* in Asia. Still unknown genomes occur in *Hordelymus, Peridictyon, Leymus, Pascopyrum* and *Thinopyrum*. Several of the studied *genomes* have no obvious connections to others, e.g. the **G** genome of *Festucopsis*.

The problems included in genome analysis should not be ignored. This encounters especially the genetic pairing regulation like the Ph genes in wheat and other species (McGuire & Dvorak 1982, Petersen 1991). The operation of these pairing promoting or pairing reducing genes disrupt the regular pairing patterns which may result in wrong conclusions on genome affinities. However, by the analysis of a large number of hybrid combinations this risk is reduced. Information on genome relationships will also in the future contribute to the understanding of species relationships in the Triticeae.

Molecular Methods

Much effort has been invested in development of new techniques for studies of phylogenetic relationships, which also have been applied in the Triticeae. These methods include biochemical techniques like electrophoresis of isoenzymes and storage proteins (cf. Jorgensen 1986, Jaaska 1992). Molecular biology has added to the richness of new, powerful, and sophisticated techniques, like RFLPs, and RAPDs both of nuclear and organellar DNA (cf. Talbert *et al.* 1991, Doebley *et al.* 1992, Dvorak & Zhang 1992, Kellogg 1992, Molinar *et al.* 1992, Terachi & Tsunewaki 1992).

Over the last years there are mainly two types of molecular investigations that have been done. (1) This group includes studies that are concentrated on a genus or a restricted group of species. Here several more or less closely related species or populations are selected. In the *Triticum/Aegilops* group Talbert *et al.* (1991) studied the repetitive DNA sequences primarily characteristic for the **S** genome and how the diploid and the polyploid species are related. *T. speltoides* (Tausch) Gren. is distinct from the other diploid **S**-genome species. The tetraploid *T. timopheevi* (Zhuk.) Zhuk. is shown to be 98 % identical showing close affinity between the **S** and the **G** genomes (Fig. 7).

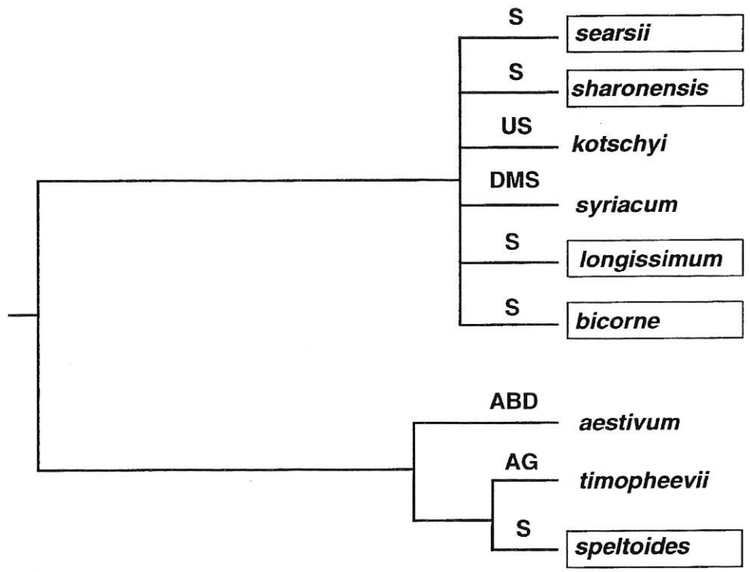

Figure 7 Variation in repetitive DNA sequences in the *Triticum/Aegilops* group. The letters represent genome designations. (Modified from Talbert et al. 1991).

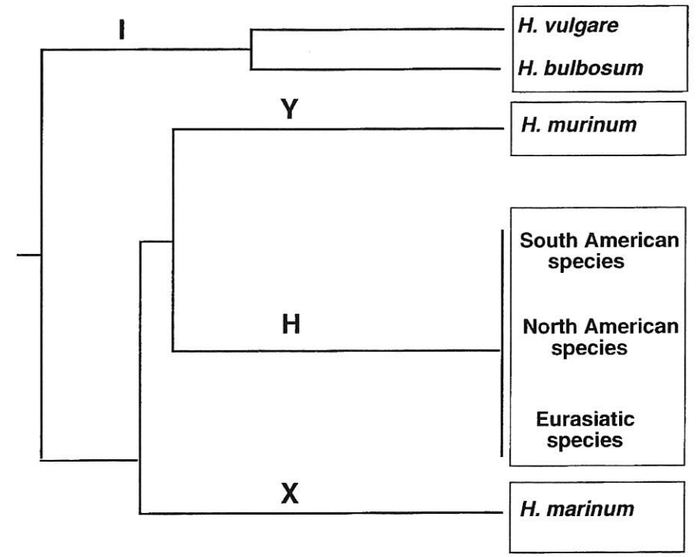

Figure 8 Variation in repetitive DNA sequences in *Hordeum*. The letters represent genome designations. (Modified from Svitashew *et al.* 1994).

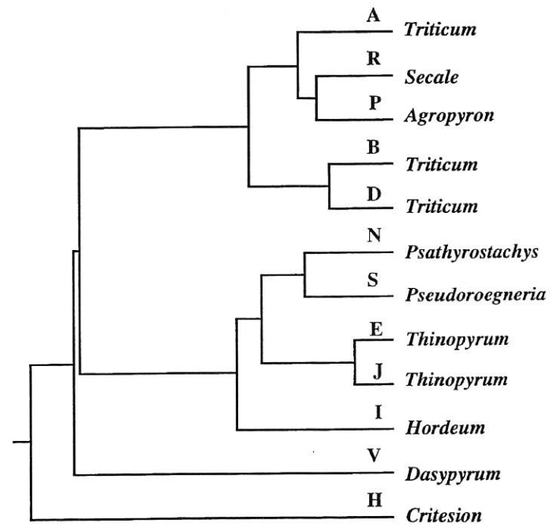

Figure 9 RFLP variation between different genera of the Triticeae. The letters represent genome designations. (Modified from Monte *et al.* 1993).

In *Hordeum* several studies with different techniques have been applied, like a cpDNA study by Doebley *et al.* (1992) showing differentiation between the four basic genomes in the group. This pattern is also evident in the study of repetitive sequences of the nuclear DNA by Svitashev *et al.* (1994, Fig. 8).

In this kind of studies it is often so that a too narrow group is chosen. Further representatives outside the group should be included. It is, for example, usually not meaningful to compare just the cereals and include none of the wild species. (2) The other approach includes usually the whole tribe. The species are chosen to represent an entire group or genus. For example, Monte *et al.* (1993) studied the RFLP variation of 21 cDNA probes from hexaploid wheat in 16 species of the Triticeae (Fig. 9). They found a good correlation between the phylogenetic tree produced by this approach and by earlier investigations.

In this type of investigations the chosen species may not at all be representative for the group or the variation within the group is not covered sufficiently. More careful considerations and planning about the material should be done before the costly techniques are applied.

In Situ Hybridization

One particular molecular technique is *in situ* hybridization or molecular cytogenetics where a probe is hybridized with chromosomes - usually in the metaphase plates. *In situ* hybridization has contributed quite a new tool to study the organization of the DNA structures in the chromosomes and affinities between species (Lapitan *et al.* 1987, Anathawast-Jónsson, K. *et al.* 1990, Heslop-Harrison 1992, Ørgaard & Heslop-Harrison 1994 a,b). It refines our tools for genome analysis and for breeding purposes.

The probes can detect: cloned sequences, chromosomal segments, whole chromosomes, and entire genomes. A special aspect is the possibility to study the meiosis and distinguish between auto- and allosyndetic pairing.

All the new techniques have added immensely to the knowledge of relationships in the Triticeae. In the future we will get new and exciting results. There are two major areas where the knowledge of relationships and phylogeny is particularly weak. (1) Many of the annual species of the Triticeae belong to small or even monotypic genera. The morphology is in most cases quite distinct. Crossing experiments and genome analysis have not added much to our understanding of the affinity to perennial groups or genomes. How are these annuals differentiated, which are their respective closest relatives and are they old or new taxa? Do the annuals have particular genetic systems which promote rapid differentiations? (2) Several of the perennials have been poorly studied mainly due to lack of material. These include several species of Asiatic *Leymus* and *Elymus*. With various methods one should study which groups of species are monophyletic and perhaps ultimately to get a better generic delimitation.

BREEDING

The breeding aspects in the Triticeae are naturally dominated by the big cereal crops, wheat, barley, and rye. In this context the elite breeding in cereals will not be discussed, but merely the utilization of a wider genepool in pre-breeding programs. The entire tribe constitute a vast genepool. Many species belong to the primary and secondary genepools of bread and durum wheats, and due to the polyploidy several species from the tertiary genepool are also used. The efforts in pre-breeding is dominated by screening for disease resistance in wild *Aegilops* and *Triticum*. Over the last decade resistance to at least 15 pathogens have been investigated and some of the sources are now included in conventional breeding programs (cf. Tosa & Sakai 1991, Eastwood *et al.* 1994, Siedler *et al.* 1994). Some work has also been devoted

to stress tolerance, mainly for salt and drought (cf. Nevo *et al.* 1993, Taeb *et al.* 1993, Dubcovsky *et al.* 1994). Among other genera of interest for wheat improvement include the annual *Dasypyrum villosum* (L.) Candargy and some perennial species like *Thinopyrum elongatum* (Host.) D.R. Dewey, *Th. bessarabicum* (Savul. & Rayss) A. Löve and *Th. intermedium* (Host) Barkworth & D.R. Dewey (cf. Blanco *et al.* 1988, Jiang *et al.* 1993, William & Mujeeb-Kazi 1993, Zhong & Qualset 1993).

Contrary to wheat, barley is a diploid organism which makes gene transfer more problematic. Only the progenitor of the crop, *Hordeum vulgare* ssp. *spontaneum* belongs to the primary genepool. It has been studied particularly for resistance to BYDV, powdery mildew and rust (cf. Jana & Nevo 1991, Jahor & Fischbeck 1993). Ssp. *spontaneum* material is at present included in at least three major pre-breeding programs (cf. Lehmann & Bothmer 1988).

Hordeum bulbosum L., which is the single species in the secondary genepool of barley (Bothmer *et al.* 1991), has since long been used in production of doubled haploids through chromosome elimination (cf. Lange 1988). Now there are also promising results with the use of *H. bulbosum* for transferring genes to barley (Pickering 1992, Xu & Kasha 1992). The first successful transfer was with a resistance gene for powdery mildew. The other wild species of *Hordeum* are more inaccessible for breeding (Bothmer *et al.* 1991).

Rye has been extensively used as a gene source for transfer of resistance genes to wheat. For breeding of rye the very closely related wild species could be utilized but so far very little efforts have been invested (Singh & Seti 1991, Izdebski 1992).

New Crops

The intergeneric hybrid between wheat and rye, triticale, is now at last established as an important cereal in some countries. It took about a century to develop triticale from the time of the first crosses. It is thus not an easy task to introduce new crops. One very interesting attempt to develop another amphiploid, putative new crop is tritordeum, i.e. the intergeneric hybrid between *Hordeum chilense* Roem. & Schult. and *Triticum*, especially durum-wheat. The first crosses were made at PBI in Cambridge some 20 years ago (Martin & Chapman 1977). The first papers included mainly hybridization, cytogenetics, and molecular studies (cf. Martin & Sanchez-Monge Laguna 1980, Padilla & Martin 1983, Schwarzacher *et al.* 1989). Later more applied approaches were performed, for example, on resistance to rust, powdery mildew and nematodes as well as on field trials (cf. Milan *et al.* 1988, Alvarez *et al.*1992, Rubiales *et al.* 1992, 1993). Another interesting new combination is wheat X *Leymus spp.* (cf. Plourde *et al.* 1993). Even if the task to establish a new crop seems frustrating further initiatives should be encouraged.

Forages

Triticeae comprises also several range and forage grasses, which are important for grazing in natural conditions in Central Asia as well as under domesticated conditions in North America. The most important species are the crested wheatgrasses (*Agropyron cristatum* (L.) Gaertn.), intermediate wheatgrass (*Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey), and Russian wildrye (*Psathyrostachys juncea* (Fisch.) Nevski). Much work is in progress concerning disease resistance, stress tolerance and yield potentials in these and other species (cf. Berdahl & Krupinsky 1987, Johnson 1991, Asay 1992, Dong *et al.* 1992, Vogel *et al.* 1993, Xu & Conner 1994, Wang 1994). Similar studies should be encouraged in other parts of the world, e.g. in Central Europe, SW Asia, and South America (cf. Salomon *et al.* 1992, Esteban *et al.* 1993).

CONCLUSIONS

There is a huge task lying in front of us for research and development in the Triticeae. To summarize some of the major topics:

Germplasm :

- enlarge collecting
- improve preservation
- increase utilization

Taxonomy:

- more monographic studies
- improve the generic delimitation

Relationships and phylogeny.

- consensus of genome designation
- study the relationships with the annuals
- study the relationships with the perennials

Breeding:

- improved technique for gene transfer
- more studies of agronomic traits

The Triticeae symposia need to get a formalized continuation. It is important that breeders and researchers meet at regular intervals to discuss this fascinating plant group. Further international cooperation in Triticeae research is also required.

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Genomes, Chromosomes, and Genes and the Concept of Homology

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ABSTRACT

The traditional application of genome analysis in phylogenetic inference is questionable. Hypotheses about phylogeny are based upon the analysis of homologous characters, existing as a consequence of common descent. The concept of homology in morphology and molecular biology is well-defined: To count as an homology any character must pass the similarity, congruence, and conjunction tests. In genome analysis homology is related to the behaviour of chromosomes during meiosis: homologous chromosomes pair, nonhomologous chromosomes do not. Thus, in genome analysis homology becomes a purely operational concept. How well does this operational concept work? And what are the relationships, if any, between this operational concept of homology and the homology concept of morphology and molecular biology?

INTRODUCTION

As a discipline genome analysis was formally founded and outlined by Kihara (1930) and has been applied extensively, not least within the Triticeae, to studies of systematics and evolution ever since. More recently, Dewey (1982: 52) stated that: "*The fundamental premise of genome analysis is that like (homologous) chromosomes pair during meiosis and unlike (nonhomologous) chromosomes do not. The corollary premise is that the level of chromosome pairing in a species-hybrid reflects the degree of relationship between the parental species.*" Thus, genome analysis is strongly dependent upon the homology of chromosomes, and hence upon the concept of homology.

The concept of homology in morphology and molecular biology is well-defined (Patterson 1982, 1988). To qualify as homologous any character must pass the three tests of homology: similarity, conjunction, and congruence. The test of similarity is intuitively the most obvious, as we would not even consider the possibility of two characters being homologous if we observed no similarity at all between them (Stevens 1984). The test of conjunction deals with the duplication of a character within an organism. e.g., because of the position of the bract scales the female cone of a conifer is considered a condenced shoot and thus homologous to the entire female cone of a cycad (Florin 1944). Because of the presence of bract scales the ovuliferous scales fail the conjunction test. The third test concerns the congruence of one homology with other homologies, and is closely linked to the principle of parsimony. Monophyletic groups are characterized by synapomorphies (=homologies) and the hypothesis of one homology is tested by presence of other synapomorphies. Failing this test may be caused by e.g., parallelism or convergence.

These three tests are equally valid in morphology and molecular biology, the only difference residing in the terminology and in the relative importance attached to the three tests (Patterson 1988). However, in genome analysis homology is being related to chromosome pairing, i.e. to the behaviour of the chromosomes during meiosis. Thus, the concept of homology has been turned into something purely operational. But how then, does this operational concept of homology relate to the homology concept of morphology and molecular biology?

HOMOLOGY AND CHROMOSOME PAIRING

Since the very early studies of chromosome pairing during meiosis it has been suggested that the pairing chromosomes were homologous (e.g., Sutton 1902, McClung 1908). This was based upon the apparent similarity of the pairing chromosomes and on their assumed descent from a male and female parent. Often it will be so

that two pairing chromosomes are truly homologous. The 4A chromosomes in one plant of hexaploid wheat are most likely homologous to the 4A chromosomes in another wheat plant. We can infer by their apparent similarity, common descent. Thus, chromosomes regarded as entities surely can be homologous. Just as chromosome arms, genes, or other well defined parts of the chromosomes can. We may run into some problems when changes such as translocations, inversions, or substitutions occur, but that will only be a matter of addressing the problem at the "correct" level. The above chromosomes 4A will no longer be homologous if one of them, because of a translocation, carries the short arm of chromosome 4D instead of its own 4AS, but the two 4AL's will still be homologous. Trying to assign a "degree" of homology to the chromosomes 4A and 4AL/DS would be absurd.

As pairing chromosomes within a species usually were homologous, the idea emerged that the degree of pairing could measure the degree of homology and further assess a level of organismal relatedness (e.g., Federley 1914, Kihara 1924, 1930). Thereby the concept of homology was changed into a purely operational one, which is still used in genome analysis. This use of homology raises two major questions.

The first question addresses the relationships between chromosome pairing, chromosome similarity, and DNA similarity. In genome analysis it is assumed that the ability of chromosomes to pair estimates an overall similarity of the total amount of DNA (e.g., Alonso & Kimber 1981, Chapman & Kimber 1992). However, less than 1% of the total amount of DNA is trapped in the synaptonemal complex (Wettstein et al. 1984). As for the similarity of the remaining 99% of DNA we have virtually no knowledge.

The extrapolation from chromosome pairing to DNA similarity is purely *ad hoc*. It is completely unknown to what extent differences in base composition influences pairing ability, both with respect to coding (e.g., genes) and non-coding sequences (e.g., the 70 % or so repetitive DNA that occurs in the Triticeae genome). Thus, the invoked relationship between pairing and DNA similarity is more an article of faith than a scientific theory.

The concept of chromosome similarity as viewed macroscopically is equally elusive. Hence it is only very rarely known, whether the chromosomes involved in the pairing in one hybrid combination are the same as observed in another combination.

Further, it is well documented that chromosome pairing is under genetic control, e.g., the Ph-gene of chromosome 5B in *Triticum* L. (e.g., Holm 1986). Functioning/non-functioning of this gene can change pairing from virtually zero to 100%. Thus, a very small change, perhaps just a one-basepair mutation, could make the interpretations from genome analysis change from total similarity to total dissimilarity between two genomes. This of course is the extreme situation, but any genetic or environmental factor (e.g., temperature [Pickering 1990] or nutrition [Bennett & Rees 1970]) having an influence on chromosome pairing will contribute so that the observed chromosome pairing does not reflect DNA similarity.

The second major question concerns the relationship of homology to phylogeny, and hence the congruence test. Previously both Kellogg (1989) and Seberg (1989) have stated that the ability of chromosomes to pair and hence inferred as homologous as defined by Dewey (1982), is the plesiomorphic character state. The ability to pair tells us only that the chromosomes/genomes have not diverged. Thus, the pairing ability of chromosomes cannot be used in phylogeny reconstruction as only apomorphic character states are informative.

Intermediate levels of chromosome pairing (= the average chiasma frequencies) assessed by genome analysis are not discrete character data but distance data, and thus cannot be transformed into character data. As such they offer no opportunity to examine notions of homology, and in phylogenetic inference they provide very little opportunity for further research (Eernisse & Kluge 1993). Thus, it remains to be proven that homology expressed as pairing ability passes any of Patterson's (1982, 1988) tests, apart perhaps the conjunction test.

THE OPERATIONAL CONCEPT OF HOMOLOGY - HOW DOES IT WORK?

What is measured in genome analysis is usually the definite number of chiasmata in a definite number of cells. Thereafter an average value of chiasmata per cell is calculated. But what does this average value represent? Assume that we in a diploid hybrid with 2n=14 chromosomes, observe cells with every number of chiasmata from one to 13, with an average frequency of 8.6 chiasmata per cell (as in the hybrid *Hordeum brachyantherum* Nevski x *H. muticum* Presl [Bothmer et al. 1986]). Most genome analysts would not put much emphasis on the observed range, but would regard the average value as an indication of a fairly high level of homology between the genomes, and consequently consider the species quite closely related. But what about the chromosome behaviour in the cells with only one chiasma or 13, respectively? One chiasma would indicate a fairly low level of pairing and little homology between the genomes, whereas 13 chiasmata would indicate a high level of pairing and homology. But these two cells nevertheless would (for all practical considerations) contain exactly the same DNA. So we must ask, what is then the biological rule that tells us that the level of "homology" or "relatedness" is given by the average value and not by any of the extremes.

It seems to become even more difficult to interpret the mean values, when looking at the chiasmata distributions in hybrids (Fig. 1, 2). One might have

expected that chiasmata distributions typically would be either binomial with the top-point equalling or close to the mean value (Fig. 2A: *Aegilops geniculata* Roth x *Triticum durum* Desf., 2B: HH 10183-1), or form distributions sloping steeply from either zero in hybrids with virtually no pairing (Fig. 2D: HH 10339-2) or from the absolute maximum number of chiasmata in hybrids or species with normal, full pairing. However, this is rarely the case. The top-point may be strongly skewed (Fig. 1A), the curve may be flattened (Fig. 2D: HH 10339-1), there may be no apparent top at all (Fig. 1B), or there may be more than one top (Fig. 1C: BB 7271a, 1D). If the distribution of chiasmata is skewed, then the average value will be either higher or lower than the most frequently occurring number of chiasmata, and the modal value would better represent the chiasmata distribution than the mean value. If all observed numbers of chiasmata per cell occur with almost the same frequency, the average value seems hardly more representative than any other value. In cases where the distribution is bimodal the average may be closer to the trough between the two peeks than to any of the maximum values (e.g., Fig. 1D: BB 7511b with an average chiasma frequency 17.4). We have most clearly observed bimodal distributions in tetraploid hybrids, and it is possible that such distributions are caused by the combination of two different pairs of genomes having different levels of pairing. If so, combining the distributions into one average chiasma frequency seems absurd.

Here we shall not attempt to answer in depth what it signifies that the average number of chiasmata deviates from the most frequently occurring number(s), but merely ask what biological relevance the average value has over any other value.

One further, serious problem in the use of average chiasma frequencies to assess phylogenetic relatedness is the variation between values that can be obtained from reciprocal hybridization and between progeny from hybrid combinations involving the same parental species. Few, if any studies, since Kihara (1929) have focused on these problems, though the observed discrepancies ought to be most alarming to any genome analyst.

In reciprocal hybrids involving *Triticum* and *Aegilops* L. (Fig. 2A) Kihara (1929) observed quite deviating patterns of chromosome pairing. In one hybrid virtually no pairing occurred, whereas in the reciprocal an average of approx. 4 chiasmata per cell were observed. Lu & Bothmer (1993b) observed significantly different pairing in reciprocal hybrids between *Elymus caucasicus* (C. Koch) Tzvelev and *E. tibeticus* (Melderis) G. Singh, and the difference would place the hybrids in each of two groups, defined by Lu (1993) to distinguish five different levels of chromosome pairing. As these levels are being interpreted as a measure of phylogenetic relatedness, it must be disturbing that reciprocal combinations give different measures of distance between the same parental species.

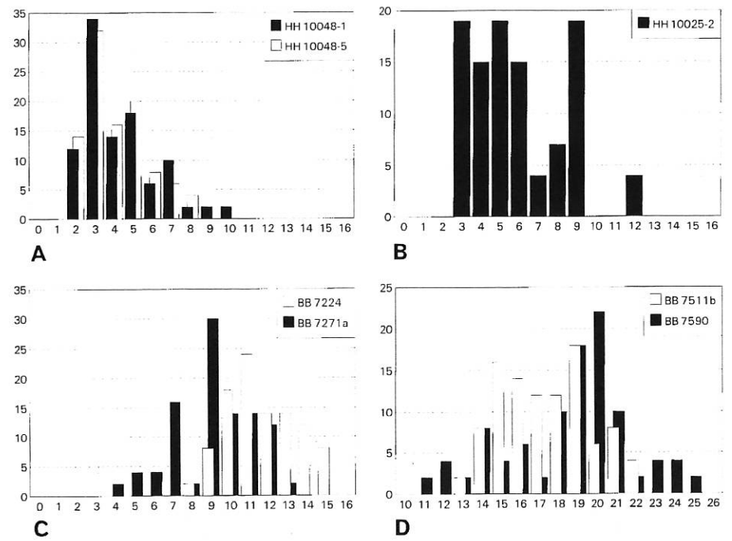

Figure 1 Distribution of chiasmata in hybrids. A, B: *Hordeum brachyantherum* (4x) x *Secale cereale*, two crosses involving different parental accessions. C: *Elymus tschimganicus* (Drob.) Tzvelev x E. *caninus* (L.) L., two crosses involving different parental accessions. D: *Elymus tschimganicus* x E. *caucasicus*, two crosses involving different parental

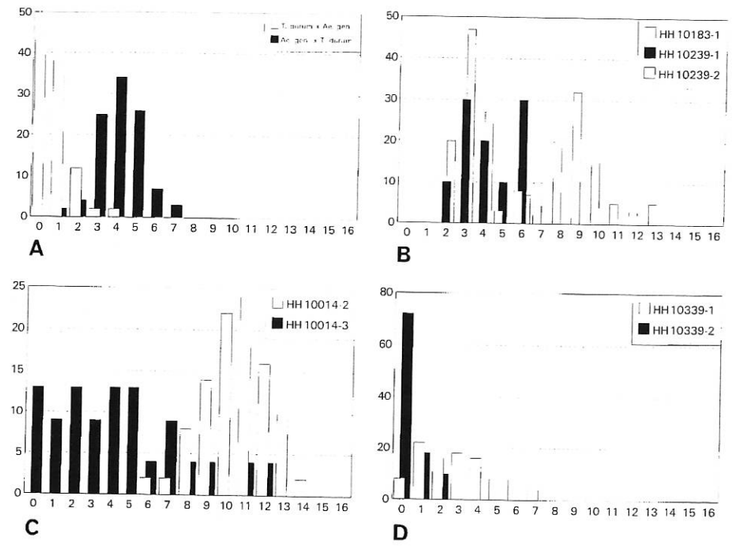

Figure 2 Distribution of chiasmata in hybrids. A: Reciprocal hybrids between *Triticum durum* and *Aegilops geniculata* (Data from Kihara 1929). B: *Hordeum procerum* x *Secale cereale*, two crosses involving different parental accessions. C: *Hordeum brachyantherum* (4x) x *Secale cereale*, two plants from the same cross. D: *Hordeum capense* Thunb. x *Secale montanum* Guss., two plants from the same cross. X-axis: Number of chiasmata; Y-axis: Percentage of cells.

Similar differences in chromosome pairing can be observed when comparing offspring from different crosses of the same hybrid combination. e.g., hybrids from two different crosses between *Elymus brevipes* (Keng) Löve and *E. tsukushiensis* Honda had average chiasma frequencies of 20.66 and 8.19, respectively (Lu & Bothmer 1993a), and offspring from one cross between *Hordeum procerum* Nevski and *Secale cereale* L. (Fig. 2B: HH 10183-1) had more than twice the high average chiasma frequency than offspring from a second cross (Fig. 2B: HH 10239) (Petersen 1991). Even individual plants from the same cross may exhibit strongly deviating patterns of chromosome pairing (Fig. 2C, D). In two hybrids between *Hordeum brachyantherum* (4x) and *Secale cereale* the average chiasma frequencies were 10.50 and 4.39, respectively (Fig. 2C) (Petersen 1991). As in these hybrids only *Hordeum* L. chromosomes take part in the pairing, one hybrid shows almost complete pairing of the *Hordeum* chromosomes (average 6.42 bivalents per cell) whereas in the other, only half of the chromosomes are paired (average 3.85 bivalents per cell) (Petersen 1991). Thus, following genome analysis the two genomes of *Hordeum brachyantherum* should in one hybrid be interpreted as almost fully homologous and in the other as only partly homologous.

If the average chiasma frequency is accepted as a measure of relatedness (= "overall similarity") between two species, this may only be extended to more inclusive groups of species by using phenetic clustering methods (e.g., UPGMA [Lu 1993]). However, that the overall similarity (and phenetic clustering) is an unsatisfactory measure of phylogenetic relationships is beyond debate.

CONCLUSION

It seems a paradox that it was Kihara (1930), who immediately after having observed great differences in chromosome pairing between reciprocal hybrids, within offspring from crosses, and even within florets from just one spike (Kihara 1929), formulated the theories and practices of genome analysis. Kihara (1929) assumed that most of the variation could be explained by the influence of environmental factors. Though this may to some extent be true, other factors, not least genetic, may be strongly influential, too. Both factors make reproducibility and comparisons a difficult matter. However, we do not here aim at speculating about possible ways in which chromosome pairing may be affected, but merely wish to demonstrate some of the patterns and magnitude of the variation that are not addressed or deliberately neglected in genome analysis. Variation obscures the biological relevance of the mean values, which are the underlying basis for genome analysis.

The conversion of chromosome pairing data into a measure of homology and phylogenetic distance is questionable on the basis of the conceptual discrepancy alone. There is no known relationship between the theoretically formulated definitions of homology in classical morphology and molecular systematics and homology defined as chromosome pairing (Moritz & Hillis 1990). As previously stressed, by e.g., Kellogg (1989) and Seberg (1989), the presence or absence of pairing may to the extent it represents states of the same character, be used in phylogenetic reconstruction. The degree of pairing, though being mathematically well-defined, can only be used in phenetics and hence it is phylogenetically incomprehensible.

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The Study on N Genome of *Leymus* Species

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ABSTRACT

Leymus Hochst. is a perennial genus of Triticeae. All species in *Leymus* have the genomes NX. The genome N is from the genus *Psathyrostachys*. Two *Psathyrostachys* species, diploid *P. huashanica* Keng ex Kuo and *P. juncea* (Fische.) Nevski (2n=14), were hybridized with allotetraploid, *Leymus secalinus* (Georgi.) Tzvelev and *L. multicaulis* (Kar. & Kir.) Tzvelev. Meiotic behavior of the synthetic hybrids was studied. The chromosome pairings indicated that one *L. secalinus* genome and one *L. multicaulis* genome were closely homologous with both *P. huashanica* and *P. juncea* genomes. The data of genomic analysis in the hybrids of *P. huashanica* crossed with *L. secalinus* and *L. multicaulis* are so similar to those in the hybrids of *P. juncea* crossed with *L. secalinus* and *L. multicaulis*, there is no significant difference between them. Both *P. huashanica* and *P. juncea* are possible donors of the N genome of *L. secalinus* and *L. multicaulis*.

INTRODUCTION

Leymus Hochst., a perennial genus of Triticeae, includes about 30 species. They are distributed in the temperate regions of Eurasia, North and South America and extend to the subtropic and the tropic alpine regions. All species in *Leymus* have the genomes N and X. Here the N genome is donated by *Psathyrostachys*.

Psathyrostachys is a small genus with no more than 10 species, about half of which have been determined to be diploid ($2n=14$) containing the N genome (Dewey, 1984). Interspecific hybrids were made among the three diploid species *P. juncea* (Fisch.) Nevski, *P. fragilis* (Boiss.) Nevski, and *P. huashanica* Keng ex Kuo. Chromosome pairing in the hybrids indicated that each species has a modified form of the N genome (Dewey and Hsiao, 1983; Bothmer et *al*., 1987; Wang, 1987; Lu et *al*., 1990). Therefore, the symbols N^j, N^f, and N^h are used for these species, respectively.

The desirability of a classification based on relationships is obvious. Cytogenetic data from species and generic hybrids are effective measures of biological relationships. Intergeneric hybrids of *Psathyrostachys juncea* with *Leymus* species have been reported. The cytological data showed that *P. juncea* was one of the original diploid parents of *Leymus* species (Dewey 1970, 1972a, 1972b; Wang et *al*., 1984). But all these studies only involved *P. juncea* as a parent. None has involved other *Psathyrostachys* species. Because each *Psathyrostachys* species has a modified form of N genome, it is worthwhile to extend the investigation to other species of *Psathyrostachys*. This paper reports successful hybridization of *P. huashanica* and *P. juncea* with *L. secalinus* and *L. multicaulis*. The genomic relationships are analyzed. The major objective was to determine whether the *P. huashanica* genome is found in *Leymus secalinus* and *L. multicaulis*.

MATERIALS AND METHODS

Leymus secalinus (Georgi.) Tzvelev (6040) was collected from Fuhai county, Xinjiang, *L. multicaulis* (Kar. & Kir.) Tzvelev (Y094) from Habahe, Xinjiang, and *Psathyrostachys juncea* (Fisch.) Nevski (Y136) fromTeilike, Xinjiang, China. *Psathyrostachys huashanica* Keng ex Kuo is an endemic species of the Huashan mountains of Shaanxi, China. All materials were grown in the field at the Triticeae Research Institute, Sichuan Agricultural University.

The *L. secalinus* and *L. multicaulis* accessions were used as female parents. The spikes of *L. secalinus* and *L. multicaulis* were emasculated and covered by cellulose bags. Several days later, artificial pollinations were made by putting newly mature anther powder into maternal florets. The 15-16-day old hybrid embryos were cultured. When the hybrid seedlings had three leaves, they were transplanted into sand pots and kept in an air conditioned room to survive the hot summer.

Spikes for cytological analysis were fixed in Carnoy's (6:3:1) solution for 24hr, then transferred into 70% ethanol and stored in a refrigerator. Slides were prepared by acetocarmine smear for cytological observation.

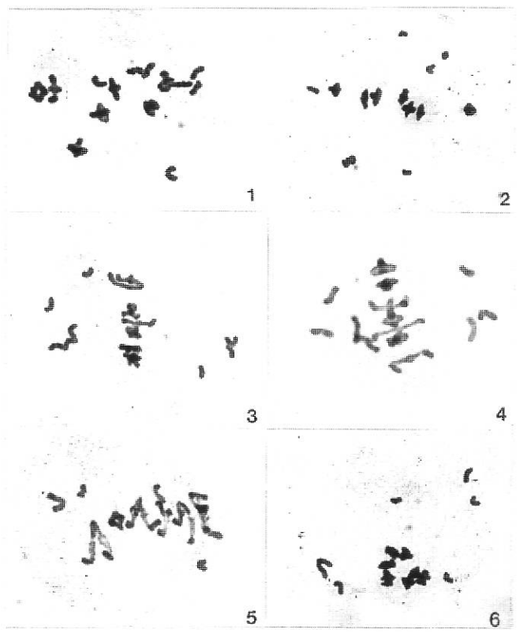
Results

The chromosome pairings at metaphase-I of pollen mother cells in both the parental species and hybrids are

listed in Table 1, and the meiotic configurations are shown in Fig. 1-6. Chromosome pairings of the parents in meiosis were very high (Table 1). Univalent and multivalents were only occasionally observed in *Leymus secalinus* and *L. multicaulis* (Table 1).

Table 1. Meiotic behaviour in parental species and hybrids; The range is given in the parentheses

Species		II					Chiasmata
or hybrids	I	ring	rod	Total	III	IV	per cell
L. secalinus	0.15 (0-2)	13.01 (8-14)	0.81 (0-4)	13.82 (13-14)		0.04 (0-1)	26.95
L. multicaulis	0.58 (0-4)	11.2 (8-14)	2.5 (0-6)	13.7 (12-14)			24.9
P. juncea		6.12 (4-7)	0.88 (0-7)	7 (7-7)			13.12
P. huashanica		3.81 (2-7)	3.19 (0-5)	7 (7-7)			10.18
L. secalinus x *P. huashanica*	7.03 (5-9)	5.91 (4-8)	1.08 (0-4)	6.99 (6-8)			12.9
L. secalinus x *P. juncea*	7.1 (5-11)	5.99 (3-7)	0.93 (0-4)	6.92 (5-8)	0.01 (0-1)		12.93
L. multicaulis x *P. huashanica*	7.3 (3-13)	4.15 (1-7)	2.54 (0-5)	6.69 (4-7)	0.086 (0-3)		12.02
L. multicaulis x *P. juncea*	7.48 (5-11)	5.26 (2-7)	1.49 (0-6)	6.75 (5-8)	0.01 (0-1)		12.03



Figs. 1-6 Chromosome pairing at metaphase-I in hybrids of *Leymus* species crossed with *Psathyrostachys* species. (1) *L. secalinus x P. huashanica,* 7 bivalents + 7 univalents. (2-3) *L. secalinus x P. juncea* : 2. 7 bivalents + 7 univalents, 3. 8 univalents + 5 bivalents + 1 trivalent. (4-5) *L. multicaulis x P. huashanica* : 4. 7 bivalents + 7 univalents, 5. 5 univalents + 5 bivalents + 2 trivalents. (6) *L. multicaulis x P. juncea*, 7 bivalents + 7 univalents.

Table 2. Chromosome pairing at the MI of the PMCs in intergeneric hybrids

Chromosome pairing IV	III	II	I	No. of cells observed	%
L. secalinus x P. huashanica					
		7	7	57	86.36
		6	9	5	7.58
		8	5	4	6.06
	Total			66	100.00
L. secalinus x P. juncea					
		7	7	59	86.76
		6	9	4	5.88
		5	11	1	1.47
	1	5	8	1	1.47
		8	5	3	4.11
	Total			68	99.99
L. multicaulis x P. huashanica					
		7	7	57	54.81
		6	9	19	18.27
		5	11	7	6.73
		4	13	1	0.96
		8	5	12	11.54
		9	3	1	0.96
	3	4	4	1	0.96
	1	6	6	5	4.81
	1	7	4	1	0.96
	Total			104	100.00
L. multicaulis x P. juncea					
		7	7	57	67.06
		6	9	19	22.35
		5	11	3	3.58
	1	6	8	1	1.18
		8	6	5	5.88
	Total			85	100.00

Leymus secalinus x *P. huashanica* had 21 chromosomes. The metaphase-I (MI) pollen mother cells of this hybrid gave a mean pairing configuration of 7.03 univalents + 5.91 ring bivalents + 1.08 rod bivalents (Table 1, Fig. 1). Chromosome pairing was examined in 66 metaphase-I cells, 57 cells, or 86.36% of total, had 7 bivalents and 7 univalents (Table 1). The metaphase-I pollen mother cells of *L. secalinus x P. juncea* gave a mean pairing configuration of 7.10 univalents + 5.99 ring bivalents + 0.93 rod bivalents + 0.01 trivalents. Of 68 metaphase I cells examined, 59 cells, or 86.76% had 7 bivalents and 7 univalents (Table 2, Fig 2). The ring bivalents were predominant. Trivalents were observed in one of the 68 cells (Fig. 3).

The metaphase-I pollen mother cells of *L. multicaulis x P. huashanica* gave a mean pairing configuration of 7.30 univalents + 4.15 ring bivalents + 2.54 rod bivalents + 0.086 trivalents (Table 1, Fig. 4-5). Chromosome pairing was examined in 104 metaphase I cells, the configuration of 7 bivalents and 7 univalents was observed in 54.81% of total cells (Table 2, Fig. 4). Chromosome pairing in the *L. multicaulis x P. juncea* hybrid averaged 7.48 univalents + 6.75 bivalents + 0.01 trivalents (Table 1). 85 metaphase-I cells examined, 57 cells, or 67.06% of the total had 7 bivalents and 7 univalents (Table 2, Fig. 6). Chromosome bridges at anaphase I and II were observed in this hybrid. PMCs with less than seven univalents were observed in all four intergeneric hybrids (Table 2, Fig. 5), which indicated that autosyndetic or homoeologous pairing occurred.

Discussion

The two diploid *Psathyrostachys* species, *P. huashanica* and *P. juncea* were crossed with *Leymus secalinus* and *L. multicaulis* to identify the N genome in *L. secalinus* and *L. multicaulis*. The average number of bivalents were 6.99 per cell in *L. secalinus x P. huashanica*, 6.92 per cell in *L. secalinus x P. juncea*, 6.69 bivalents per cell in *L. multicaulis x P. huashanica*, and 6.75 bivalents per cell in *L. multicaulis x P. juncea*, which indicated that one *L. secalinus* and *L. multicaulis* genome was closely homologous with the *P. huashanica* and *P. juncea* genome. Fewer than seven univalents were observed in these four cross combinations, which showed either autosyndetic or homoeologous pairing occurred.

Genomic and phylogenetic relationships of species can be supported by observation on F_1 hybrids whose parents include one common diploid tester. Therefore, phylogenetic studies of a genus with a higher ploidy level often commence with the establishment of the genomic relationships to diploid species. Many cytogenetic investigations have been carried out on intergeneric hybrids between species of *Leymus* and *P. juncea* (Dewey 1970, 1972a, 1972b; Wang & Hsiao 1984). Meiotic pairing in the hybrid *L. mollis x P. juncea* demonstrated that an N genome is present in *L. mollis* (Wang & Hsiao 1984). Cytological data have further shown that the North American *L. ambiguus* (syn: *Elymus ambiguus*) is an allotetraploid species with one N genome (Dewey 1976 used to designate N as J) closely homologous with the genome of *P. juncea* (syn: *Elymus junceus*), suggesting that *P. juncea*, or a precursor of *P. juncea*, was one of the original diploid parents of *L. ambiguus*. Dewey (1976) concluded that the N genomes in *P. juncea* and *L. ambiguus* are so nearly alike that one need not look beyond *P. juncea* for the source of the *L. ambiguus*

N genome. Chromosome pairing in the synthetic triploid hybrid *P. juncea x L. innovatus* leaves little doubt that one of the *L. innovatus* genomes came from *P. juncea*, either directly of indirectly (Dewey 1970). Cytological data on some *Leymus* species crossed with *P. juncea* led to the conclusion that *P. juncea*, or a precursor of *P. juncea*, was one of the original diploid parents of *Leymus* species (Dewey 1970, 1976; Wang & Hsiao 1984. However, each *Psathyrostachys* species with a modified form of N genome has been identified, at least in *P. huashanica, P. juncea* and *P. fragilis* (Dewey and Hsiao 1983; Bothmer it al. 1987; Wang 1987; Lu *et al*. 1990). Zhang & Dvorak (1991) examined variation in 26 repeated nucleotide sequence families isolated from four species of the Triticeae to investigate the origin of the tetraploid species of *Leymus*. Their results leave no doubt that the N genome of *Psathyrostachys* is in *Leymus*, and suggesting that it is currently unknown which *Psathyrostachys* species were involved in the hybridization that gave rise to *Leymus*. In the present study, data of meiotic pairing in the hybrids of *P. huashanica* crossed with *L. secalinus* and *L. multicaulis* are so similar to those in the hybrids of *P. juncea* crossed with *L. secalinus* and *L. multicaulis* that one cannot determine if the N genome in *L. secalinus* and *L. multicaulis* originated from *P. huashanica* or from *P. juncea*. It is not excluded that *P. huashanica* or another *Psathyrostachys* species also are possible donors of the *Leymus* N genome. Polyploid species may have complex origins derived from a single or multiple genomic donors. In phylogenetic studies, it is important to use a broad set of *Psathyrostachys* and *Leymus* species. With more species involved, more detailed information can be obtained, and consequently, a greater resolution of the relationships between *Psathyrostachys* and *Leymus* may be provided.

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Experimental Hybridization and Genome Analysis in *Elymus* L. Sect. *Caespitosae* and Sect. *Elytrigia* (*Poaceae: Triticeae*)

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ABSTRACT

Crossing experiments were performed between and within taxa of *Elymus* sect. *Elytrigia* and sect. *Caespitosae* from Iran and two taxa from Central Asia and China. The hexaploid *Elymus repens* (genomic constitution SSH) was crossed with the octoploid *E. elongatiformis*. The chromosome associations at meiosis show that *E. elongatiformis* possesses the SSH genome of *E. repens* as well as and additional genome of unknown origin. Crosses between different accessions of *E. libanoticus* (genomic constitution S) from W., NW. and N. Iran as well as crosses between *E. libanoticus* and accessions morphologically assignable to *Elytrigia gracillima* and *Elymus sosnovskyi* should therefore be merged into *E. libanoticus*. Crosses between *Elymus libanoticus* and the diploid *Elytrigia geniculata* ssp. *ferganensis* and *Elytrigia strigosa* ssp *aegilopoides* showed a high degree of meiotic pairing (c-values 0.6-0.8) confirming that these taxa have the genomic constitution S, as reported by Löve. The pollen fertility was zero in both hybrids. The configurations at metaphase I in a hybrid between *E. libanoticus* and *E. pertenuis* (genomic constitution SP) indicate that the two species may share the same version of the S genome.

INTRODUCTION

The study deals with crossing experiments within *Elymus* sect. *Caespitosae* (Rouy) Melderis and sect. *Elytrigia* (Desv.) Melderis, belonging to *Pseudoroegneria* and *Elytrigia s. str.*, respectively, in the classification based on genomic constitutions (Löve 1984). In accordance with Melderis (1980, 1985) and Assadi & Runemark (1995), a relatively broad generic concept is used.

MATERIALS AND METHODS

The species used in the crossing experiments are shown in Table 1.

Elytrigia geniculata ssp. *ferganensis* and *Elytrigia strigosa*

Table 1. Genomic constitutions of *Elymus* species used in the crosses.

Taxa	2n	Genomes	References	Origin
Elymus repens (L.) Gould	42	SSH	Assadi & Runemark 1994	Iran
—*elongatiformis* (Drobov) Assadi	56	SSHX	This study	Iran
—*libanoticus* (Hackel) Melderis	14	S	Dewey 1972	Iran
—*pertenuis* (C. A. Meyer) Assadi	28	SP	Assadi 1994a	Iran
Elytrigia geniculata (Trin.) Nevski ssp. *ferganensis* (Drobov) Tzvelev	14	S	This study	C. Asia
—*strigosa* (Bieb.) Nevski ssp. *aegilopoides* (Drobov) Tzelev	14	S	This study	C. Asia & China

ssp. *aegilopoides* are probably members of *Elymus* sect. *Caespitosae (Pseudoroegneria* according to Löve's (1984) classification based on genomic constitutions) but, since they belong to a critical species complex (Tzvelev 1976) which has not yet been satisfactorily revised, the author at present refrains from publishing new combinations under *Elymus*.

Information on the origin of the accessions used is available from the author on request. Voucher specimens are deposited in TARI and LD. For the methods used in seed germination, vernalization, mitotic and meiotic studies, crosses and pollen fertility tests, see Assadi & Runemark (1995). The c-values were calculated according to Wang (1989).

RESULTS

All successful crossing combinations are given in Table 2. The hybrid plants grew well and no hybrid weakness was observed. Table 3 shows the mean chromosome associations at meiotic metaphase I as well as chiasma frequencies and pollen fertility (percentage of stainable pollen grains) in the crossing combinations.

Elymus elongatiformis x E. repens

The parents are morphologically distinct (Assadi 1995a). *Elymus elongatiformis* is octoploid and *E. repens* is hexaploid. The hybrid was morphologically closer to *E. elongatiformis*, with lax spikes, ciliate sheaths, obtuse and mucronate glumes and lemmas, and mid spike internodes c. 7 mm long. The chromosome number was 2n=49. The anthers dehisced and the pollen fertility was 72%. A mean of 7.12 univalents, 20.68 bivalents and 0.14 multivalents and 38.18 chiasmata was observed in PMCs at metaphase I. Six to 12 lagging univalents were observed at anaphase I.

Elymus libanoticus intraspecific crosses

Elymus libanoticus is diploid and shows intraspecific variation in glume shape, stem indumentum and leaf width. Collections with narrow, 3-nerved glumes with an acuminate-subulate apex were similar to *E. sosnovskyi* and collections with thin, filiform leaves were similar to *Elytrigia gracillima* (Nevski) Nevski (e. g. accession H3729) described from the Caucasus. Accessions from N., NW., and W. Iran, representing different morphological variants, were used in the crossing experiments. All six crosses gave rise to vigorous hybrids, with pollen stainability ranging from 70-99%. A mean of 6.50 to 7.00 bivalents was observed at metaphase I. The chiasma frequency varied from 10.98 to 13.32.

Elymus libanoticus x E. pertenuis

Elymus libanoticus is a diploid with the genomic constitution S and *E. pertenuis* is a tetraploid with the genomic constitution SP. The hybrid was morphologically intermediate between the parents. The glumes were pointed, a possible influence from the P genome. The anthers did not dehisce and the pollen fertility was zero. The hybrid was triploid with an average of 6.36 univalents, 6.18 bivalents and 0.76 trivalents. Four lagging univalents were observed at anaphase I.

Table 2. Results of the crossing program in *Elymus* sect. *Elytrigia* and sect. *Caespitosae* (percentages are based on the number of flowers crossed).

Combinations	No. of combinations	No. of flowers	Seed set %	Embryo %	Plants %
Sect. *Elytrigia*					
Elymus elongatiformis x E. repens	1	18	33	-	17
Sect. *Caespitosae*					
E. libanoticus x E. libanoticus	6	135	19	15	15
E. libanoticus x E. pertenuis	1	26	77	19	23
E. libanoticus x E. sosnovskyi	1	12	17	17	8
E. libanoticus x Elytrigia geniculata ssp. *ferganensis*	1	18	67	61	44
E. libanoticus x Elytrigia strigosa ssp. *aegilopoides*	2	60	52	-	33
Elytrigia strigosa ssp. *aegilopoides* x *Elymus libanoticus*	1	20	25	-	5

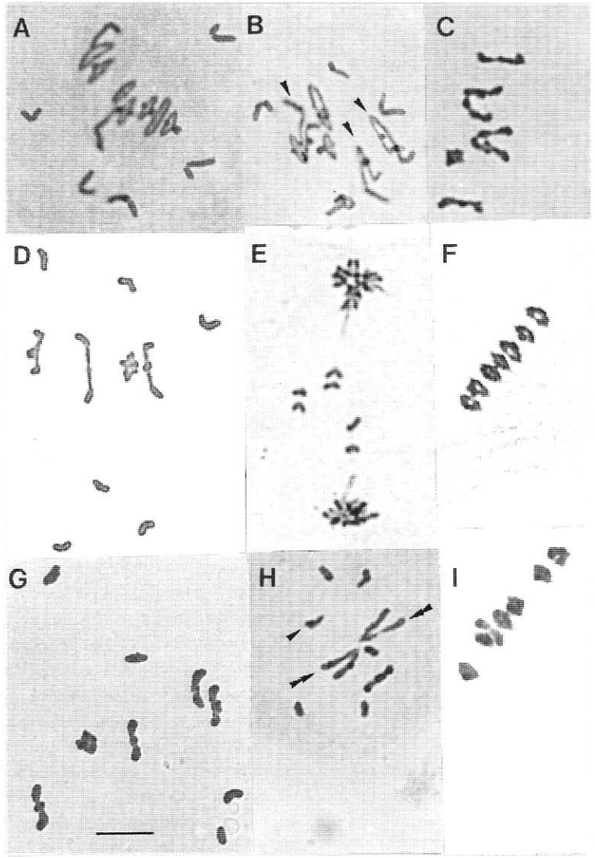

Figure 1 Meiotic configurations at metaphase I (A-D & F-I) and anaphase I (E) in *Elymus* species. (A-B): *E. libanoticus x E. pertienuis* (2n=21) with 7 univalents and 7 ring bivalents in A, and 6 univalents, 3 ring bivalents and 3 trivalents indicated by arrows in B. (C-E): *E. libanoticus x Elytrigia geniculata ssp. ferganensis* (2n+14) with 7 bivalents (6 rods and 1 ring) in C, 6 univalents and 4 bivalents (3 rods and 1 ring) in D, and 6 lagging univalents in E. (F): *Elymus sosnovskyi x E. libanoticus aegilopoides* with 4 univalents and 5 bivalents (4 rods and 1 ring) in G, 4 trivalents indicated by double arrows in H, and 7 bivalents (1 rod and 6 rings) in I. —Bar+ 10 mm.

Elymus libanoticus x E. sosnovskyi

Elymus sosnovskyi was described from material from a locality near the Iranian border in Turkey. It has been distinguished from *E. libanoticus* by its narrower 3-veined glumes with an acuminate-subulate apex (see Melderis 1985). The accession (H3741) used in the cross was collected very close to the type locality of *E. sosnovskyi*. Two hybrid combinations (representing reciprocal crosses) were produced. The hybrids were vigorous and had pollen stainability of 97 and 94%, respectively, and a mean of 6.98-7.00 bivalents (chiasma frequencies of 13.34 and 13.48) were observed at metaphase I.

Elymus libanoticus x Elytrigia geniculata ssp. *ferganensis*

The two taxa are allopatric, with *E. libanoticus* occurring in Lebanon, Turkey, Iraq, Iran and the Caucasus, while the other taxon is confined to Central Asia. Both taxa are diploids. The hybrid is morphologically closer to *Elytrigia geniculata* than to *E. libanoticus* and has scabrous culms and rachis, and lanceolate glumes with a broad membranous margin. The anthers did not dehisce and pollen fertility was zero. A mean of 2.95 univalents, 5.50 bivalents and 0.02 trivalents and a chiasma frequency of 8.62 was found at metaphase I. In 9 of the 50 cells studied 7 bivalents were observed.

Elymus libanoticus x Elytrigia strigosa ssp. *aegilopoides*

Elytrigia strigosa ssp. *aegilopoides* occurs in Siberia, Central Asia and China, far from the distibutional area of *Elymus libanoticus*. Both taxa are diploids. The hybrids were intermediate between the parents and vigorous. The anthers did not dehisce and the pollen fertility was zero. Three crossing combinations, including a reciprocal one, were made. A mean of 6.26, 5.52, and 6.52 bivalents and a chiasma frequency from 8.36 to 11.24 were observed in the hybrids.

DISCUSSION

Sect. *Elytrigia*

Elymus repens is hexaploid with the genomic constitution SSH (Assadi & Runemark 1995), while *E. elongatiformis* is octoploid. A mean of 7.12 univalents in the chromosome association of the hybrid *E. repens x elongatiformis* indicates that *E. elongatiformis* has the same genomic constitution as *E. repens* plus an additional unknown genome. Therefore, the genomic constitution of *E. elongatiformis* is designated as SSHX. The genomic configuration of the hybrid in the present study agrees with that given by Dewey (1980).

Sect. *Caespitosae*

All the taxa of the section used in the crosses belong to the genomically-defined genus *Pseudoroegneria* (cf. Löve's (1984) classification) which consists of c. 15 species in Asia and W. North America. According to Dewey (1984), hybrids between diploid species of *Elymus* have an almost complete bivalent pairing at metaphase-I but are completely sterile, indicating different versions of the same basic genome (S).

Elymus libanoticus is a morphologically variable diploid. Meiotic pairing was regular or almost regular and pollen fertility was high in the seven crosses between accessions from N., NW., and W. Iran, which included morphological variants similar to *Elytrigia gracillima* and *Elymus sosnovskyi*. The results of the present study indicate that *Elytrigia gracillima* and *Elymus sosnovskyi* should be included in *E. libanoticus* (see Assadi 1995b).

Elymus libanoticus was also crossed with the diploid *Elytrigia geniculata* ssp. *ferganensis* and *Elytrigia strigosa* ssp. *aegilopoides*. At metaphase-I means of 5.50 to 6.52 bivalents were observed and c-values ranged from 0.6 to 0.8. The complete pollen sterility of the hybrids, as well as a somewhat incomplete meiotic pairing, indicates that the *Elytrigia* species have different versions of the S genome compared to *E. libanoticus*.

Diploid *Agropyron cristatum* (L.) Gaertner, with the genomic constitution P, has been recorded from NW. Iran (Dewey and Asay 1975). *Elymus libanoticus*, with the genomic constitution S, has a relatively large distributional area from Lebanon to Turkey, Iraq, W., NW., and N. Iran and the Caucasus. *Elymus pertenuis*, with the genomic constitution SP, is confined to the Caucasus, NW., and W. Iran. From the present-day distribution pattern it seems reasonable to assume that *E. pertenuis* is an amphidiploid between equivalents to *E. libanoticus* and diploid *Agropyron cristatum*. The high number of bivalent in the hybrid *E. libanoticus x E. pertenuis* supports the suggested amphidiploid origin of *E. pertenuis*. However, this evidence is not conclusive, since the meiotic pairing may have been influenced by homoeologic pairing between chromosomes of the S and P genomes (see Wang 1989).

Table 3. Meiotic configurations and pollen fertility in the different hybrid combinations within and between *Elymus* species. Haploid genomic constitutions are given in parenthesis.

Name of parents and accessions ♀ x ♂	No. of cells	Mean and range of chromosome association and chiasmata/cell							Pollen %
		I	II			III	IV	Chiasmata	
			Total	Rods	Rings				
Elymus elongatiformis x E. repens (SSHX x SSH)									
H3727 x H3736	50	7.12 (1-13)	10.68 (19-24)	3.66 (0-9)	17.02 (11-20)	0.04 (0-1)	0.10 (0-1)	38.18 (31.43)	72
Elymus libanoticus x E. libanoticus (S x S)									
H3729 x H3751	50		7.00 (7)	1.20 (0.3)	5.8 (4.7)			12.80 (11-14)	93
H3729 x H3755	50	0.12 (0-2)	6.94 (6-7)	1.32 (0-4)	5.62 (3-7)			12.56 (10-14)	88
H3735 x H3754	50	0.12 (0.2)	6.94 (6-7)	0.68 (0-3)	6.26 (4-7)			13.20 (11-14)	89
H3755 x H2729	50		7.00 (7)	0.78 (0-3)	6.22 (4-7)			13.22 (11-14)	70
H3778 x H3735a	50		7.00 (7)	0.68 (0-3)	6.32 (4-7)			13.32 (11-14)	93
H3778 x H3787	50	1.00 (0-6)	6.50 (4-7)	2.02 (0-4)	4.48 (1-7)			10.98 (10-14)	99
Elymus libanoticus x E. pertenuis (S x SP)									
H3755 x H3733	50	6.36 (3-9)	6.18 (3-8)	1.16 (0-3)	5.02 (3-8)	0.76 (0-3)		12.74 (10-16)	0
Elymus libanoticus x E. sosnovskyi (S x S)									
H3755 x H3741	50		7.00 (7)	0.66 (0-3)	6.34 (4-7)			13.34 (11-14)	94
Elymus sosnovskyi x E. libanoticus (S x S)									
H3741 x H3751	50	0.04 (0-2)	6.98 (6-7)	0.48 (0-3)	6.50 (4-7)			13.48 (11-14)	97
Elymus libanoticus x Elytrigia geniculata ssp. *ferganensis* (S x SP									
H3751 x H10230	50	2.94 (0-8)	5.50 (3-7)	2.42 (0-6)	3.08 (0-7)	0.02 (0-1)		8.62 (5-14)	0
Elymus libanoticus x Elytrigia strigosa ssp. *aegilopoides* (S x S)									
H3755 x H10094	50	1.14 (0-8)	6.26 (3-7)	2.34 (0-6)	3.92 (1-6)	0.06 0-2)	0.04 (0-1)	10.42 (5-13)	0
H3754 x H7712	50	2.84 (0-8)	5.52 (1-7)	2.76 (0-5)	2.76 (0-5)	0.04 (0-2)		8.36 (3-11)	0
Elytrigia: strigosa ssp. *aegilopoides x Elymus libanoticus* (S x S)									
H10094 x H3729	50	0.96 (0-4)	6.52 (5-7)	2.80 (0-7)	4.72 (0-7)			11.24 (5-14)	0

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Genome Symbols in the Triticeae (Poaceae)

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ABSTRACT

A system for the application of nuclear genome symbols in the tribe Triticeae is proposed. It is based mainly on prevailing symbols. In agreement with this, the system uses individual upper case letters as symbols in the first place. Since the number of basic nuclear genomes in the Triticeae exceeds the number of single letters in the Roman alphabet, some basic genomes are designated with an upper case letter followed by a lower case letter, e.g. **Ns** for the genome of *Psathyrostachys*. Superscripts in small letters are used when modified versions of a basic genome are referred to, e.g. $\mathbf{H}^{\mathbf{p}}$ for the genome found in *Hordeum pusillum*. Unknown or equivocally identified genomes are designated by **X** followed by a lower case letter, e.g. **Xu** for *Hordeum murinum*. Underline of the relevant genome symbol can be used to indicate the origin of the cytoplasm.

PROPOSAL

Classification of the Triticeae based on genome relationships has over the years been a matter of controversy, especially between taxonomists and cytogeneticists (Löve 1984, Baum et al. 1987, Gupta and Baum 1989, Kellogg 1989, Seberg 1989). Today there is, however, no disagreement as to the conceptual ideas of genomes *per se* as defined by several authors (Löve 1982, Alonso and Kimber 1983, Kimber and Zhao 1983, Dewey 1984). In the Triticeae the genomes of the various genera, or groups of species, are more or less similar as indicated by the variation in chromosome pairing ability at meiotic metaphase I in interspecific or intergeneric hybrids. The genomic affinities may vary from complete pairing, i.e., homology, to no pairing, i.e, non-homology, with various intergrades, i.e., homoeology.

One practical aspect which has created problems among Triticeae researchers is the designations of individual basic genomes. Traditionally, each genome has been designated with a single, upper case letter A-Z. Because of the large number of basic genomes in Triticeae, the number of letters in the Roman alphabet is insufficient for covering all basic genomes of the tribe. Further, various authors have used different symbols for the same genome and in some cases different basic genomes have been designated with the same symbol. Especially, there have been confusion between the genome designations used by scientists studying wheat and related species, and those used by researchers working with other groups in the Triticeae. Moreover, various authors have assigned the letters X and Y to the unidentified genomes of several species or unrelated groups of species. Since the knowledge of genome relationships in the tribe and the need to use intergeneric hybridization for cereal improvement are rapidly increasing, there is an increasing demand for a standardization of the genome symbols.

In this paper we propose a system of assigning basic genome symbols that may be acceptable to all scientists working with the Triticeae. Instead of proposing a

Table 1. Genome symbols in the Triticeae

Genus or Species	Previous designation	Reference	Suggested designation	Reference
Agropyron	P	Löve 1984	**P**	
Heteranthelium	Q	Löve 1984	**Q**	
Crithopsis	K	Löve 1984	**K**	
Taeniatherum	T	Löve 1984	**Ta** [1]	
Hordeum vulgare	I	Löve 1984	**I**	
H. bulbosum	H	Löve 1984	**I**	Dewey 1984
H. marinum	H	Löve 1984	**Xa** [2]	Bothmer et al. 1986
H. murinum	H	Löve 1984	**Xu**	Bothmer et al. 1987, 1988a,b
other *Hordeum* species	H	Löve 1984	**H**	
Hordelymus	HT	Löve 1984	**XoXr** [3]	Bothmer et al. 1994
Festucopsis	G	Löve 1984	**L** [4]	
Peridictyon sanctum	(in *Festucopsis*)	Löve 1984	**Xp** [4]	Seberg et al. 1991
Australopyrum	W	Löve 1984	**W**	
Pseudoroegneria	S	Löve 1984	**St** [1]	
P. pertenuis	SP	Löve 1984	**StP**	Wang et al. 1986; Assadi
P. deweyi	SP	Jensen et al. 1992		
P. geniculata ssp. scythica	SS	Löve 1984	**E^eSt**	Liu & Wang 1993b
Psathyrostachys	N	Löve 1984	**Ns**	
Thinopyrum bessarabicum	J	Löve 1984	**E^b** [6]	Wang 1985
T. junceiforme	JJ	Löve 1984	**E^bE^e**	Liu & Wang 1992
T. sartorii	JJ	Löve 1984	**E^bE^e**	Liu & Wang 1992
T. distichum	JJ	Löve 1984	**E^bE^e**	Liu & Wang 1993a
T. junceum	JJJ	Löve 1984	**$E^bE^bE^e$**	Liu & Wang 1993a
Lophopyrum elongatum	E	Löve 1984	**E^e** [6]	Wang 1985
L. caespitosum	EE	Löve 1984	**E^eSt**	Liu & Wang 1989, 1993b
L. curvifolium	EE	Löve 1984	**E^bE^b**	Liu & Wang 1993a
L. nodosum	EE	Löve 1984	**E^eSt**	Liu & Wang 1993b
L. scirpeum	EE	Löve 1984	**E^eE^e**	Liu & Wang 1993a
Trichopyrum	ES	Löve 1984	**E^eSt**	
T. intermedium	EES		**E^eE^eSt**	Liu & Wang 1993b
			E^bE^eSt	Xu & Conner 1994
Elymus sibiricus	SH	Löve 1984	**StH**	
E. caucasiucus	SH	Löve 1984	**StY** [5]	Jensen & Wang 1991
E. drobovii	SH	Löve 1984	**StHY**	Dewey 1980
E. batalinii	SH	Löve 1984	**StPY**	Jensen 1990
E. scabrus	SH	Löve 1984	**StWY**	Torabinejad & Mueller 1993
E. transhyrcanus			**StStH**	Dewey 1972
Kengyilia	(included in *Elymus*)	Löve 1984	**StPY**	Yen & Yang 1990
Leymus	JN	Löve 1984	**NsXm** [7]	Zhang & Dvorak 1990
				Wang & Jensen 1994
Elytrigia	SX	Löve 1984		
E. repens			**StStH** [8]	Assadi & Runemark 1994
				Vershinin et al. 1994
Psammopyrum	GJ	Löve 1984	**LE**	
Pascopyrum	SHJN	Löve 1984	**StHNsXm** [7]	Zhang & Dvorak 1990
Crithodium	A	Löve 1984		
Triticum monococcum			**A^m**	Dvorak et al. 1993
T. urartu			**A^u**	Dvorak et al. 1993
Sitopsis	B		**S**	Kimber & Tsunewaki 1988
Aegilops speltoides			**S**	
Ae. bicorn			**S^b**	

Table 1. Genome symbols in the Triticeae

Genus or Species	Previous designation	Reference	Suggested designation	Reference
Ae. longissimum			**S^l**	
Ae. sharonensis			**S^l**	
Ae. searsii			**S^s**	
Orrhopygium	C	Löve 1984		
Aegilops caudata			**C**	
Patropyrum	D	Löve 1984		
Aegilops tauschii			**D**	
Comopyrum	M	Löve 1984		
Aegilops comosa			**M**	
Amblyopyrum	Z	Löve 1984		
Aegilops mutica			**T**	Kimber & Tsunewaki 1988
Chennapyrum	L	Löve 1984		
Aegilops uniaristata			**N**	Kimber & Tsunewaki 1988
Kiharapyrum	U	Löve 1984		
Aegilops umbellutata			**U**	
Secale	R	Löve 1984	**R**	
Dasypyrum	V	Löve 1984	**V**	
Eremopyrum	F	Löve 1984	**F, Xe** [9]	Frederiksen & Bothmer 1989
Henrardia	O	Löve 1984	**O**	
Gigachilon	AB	Löve 1984	**A<u>B</u>**	Kimber & Tsunewaki 1988
Triticum durum	AB	Löve 1984	**A^u<u>B</u>**	Dvorak et al. 1993
T. timopheevii	AB	Löve 1984	**A^u<u>G^l</u>**	Dvorak et al. 1993
T. zhukovskyi	AAB	Löve 1984	**A^mA^u<u>G^l</u>**	Dvorak et al. 1993
Triticum	ABD	Löve 1984	**A^u<u>B</u>D**	
*T. ventricosum**	DM	Löve 1984	**<u>D</u>N**	Kimber & Tsunewaki 1988
T. recta **	MMU	Löve 1984	**<u>U</u>MN**	Kimber & Tsunewaki 1988
			<u>U</u>MX	Yen & Kimber 1992
T. syriacum			**<u>D</u>MS**	Kimber & Tsunewaki 1988
			<u>D</u>$^cS^sX$	Zhang & Dvorak 1992
Aegilemma	BU	Löve 1984	**<u>US</u>**	Kimber & Tsunewaki 1988
Aegilops variabilis			**<u>US</u>^{l}**	Zhang et al. 1992
Cylindropyrum	CD	Löve 1984	**<u>CD</u>**	
Aegilops cylindrica			**<u>CD</u>**	
Aegilopodes	CU	Löve 1984	**<u>UC</u>**	Kimber & Tsunewaki 1988
Aegilops triuncialis			**<u>UC</u>**	
Gastropyrum	DM	Löve 1984	**<u>D</u>M**	
Aegilops crassa (4x)			**<u>D</u>^{c}Xc**	Zhang & Dvorak 1992
Ae. crassa (6x)			**D<u>D</u>^{c}Xc**	Zhang & Dvorak 1992
Aegilonearum	DMU	Löve 1984		
Aegiops juvenale			**<u>D</u>^{c}ZcU**	McNeil et al. 1994
Aegilops	MU	Löve 1984	**<u>UM</u>**	Kimber & Tsunewaki 1988
Aegilops ovata			**<u>UM</u>**	
Ae. biuncialis			**<u>UM</u>**	
Ae. columnaris			**<u>UM</u>**	
Ae. triaristata			**<u>UM</u>N**	

*included in *Gastropyrum* by Löve (1984).
**inclucded in *Aegilops* by Löve (1984).
[1] to [9] see comments.

completely new system, the suggested system builds on the most prevalent, presently used designations. Only when there is an overlap or a controversy between various systems do we suggest new symbols or change of symbols. The symbols proposed (Table 1) are basically those used by Löve (1984) in his classification with minor modifications (e.g. Kimber and Tsunewaki 1988). Löve's system is based on the prerequisite that a genus should consist species of the same genome constitution. His system of nomenclature is not endorsed here for a formal taxonomic classification system of the Triticeae. We use it merely as a framework for listing different basic genomes and combinations of genomes.

We propose the following basic rules for the designations of genome symbols in the Triticeae:

1. Genome symbols should be written in **bold face**.
2. Different basic genomes in Triticeae (with x=7), defined as having less than 50% of complete meiotic pairing, i.e. c 0.5, in a diploid hybrid in the absence of the Ph or other pairing promoter/suppressor gene effect, should be designated with different symbols.
3. Single upper case letters of the Roman alphabet (A-Z) should, as far as possible, be used as symbols for basic genomes (see Table 1).
4. Since all upper case letters of the alphabet are now occupied, additional basic genomes should be designated by an upper case letter followed by a lower case letter.
5. The genome designation of a polyploid taxon should be given as a combination of the symbols of the constituent basic diploid genomes.
6. Unknown or unverified genomes should be designated with the letter **X** followed by a lower case letter (e.g., **Xu** for *Hordeum murinum*). When a genome has been sufficiently identified as distinct from all other established basic genomes, it should be given a permanent basic genome symbol.
7. The letter **Y** has previously been used to designate unknown genomes. However, it has been extensively used as the designation of one basic genome present in some species of the polyploid genus *Elymus*. The diploid donor species for **Y** has not yet been identified. We propose that the designation **Y** is retained for this basic genome.
8. Modified versions of a basic genome should be designated by superscripts in small letters indicative of the species carrying such modified genomes. Further modifications may be indicated by superscripted numeric numbers.
9. When previously unrecogized basic genomes are identified, genome symbols should be assigned in accordance with this system.
10. A genome symbol may be underlined to indicate the origin of the cytoplasm of an alloploid species.
11. From this date (1996) on, the designations given in Table 1 should have priority over younger ones.

Comments (cf. Table 1)

1. The symbols **S, T, N** and **G** have been used in two different senses (cf. Love 1984, Kimber and Tsunewaki 1988). Therefore, the three former symbols are replaced by the symbols **St, Ta,** and **Ns**, to designate the genomes of species of the genera *Pseudoroegneria, Taeniatherum* and *Psathyrostachys*, respectively. For **G**, see comment 4 below.
2. The genomes of *Hordeum marinum* and *H. murinum* are given the symbols **Xa** and **Xu**, respectively, to indicate that they are different enough from **H** to deserve different basic genome symbols, but still being imperfectly known.
3. The two genomes in *Hordelymus* have not been unequivocally identified, thus they are temporarily assigned the symbols **Xo** and **Xr**.
4. Since the new genus *Peridictyon* split from *Festucopsis* by Seberg et al. (1991) has not been studied by chromosome pairing, the genome is assigned **Xp** for now. *Festucopsis* is not the donor of the **G** genome in *Triticum timopheevi*; therefore, the genome symbol of *Festucopsis* is changed to **L**.
5. **Y** is retained as the symbol for a basic genome of an unidentified diploid species that contributed a genome to some species of the polyploid genus *Elymus*.
6. The **E** genome is present in *Thinopyrum*, *Lophopyrum*, and *Trichopyrum* (Liu and Wang 1992, 1993a, 1993b) in combination with **J** and **St**. Because **J** is closely related to **E** (Wang 1985) and **E** has been extensively used by wheat workers (Dvorak 1980), we propose the change of **J** to **E**. Most existing evidence (for reservation, see Jauhar 1990) from diploid c values, the triploid trivalent frequency, triploid x values, the multivalent frequency in amphidiploids, and *in situ* hybridization results indicates that **J** and **E** are closer to each other than the genomes of *Hordeum vulgare* and *H. bulbosum*. Because the genomes in these two *Hordeum* species have the same basic genome symbol, we must also use a single basic genome symbol for *Thinopyrum bessarabicum* and *Lophopyrum elongatum*.
7. Because the presence of **J**(=**E**) in *Leymus* and *Pascopyrum* is in doubt (Zhang and Dvorak 1991, Wang and Jensen 1994), it is proposed to replace **JNs** with **NsXm** until **Xm** is experimentally identified.
8. Newer results indicate that the formerly unknown X genome in *Elytrigia repens* is actually an **H** genome (Assadi and Runemark 1995, Vershinin et al. 1994). The genome combination of *E. repens* is thus **StStH** and is identical to a group of species in *Elymus*.
9. The genus *Eremopyrum* probably comprises two different genomes (Sakamoto 1979). They are assigned the symbols **F** and **Xe**.

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Physical Mapping of Micronutritional Genes in Wheat-rye Translocations

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ABSTRACT

In rye (*Secale cereale* L.), there are loci on chromosome arm 5RL which give rise to increased copper (Cu)- and iron (Fe)-efficiency, respectively. Four different wheat-rye translocations each harboring a terminal segment of different size of the rye chromosome arm 5RL were identified by test crosses and Giemsa-banding: 'T29' (5AS.5RL), 'T63' (5BS.5BL-5RL), 'Vhn' (4BS.4BL-5RL) and 'Cor' (4BS.4BL-5RL). The translocation break points were detected by chromosome painting technique GISH and the sizes of the rye chromosome segments involved were determined by computer image analysis. The Cu-efficiency gene *Ce* was physically mapped to the terminal region of 5RL, and the genes for mugineic acid and for hydroxymugineic acid synthetases involved in the strategy II of Fe-efficiency control to two intercalary regions of 5RL. In all wheat-rye translocation lines the *Ce* gene is linked to the dominant hairy neck character (*Ha1*) from rye. This morphological trait and the RFLP probe 'WG199' as well can serve as proper markers for a marker-based large-scale selection in wheat breeding.

INTRODUCTION

Cereals differ considerably in their efficiency to acquire and/or metabolize micronutrients (Snowball and Robson 1984, Podlesak *et al*. 1990). Genes influencing the micronutritional system are clustered on the homoeologous chromosome groups 4 and 5 (Mori and Nishizawa 1989, Schlegel *et al*. 1991). In rye (*Secale cereale* L.), loci on chromosome arm 5RL were found to control the response to Cu- and Fe-shortage stress (Graham *et al*. 1987, Mori *et al*. 1990, Schlegel *et al*. 1993). These genes may be used as suitable sources for crop improvement by chromosome engineering in alien species, especially in wheat for cultivation on marginal soils (Graham 1984). Here, we report the physical mapping of the Cu-efficiency gene *Ce* to the terminal and the genes for mugineic acid and for hydroxymugineic acid synthetases (*Mas* and *Hmas*) involved in Fe-efficiency control to the intercalary regions of 5RL. We also present the development of genetical and molecular markers as the main prerequisite for a marker-based large-scale selection of micronutritionally efficient genotypes in plant breeding programs.

MATERIALS AND METHODS

Plant genotypes were obtained from a cytogenetic tester stock collection of the Cereals Cytogenetics Group in Gatersleben: The rye, *Secale cereale* L., 'PC361' is an inbred line and originated from a selected self-fertile mutant of 'Petkus Spring'. The wheat, *Triticum aestivum* L., 'Chinese Spring' came from the Gatersleben Germplasm Bank. The wheat-rye translocation line (WRT) 'T29' harbouring the 5AS.5RL chromosome was kindly provided by T. Miller (Norwich, UK). The WRTs 'T63' (5BS.5BL-5RL) and 'Cor' (4BS.4BL-5RL) were kindly provided by J. P. Gustafson (Columbia, USA). The WRT 'Vhn' arose from a single selection of the wheat 'Viking' and carries the 4BS.4BL-5RL chromosome.

Copper efficiency was analysed on 40 plants per line and variant grown in pots in the greenhouse as described by Schlegel *et al*. (1991), except that the copper treatments were modified to 3 mg Cu per pot (deficiency variant) and 60 mg Cu per pot (sufficiency variant). At maturity, the three main spikes of each plant were harvested for grain yield (GY) measurements. Statistical calculations were evaluated using the F- and the t-Test.

Iron efficiency was analysed on 35 plants per line and

variant germinated and grown for 7 days in deionized water followed by a hydroponic culture in nutrient solutions according Römheld and Marschner (1986) with either Fe-absence (deficiency variant) or presence of 100 M Fe-EDTA (sufficiency variant) for 21 days. The chlorophyll was extracted (Arnon 1949), and the contents of chlorophylls A and B per mg fresh matter were determined using a DU650 spectrophotometer (Beckman).

Phytosiderophores (PSs) were analysed from root exudates of three successive collections by HPLC (Mori *et al*. 1987) and the portions of DMA (2'-deoxymugineic acid), MA (mugineic acid) and HMA (3-hydroxymugineic acid) determined. Exudates were collected after the Fe-deficiency chlorosis became visible in the plants cultivated in a continuously aerated nutrient solution as described by Marschner *et al*. (1987).

Chromosome painting was accomplished by GISH (genomic *in situ* hybridization) to squashed mitotic metaphases and interphase nuclei as well. Per slide, 0.1 g of labelled total genomic DNA of rye together with 3 g unlabelled wheat DNA as competitor were applied in 50 % formamide, 2xSSC, 0.1 % SDS, 10 % dextran sulfate. Slides were washed to a stringency greater than 85 %. Conditions of labelling, hybridization and detection were chosen as described in detail by Heslop-Harrison *et al*. (1991).

RFLP analyses were carried out using Southern blot hybridizations with the 3.3 kbp long, low copy, genomic probe WG199 (Heun *et al*. 1991) onto Pst I-, Dra I- and Sst I-digested total DNA of the plant genotypes mentioned above under the experimental conditions described by Anderson *et al*. (1992).

RESULTS AND DISCUSSION

Although, in comparison to wheat, rye cultivars are preferably planted on light, sandy clay soils with bad nutrient supply, severe iron shortage induced a considerable decrease in fresh matter production of young rye shoots (Tab. 1). Whereas, the grain yields of rye (PC361) demonstrated a higher tolerance against copper shortage than those of wheat (CS) did. Moreover, the presence of rye chromatin of the 5RL arm improves the copper efficiency in each WRT (Tab. 1). That indicates the presence of the *Ce* gene in each WRT. The difference between the decrease in fresh matter production of rye (46 %) and the decrease in that of wheat (26 %) is highly significant. The wheat-rye translocations (WRTs) reduced their production of fresh matter more than the "translocation-free" 'Chinese Spring' wheat and also more than rye, except the 'T63' line which behaved in an intermediate way between wheat and rye (Tab. 1). Obviously, the decrease reaction to iron shortage stress in the WRTs was enhanced beyond the level of rye itself by either the presence of the rye and/or absence of the wheat chromatins. At sufficient iron supply, however, the translocated segments of the 5RL arms evidently accelerated the growth of the WRTs during the first weeks. Therefore, these WRT types can apparently be used to improve the seedling emergence in wheat.

Since the shoot fresh matter amount does not solely reflect iron efficiency, the symptom of mild chlorosis (Marschner *et al*. 1987) was substantiated by determining the chlorophyll contents (Tab. 1). The response to Fe-shortage varied among the WRTs (36 - 1 %). Their efficiencies were elevated by genes from the 5RL arm. This gradation fits nicely with the results from the analyses of

Table 1. Grain yield (GY), fresh matter (FM) and chlorophyll contents of shoots GY determined from mature plants; FM and chlorophyll (A+B) measured from 28 day old plants; [a] 60 mg Cu/pot; [b] 3 mg Cu/pot; [c] 100 uM Fe-EDTA; [d] no iron.

	Genotype					
	PC361	CS	T29	T63	Vhn	Cor
g GY/main spikes [a]	3.11	4.41	4.54	3.93	5.84	4.27
g GY/main spikes [b]	2.67	0.79	2.72	2.71	4.32	2.86
% GY decrease	14	82	40	31	26	33
mg FM [c]	289	633	1202	1093	1030	930
mg FM [d]	160	467	470	688	448	502
% FM decrease	46	26	61	37	56	46
ng(A+B)/mg FM [c]	729.9	650.53	405.4	408.41	547.12	569.01
ng(A+B)/ mg FM [d]	271.71	510.82	348.97	405.24	497.75	363.92
% (A+B) decrease	63	22	14	1	9	36
A:B ratio [c]	4.1:1	2.7:1	1.8:1	2.1:1	1.4:1	1.7:1
A:B ratio [d]	4.3:1	4.1:1	4.0:1	4.1:1	4.0:1	4.4:1

Table 2. Phytosiderophore (PS) exudation after Fe-shortage in root dry matter (DM) DMA: 2'-deoxymugineic acid; MA: mugineic acid; HMA: 3-hyroxymugineic acid.

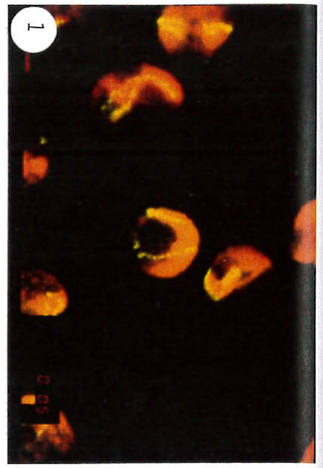
	Genotype					
	PC361	CS	T29	T63	Vhn	Cor
g root DM	0.479	1.351	1.198	0.817	3.670	1.046
µmol PS/g root DM	6.71	32.84	28.72	26.20	42.10	34.21
µM DMA	5.62	1154.48	*	1352.40	2843.38	2408.82
µM MA	23.26	0	*	133.62	44.77	0
µM HMA	234.73	0	*	33.00	0	0

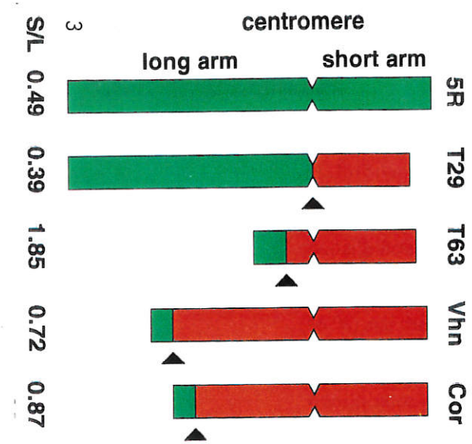
exudated Phytosiderophores (Tab.2). 'T63' shows the highest HMA and MA exudation of the WRTs investigated and the lowest decrease of chlorophyll content (Tab. 1), while 'Cor' reacts like the control 'Chinese Spring' with respect to MA and HMA, but with more than a doubled production of DMA (Tab. 2) connected with the highest decrease of chlorophyll content after rye (Tab. 1).

PSs are essential parts of the strategy II system for the mobilization of Fe^{III} ions via chelating in the rhizosphere and transmission into the apical root zones (Römheld and Marschner 1986, Marschner *et al*. 1986, 1987, Treeby *et al*. 1989). The genes of the synthetases for MA and HMA ('*Mas*' and '*Hmas*') were localized together on chromosome 5R by Mori *et al*. (1990). The translocation points of WRTs 'T63', 'Vhn' and 'Cor' "break" this linkage group and enable the genes to be physically mapped to two defined interstitial regions. GISH was used to paint the different 5RL chromosome segments of rye in the alien genomic background of the wheat in interphases (Fig. 1) and metaphases (Fig. 2) of the WRTs. The wheat translocation segments were identified by test crosses and Giemsa-banding. The translocation break points were confirmed by computer image analysis and fixed the sizes of the 5RL-segments as follows: 'T29' = 1 Plu (=100 cPlu), 'T63' = 13.5 cPlu, 'Vhn' = 8.4 cPlu, and 'Cor' = 8.2 cPlu of the 5RL arm.

Assuming that the full length of a 5RL chromosome arm as the reference comprises 1 Plu (Physical length unit), the size of the region accounts for 0.2 cPlu (centiPlu) for *Hmas*, 8.2 cPlu proximal to the chromosome end. For *Mas* the region size is 5.1 cPlu, 8.4 cPlu proximal to the chromosome end (Fig. 3 and 4).

The genes *Ha1* and *Ce* are linked on the translocated chromosome segments from rye. In all WTRs the hairy neck character is expressed together with the improvement of copper efficiency (Tab. 1), though with different intensity. This can be attributed to the different biological origins of the 5RL segment donors. Nevertheless, the hairy neck character marks an increased Cu-efficiency in every WRT, even within the smallest segment of the 'Cor' line (Tab. 1 and Fig. 5b). To omit labour consuming pot experiments for direct Cu-efficiency selection and waiting till heading for hairy neck screening, a suitable RFLP probe was used as a molecular marker for both genes, *Ce* and *Ha1*. Fig. 6 shows the polymorphic bands of the 'WG199' probe onto Pst I digested DNA of 'Chinese Spring' (W), 'PC361' (R), 'T29' (a), 'T63' (b), 'Vhn' (c) and 'Cor' (d). A 5.5 kb fragment in (b) marks the rye segment of 'T63'. In the other WRTs, however, the rye specific fragments were polymorphic (7.8 kb in (c) and (d)). An 8 kb fragment was observed in wheat and all WRTs except (d) and is therefore located at the distal end of 4BL. The presence of both fragments, the 7.8 kb rye specific and the 8.0 kb wheat specific fragment in (c) indicates, that the rearranged 4BS.4BL-5RL chromosome of 'Vhn' includes a duplication of an evolutionary modified fragment (Devos et al. 1993). Thus, the more than double of DMA production compared to Chinese Spring (Tab. 2) could be interpreted as dosage effect. Because of the cosegregation of the 'Vhn' specific fragment obtained with WG199 in an F_2 derived from (Vhn x wheat) with the *Ha1* this probe is a molecular marker for Cu-efficiency as well.





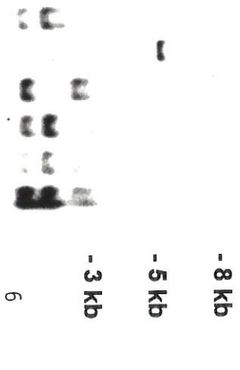


Fig. 1: bright yellow strings label the painted rye arm pairs in T29 interphases.

Fig. 2: T63 metaphase with brightly yellow painted terminal rye segments.

Fig. 3: Karyogramme of 5R and translocated chromosomes, wheat chromatin is labelled in red, while the rye segments involved are labelled green. Arrow heads mark break points.

Fig. 4: Examples of 'painted chromosomes' from the different WRTs.

Fig. 5a: Spike morphology of 'Chinese Spring' and WRTs. Notes: tip-awned ear of T29 indicates the lost of the awn inhibitor gene *'B1'*. All WRTs show the hairy neck character from rye (*'Ha1'*).

Fig. 5b: Magnification of the peduncles of the same plants.

Fig. 6: Southern blot of Pst I digested DNA of 'Chinese Sping' (W), 'PC361' (R), 'T29' (a), 'T63' (b) 'Vhn' (c) and 'Cor' (d) probed with 'WG199'.

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Chromatin Characterization in *Dasypyrum*

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ABSTRACT

An open pollinated natural population and an inbred line of *Dasypyrum villosum* were cytologically examined. Nuclear DNA content, chromosomal distribution of the C-banded heterochromatin and the chromosomal site of action of restriction endonucleases were investigated. The results demonstrate that in *D. villosum* two classes of heterochromatin exist with different chromosomal location and reacting properties. One fraction of heterochromatin appears to be more affected by individual variation than the other. Preliminary examination of the chromosomal characteristics of *D. breviaristatum* indicate that the evolution of this latter species involved a more complicated process than the simple duplication of its chromosome number.

INTRODUCTION

The genus *Dasypyrum* comprises two Mediterranean species: *D. villosum* (L.) Candargy (formerly *Haynaldia villosa* Schur), annual, 2n= 2x= 14, widely spread in the coastal areas of the Mediterranean region, and *D. breviaristatum* (Lind. f.) Frederiksen (formerly *D. hordaceum* Candargy), perennial, 2n= 4x= 28, whose range is limited to Morocco, Algeria and Greece (Frederiksen 1991).

Dasypyrum villosum is considered an important source of genes for powdery mildew resistance, seed storage protein content and quality (De Pace et al. 1988). *Dasypyrum breviaristatum* is thought to be an autotetraploid having the same genomes as *D. villosum* (Frederiksen 1991).

Natural populations of *D. villosum* produce two types of caryopses, yellow and brown, in the same ear. The inheritance of the seed color does not show any dominance effect, nor does it follow Mendelian segregation. Mature plants developed from the two types of seeds do not show evident morphological differences; both of them are able to produce ears with yellow and brown caryopses.

Analysis of interphase nuclei chromatin organization, by use of densitometric determination at different thresholds of optical density, and of chromosomal heterochromatin distribution by means of C-banding, fluorochromes Hoechst 33258, DAPI, CMA, and Ag-NOR staining, were applied for characterizing heterochromatin. To reach a better level of understanding, *in situ* restriction endonuclease digestion was performed on an inbred line of *D. villosum*. Finally a preliminary characterization of *D. breviaristatum* chromatin was started.

MATERIALS AND METHODS

A natural population of *D. villosum* was collected near Campobasso (central Italy); the caryopses of this population exhibited yellow and brown color. From a natural population collected near Bari (south Italy) an inbred line (GHA 01) was derived by selfing, in each generation, a plant derived from a single seed of a selfed ear of the former generation. The process was carried out for eight generations, with the residual heterozigosity estimated below 1%.

Seeds were germinated in Petri dishes in the dark at 21° C. For cytophotometric analyses, root tips were fixed in ethanol-acetic acid (3:1, v/v); squashes were made after digestion in pectinase and staining with Feulgen. Squashes of *Vicia faba* were concurrently stained as internal standards. Absorption was measured using a Leitz MPV3 integrating microdensitometer. Feulgen DNA absorption of chromatin fractions with different condensation level was determined by measurements of one and the same nucleus, after selecting different thresholds of optical density in the instrument according to the method discussed in Cremonini *et al.* (1992). The instrument reads all parts of the nucleus where the optical density is greater than the preselected limit, regarding those below this limit as a clear field. The value of Feulgen absorption at 3 (arbitrary units) thresholds of optical density is the total value (100) of Feulgen absorption. Measurements carried out on the same nucleus at different thresholds of optical density were expressed as a percentage of the initial value.

For chromosome banding, roots were excised and treated overnight with ice cold distilled water and fixed in ethanol-acetic acid (3:1, v/v). C-banding, Ag-NOR and fluorochromes staining were performed as described in Galasso and Pignone (1992)

In situ digestion with restriction endonucleases (RE-banding) was carried out according to Mezzanotte *et al.* (1983) using the following enzymes: Alu I, Dde I, Taq I, Dra I, Eco RI, and Hae III.

Table 1. Mean absorption, DNA amount and nuclear area of yellow and brown seeds of *D. villosum*

Caryopses	Absorption (a.u. ± S.E.)	DNA content per (4C nucleus (pg)	Nucleus area (μm^2 ± S.E.)
Yellow	2615 ± 32.4	23.7 ± 0.3	330 ± 4.7
Brown	2033 ± 25.1	19.1 ± 0.2	249 ± 3.0

RESULTS AND DISCUSSION

DNA content

Yellow and brown seeds showed different nuclear DNA content: 23.7 pg and 19.1 pg in 4C interphase nuclei respectively (Table 1). The data clearly demonstrated a significant amount of variation in the chromatin organization of the two types of caryopses. The Feulgen absorption of brown seeds is reduced to nothing at 21 thresholds of optical density and the Feulgen absorption of yellow seeds is reduced to nothing at higher thresholds of optical density of 24 (Figure 1).

A mathematical elaboration based on the Simpson's rule, allows one to determine the inflection point of the two curves (Figure 1). This point allows us to distinguish two areas in each curve, the first being the integral of the half-curve left of the inflection point and the other, the integral of the half-curve to the right of the same point. The integral calculation was carried out on the two semi-areas (Figure 2). While the values of the left areas are rather similar (454 and 448, yellow and brown respectively), the values of the right areas are different in the two types of

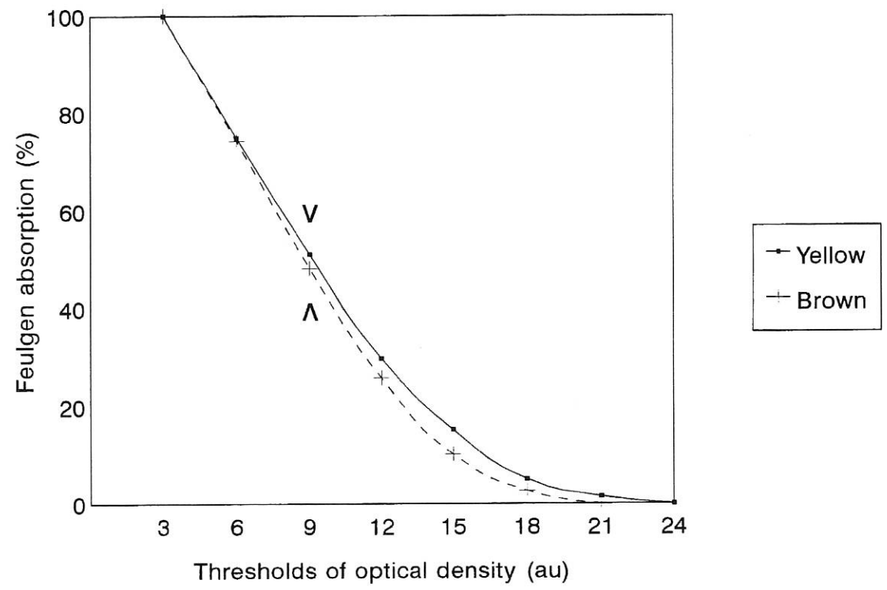

Figure 1 Absorption curves from nuclei of yellow and brown seeds of *D. villosum*; the inflection point is indicated (v)

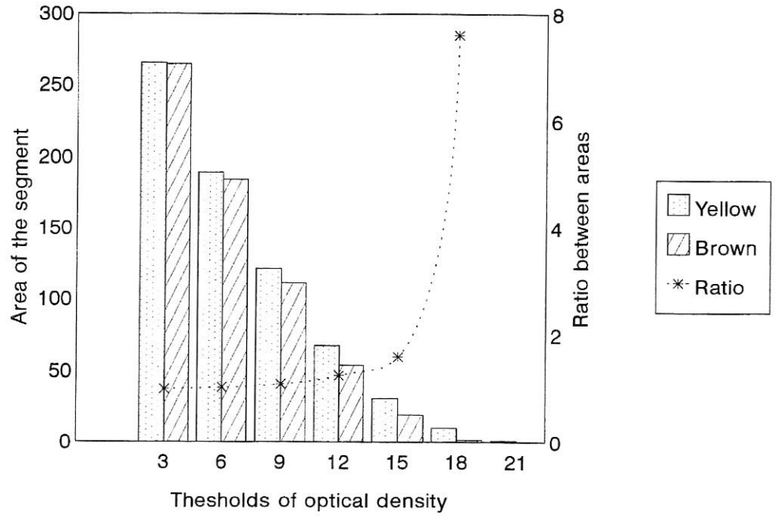

Figure 2 Areas of the absorption curves of yellow and brown seeds of *D. villosum*; the ratio between areas is shown with a dotted line

caryopses, 229 and 184 for yellow and brown, respectively. Moreover, it is interesting to point out that the ratio between these latter areas is 1.244, similar to that between the total DNA contents. The data indicate that the difference in DNA amount only regards the condensed fraction of the genome, that is the heterochromatin.

The analyses at different thresholds of optical density were carried out on meristematic cells in order to make photometric results comparable with those from chromosome analyses. The percentage of heterochromatin is higher in meristematic cells than in differentiated ones indicating underreplication of heterochromatin during differentiation (Bassi 1990 and references therein).

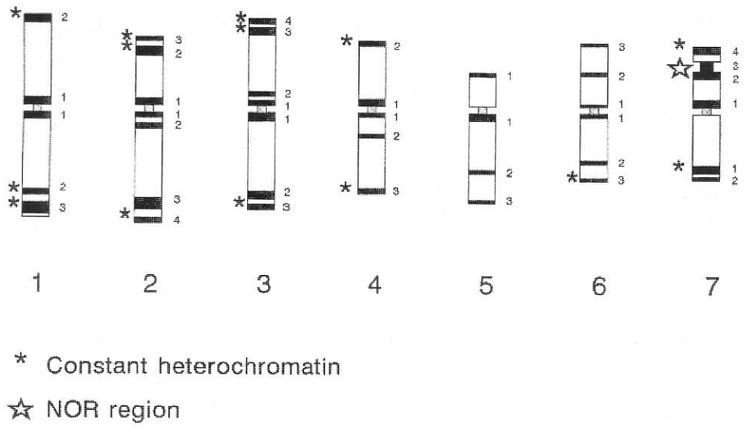

Figure 3 General karyotype of *D. villosum*; the more constant bands are indicated.

Heterochromatin detection and classification

The general karyotype of *D. villosum* (Figure 3) is similar to that reported by Friebe *et al*. (1987) and Linde-Laursen and Frederiksen (1991). The techniques of differential chromosome staining allowed the identification of each pair of chromosomes, although a high degree of variation was observed within homologous chromosomes of different individuals of the same population. The general karyotypes of the two types of caryopses did not change too much and only few differences could be evidenced. C-banding revealed a large amount of chromosomal heterochromatin as well as a high degree of variation. Hoechst 33258 and DAPI produced the same banding pattern. These fluorochromes induce occasional dots in the centromeric areas while revealing intensely stained bands in the telomeric regions, except on chromosome 6 (Figure 3). They allowed the identification of only a part of the C-bandable heterochromatin. This fraction was uniformly distributed and did not show any difference in yellow and brown caryopses. Finally only the NOR region was identified with both CMA and Ag-NOR staining.

It is worth noting that the polymorphism occurs on different bands in yellow and brown types. Bands that are stable in one type of caryopse may be polymorphic in the other. The polymorphism generally occurs on bands that are not evidenced with fluorochromes, with some exceptions. This might indicate that the different classes of heterochromatin are involved at a different level in the polymorphism.

It is also interesting that on metaphase chromosomes banding techniques do not bring out striking evidence of variation in the level of heterochromatinization between yellow and brown types. The variation observed does not account for a 20% difference in heterochromatin content as cytophotometrically determined.

It has to be considered that the binding of Giemsa to C-bands is non-stoechiometric, therefore bands with similar appearance could possess different amounts of heterochromatic DNA, moreover, C-banding and cytophotometry consider cells in different functional stages. Nuclear hetrochromatin represents unexpressed DNA and a difference in heterochromatin amount reflects differences in genome expression, whereas chromosomal heterochromatin represents an alternative level of packaging DNA into chromosomes. The fact that densitometric curves do not differ in the euchromatic fraction show that there is no difference in genome expression between brown and yellow types and, therefore, all the extra DNA in yellow type is unexpressed DNA.

RE-banding

In situ digestion with restriction endonucleases followed by staining with Giemsa or with DNA-specific fluorochromes may be an intermediate approach between chromosome banding and the molecular level, capable of investigating the DNA base composition and the chromatin organization of specific chromosomal regions.

RE-banding revealed different patterns in relation to the enzyme used; some produced positive bands (Alu I, Dde I, Taq I, Hae III) other negative bands (Dra I, Eco RI). It is interesting to note that each enzyme had a characteristic pattern which differed from the other enzymes for the same bands. The action of REs does not appear to be in relation to the DNA base composition alone, as it can be demonstrated by the staining of digested chromosomes with DAPI. All areas that after RE digestion followed by Giemsa staining appeared as gaps showed a dull appearance also with DAPI; these areas are located centromerically and interstitially. Conversely, telomeric bands, which in undigested preparations often display DAPI bands, show a different reaction according to the enzyme used. They show a brilliant fluorescence after digestion with Alu I, Dde I, Taq I, and Hae III, but an indifferent reaction after digestion with Eco RI and Dra I. Taking into account the action of REs on the above mentioned heterochromatin classes, it is possible to detect different subclasses of heterochromatin in *D. villosum*.

$C^+/DAPI^+$ (class 1): This AT-rich heterochromatin, localized mostly in telomeric areas, generally shows RE^+ reaction. RE^- reaction in class 1 heterochromatin is present only in a few centromeric areas and never in telomeric ones. This implies that: a) centromeric and telomeric areas possess two different sub-classes of heterochromatin, as is also suggested by their appearance as large fluorescent blocks at the telomeres as opposed to very thin bands at the centromeres, b) centromeres contain high frequency specific RE base sequences, and c) chromatin organization is a critical factor in permitting or prohibiting RE action, as hypothesized above.

C^+ only and no reaction with fluorochromes (class 2): the RE^+ reaction indicates that this heterochromatin does not seem to be enriched in AT- or GC- base pairs nor does it contain appreciable amounts of specific DNA base sequences. By contrast class 2 areas showing RE^- reaction would contain, possibly with a high interspersion frequency, the base sequence target of specific REs. Even in this class of heterochromatin it is noteworthy that RE^- reaction is present only at centromeres and never at telomeres, which are again shown to be very resistant structures.

Finally, the NOR region positive to silver staining (CMA^+ only) is localized on chromosome 7. This area, affected to a limited extent by all REs, is cleaved by Hae III, which produces a marked RE^- reaction. The cleavage and extraction of GC-rich DNA (CMA^+, Sumner 1990 and references therein) from this area by Hae III (restriction target GGCC) is not surprising considering that in this case the NOR does not show a C^+ reaction, thus indicating that this chromatin possesses a further organization level.

D. breviaristatum

Our C-banding results are similar to those reported by Linde-Laursen and Frederiksen (1991). C-banding produces a rather complex pattern: bands are generally distributed at centromeric and interstitial positions; few and thin bands are seen at telomeric positions. The very high level of polymorphism observed within some chromosome pairs is clearly the result of the strict allogamic reproductive habit of this species. Preliminary attempts to produce fluorochrome staining did not produce good results and indicate the absence of the bright $DAPI^+$ telomeric blocks seen in *D. villosum*. This might appear in contrast with the hypothesis that *D. breviaristatum* is an autotetraploid species possessing the same genome as *D. villosum*. Nevertheless one has to consider that; a) the examined population might be poor in that class of heterochromatin, b) during the establishment process, following the evolution of the new polyploid, that class of heterochromatin has been specifically lost or restructured as a consequence of a phenomenon similar to amphiplasty, and c) differences in chromatin organization and distribution may be in relation to the perennial habit of this species. Studies are in progress to better clarify this point.

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The Mechanism of the Origination of Auto-allopolyploidy and Aneuploidy in Higher Plants Based on the Cases of *Iris* and *Triticeae*.

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ABSTRACT

Cytomixis is a natural process of chromatin exchange among cells. In *Iris confusa* and *I. japonica*, the synchronized cytomixis takes place between PMC's during a stage just before meiosis. This process produces euploid and aneuploid offspring. The chromosome number of a fertile diploid plant is 30 (2n). Most accessions of *I. confusa* and *I. japonica* are sterile aneuploids. The chromosome numbers are varied, ranging 2n = 28 to 60. In Triticeae cytomixis plays an important role in spontaneous chromosome doubling or redoubling, resulting in the origin of auto-allopolyploidy and aneuploidy. We have obtained amphidiploid plants by spontaneous chromosome doubling. These plants indicate indirectly that cytomixis takes place in the macrosporocytes, giving rise to high level auto-allopolyploid Triticeae species.

INTRODUCTION

The phenomenon of cytomixis was discovered by Arnol'de in 1900. Gates (1911) studied this phenomenon in *Oenothera* and designated the term "cytomixis" to describe chromatin material migrating through the plasmodesmata into neighboring cells. The question of whether cytomixis is an abnormal artificial behavior or a natural behavior of chromatin material has long been debated. Lou *et al.* (1962) observed cytomixis in living cells, Cheng *et al.* (1956) observed cytomixis using electronmicroscopy, and Yen *et al.* (1993) observed cytomixis taking place between two untreated fresh pollen mother cells of the *Roegneria ciliaris x Psathryostachys huashanica* F_1 hybrid under phase contrast microscopy. These workers proved that cytomixis is a natural process where chromatin exchange occurs among cells. Yen *et al.* (1993) and Sun *et al.* (1993) reported that intergeneric hybrids of Triticeae had some special cell structure formations, including the conjugation opening and conjugation tube besides the plasmodesma. The resting stage nuclei, chromatin masses, chromonemata, or chromosomes can migrate through these structure into immediate neighboring cells before, during or after meiosis in the hybrids of *Roegneria ciliaris x Psathyrostachys huashanica* and *Triticum aestivum x Psathyrostachys huashanica*. Multipolar division and coenocytes also occurred in these hybrids. Yen *et al.* (1992) pointed out multipolar division might be caused by the multipolar zones of synchronized nuclei in the coenocyte. We speculate that this kind of PMCs can not form normal tetrads and degenerates. Conversely, synchronized nuclei in some PMCs fuse together first, then it is followed by normal bipolar division in a few PMCs, where the normal tetrad might be produced. If this is true, the spontaneous chromosome doubling or redoubling might have occurred. If this process takes place in the macrosporocyte, a fertile egg cell and synergid nuclei should be produced. Fertile pollen grains are produced by the same spontaneous chromosome doubling or redoubling in the microsporocyte of the same floret. There is a chance that chromosome doubled or redoubled egg cells could develop a plant by parthenocarpy. The present paper reports on the origin of allopolyploidy, euploidy, and aneuploidy in *Iris* and Triticeae taxa.

MATERIALS AND METHODS

The accessions of *Iris confusa* Sealy and *I. japonica* Thunberg collected in China and Japan are shown in Table 1. The hybrids of Triticeae are shown in Table 2. Root tips were collected at 11 o'clock in the morning, and keep in the refrigerator at 4° C overnight, then fixed in Carnoy's fluid for 48 hours. Root tips were then transferred to 70% alcohol and stored until analyzed. PMC's for cytology were collected and treated in the same way as the root tips. Slides were prepared for cytological studies by means of

Table I: Observation on evansia irises of Far East, Asia.

species	accession number	locality	chromosome number	fertility
Iris confusa	1	Baimaqiao, Rongjing, Sichuan, China	42	-
	2	Siping, Rongjing, Sichuan, China	30	+
	3	Siping, Rongjing, Sichuan, China	34	-
	4	Jizigang, Yaan, Sichuan, China	28	-
	5	Juejiping, Tianquan, Sichuan, China	30	+
	6	Juejiping, Tianquan, Sichuan, China	36	-
	7	Xingou, Tianquan, Sichuan, China	30	+
	8	Xingou, Tianquan, Sichuan, China	34	-
	9	Dechang, Sichuan, China	28	-
	10	Kunming, Yunnan, China	30	-
Iris japonica	11	Kyoto, Japan	54	-
	12	Kyoto, Japan	36	-
	13	Kyoto, Japan	56	-
	14	Okayama, Japan	34	-
	15	Yokahama, Japan	28	-
	16	Laobanshan, Yaan, Sichuan, China	60	-
	17	Jinjiguan, Yaan, Sichuan, China	36	-
	18	Dujiangyan, Sichuan, China	32	-
	19	Jiuionggou, Chongqing, Sichuan, China	42	-
	20	Neijiang, Sichuan, China	38	-
	21	Zigong, Sichuan, China	46	-
	22	Wanxian, Sichuan, China	36	-
	23	Dali, Yunnan, China	30	+
	24	Changsha, Hunan, China	34	-
	25	Nanjing, Jiangsu, China	52	-
	26	Hangzhou, Zhejiang, China	42	-

Table 2: Observation on somatic chromosome number of the F_1 hybrid of *Elymus caninus* x *Hordeum vulgare*.

F_1 hybrid	number of chromosomes per cell												
	30	40	41	42	43	44	45	56	47	48	49	50	51
1	-	1	-	4	-	-	-	-	-	-	-	-	-
2	-	-	6	3	-	-	-	-	-	-	-	-	-
3	-	2	1	9	-	-	-	-	-	-	-	-	-
4	-	-	-	1	-	-	-	-	-	-	8	-	1
5	1	-	-	6	-	1	1	-	-	-	-	-	-

acetocarmine smear. Before squashing the root tips, the intercellular substance between the cells is dissolved by 1N hydrochloric acid solution, in a 60° C incubator for 5 minutes.

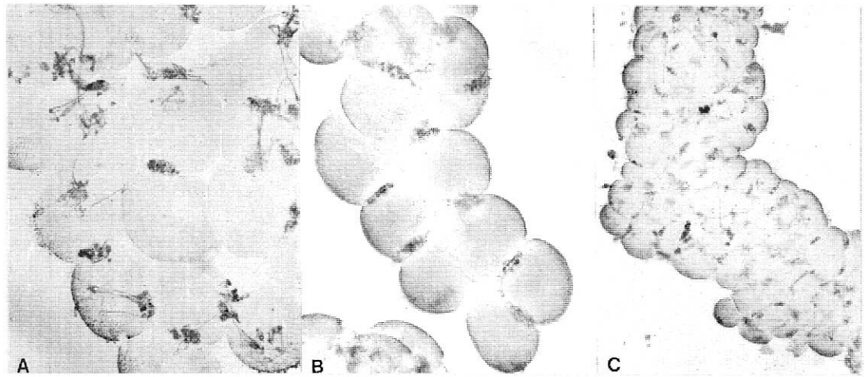
RESULTS AND DISCUSSION

Iris japonica Thunberg and *I. confusa* Sealy

Iris japonica is native to China and Japan. *Iris confusa* has an erect cane-like stem which is different from *I. japonica*. It is a native plant of southwest China, where it inhabits high mountain meadows. All accessions of *I japonica* observed were sterile, with only a few abnormal capsules found. All the accessions were studied cytologically. They are all aneuploids except one autotetraploid accession found from Yaan (Table I). All plants have a cytomixis stage just before meiosis. The synchronized cytomixis takes place among PMCs evenly (Fig. 1:A. B. C.). Sinoto (1921) and Yasui (1939) observed cytomixis in *I. japonica*. Yasui believed that *I. japonica* was a triploid, she followed Kazo (1929), and Simonet (1934). Kihara (1982) also agreed with them. They did not point out the universality of cytomixis in this species and did not point out how cytomixis caused the chromosome number to vary among different accessions. We observed cytomixis in microsporocytes. The normal pollen grains accompanied many abnormal and functionless pollens in the same anther lobe. However, the diploid plants produced normal capsules and bore normal seeds. Most seedlings from these seeds are aneuploids. In natural vegetation, more than 90 percent of accessions were aneuploids. Our results of cytological observations are shown in Table I. Between *I. confusa* and *I. japonica*, there were intermediate forms observed in morphology and cytology. Diploid *I. confusa* has a small geographical distribution on the high mountain meadows of Sichuan, Yunan and Guangxi Provinces. The distribution of the aneuploid *I. japonica* is much larger. It occupies all the subtropic regions of the Far East, from the Himalaya valley to Japan. An accession of *I. japonica* from Dujiangyan City was found to have two normal and five abnormal seeds in 0.35% of its capsules. The normal seeds proved to be aneuploids with chromososome number of 36 and 40. Cytomixis plays an important role in chromosomal aberrations, which is bound to create cytological and morphological diversity in these evansia irises. Natural selection seeks out the adaptive accessions.

Triticeae

Within the Triticeae taxa there exists a high level of auto-allopolyploids which have multiple genomes, such as *Leymus angustus* (Trin.) Pilger. Chromosome numbers of 2n=28, 42, 56, 70 and 84 have been reported for this species (Bowden, 1957; Sun *et al.*, 1990). In the hexaploid plant of *L. angustus*, the genomes must be unevenly doubled, although the origin of these various cytotypes could be quite different, they could be accomplished much easier through cytomixis of complete nuclei which fuse together. This mechanism was suggested earlier (Yen *et al.*, 1993; Sun *et al.*, 1993). Recently, we obtained F_2 hybrid plants, which were derived from a cross of *Elymus caninus* (L.) L. x *Hordeum vulgare* L. The F_1 hybrid is a normal amphihaploid which has 21 chromosomes comprised of the S, H and I genomes. Cytomixis took place in PMCs (Fig. 2B. C.). Fig. 2A shows that a PMC has an outgrowth. We can see a new bud developing again on the old one. As a result of cytomixis, chromosome numbers varied among PMCs. Fig. 2D shows that only 4 chromosomes are present in a small PMC. Fig. 2E, shows 14 chromosomes in the PMC, and Fig. 2F shows that it has 19 chromosomes slightly less than the normal cell. Fig. 2G, shows a small pollen grain which has no chromatin material (arrowed). Pollen has more chromatin bodies than usual. Every year, this plant bears some seeds (30%). Most of these seeds are shrunken and have no viabity. The normal seeds and seedlings had mainly 42 chromosomes (Table 2). The endosperm of these seeds were well-developed, but most of the seeds had no embryo. F_1 and F_2 plants had dehiscent anthers, which contained some viable pollen grains when stained with iodine. The F_2 plant looked like an amphidiploid which had 42 chromosomes. If the F_2 is an amphidiploid and breeds true, its origin is either by nondisjunction division of anaphase I followed by normal fertilization or by cytomixis followed by parthenogenetic reproduction. Fortunately, this perennial hybrid produces seed every year. Most of the F_2 plants proved to be euploids with 42 somatic chromosomes (Fig. 2H), along with fewer aneuploids formed. Some F_2 plants, had many univalents (Fig. 3A) and multivalents in their PMCs at MI (Fig. 3B). This kind of F_2 euploid is frequently produced (more than 0.3%). Thus, *Elymus caninus x Hordeum vulgare* is a good example to demonstrate that cytomixis plays an important role in chromosome multiplication, aberration and production of euploid and aneuploid offspring. Sarvella (1958), Bell (1964), Marechal (1963), Salesses (1970), and Kundu *et al.* (1988) noted that cytomixis contributed to the production of aneuploidy gametes, but they did not discuss the chance for amphipolyploid to be originated through the process of cytomixis. Cheng *et al.* (1975) pointed out that cytomixis could be caused by chromosomal aberration and chromosome multiplication or diminution. Kundu and Sharma (1988) wrote, "however, this is of limited significance because of the absence of viable seed setting". But, our observation of *Iris confusa, Iris japonica* and the hybrids of *Triticum aestivum* L. cv. 'Xiaoyuermai' x *Secale cereal* L. winter rye (Luo, Yen and Yang, 1992) and *Elymus caninus* L. x *Hordeum vulgare* L. showed that they did bear good viable seeds in nature. These cases suggested the evolutionary significance of cytomixis.



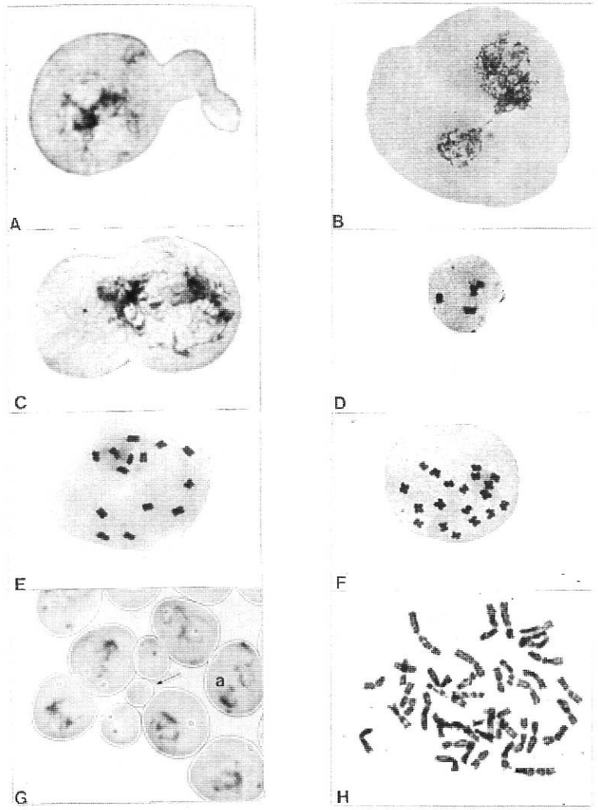


Figure 1. The cytomixis stage of PMCs in *Iris japonica* Thunberb. (A). *I. japonica* from Kyoto, Japan. (B). *I. japonica* from Dujiangyan City, Sichuan, China. (C). *I. japonica* from Nanjing, China.

Figure 2. Cytological observation on the hybrid of *Elymus caninus* (L.) *L. x Hordeum vulgare* L. (A). A PMC of hybrid shows secondary budding (conjugation tube). (B). Cytomixis takes place through plasmodesma between two PMCs. (C). Cytomixis takes place through a big conjugation opening it seems to be cell fusion. (D). A small PMC of F_1 cybrid has 4 chromosomes. (E). A PMC of F_1 hybrid has 14 chromosomes. (F). a PMC of F_1 hybrid has 19 chromosomes. (G). A group of young pollen grains have diferent amount of chromatin materials. A small one has no chromatin material (arrowed). Pollen grain a, has a large amount of chromatin materials much more than usual. (H). A somatic cell has 42 chromosomes which was observed in the root tip of F_2 hybrid plant.

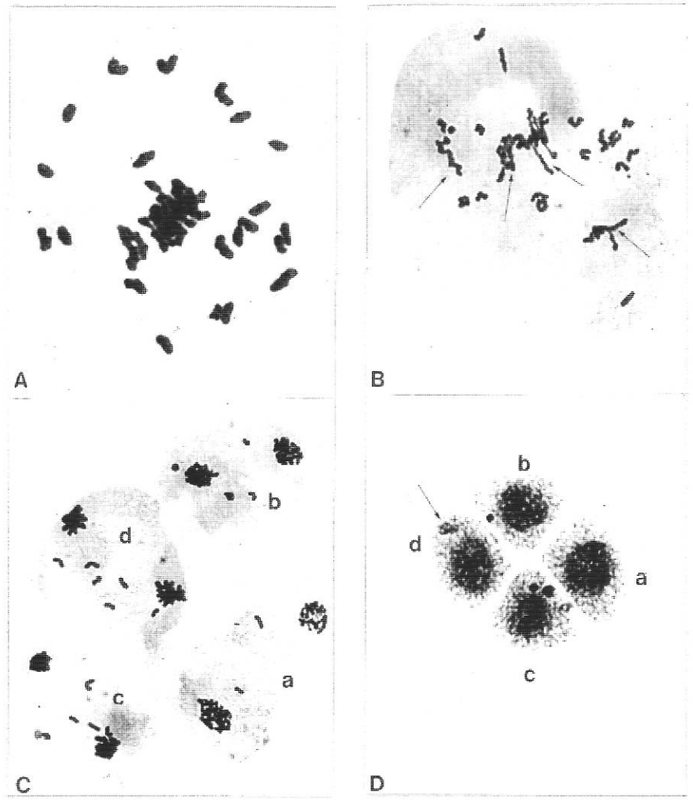


Figure 3. Meiotic behavior of F_2 hybrid of *Elymus caninus* (L.) *L. x Hordeum vulgare* L. (A). A PMC at MI, it has 26 univalents. (B). A PMC at MI, it has multivalents (arrowed), they are separated in the cell. (C). The PMCs at anaphase I show different amount of lagging chromosomes. The cell a, has one pair; cell b and c have two pairs and cell d, has three pairs. (D). The tetrad of F_2 hybrid, a is normal cell, b has one micronucleous, c has two micronuclei and d has a small group of extra late concentrated chromonemata.

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Procedures for Transfer of Agronomic Traits from Alien Species to Crop Plants

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ABSTRACT

The steps involved in the transfer of alien genetic variation will be outlined and the impact of recent technologies on improving the efficiency of the process will be discussed. The selection of parents is the first step; it is critically important that each selection be carefully screened for maximum expression of the desired trait. The crossing process is becoming increasingly more efficient with improved efficiency of growth regulators and embryo rescue media. Doubled haploid methods are being used to facilitate the production of chromosome addition lines. Molecular methods such as RFLPs, RAPDs, chromosome banding, and *in situ* hybridization add an increased level of resolution to the identification of chromosome additions and the monitoring of introgressed chromosome segments. Emerging technologies such as monocot transformation, chromosome-specific libraries, and transposon tagging may soon replace some of the traditional methods of gene transfer.

INTRODUCTION

Plant breeding effort is directed at the production of cultivars showing improvement in yield plus improvements in any number of agronomic traits is an ongoing and upwardly incremental process. Improved cultivars of every crop are being released at a steady pace. All such cultivars are regarded as elite germplasm. The major prerequisite for the plant breeding process is a continued supply of genetic variability. For most traits, ample variability exists in the primary gene pool.

The first consequence of ranging into the secondary gene pool obtain desirable agronomic traits is dilution of the elite germplasm. Repeated backcrossing to the elite recurrent parent becomes necessary to restore the original cultivar. Therefore wide crossing for purposes of gene transfer is done as a last resort when the variability for a particular trait is exhausted or non-existent in the primary gene pool. Examples of traits that show low variability in primary gene pools are resistance to BYDV, streak mosaic, *Fusarium* head blight in wheat, and net blotch-root rot in barley. Other traits that are being sought in alien species with the objective to transfer to wheat are perennial habit, resistance to leaf stem and stripe rust, curl mite resistance, increased protein content, yellow semolina color, and apomixis. In addition there is limited variability and ample room for improvement to tolerance to abiotic stresses such as cold, drought, heat, and salinity in wheat, barley and rye. There are some 400 species and 25 genera in the Triticeae tribe and the scope for intergeneric hybridization is immense. Numerous intergeneric hybrids within the tribe have already been made with wheat (Miller, 1987; Pienaar, 1990) and barley (Fedak, 1992).

BREEDING

Germplasm - parents

The first and probably single most important criterion determining the success of a wide crossing program is the critical screening of the parental material. Sources of alien germplasm are the genebank networks and the variability in the wild that can still be collected. In the case of the latter there is considerable knowledge available on the natural distribution of Triticeae species plus the types of stresses they encounter. This offers some clues as to the geographical distribution of the traits in question. Natural selection pressures seem to have been effective in concentrating certain types of variability.

Whatever the source of variability, whether botanical collection or gene bank, every accession of every species needs to be thoroughly screened to ensure the maximum expression of the trait in question (Sharma *et al.*, 1984). This trait may be polygenic in inheritance or the source could be heterozygous and thus complicate the transfer procedure. Problems of trait expression are often encountered in the progeny of wide crosses so every precaution should be taken in selecting the best possible parents.

Selection for abiotic stress resistance is usually done in *in vitro* cultures while selection for disease resistance has been done by exposure to the organism itself. Molecular tags and known sequences for specific genes will be useful in the future. For traits such as storage protein genes, cloned probes have become powerful tools for screening parents for variability, for example, hordein genes in barley (Bunce *et al*., 1986), gliadin genes in bread wheat (Bartels *et al*., 1986), and gliadin and glutenin PCR probes in wheat (D'Ovidio *et al*., 1992).

Crossing

It is now believed by some researchers involved in intergeneric hybridization within the Triticeae that hybrids can be made between virtually any two species within the tribe, Most species can be crossed onto wheat in particular and many onto barley and rye. The most important condition is that sufficient numbers of accessions of a particular species be evaluated. The other factors involved are the pre and post-pollination applications of growth regulators. For example, it was shown that whereas the post-pollination application of GA_3 to barley enhances seed set and embryo differentiation, 2,4-D is more effective than GA_3 on wheat following wheat x maize pollination for haploid production (Fedak *et al*., 1994). Embryo rescue media are becoming more complex but also more effective. In some cases immature ovules at two days post pollination can be rescued on the more complex media (Comeau *et al*., 1992). The nurse culture technique (Kruse, 1973), though quite labor intensive, was effective in rescuing hybrid embryos from *Hordeum* x *Secale* crosses (Fedak, 1979). Employing such techniques a total of 300 intergeneric hybrids in *Hordeum* have been reported (see Fedak, 1992 for summary) and probably an equal number involving wheat (Pienaar, 1990).

Genome analysis

The relationship between species (and to a certain extent between genomes) in the Triticeae has been the subject of decades of ongoing study. Species relationships *per se* can be deduced from analysis of the parental species prior to making hybrids by employing techniques such as C banding (Linde-Laursen *et al*., 1992), N banding (Gecheff *et al*., 1994), and isozyme analysis (Hart, 1987), RFLP analysis using repetitive sequence probes (Molnar *et al*., 1989; Gupta *et al*., 1989; Appels *et al*., 1989). Techniques have also been developed to conduct sequential banding and *in situ* hybridization for mapping of euchromatic and heterochromatic regions of wheat chromosomes (Jiang and Gill, 1993b).

The extent of meiotic chromosome pairing will provide indications of homoeology between parental genomes in the hybrid for another estimate of genome and hence species relationships. In hybrids involving crop and wild species, meiotic data also provide estimates of species relationships. The amount of chromosome pairing is an indication of the amount of recombination that might be expected and hence potential gene transfer. In hybrids within the Triticeae especially involving polyploid species, it has been virtually impossible with conventional staining methods to distinguish between autosyndetic and allosyndetic pairing. The concept of *in situ* hybridization using fluorescent- labelled total genomic DNA as a probe with genome blocking has only recently been reported as a means of identifying component genomes at meiosis (King, 1993). Fluorescent *in situ* hybridization using labelled total genomic DNA of one species and blocking DNA from the other species on somatic chromosome preparations has been employed for several years to distinguish firstly, component genomes in a hybrid and secondly detect any piece of introgressed alien chromatin such as wheat-alien addition substitution and translocation lines (for review see Jiang and Gill, 1994). Individual genomes and introgressed segments can be differentiated by direct labelling of component and DNA (Anamthawat-Jonsson and Reada, 1995), as a more efficient alternative to genome blocking.

Chromosome doubling

The old standard methods of chromosome doubling through the use of spindle fibre suppressants such as colchicine and methotrexate are still in use today. Chromosome doubling of intergenomic hybrid plants regenerated from callus cultures has been reported (Fedak and Grainger, 1986; Wang, 1992). The success rate in producing amphiploids from intergeneric hybrids in the Triticeae has generally been quite low. In hybrids involving *Hordeum* species or cultivars the success rate has been negligible although there are indications that colchicine response is genotype dependent as evidenced by the amphiploid obtained from the *H. californicum* x Chinese Spring hybrid (Fedak, 1987).

Backcrossing

The ideal method of achieving gene transfer from an alien species into a crop plant is to backcross the hybrid or amphiploid to the crop plant until the complete series of addition lines is produced. Even if chromosome doubling treatments on the hybrid are not effective, it is usually possible to backcross onto the hybrid, particularly hybrids involving wheat as one of the parents. This is usually mediated by the production of restitution nuclei in the hybrid. In some cases, tens of thousands of florets had to be pollinated to obtain a backcross on the hybrid involving wheat x *Elymus angustus* (A. Plourde, p.c.). Negligible success has been reported in trying to obtain backcrosses with any hybrids involving barley cultivars or *Hordeum* species. Partial amphiploids (with 2n=56 and having one alien genome) are one, albeit rare, product of backcrossing wheat onto wheat-6x *Thinopyrum* hybrids (Cauderon, 1966; Pienaar, 1988). Otherwise backcrossing is continued until the entire series of alien addition lines are produced in the crop plant background. Several effective methods of rapidly obtaining disomic addition lines involve the crossing of a BC_1 plant back onto an amphiploid (Lukashewski, 1988) or by producing haploids with an additional chromosome, then doubling the chromosome number (Fedak, unpublished). Such a situation was encountered in attempting to produce disomic addition lines of *H. californicum* in a Chinese Spring background. No disomic additions were recovered in the selfed progenies of monosomic additions presumably because of a lack of transmission of the monosome through the pollen.

Even though barley tolerates trisomy, telotrisomy, triploidy, tetraploidy and a few cases of tetrasomy, it does not appear to tolerate the addition of alien chromosomes or genomes. For example, triploid hybrids containing 14 barley and 7 rye chromosomes have been produced (Fedak and Nakamura, 1982), but such plants were sterile and further backcrosses to barley to produce rye addition lines were not successful. Similarly, numerous interspecific hybrids were produced with barley (Jacobsen and Bothmer, 1981), but with the exception of hybrids with *H. spontaneum* x *H. bulbosum*, backcrossing with other barley interspecific hybrids has not been successful.

Identification of critical chromosome additions

The identification of addition lines involves the determination of the homoeologous relationship of the additional chromosome and also the identification of the addition line carrying the trait in question. The homoeology of the added chromosome can be determined by employing various markers with known chromosome locations such as isozymes (William and Kazi, 1993; Hart, 1987; Forster *et al*., 1987), C banding (Dhaliwal *et al*., 1990), RFLP markers (Anderson *et al*., 1992; Sharp *et al*., 1989), RAPD markers (Penner *et al*., 1993), STS markers (Talbert *et al*., 1994), or specific banding patterns (Gill, 1987; Jiang and Gill, 1993b).

Some examples of identification of addition lines carrying specific traits include: chromosome 7 addition of *Th. intermedium* provides resistance to BYDV (Brittel *et al*., 1988), additions of chromosomes of *Th. elongatum* provides salinity tolerance (Dvorak *et al*., 1985), chromosome 5J of *Th. junceum* provides salinity tolerance (Forster *et al*., 1988), addition lines of chromosome $1H^{ch}$ of *Hordeum chilense* added to wheat provide resistance to the root-knot nematode (Perron-Dedryver et al., 1990) and *E. ciliaris* chromosomes $1S^c$ or $1Y^c$ restore fertility to alloplasmic euploid wheat with *E. ciliaris* cytoplasm (Jiang *et al*., 1993). A compendium of wheat-*Thinopyrum* addition, substitution, and translocation lines has been published (Shepard and Islam, 1988).

Induction of recombination

Although all the species within the Triticeae originated from a common ancestor and share basic genomes that may or may not have undergone chromosome rearrangements, chromosome pairing may be under genetic control so that homoeology is not fully expressed. The best known example is the *Ph* locus of wheat that restricts chromosome pairing to strictly bivalent formation (Sears, 1976) so that any homoeologous pairing must be induced. A *Ph* mutant has also been isolated in durum wheat (Georgi, 1978). There are several standard methods of induction of homoeologous chromosome pairing in wheat. In wheat itself there are aneuploids such as nulli 5B and mutations at the *Ph* locus (Sears, 1984) that permit pairing of wheat with homoeologous alien chromosomes. Sears (1973) was the first to use the above tools to transfer leaf rust resistance from a *Th. ponticum* chromosome 7 substitution to chromosome 7D of wheat. Numerous other researchers were able to use the same tools to induce meiotic crossing-over and transfer of traits from wheat to alien chromosomes (See Pienaar, 1990 for summary of wheat-*Thinopyrum* transfers).

The genomes of certain *Aegilops* species such as *Ae. speltoides* are known to suppress the *Ph* system of hexaploid wheat and thus permit homoeologous pairing. By this means translocations between *Th. intermedium* and wheat chromosomes were induced (Ortiz *et al*., 1986).

Radiation with X or gamma-rays of seed or poffen of hybrids, partial amphiploids, and addition or substitution lines have induced recombinations and gene transfers. Numerous examples are listed in the review by Pienaar (1990). A more recent method for recombination induction involves the production, extended proliferation, and maintenance of callus induced from vegetative or reproductive parts of hybrids or aneuploid plants. Plants regenerated from such callus were shown to contain numerous chromosome translocations (Fedak, 1990).

Spontaneous translocations between wheat and alien chromosomes do occur at a low frequency at meiosis and several examples of transfers of useful genes have been documented (See Pienaar, 1990 for summary).

Screening of progenies

The induction of interchanges produces vast numbers of critical and non critical chromosome translocations. The usual large numbers of derivatives must be screened to isolate those carrying the desired gene(s). The usual approach is to inoculate with the specific pathogen, expose to a strong selection pressure, or electrophoretically analyze progeny for a particular value-added trait. Rapid screening methods are continually being devised to expedite this process.

Molecular tags are rapidly being developed for numerous agronomic traits, particularly those that are

simply inherited, eg., disease resistance loci (Hinze *et al.*, 1991; Penner *et al.*, 1993), biochemical loci (Kilian *et al.*, 1994), and value-added traits (Reddy and Appels, 1993). Such markers can rapidly be developed as particular needs arise. Desirable genotypes can be selected based on molecular markers rather than selecting for the trait itself. The advantage of such markers is the ability to screen for traits that are recessive, difficult to score, obscured by other traits.

Linkage maps have been assembled from RFLP markers in various crop plants, including wheat (Gale *et al.*, 1990; Anderson *et al.*, 1992), barley (Huen *et al.*, 1991; Graner *et al.*, 1991; Kleinhofs *et al.*, 1993), and rye (Wang *et al.*, 1991). These are being combined with isozyme, biochemical, and morphological markers in order to maximize coverage. Such maps are becoming a valuable tool in screening and mapping polygenic traits as quantitative trait loci (QTL) (Hayes *et al.*, 1993). Such tags will permit the monitoring of such loci during their transfer and integration. RAPD markers are a cheaper and simpler method of providing gene tags. They function by amplifying a sequence that appears as an electrophoretic band, that is closely linked to a gene in question (Penner *et al.*, 1993; Procunier *et al.*, 1994). Alternatively, the primer can be an actual base sequence of the transferred chromosome segment or particular gene. An example of the latter is a sequence of a virus coat protein gene that can be used on slot blots to detect the presence of BYDV in recombined progenies (Ouellette and Fedak, unpublished). Another example of a rapid screening technique is the use of monoclonal antibodies to screen for various metabolites such as the toxin produced by *Fusarium graminearum* (Sinha and Savard, unpublished).

Detection of alien chromatin

The objective in transferring traits from alien sources into crop plant chromosomes is to transfer the trait with minimal amounts of additional chromatin. With traditional staining methods it has been virtually impossible to detect the amount of alien chromatin that was translocated. The *in situ* hybridization techniques applied to somatic chromosomes of derivatives have effectively identified the integrated chromatin.

For example, the resistance to Hessian fly located on a 6RL rye telocentric chromosome addition was transferred to a terminal site on wheat chromosomes 6BS and 4BS following pollen irradiation (Mukai *et al.*, 1993) and to an interstitial location on wheat chromosome 4BS. This was achieved by sequential C banding and *in situ* hybridization using highly repetitive rye DNA probes. The size of the translocated segment was revealed in each case.

In similar fashion, the segment of *Th. intermedium* chromosome 7A carrying the leaf rust resistant gene *Lr38* was found to be translocated to wheat chromosomes 2AL, 5AS, 1DL, 3DS and 6DL in the different lines that were analyzed (Friebe *et al.*, 1993). The translocations were induced by Co^{60} treatment of a wheat-*Thinopyrum* addition line. Similarly a segment of a chromosome carrying wheat streak mosaic virus resistance was transferred from a wheat - *A. elongatum* disomic substitution line carrying chromosomal Ae-1L onto wheat chromosome 4DL (Jiang *et al.*, 1993).

The use of *in situ* hybridization adds a greater degree of resolution to cytogenetic studies. For example it was shown that what had previously been assumed to be a Robertsonian translocation based on ditelo analysis of a translocation line (Whelan and Hart, 1989) actually involved a proximal portion of the opposite arm (Kim *et al.*, 1992, 1993a).

A study of two wheat derivatives with radiation induced leaf rust resistance from *Th. ponticum* (Kim *et al.*, 1993b) showed that the lengths of the translocated segments were vastly different and that intercrosses between the lines and further *in situ* hybridization would be warranted to reduce the size of the translocated segment. It was also shown that considerable discrepancy existed between the physical map based on *in situ* hybridization patterns and the linkage map based on RFLP markers.

Current activities

There are a number of ongoing research activities of a genetic-cytogenetic nature but with a molecular base. These are going to provide more precise basic information about genetic-cytogenetic nature of relationships of Triticeae species, genome structure and evolution, and chromosome synteny. Additional techniques will facilitate gene flow from constituent members of the tribe into crop plant members.

Genomic *in situ* hybridization combined with RFLP maps of the major crops will provide better indications of genome structures within these crops in terms of inter and intragenomic translocations at the diploid parent level and derived polyploid level. An indication of such findings is the extensive interchromosomal translocations already detected in rye (Liu *et al.*, 1992). This will facilitate studies of species and crop plant evolution. The comparative RFLP mapping that is already underway will broaden the knowledge of syntenic relationships across species and crop plants. Since gene order is conserved across many species the location of a gene in a domestic species may be used to predict the location in a wild species. As gene tagging and map-based cloning techniques develop, this information will be useful especially for species where sexual hybridization is difficult.

Over the past year and following considerable effort, several labs have now reported the stable transformation of both wheat (Weeks *et al.*, 1993; Bilang *et al.*, 1993; Becker *et al.*, 1994; Nehra *et al.*, 1994) and barley (Wan and Lemaun, 1994). These achievements can probably be regarded as the most significant achievements in crop plant genetics in the past few decades. This will open up the possibilities of gene flow from any living organism into crop plants. The genome of rice may become the major source to circumvent gene isolation from the large and complex genomes of wheat and barley. The production of chromosome- specific libraries through microdissection (Albani *et al.*, 1993) will produce saturated RFLP maps for possible applications of map-based cloning.

Gene tagging with RFLPs and RAPDs will provide molecular markers for ever increasing numbers of genes. The RFLP markers are being converted to STS for PCR use while RAPDs are being converted to SCARS to provide extended applications over genotypes. The gene tags will permit the pyramiding of various gene combinations.

Traits such as tolerance to abiotic stresses, levels of value-added traits, and yield and its components are generally regarded to be controlled by polygenic systems. The production of RFLP maps of ever-increasing density will permit for the first time a better understanding of the QTL phenomenon, the chromosomal location of these factors, and a method of monitoring their manipulation.

With transformation now a reality, the incorporation of transposable elements into wheat and barley should be a possibility and thus an alternate method of gene tagging. Map-based cloning of genes from barley and wheat will undoubtedly be difficult because of the large genome size and large proportion of highly repetitive sequences, so rice may be an alternate source of genes for transformation of these two crops. Rice carries a much smaller genome that has been well mapped. The above are a few examples of techniques that are already being applied. New methods are bound to arise from the above that will be quicker, cheaper, and more discriminatory.

Although the technologies mentioned above have all been reported, they are still not routinely applied. RFLP maps have identified location of QTLs and quantitative loci to chromosome regions or linkage groups, for map-based cloning markers that flank the gene at distances of about 1 centimorgan are required. Very few such tight linkages have been reported. Transformation and stable integration of traits has been reported for all the major cereal crops, the frequencies are low and only a small proportion of transformations are stable. "Fine-tuning" of the above technologies is essential before they become routine.

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Perennial Lymegrass (*Leymus arenarius* and *L. mollis*) as Potential Crop Species for Northern Latitudes

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INTRODUCTION

The perennial lymegrass

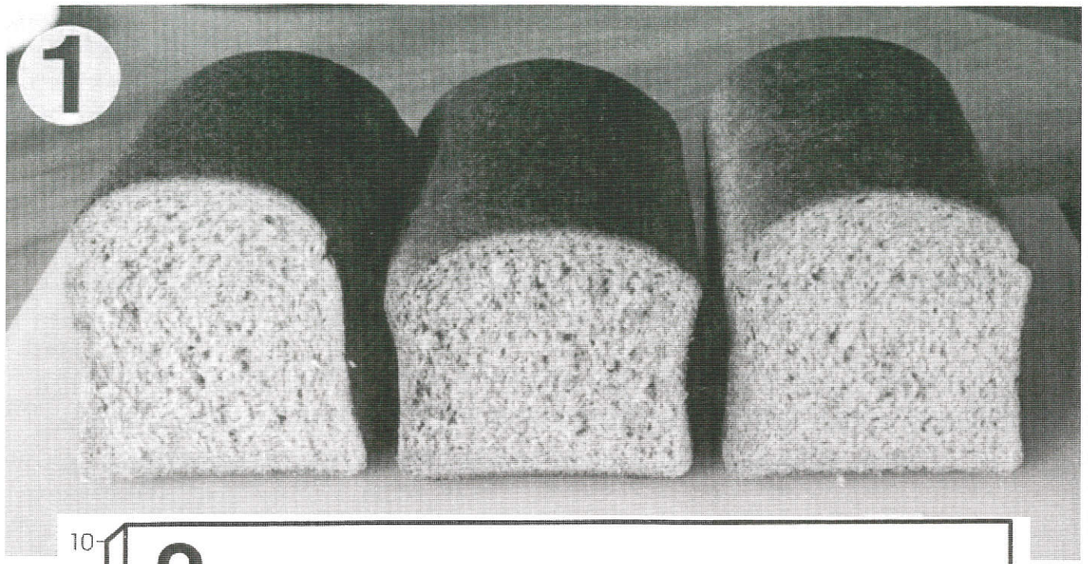
The most common species of lymegrass in the northern circumpolar regions are the European *Leymus arenarius* (L) Hochst. and the American *L. mollis* (Trin.) Pilger (Löve and Löve, 1975). These perennial and rhizomatous species of the tribe Triticeae tend to colonize coastal areas, but inland populations are also found. Morphologically the two species are so similar that it is often difficult to differentiate them, whereas cytologically they have different chromosome numbers. *Leymus arenarius* is octoploid with 2n=8x=56 and *L. mollis* is a tetraploid species with 2n=4x=28 (Löve and Löve, 1948; 1975), but both species share the same basic genome constitution (NX; Wang and Jensen, 1994). These two closely related species are geographically separated: *L. arenarius* is found in northern Europe, from Lapland and north-west Russia, Scandinavia and the countries along the Baltic Sea, to central Europe, and from England, Scotland, Faroe Islands to Iceland; whereas *L. mollis* is found in Greenland and the north American continent, on the shorelines of both the Atlantic and Pacific coasts, in the Canadian Arctic and Alaska (Sigurbjörnsson, 1960; Barkworth and Atkins, 1984). The species also coexist in some places due to natural or intentional introductions, for example *L. arenarius* in southern Greenland and Canada (Ahokas and Fredskild, 1991) and *L. mollis* recently introduced in Iceland. The significance of such extensive distribution is that the potential cultivation areas for lymegrass, given domestication, are enormous. At present most of these areas are not suitable for common crop species like wheat or barley.

The aim of the present study is to improve the perennial lymegrass (*L. arenarius* and *L. mollis*) for cultivation as potential cereal crop for Iceland as well as for other regions of native lymegrass distribution. The study will also provide cereal breeders with broader genetic resource containing several characters of the wild species such as tolerance to extreme environments and perhaps resistance to pathological diseases.

The use of lymegrass for bread-making

Lymegrass has a long history of use for human consumption. Earliest records of lymegrass in Iceland date back to the Icelandic sagas. Carbonized remains of the grass have been discovered in Viking archeological sites, especially in Iceland and Greenland, as well as an increase in *Leymus* pollen with the Viking homesteads in Newfoundland (see Griffin and Rowlett, 1981). In Iceland, lymegrass (*L. arenarius*) grains were used as a source of bread flour until the 19th century, and in the south coast areas of Vestur-Skaftafellsssla the local production of lymegrass flour was apparently sufficient that no other flour was imported (Sigurbjörnsson, 1960). Grains of *L. mollis* were also used by North-American Indians, while these of *L. arenarius* were sometimes gathered in north Russia for the same purpose (in Klebesadel, 1985).

The quality of lymegrass flour for bread-making was known to be high, and some reported that products made out of lymegrass flour were even better than from any imported flour at that time (e.g. in Hooker, 1813). But the characteristics of bread-making are unknown by the present standards. In collaboration with the Flour Milling and Baking Research Association at Choleywood (UK), we investigated the quality of lymegrass flour. Lymegrass grains were obtained from Eyrarbakki population in south



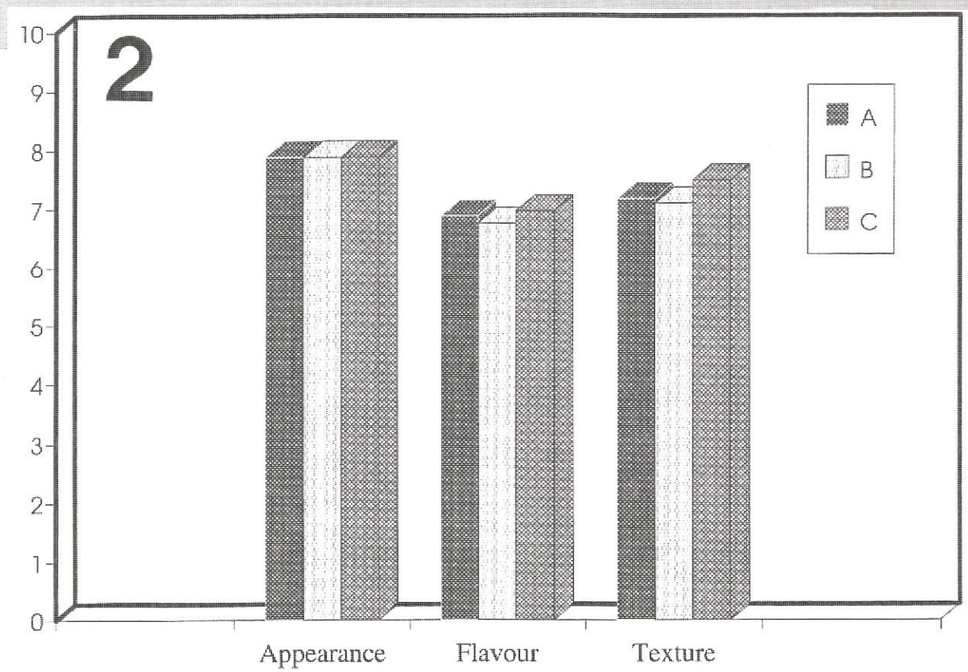


Figure 1. Bread made from different mixtures of whole-meal wheat flour from Canadian winter wheat variety and lymegrass flour milled from grains of *Leymus arenarius* havested in Eyrarbakki, south Iceland: (A) 100% wheat flour, (B) 85% wheat flour and 15% lymegrass flour, and (C) 80% wheat flour, 15% lymegrass flour and 5% gluten. The baking was prepared by Flour, Milling and Bread Research Association at Choleywood, UK.

Figure 2 Results of taste test of the bread in Fig. 1, conducted by Food Research Department, Icelandic Agricultural Research Institute. the evaluation was given as values from 9 to 10, where 0: unacceptable, 5: neither good nor bad, and 10: exceptionally good. The means of 25 independent evaluations are presented here. The standard deviation for all three characters is small, between 1.0 and 1.5.

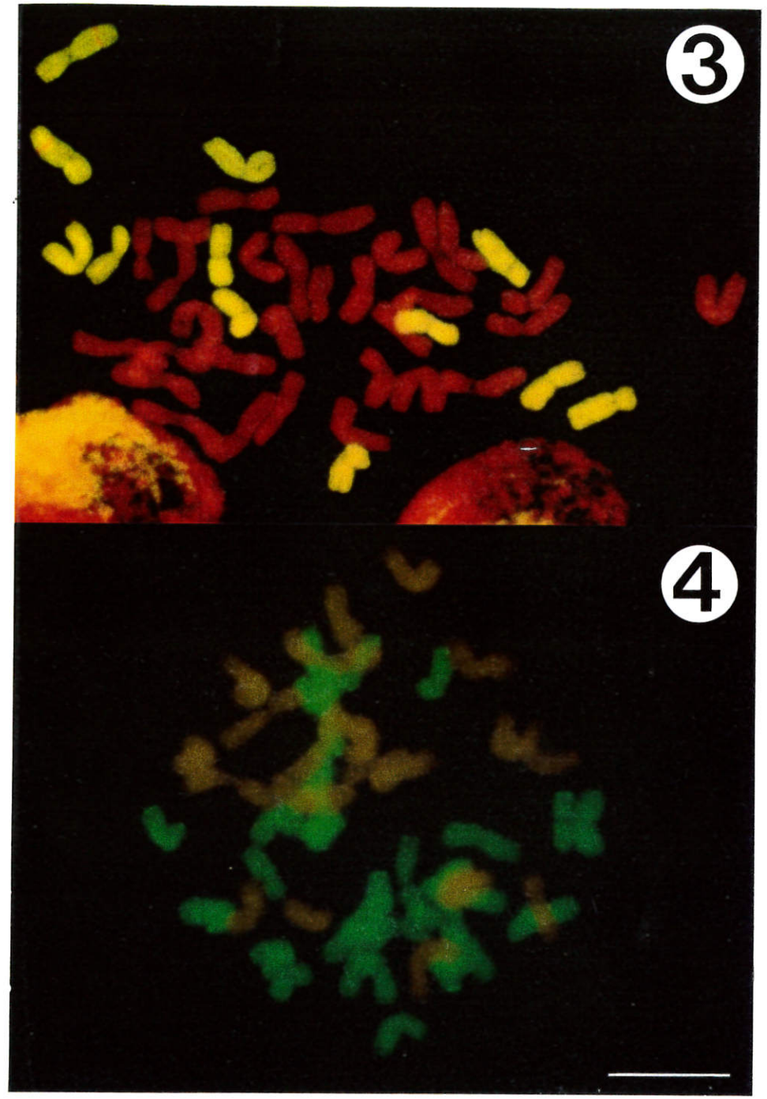


Fig. 3. A metaphase cell of wheat x lymegrass partial amphiploid PI442574 showing 12 yellow-green FITC fluorescing chromosomes originated from lymegrass and 30 red propidium iodide stained wheat chromosomes.

Fig. 4. A metaphase cell of wheat x lymegrass hybrid (*Triticum aestivum x Leymus arenarius*) showing a haploid set of 28 green FITC fluorescing lymegrass chromosomes and a haploid set of 21 red-brown rhodamine fluorescing wheat chromosomes. Scale bar: 10 µm.

Iceland. The grains were mechanically harvested and threshed, using the facilities developed for seed production for land reclamation purposes (Greipsson and Davy, 1994). Icelandic lymegrass (*L. arenarius*) has relatively large grains, about on third to half the dry weight of wheat grains, depending on accessions, and are twice as large as most samples of *L. mollis*. Whole grains were milled and the flour was used in the baking experiment as described in Fig. 1. Three different breads were made: (A) all wheat bread. (B) wheat bread containing 15% lymegrass and (C) wheat bread containing 15% lymegrass and 5% pure gluten, each bread in triplicate. Some of the results have been obtained and among them are the taste testing (Fig. 2) and chemical analysis of the breads. The breads made from wheat and lymegrass flour mixtures (B and C) were similar to that of the high quality wheat bread (A), in appearance, flavor and texture (Fig. 2). In general, all three breads were highly acceptable. In addition, the breads containing lymegrass flour were described as having favorable characters as nut-like flavor and yellowish color. The bread made from wheat and lymegrass flour mixture (B) was more flat than the control bread (A), but when supplemented with pure gluten (C), its physical quality was recovered. However, all the breads had high protein content and good nutritional and dietary quality. The high protein content was found in both the wheat and the lymegrass flour. The wheat variety used in this experiment has exceptionally high total protein (17% dry weight) and lymegrass flour contains about 19%, whereas flour of most wheat varieties contains between 9% and 14% protein (Reykdal, 1993). The present study also shows that the lymegrass flour has significantly higher mineral content, especially calcium, potassium and iron, than all other cereal flour, while its fatty acid content is lower than in other cereals. The overall quality of lymegrass flour, however, appears to be variable among the accessions and further studies will be important for selection of the breeding materials.

BREEDING

Wide-hybridization for simultaneous improvement of lymegrass and wheat

Bread wheat (*Triticum aestivum* L. em. Thell.) is used in the wide-crossing program aiming to transfer important crop characteristics into lymegrass, for example physical quality of bread-making and grain size. Wheat and lymegrass wide-hybrids have been made, from both *L. arenarius* and *L. mollis*. The hybrids will be used for developing amphiploids and for back-crossing with the lymegrass parents, aiming to produce lymegrass breeding lines containing crop characters of wheat while maintaining characters of the wild species such as perenniality and adaptability to sub-Arctic environments.

The wide-hybrids can also be used for wheat improvement. In contrast to the new breeding program for lymegrass described here, transfers of characters from wild species to crops have been extensively practiced (e.g. Gale and Miller, 1987). Several Triticeae species have been involved in wheat improvement - for example, rye (*Secale cereale*) in wheat cultivars 1B/1R (reviewed in Heslop-Harrison et al., 1990), wild barley (*Hordeum chilense*) adding nematode resistance to wheat (Person-Dedryver et al. 1990) and *Agropyron* for rust disease resistance (Friebe et al., 1992). *Leymus*, especially Asiatic species like L. *racemosus* and L. *multicaulis*, has also received much attention for wheat breeding (Mujeeb-Kazi and Rodriguez, 1981; Dong et al., 1986; Plourde et al., 1989), and wheat breeding lines containing *Leymus* chromosomes have been identified. Several traits of *Leymus* have been targeted, especially the resistance to virus and fungal diseases. The relevance of the present study to wheat breeding is that the sub-Arctic lymegrass species (i.e. *L. arenarius; L. mollis*), which has been little exploited, can add to the genetic diversity of crops via the wide-hybrids wheat x lymegrass.

Wide-hybrids involving wheat (both tetraploid and hexaploid species) and several species of *Leymus* were made in the early 1960's (e.g. Tsitsin, 1965; D. Dewey, unpublished), and a few amphiploid lines deriving from these hybrids are still maintained. We have obtained two partial amphiploid lines for cytogenetic and breeding purposes: "AD99" from Professor Arnulf Merker, Swedish University of Agriculture at Uppsala, Sweden, and "PI442574/Dewey" from Professor Bikram Gill, Wheat Genetics Center, Kansas State University, USA. The AD99 is derived from back-crossing of the hybrid *T. durum x L. mollis* to bread wheat, while the PI442574 is derived from a cross between a *Triticum* species and *L. arenarius*. Both lines have been maintained for more than ten generations. We found that both amphiploids had 42 chromosomes, 12 of which have originated from *Leymus*, while 30 chromosomes have wheat origin (Fig. 3). One of these lines, AD99, was shown to have high resistance to mildew and leaf rust (Fatih, 1983; Merker, 1992). Although these materials are not suitable for Icelandic climates, they are valuable for genetic studies and breeding. Methods including *in situ* karyotyping and chromosome mapping will allow identification of chromosomes carrying important agronomic characters, while the plant materials can be used for further back-crossing to either lymegrass or wheat.

We made new wide-hybrids between wheat and lymegrass in the summer of 1993. The seed parent used in the wide-crossing was hexaploid bread wheat *T. aestivum* cv. Sicco (CS/5B) provided by Cambridge Laboratory, John Innes Center, Norwich, UK. The pollen parents were the tetraploid American lymegrass *L. mollis* originating from Alaska Peninsula and the octoploid European lymegrass *L. arenarius* collected from a wild stand in Reykjavík. The crosses were conducted in an unheated glasshouse at Korpa Experimental Station in Reykjavík. The method of crossing and embryo rescue followed Laurie and Bennett (1986). About 3% of the developed ovaries contained embryos. The hybrids were treated with colchicine and the regenerated plants have been grown to flowering. The mature hybrids showed vigorous vegetative growth and rhizomatous habit. Cytological study confirmed the hybridity and the colchicine doubling of chromosomes. Root-tip chromosomes were analyzed using genomic *in situ* hybridization (Schwarzacher it al., 1989; Anamthawat-Jónsson et al., 1990), which was modified using pre-annealing of two differently labeled genomic DNA probes (Anamthawat-Jónsson et al., unpublished) and rapid *in situ* hybridization protocol (Reader et al., 1994). All hybrids before colchicine treatment showed haploid chromosome number of wheat and lymegrass genomes - *T. aestivum x L. mollis*, 5x-35; *T. aestivum x L. arenarius*, 7x=49 (Fig. 4), where 21 chromosomes originated from wheat and 14 or 28 chromosomes from *L. mollis* and *L. arenarius* respectively. No elimination of chromosomes were observed. Colchicine treated plants showed high proportion of diploid root-tip cells. Molecular cytogenetic studies will be important in the further breeding work, especially to follow chromosome behavior and recombination during the stabilization of amphiploids and to identify transfer of chromosomes carrying genes of interest during the production of lymegrass breeding lines.

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Progress in Polyhaploid Production Techniques of Hexaploid Wheat through Wide Crosses

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ABSTRACT

Polyhaploid production techniques of hexaploid wheat (*Triticum aestivum* L.) through wide crosses were evaluated in terms of pollen sources, 2,4-D application, embryo rescue and chromosome retention. Pollen sources included *Hordeum bulbosum* L., *Zea mays* L., *Pennisetum glaucum* (L.) R. Br., *Sorghum bicolor* (L.) Moench, and *Tripsacum dactyloides* (L.) L. Maize-mediated polyhaploid production was more stable than the other methods because of a lesser genotypic influence on embryo formation. Application of 2,4-D onto wheat after pollination was critical to promote seed setting and embryo formation in all cross combinations. Embryo rescue was necessary at an appropriate embryo developmental stage to obtain plant regeneration. Paternal chromosomes were eliminated by the stage of active growth of the polyhaploid seedlings. Polyhaploid production frequencies ranged between 10 and 20% of pollinated wheat florets, suggesting germplasm genotypic effects.

INTRODUCTION

The main objective of wide hybridization in the tribe Triticeae is alien genetic transfer, which is facilitated by tissue culture and cytogenetic manipulation (Sharma and Gill 1983, Mujeeb-Kazi and Asiedu 1990). Chromosomal stability of wide hybrids is dependent on cross combinations. Failure of alien genetic transfer after fertilization, as a consequence of the preferential elimination of chromosomes of one parent, was first reported in *Hordeum* hybrids (Symko 1969, Kasha and Kao 1970). Artificial rescue of haploid embryos was required since endosperm did not develop in most of seeds set. Monoploid production technique of barley (*Hordeum vulgare* L., 2n=2x=14) through crosses with *H. bulbosum* L. was developed in the 1970's (Jensen 1976).

Intergeneric crosses of hexaploid wheat (*Triticum aestivum* L., 2n=6x=42) with *H. bulbosum* also produced immature embryos capable of regenerating polyhaploids (3x=21) wheat plants (Barclay 1975). A polyhaploid production technique using *H. bulbosum* crosses was reviewed and its application restricted to cross-compatible wheat genotypes (Inagaki 1990). This paper reviews the development of various techniques for polyhaploid production of hexaploid wheat through wide crosses with the members of the Gramineae family.

BREEDING

H. bulbosum crosses

Cross-compatibility between hexaploid wheat and *H. bulbosum* depends on both parental genotypes (Snape *et al.* 1979, Falk and Kasha 1981, Inagaki and Snape 1982, Sitch and Snape 1986). The dominant genes *Kr1* and *Kr2*, located on wheat chromosomes 5B and 5A, respectively, control the cross-incompatibility (Falk and Kasha 1983, Sitch *et al.* 1986). The absence of fertilization in cross-incompatible wheat genotypes is attributed to the failure of pollen tube to penetrate the embryo sac (Snape *et al.* 1980, Sitch and Snape 1987a). Therefore, at present, wheat genotypes used for polyhaploid production are restricted to those that are cross-compatible with H. bulbosum. These wheat genotypes presumably have their origin in Asia (Falk and Kasha 1981, Inagaki and Snape 1982, Li and Hu 1986). According to the genealogical pedigrees of Japanese cultivars (Fukunaga and Inagaki 1985), wheat cultivars derived from the hybrid progenies of local varieties are highly cross-compatible (Inagaki 1986). On the other hand, *H. bulbosum* genotypes with heterogeneity show great variation in their ability to affect cross-compatibility of wheat. However, *H. bulbosum* genotypes that may be sufficiently cross-compatible with all wheat genotypes have not yet been found (Inagaki 1986, Sitch and Snape 1986).

When the cross-compatible wheat genotypes are used for crosses, the production efficiency is enough to obtain

200 polyhaploids from 1000 wheat florets (40 spikes) pollinated by *H. bulbosum* (Inagaki 1989). It also takes 26-30 weeks from crossing to harvest of doubled haploid grains by using environment-controlled facilities. This technique requires further development because of the restriction of wheat germplasm.

Application of plant growth regulators

Reduction of the cross-incompatibility barrier of hexaploid wheat has been attempted by means of some synthetic plant hormones. Marshall *et al* (1983) reported that the application of a 2,4-dichlorophenoxyacetic acid (2,4-D) solution onto emasculated wheat florets prior to pollination induced parthenocarpic seed development and increased ovule size due to cell expansion. This however did not have a favorable effect on seed setting in crosses with *H. bulbosum*. To avoid physiological damage of 2,4-D on wheat florets, Inagaki (1986) adopted a method to inject repeatedly a 2,4-D solution (100 mg/l) in the wheat culms from emasculation to pollination. The development of wheat caryopses after *H. bulbosum* pollination was similar to that observed during self-pollination. The embryo formation frequency was twice that of the control when a cross-compatible wheat genotype was utilized. This crossing method also produced embryos with a cross-incompatible genotype at very low frequencies, suggesting that the 2,4-D application enhanced seed and embryo development after fertilization rather than increasing fertilization itself. Effectiveness of the 2,4-D treatment was further confirmed during production of wheat x barley hybrids (Koba and Shimada 1991, Riera-Lizarazu *et al.* 1992a). Other hormones such as indole-3-acetic acid and gibberellic acid did not reduce the cross-incompatibility mechanism (Falk and Kasha 1982).

Pollen source

Alien genetic transfer to hexaploid wheat has been attempted in crosses with the members of the *Panicoides* subfamily (Zenkteler and Nitzsche 1984). Cytological evidence indicates that the fertilization of wheat with maize (*Zea mays* L.) pollen was successful, irrespective of the presence of *Kr* gene(s), and produced hybrid zygotes (Laurie and Bennett 1986, 1987a). In these zygotes, the maize chromosomes were rapidly lost within two days after pollination (Laurie and Bennett 1987b), necessitating artificial rescue of proembryos at early developmental stages (Laurie and Bennett 1988a). Suenaga and Nakajima (1989) found an enhancing effect of 2,4-D treatment on the embryo development in wheat caryopses. Inagaki and Tahir (1990) demonstrated that maize pollination of, and subsequent 2,4-D treatment onto wheat also resulted in production of wheat embryos capable of regenerating polyhaploid plants, even for wheat varieties that were cross-incompatible with *H. bulbosum*. Genotypic differences in embryo formation frequency were significant only for the wheat parent. Polyhaploid production through maize crosses has been further confirmed using diverse wheat varieties (Inagaki and Tahir 1990, Laurie and Bennett 1991). Some species related to maize, like teosinte (*Z. mays* L. spp. *mexicana*) and eastern gamagrass (*Tripsacum dactyloides* (L.) L.) are alternative pollen sources for wheat polyhaploid production (Ushiyama *et al.* 1991, Riera-Lizarazu and Mujeeb-Kazi 1993). For increasing flexibility to handle wheat materials, detaching the wheat spikes pollinated with maize and culturing them in a nutrient solution containing 2,4-D was developed (Ushiyama *et al.* 1991, Riera-Lizarazu *et al.* 1992b).

In sorghum (*Sorghum bicolor* (L.) Moench) and pearl millet (*Pennisetum glaucum* (L.) R. Br.) crosses, successful fertilization and elimination of paternal chromosomes from hybrid zygotes were observed (Laurie and Bennett 1988b, Laurie 1989b). Efficient formation of polyhaploid embryos in these crosses indicated that the 2,4-D application was essential (Ohkawa *et al.* 1992, Inagaki and Mujeeb-Kazi 1994a). Significant embryo formation frequency differences existed among wheat varieties. Sorghum crosses however, expressed a strong genotypic barrier to embryo formation (Inagaki and Mujeeb-Kazi 1994a). Therefore, the maize-mediated polyhaploid production appears more stable than the other methods because of its lesser genotypic effect on embryo formation.

A technique of storing pollen for long periods is helpful for crosses onto wheat parents without having to synchronize flowering times of both parents. Long-term pollen storage at ultra-low temperatures is feasible in maize (Barnabas and Rajki 1981) and pearl millet (Hanna 1990). Dried maize pollen with 10 to 12% water content, stored for three months at -80°C produced embryos on wheat at half the frequency of fresh pollen (Inagaki and Mujeeb-Kazi 1994b). Stored maize pollen can thus be used for wheat polyhaploid production when and where fresh pollen is not available.

Polyhaploid production efficiency

A technique of polyhaploid production in hexaploid wheat through wide crosses consists of two steps; hybridization and embryo rescue. A factor affecting polyhaploid production efficiency was the developmental stage of the wheat florets at crossing. This was critical in crosses with *H. bulbosum* (Sitch and Snape 1987b), maize (Laurie 1989a) and pearl millet (Inagaki and Bohorova 1994). Environmental conditions of humidity and temperature also affected embryo formation frequency in crosses with *H. bulbosum* (Inagaki and Snape 1982, Inagaki 1986, Sitch and Snape 1987c). Further, the developmental stage of wheat embryos formed was critical with respect to plant regeneration frequency in crosses with *H. bulbosum* (Inagaki 1985) and pearl millet (Inagaki and Bohorova 1994). In general, pollination at an early developmental stage of wheat florets under a high level of humidity and temperature resulted in higher frequencies of embryo formation. Artificial rescue at a suitable embryo developmental stage was required to attain a higher frequency of plant regeneration. These procedures are routinely utilized in other Triticeae wide crosses (Mujeeb-Kazi *et al.*, 1987, 1989).

Crosses of diverse hexaploid wheat varieties with maize resulted in embryo formation frequencies of 32.7 % (Laurie and Reymondie 1991), 21.7% (Inagaki and Tahir 1990) and 15.6%, 43.7% and 60.7%, respectively. These values are comparable to those obtained for the *H. bulbosum* crosses. Consequently, the polyhaploid production frequency with maize ranged from 20.1% to 9.5%, suggesting some genotypic effect of the wheat and maize germplasm used for crosses.

Cytological examination of the regenerated wheat plants indicated that most of them were euhaploids with 21 chromosomes (Inagaki and Tahir 1990, Riera-Lizarazu *et al.* 1992b, Inagaki and Mujeeb-Kazi 1994a). Retention of paternal chromosomes was detected at the proembryo stage in crosses with maize and sorghum (Laurie and Bennett 1986, 1988a), at the tillering stage of plants from crosses with pearl millet and maize (Ahmad and Comeau 1990, Comeau *et al.* 1992), and at the doubled haploid plant stage derived from the crosses with *H. bulbosum* (Inagaki 1987). These facts do not eliminated the possibility of translocating chromosome segments of pollen parents to the wheat genome through somatic associations. However, variations in agronomic traits of doubled haploids were negligible in *H. bulbosum* (Inagaki 1987, Snape *et al.* 1988) and maize (Laurie and Snape 1990, Suenaga and Nakajima 1993a) crosses. Significant distortion of segregation ratios in the doubled haploids derived from hybrid progenies was not observed in *H. bulbosum* (Inagaki and Tahir 1991, Inagaki and Egawa 1994) and maize (Suenaga and Nakajima 1993b) crosses.

CONCLUSION

Two decades were spent to establish a technique for producing polyhaploids from diverse genotypes of hexaploid wheat through wide crosses. Significant technical developments were attributed to pollen selection from different subfamilial species and the application of 2,4-D. At present, doubled haploids derived from hybrid progenies can be used as materials of recombinant inbred lines in genetic analyses and breeding purposes. However, it needs more development to extend this technique to the related crop species. Polyhaploid production in durum wheat by maize crosses was demonstrated (Riera-Lizarazu *et al.* 1992b), but dependent upon its genotypic variation (Inagaki and Tahir unpublished).

The initial objective of wide crosses is to augment the genetic diversity of the existing germplasm. Process of fertilization and embryo formation have been already confirmed in crosses of hexaploid wheat with maize, sorghum and pearl millet. The third step in producing symmetrical or asymmetrical hybrids is to develop a technique for retaining some paternal chromosomes in these distant hybrids. Cytogenetic manipulation and molecular approaches will be increasingly required to materialize this step.

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Prospects for Gene Introgression from *Hordeum bulbosum* L. into Barley (*H. vulgare* L.).

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INTRODUCTION

Hybridizations between *Hordeum vulgare* L. (cultivated barley) and *H. bulbosum* L. have been performed over many years with two aims. First, the production of doubled haploid barley cultivars (Kasha and Kao 1970); second, the transfer of desirable traits such as resistance to fungal and viral pathogens from the wild species into barley. Apart from the report of an occasional recombinant (Xu and Kasha 1992; Pickering *et al* 1994), successful gene introgression has been hampered by several barriers. In this report we will describe recent progress in overcoming these barriers.

BREEDING

Incompatibility

Incompatibility that occurs in crosses between *H. vulgare* and *H. bulbosum* before fertilization (pollen tube inhibition, Pickering and Hayes 1976) or after fertilization (endosperm breakdown) has been resolved, respectively, by selecting particular parental genotypes in the crossing program (Pickering 1980) and using conventional embryo rescue techniques.

Chromosome instability

After fertilization, *H. bulbosum* chromosomes are usually eliminated from the immature embryo (Kasha and Kao 1970), especially when genome ratios of 1V : 1B occur (where V and B are the *H. vulgare* and *H. bulbosum* genomes, respectively). *H. bulbosum* chromosome retention can be promoted by manipulating the genotype and by allowing embryos to develop below 17.5°C (Pickering 1985). This has simplified diploid VB hybrid production, and even enabled triploid VVB (2V : 1B) hybrids to be obtained directly from crosses of *H. vulgare* (4x) x *H. bulbosum* (2x) (Pickering 1991a). Conversely, VBB (1V : 2B) hybrids from *H. vulgare* (2x) x *H. bulbosum* (4x) crosses can, as expected, frequently be regenerated.

Chromosome pairing

Pairing between *H. vulgare* and *H. bulbosum* homoeologues was inexplicably absent in VVB hybrids described by Pickering (1991a), compared with those VVB hybrids reported by Pohler and Szagat (1982). In VB and VBB hybrids allosyndesis is variable and influenced by parental genotype (Thomas and Pickering 1985; Xu and Snape 1988; Pickering 1992) making it possible to identify high-pairing hybrids. Since environmental conditions affect seed development (Thörn 1992) and chromosome stability in *H. vulgare* x *H. bulbosum* crosses (Pickering 1985), the influence of temperature on chromosome pairing in VB and VBB hybrids was studied. To promote allosyndesis, a temperature of 21°C was more suitable than 15°C during meiosis (Pickering 1990) but, as temperatures greater than 17.5°C also induce *H. bulbosum* chromosome elimination (Pickering 1985), optimum temperatures should be determined for particular hybrid combinations to combine maximum chromosome retention and chromosome pairing.

Hybrid infertility

A pre-requisite for using *H. vulgare* x *H. bulbosum* hybrids in conventional breeding programs is fertility. VB hybrids possess indehiscent anthers and have not proved useful even after obtaining rare backcross seed. Fertility can be induced by doubling the chromosome number to create tetraploid VVBB hybrids. Selfed progeny from the VVBB hybrids usually resemble their *H. vulgare* parents or maintain their hybridity, but occasional recombinants or 'modified' barley plants have been obtained (see final section). Although VVB hybrids are moderately fertile, their value for gene introgression is limited because of low allosyndetic pairing (Pickering 1991a). Until recently, only indehiscent anthers were found in VBB hybrids, but when *H. vulgare* (2x) was pollinated with *H. bulbosum* (4x) that had been derived from colchicine-treated diploid *H. bulbosum* genotypes, partially fertile VBB hybrids were obtained (Pickering 1988). It is possible that the colchicine treatment induced anther dehiscence in the VBB hybrids, since colchicine can cause heritable disturbances to plant development (Hague and Jones 1987) and gametogenesis (Hassan and Jones 1994). The VBB hybrids have been successfully backcrossed to barley and chromosomally engineered plants and recombinants identified (see final section).

Crossing over

Because of the relative scarcity of *H. vulgare* - *H. bulbosum* recombinants among progeny from fertile hybrids (Lange and Jochemsen 1976), crossing over between paired homoeologues may be very low. To investigate this possibility, a paracentric inversion in barley was crossed with a diploid *H. bulbosum* genotype and several VB hybrids were obtained. These hybrids were cytologically analysed and compared with a barley inversion heterozygote (Pickering 1991b). In the latter, an inversion loop is formed, and when crossing over occurs within the loop, several anomalies can be observed at meiotic anaphase I and II comprising bridges and/or fragments. The proportions of bridges and fragments vary according to the frequency of crossovers and the length and location of the inversion. Aberrations in the VB hybrids occurred less frequently (1.0%) than in the barley inversion heterozygote (12.3%), but their presence indicated that crossovers between the parental chromosomes occasionally took place. However, the rarity of this event constitutes a considerable barrier to obtaining recombinants, and no satisfactory method of inducing crossing over has yet been found in *H. vulgare* x *H. bulbosum* hybrids.

Certation (Pollen tube competition)

In backcrosses of hybrids to *H. vulgare*, competition between pollen grains of different chromosomal constitution derived from hybrids remains a problem. In VBB hybrids, gametes with seven *H. bulbosum* chromosomes should predominate but different combinations of *H. vulgare* and *H. bulbosum* chromosomes are also likely to arise, according to the observed meiotic configurations (Lange 1971; Xu and Snape 1988). Fertilization of *H. vulgare* eggs by gametes with seven *H. bulbosum* chromosomes would lead to haploid *H. vulgare* plant formation after *H. bulbosum* chromosome elimination. However, *H. vulgare* haploids were less commonly observed than diploid *H. vulgare* plants among progeny from *H. vulgare* x VBB crosses (Pickering 1992), and fertilization must have been effected preferentially by gametes containing seven *H. vulgare* chromosomes. To overcome this problem, reciprocal crosses (VBB x *H. vulgare*) were attempted, but success has been limited. To avoid conventional hybridizations altogether, androgenesis was carried out by culturing anthers from various hybrid combinations, but only those hybrids that possessed dehiscent anthers yielded positive results (Pickering and Fautrier 1993). Seven viable green plants were regenerated that included (i) an aneuploid comprising 14 *H. vulgare* chromosomes + 1 acrocentric *H. vulgare* chromosome 4(4l) and one *H. bulbosum* chromosome; (ii) a 14-chromosome plant similar to a VB morphologically but containing 6 *H. vulgare* + 8 *H. bulbosum* chromosomes. This plant was backcrossed to barley and two plants involving double and triple monosomic substitutions of *H. vulgare* chromosomes by their *H. bulbosum* homoeologues were obtained.

Progress and future prospects

Despite the obstacles preventing consistent gene introgression between *H. vulgare* and *H. bulbosum*, 40 plants from backcrosses of VBB hybrids to barley have been produced that involve the single, double and triple monosomic substitutions of barley chromosomes 1(7l), 2(2l), 3(3l), 4(4l), 6(6l) and 7(5l) by their *H. bulbosum* homoeologues (Pickering *et al* 1994). Retention of the *H. bulbosum* chromosome in the substitution plants can be promoted by growing plants in a suitable environment (15°C; Pickering 1994). The most fertile and frequent plants to arise are those having barley chromosomes 1(7l) or 6(6l) substituted, and recombinants have been identified among selfed progeny. Transfer of single genes from *H. bulbosum* has also been reported following backcrosses of VBB hybrids to barley (Xu and Kasha 1992; Timmerman *et al* 1993). The use of tetraploid hybrids has not been as fruitful. Szigat and Pohler (1982) selected 'modified' barley plants from BBVV backcross progeny, and introgression of *H. bulbosum* DNA into a plant with pubescent leaf sheaths was confirmed using a repetitive sequence molecular probe - pSc119.2 (Pickering, Smart and Melz, unpubl). Michel *et al* (1994) described a plant with a single mildew resistance gene and a plant with barley mild mosaic virus (BaMMV) resistance, following screening of selfed progeny from a VVBB hybrid. Based on electrophoretic evidence, the transferred *H. bulbosum* DNA of the BaMMV resistant plant is located on barley chromosome 6(6l).

CONCLUSION

From the results presented above, we conclude that interspecific gene transfer from *H. bulbosum* into *H. vulgare* is possible, but difficult. The techniques are labour intensive and time consuming, but the introduction of novel traits and disease resistances into barley has made this program worthwhile.

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Breeding Potential of Durum Wheat Landraces from Jordan III. Rate and Duration of Grain Fill

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ABSTRACT

Grain fill of durum wheat coincides with terminal drought and high temperature stress in the Mediterranean region. Genotypic variation for rate and duration of grain fill was studies in 250 landrace durum wheat genotypes collected in Jordan. A quadratic polynomial was used to describe the relationship between kernel weight and accumulated growing-degree-days from anthesis to maturity. Fitted curves were employed to estimate rate and duration of grain fill. Genotypic differences were found for both traits. Genotypes with high grain filling rate and high kernel weight were identified. Based on grain yield per spike, spikelet fertility, 1000-kernel weight, rate and duration of grain fill, four clusters were identified in this germplasm collection. Correlations between these traits were inconsistent across these clusters, however, rate and duration of grain fill were not correlated across clusters, suggesting that high rate and short duration of grain fill can be combined in one genotype. Canonical discriminant analysis confirmed univariate analysis of variance and resulted in 95% correct classification of genotypes.

INTRODUCTION

Final grain weight is one of the most important yield components in wheat (*Triticum* spp.). It is determined, to a large extent, by rate and duration of grain fill (Gallagher *et al*., 1976; Jones *et al*., 1979). Wheat grown under arid and semiarid Mediterranean environments often undergoes prolonged periods of water and heat stress during grain fill (Acevedo, 1991). Grain yields of durum wheat in Jordan are more closely associated with variation in precipitation than with variation in temperature (Jaradat, unpublished data). After seed number has been determined, cereal grain yields become proportional to kernel weight (Wiegand and Cuellar, 1981), which is a function of the rate and duration of grain fill (Gallagher *et al*., 1976). Rate of grain fill, which is dependent upon the number of endosperm cells formed during the first two weeks after anthesis (Acevedo, 1991; Lengile and Chevalier, 1992), increasees only moderately with increased temperature, but duration of grain fill has a strong negative response to increasing temperature (Bruckner and Frohberg, 1987; Wiegand and Cuellar, 1981) and a strong positive response to available soil moisture (Bruckner and Frohberg, 1987; Wong and Baker, 1986).

Wiegand and Cuellar (1981) suggested that genetic variability in grain fill rate should be searched for and exploited in wheat improvement programs because genetic factors largely determine grain fill rate and environmental factors largely determine grain fill duration. High rate and short duration of grain fill may contribute to higher kernel weights and yields in cultivars developed for short growing environments (Gebeyehou *et al*., 1982) and environments prone to sever postanthesis stress (Wiegand and Cuellar, 1981) such like the Mediterranean environment.

Lengthening of the grain fill period through earlier heading and flowering (Acevedo, 1991; Wong and Baker, 1986) and identification and incorporation of drought tolerance traits that would allow photosynthesis and grain growth to continue under drought (Bruckner and Frohberg, 1987) are alternative strategies for achieving higher kernel weights and yields under drought stress. Early maturity is a desirable trait in cereal crops growing under arid and semiarid environments. Because efforts to reduce time to maturity often result in reduced grain yield (Wong and Baker, 1986), a thorough understanding of the developmental aspects of time to maturity may assist in developing early maturing cultivars with acceptable grain yield.

Landrace genotypes of durum wheat from Jordan have been evaluated for five developmental traits (Jaradat, 1991). These included days to booting, days to anthesis, days to heading, and days to maturity. Filling period was estimated as the difference between days to anthesis and days to maturity. Landrace genotypes with different

combinations of early, medium and late days to heading, days to maturity and filling period were identified in the Jordanian material. Landrace genotypes with medium-late days to heading and along filling period gave the highest grain yields. A thorough understanding of the grain filling process in these genotypes may be very helpful in attempts to select or to breed for increased grain yields and early maturity in durum wheat under the drough-prone Mediterranean environment.

The objectives of this research were to (1) evaluate genotypic variation for rate and duration of grain fill in a diverse set of landrace genotypes of durum wheat collected from Jordan and (2) examine relationships between estimated grain fill parameters and genotypic productivity.

MATERIALS AND METHODS

A total of 250 landrace genotypes, representing all possible combinations of early, medium and late days to heading, days to maturity and filling period, were used in this study. The local durum wheat cultivar, Hourani 27, was used as a check. Each landrace genotype was grown on a 1-m^2 plot, with two replicates. The local check was planted in every tenth plot. The experiment was conducted at the Research Station of Jordan University of Science and Technology (32.50 N, 36.00 E, 550 m above sea level).

At anthesis, the first 60-70 spikes that extrude anthers from central florets were tagged in each plot. Samples of five tagged spikes were collected twice a week from each plot beginning one week after anthesis and continued past harvest maturity. Each sample was oven dried at 80 C, then hand threshed. Number of spikelets per spike, grain dry weight and kernel number were determined for each spike and the latter two were used to calculate average kernel weight. Number of fertile florets per spikelet were estimated using number of spikelets and number of seed per spike. Accumulated growing-degree-days (GDD) from anthesis was used as a time scale during the grain fill period because the rate of wheat development is determined largely by temperature (Bruckner and Frohberg, 1987; Wiegand and Cuellar, 1981). A base temperature (Tb) of 5 C was used to calculate daily degree-days as follows:

Tn=(Tmax + Tmin)/2 - Tb, where Tmax and Tmin are daily maximum and minimum temperature, respectively. For each landrace genotype, the relationship between grain weight and accumulated GDD from anthesis was described by fitting a quadratic polynomial of the form: $W=a+bt+ct^2$, where W is grain weight (mg), t is time in GDD, and a, b and c are regression coefficients (Darroch and Baker, 1990). Rate of grain fill was expressed as mg $kernel^{-1}GDD^{-1}$, and duration of grain fill as accumulated GDD from anthesis to physiological maturity. Time to physiological maturity was defined as the time (in GDD) required for the attainment of maximum dry weight (Darroch and Baker, 1990).

A K-means clustering procedure was employed to cluster the 250 landrace genotypes into a maximum number of clusters significantly different for all measured variables and estimated parameters in this study. Correlation analyses were carried out, for each of 4 clusters identified in the previous step, and were used to examine relationships betweed measured variables and estimated parameters. A canonical discriminant analysis was performed using clusters as the classification criterion, then data was plotted according to the first two functions in this analysis. SAS procedures (SAS Institute, 1985) wereused for statistical analysis.

RESULTS AND DISCUSSION

The quadratic polynomial used to describe grain growth during the grain filling period, provided an excellent description of grain fill in this germplasm collection.

Table 1. Mean separation among four clusters of durum wheat landrace genotypes, collected from Jordan, for grain yield (g per spike), spikelet fertility (SF), 1000-kernel weight (TKWT, mg), growing degree days (GDD) and rate of grain fill (R, mg $kernel^{-1}$ GDD^{-1}).

Variable	Cluster 1	2	3	4
Number of acc'ns	91	69	19	71
Grain Yield	1.62b*	1.42c	1.55b	1.73a
Spikelet fertility	2.01a	1.68c	1.89b	2.02a
1000-kernel weight	47.1b	50.1a	52.2a	40.7c
Predicted 1000-kwt	48.3	51.8	50.7	43.2
Growing Degree Days	750c	789b	720d	810a
Rate of grain fill ($R \times 10^2$)	4.7c	4.9b	5.3a	4.2d

* Cluster means, within each trait, followed by the same letter do not differ significantly (Tukey, 0.05)

Table 2. Pairwise phenotypic correlation coeffecients among 6 traits measured on 4 clusters of durum wheat landrace genotypes from Jordan.

	R	GDD	FF	TKWT	GY
R	1	.048ns*	.183ns	.694	.548
	1	.005ns	.450	.365	.602
GDD	.037ns	1	.001ns	.034ns	.015ns
	.102ns	1	-.061ns	-.081ns	-.023ns
SF	-.050ns	.230	1	.154ns	.845
	.387	.101ns	1	.272	.882
TKWT	.314	.172ns	.562	1	.517
	.675	.682	.448	1	.447
GY	.207	.331	.892	.833	1
	.633	.236	.873	.682	1

* All pairwise correlation coeffecients were highly significant (P<0.001), unless otherwise indicated. Clusters 1&2 above diagonal, clusters 3 & 4 below diagonal. See Table 1 for abbreviations.

Coefficient of determination (R^2) values (range 0.87 to 0.95) suggest that kernel weight and GDD data fit the model well. This conclusion is supported by earlier findings by Darroch and Baker (1990). Results of the analysis of variance and mean separation for GDD and rate of grain fill among all 4 clusters suggest that these two parameters were estimated with high precision. Another supporting evidence of this accuracy is the high correlation between actual and predicted 1000-kernel weight (r=0.93; P; see Table 1).

Landrace genotypes in Cluster 3 (n=19) had the fastest rate of GF and the heaviest kernels. Rate of grain fill ranged from .044 mg kernel^{-1} GDD^{-1} in cluster 4 to 0.052 mg kernel^{-1} GDD^{-1} in cluster 3 and averaged 0.047 mg kernel^{-1} GDD^{-1} across landrace genotypes. Duration of grain fill averaged 757 GDD and ranged from 720 in cluster 3 to 810 in cluster 4. These data indicate that substantial genotypic variation exists for both parameters within this germplasm collection. However, longer grain fill period may not be a promissing strategy to increase grain yield under the Mediterranean environment because high temperature during the grain fill period tends to stop grain growth prematurily and force wheat to physiological maturity (Sayed and Ghandourah, 1984; Bruckner and Frohberg, 1987).

Interrelationships between both parameters of grain fill and each of grain wieght/spike (GY), 1000-kernel weight (TKWT) and spikele fertility (SF) were expressed in terms of phenotypic correlations among their mean values (Table 2). Rate and duration of grain fill were not correlated across clusteres, suggesting that there is no genetic barrier to the simultaneous change of both in a breeding program. A supporting evidence was reported by Gebeyehou *et al.* (1982). Grain yield per spike was positively and

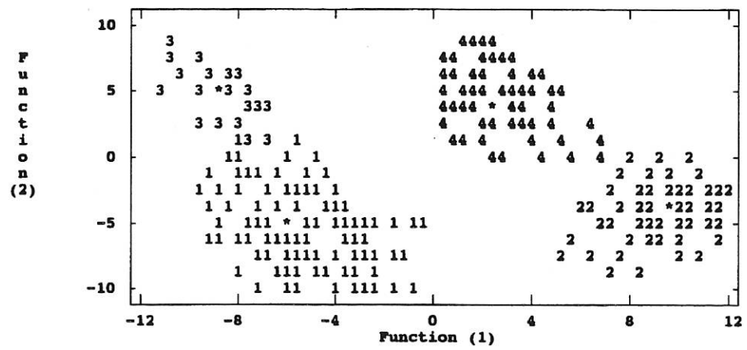

Figure 1. Canonical discrimination analysis of durum wheat landrace genotypes from Jordan (*cluster centroid).

significantly correlated with rate of grain fill in two of the four clusters (Table 2). However, associations of grain yield with 1000-kernel weight and with rate of grain fill were stronger across clusters, thus confirming earlier results in durum (Gebeyehou *et al.*, 1982) and bread wheat (Sayed and Ghandourah, 1984) especially under warm dry conditions, where grain filling duration was significantly correlated with maximum grain weight and with rate of grain filling. It can be postulated that high rate of grain fill with relatively short duration of grain fill appears to be a desirable risk-reducing pattern of grain fill in environments in which the growing season is shortened by terminal stress (Bruckner and Frohberg, 1987). Moreover, selection for higher rate of grain fill and kernel weight without lengthening grain fill duration could be possible. Rate, but not duration of grain fill, was positively and significantly correlated with 1000-kernel weight; however, the intensity of this association varied among clusters (Table 2). This finding confirms results obtained by Bruckner and Frohberg (1987) where rate, but not duration, of grain fill was closely associated with kernel weight, and by May and van Sanford (1992) where kernel growth rate was significantly correlated with effective filling period (r=-0.35; P) in one breeding population, but not in another (r=-0.03; ns).

Canonical discriminant analysis produced a reduced dimension model to effectively indicate measured differences among clusters. It resulted in satisfactory discrimination (95% correct classifiacation) between clusters (Fig. 1). This analysis was based on grain yield per spike, spikelet fertility, 1000-kernel weight, duration and rate of grain fill. The first function was mainly associated with spikelet fertility, 1000-kernel weight and duration of grain fill, and explained 72% of total variance. The second function was mainly correlated with rate of grain fill and grain yield per spike and explained 24% of total variance.

Genetic variation exists in this germplasm collection for both rate and duration of grain fill. These results suggest that kernel weight can be improved simply because it was more closely associated with rate of grain fill than with duration of grain fill. Genotypes with high yield potential, high rate, and short duration of grain fill can be developed or selected from this germplasm collection.

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Breeding Potential of Durum Wheat Landraces from Jordan IV. High Molecular Weight Glutenin Subunit Variation.

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ABSTRACT

Variation in high molecular weight glutenin subunit composition among 177 durum wheat genotypes, derived from a collection of durum wheat landraces from Jordan, was investigated using one-dimensional sodium dodecyl sulphate polyacrylamide gel electrophoresis. A total of 22 alleles, in addition to the null allele, Glu-A1c, were identified; three and seven novel variants were identified at the Glu-A1 and Glu-B1 loci, respectively. The null allele, Glu-A1c, had the highest (76.1%) frequency, followed by Glu-B1b (34.7%). Two loci at the Glu-B1 locus were lacking, these were Glu-B1c and Glu-B1i. Glu-A1b was present with low (6.7%) frequency in this collection, however, it might have a positive effect on gluten strength of the end products of durum wheat. Polymorphism (H_e) at the Glu-A1 and Glu-B1 loci averaged 0.2610.04 and 0.7330.02, respectively. H_e for Glu-A1 was negatively (r=-0.467; P) correlated, while H_e for Glu-B1 was positively (r=0.615; P), correlated with altitude of collection site. However, both H_e estimates were positively and significantly correlated with rainfall quotient.

INTRODUCTION

BRANLARD et al. (1989) pointed out that our present knowledge of high molecular weight (HMW) glutenin subunit variation in durum wheat (*Triticum durum* Desf.), as compared to that of bread wheat (*Triticum aestivum* L.), is very limited. Nevertheless, new information on these storage proteins is emerging from studies on durum wheat landraces (van Hintum and Elings, 1991) and improved cultivars (du Cross, 1987; Margiotta *et al.*, 1988; Ng *et al.*, 1989). In a recent review, Perreno and Porceddu (1990) concluded that genetical and biochemical studies, carried out on durum wheat accessions collected from several Mediterranean countries, revealed the presence of a broad genetic diversity of HMW glutenins. This variation is due to allelic genes which occur at two compound loci, i.e., Glu-A1 and Glu-B1 (Payne and Lawrence, 1983).

Studies on the HMW glutenin subunits provided useful information on genetic variation in the evolution and domestication of wheat (Galili and Feldman, 1983); enhanced the genetic variability available to improve its industrial quality (Vallega abd Waines, 1987; du Cross, 1987; Ng *et al.*, 1989), were instrumental in the assessment of genetic diversity of wild wheat (Levi and Feldman, 1988), domesticated landraces (Lagudah *et al.*, 1987; van Hintum and Elings, 1991), and improved durum wheat cultivars (Ng *et al.*, 1989; Branlard *et al.*, 1989).

This paper reports on the Glu-1 allele composition of landrace genotypes of durum wheat from Jordan, which are genetically diverse for developmental (Jaradat, 1991a) and morphological and yield-related traits (Jaradat, 1991b).

MATERIALS AND METHODS

A total of 177 landrace genotypes, derived from a collection of durum wheat landraces from Jordan (Jaradat, 1991a), were used in this study. Landrace genotypes were grouped according to agroecological characteristics of their collection sites. Rainfall quotient, which combines rainfall and mean maximum temperature effects, mean minimum temperature and elevation of collection sites, were used in characterizing collection sites (N.A.J., 1984). A total of 42 collection sites in 6 agroecological zones were identified (Table 2). Four zones (Irbid, Karak, Tafilah and Shoubak) were found within the Mediterranean semiarid bioclimate and the remaining two (Ajlun and Salt) were found within the Mediterranean semihumid bioclimate. Total proteins were extracted from ground kernels of each landrace genotype and fractionated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using 17.5% polyacrylamide gels as described by Ng and Bushuk (1987). The Canadian bread wheat cultivar "Marquis" (genotype: Glu-A1a, Glu- B1c and Glu-D1d) was used as a reference in each gel. Gels were fixed and stained following the procedure of Blakesley and Boezi (1977). Identification and nomenclature of the HMW glutenin subunits followed the systems of Payne and Lawrence (1983) and Vallega and Waines (1987). Allelic frequencies and genic diversity (H_e) at Glu-A1 and Glu-B1 loci were estimated (Nei, 1972). Diversity indices, by agroecological zone, were subjected to analysis of variance. Spearman correlations were computed between all variables and multiple regression analysis was employed to determine whether agroecological factors were associated with allelic or genic diversity.

RESULTS AND DISCUSSION

Five HMW subunits, in addition to the null allele, Glu-A1c, were detected at the Glu-A1 locus (Table 1). Two of the five HMW subunits have been previously identified (Branlard *et al.*, 1989) in durum wheats, the remaining three subunits could be explained by assuming three new Glu-A1 alleles. The nomenclature of Vallega and Waines (1987) was utilized for these alleles. The new alleles accounted for 4.5% of total allelic frequency at the Glu-A1 locus. Glu-A1c, the null allele, had the highest frequency (76.1%), while the frequency of Glu-A1a and Glu-A1b were 12.7 and 6.7%, respectively. Ng *et al.* (1988) reported that all Canadian durum wheat cultivars contain the null allele Glu-A1c, which is also the most commonly occuring allele in commercial durum wheats grown throughout the world. Glu-A1b has a positive effect on gluten strength as speculated by du Cross (1987).

Seventeen HMW subunits were detected at the Glu-B1 locus (Table 1). Ten of these subunits have been previously described by Payne and Lawrence (1983) and Branlard *et al.* (1989). The remaining seven subunits could be explained by assuming five new alleles at the Glu-B1 locus. These five alleles accounted for 9.1% of total allelic frequency at this locus. Frequencies of the remaining alleles ranged from 1.2 (Glu-B1h) to 34.7% (Glu-B1b). This collection was lacking alleles Glu-B1c and Glu-B1i, and the frequency of Glu-B1a (2.9%) is low, however, this frequency is reasonably higher than the one (0.8%) reported by Branlard *et al.* (1989).

The frequencies of alleles in the collection were compared according to geographical distribution. Four alleles (Glu-A1c, Glu-B1b, Glu-B1d, and Glu-B1e) were common and widely distributed. The alleles Glu-A1a and Glu-A1b were common in only two restricted zones. All new alleles at the Glu-B1 locus were rare and restricted to the southern part of the country, especially with high (1000 m above sea level) elevation. Finally, the Glu-B1a, Glu-B1f, Glu-B1h and all new alleles at the Glu-A1 locus were rare and appeared in at least four of the six agroecological zones.

Polymorphism (H_e) at the Glu-A1 locus ranged from 0.0930.08 to 0.5780.04, and averaged 0.2610.04, whereas H_e at the Glu-B1 locus ranged from 0.6250.04 to 0.8390.02 and averaged 0.7330.02 (Table 2). Average H_e over both loci was 0.6090.025. Two of the agroecological zones (Salt and Shoubak in Table 2) exhibited very low diversity indeces for Glu-A1 due to the high frequency of the null allele, Glu-A1c.

Table 1. Allelic frequency at 2 glutenin loci for 177 landrace genotypes of durum wheat collected from Jordan.

Locus	Allele	Frequency (%)
Glu-A1	a	12.7
	b	6.7
	c	76.1
New alleles	I	1.5
	II	0.7
	III	2.3
Glu-B1	a	2.9
	b	34.7
	d	21.1
	e	27.5
	f	3.5
	h	1.2
New alleles	I	0.5
	II	1.2
	III	0.7
	IV	3.8
	V	2.9

Table 2. Characteristics of 6 ecogeographical zones and H_e estimates of two Glu-1 loci for 177 durum wheat landrace genotypes collected from Jordan.

No.	Zone		Long.	Lat.	Alt.	H_e Glu-A1	H_e Glu-B1
1	Irbid	Min.	35 40	32 30	450	0.145±0.07	0.646±0.09
		Max.	36 00	32 39	675		
2	Ajlun	Min.	35 35	32 24	700	0.366±0.09	0.747±0.03
		Max.	36 04	32 30	1000		
3	Salt	Min.	35 42	32 11	600	0.076±0.05	0.625±0.04
		Max.	35 54	32 22	1100		
4	Karak	Min.	35 44	32 00	620	0.255±0.09	0.735±0.04
		Max.	35 47	32 08	980		
5	Tafilah	Min.	35 41	31 17	700	0.578±0.04	0.839±0.02
		Max.	35 51	31 50	960		
6	Shoubak	Min.	35 28	31 04	1080	0.093±0.08	0.780±0.02
		Max.	35 41	31 04	1600		
Average						0.261±.040	0.733±.02

Analysis of variance for H_e of both loci revealed significant differences among agroecological zones. A larger portion (77%) of total variance in H_e for Glu-B1 was found within agroecological zones as compared to 55% for Glu-A1 (Table 3).

Altitude (750 m above sea level) of collection sites was a major factor in influencing H_e estimates for both loci (Table 4). H_e for Glu-A1 was negatively and significantly (r=-0.467;P) correlated with altitude of collection sites. On the other hand, H_e for Glu-B1 was positively and significantly (r=0.615; P) correlated with altitude of collection site. Both diversity indices for Glu-A1 and Glu-B1 loci were positively and significantly correlated with rainfall quotient (Table 4). Earlier findings in bread wheat (Laghudah *et al.*, 1987) indicated that variation occurs at the Glu-B1 locus in both the altitudinal set and geographical sites of landrace collections whereas allelic variation at the Glu-A1 locus was only found at the geographical set of the collection sites.

Altitude and rainfall quotient (Q) of collection sites explained 34.9% of the variability in glutenin diversity. However, when only genotypes collected from sites 750 m above sea level were considered, both altitude and Q explained 43.5% of the variability in glutenin diversity. When each locus was considered separately, R^2 values for Glu-A1 and Glu-B1 were 49.8 and 21.4%, respectively (Table 5).

Table 3. Analysis of variance for H_e estimates for Glu-A1 and Glu-B1 loci in 177 landrace genotypes of durum wheat from Jordan.

Source of variation	Glu-A1 MS	Glu-A1 % Variance	Glu-B1 MS	Glu-B1 % Variance
Among Zones	0.283 **	45	0.047**	23
Within zones	0.041	55	0.015	77

*, ** :significant at the 5% and 1% levels of probability, respectively.

Table 4. Pairwise correlation coefficients between H_e estimates for two Glu-1 loci and each of altitude and rainfall qoutient (Q) of collection sites of 177 landrace genotypes of durum wheat from Jordan.

H_e	Altitude (m) <750	Altitude (m) >750	Rainfall qoutient (Q)
Glu-A1	0.071 ns	-0.467*	0.563 **
Glu-B1	0.296 ns	0.615 **	0.451 *

ns: not significant, *, **: significant at the 5 and 1% levels of probability, respectively.

Table 5. Multiple regression analysis for H_e estimates for each of Glu-1, Glu-A1 and Glu-B1 loci in a collection of durum wheat landrace genotypes from Jordan.

Source of variation	MS Glu-1	MS Glu-A1	MS Glu-B1
Regression	0.370	0.263	0.043
Residual	0.072	0.041	0.013
P	<0.012	<0.005	<0.05
R^2	43.5%	49.8%	21.4%

Glutenin diversity index for durum wheat landraces collected in Syria, was found to be highly correlated with geographical and climatological characteristics of their collection sites; similarly, it was highly correlated with a phenotypic diversity index based on ten phenological and morpholgical traits (van Hintum and Elings, 1991). However, other studies reported no significant differences in allelic frequencies of HMW glutenins due to geographical locations of bread wheat landraces from Afghanistan (Laghudah *et al.*, 1987) or from Nepal (Margiotta *et al.*, 1988).

This collection of durum wheat landrace genotypes from Jordan presents a wealth of quantitative and qualitative diversity for Glu-1 locus in durum wheat, as compared with a total of 18 different alleles identified in 502 durum wheats (Branlard *et al.*, 1987).

Quantitative and qualitative variation in HMW glutenin subunits of these landrace genotypes of durum wheat can be exploited in wheat breeding programs (Lukow *et al.*, 1992), and will be useful in developing countries for specialty end-use cultivars of durum wheat.

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The Evaluation On Crossabilities of Chinese Wheat Landraces

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ABSTRACT

The total of 865 accessions of Chinese bread wheat landraces (*Triticum aestivum* L.) has been investigated on their crossabilities with rye (*Secale cereale* L.), of which 121 landraces showed similar crossability to Chinese Spring, 50 accessions had much higher crossability than Chinese Spring, and 693 varieties were non-crossable with rye or had lower crossability than Chinese Spring. The analysis on the geographical distribution indicated that the landraces with high crossability occurred in most parts of China. Some utilization of high crossability resources was also discussed.

INTRODUCTION

Since the first study on crossability of wheat (*Triticum aestivum* L.) with rye (*Secale cereale* L.) by Backhouse (1916), much attention has been given to the character in its genetic structure and the agricultural application (Lein, 1943; Riley and Chapman, 1967; Falk and Kasha, 1981; Zeven, 1987; Luo *et al*., 1992, 1993a, 1993b, 1994). Lein (1943) suggested that there were two pairs of gene controlling the crossability of bread wheat with rye. Sasaki and Wada (1966), and Riley and Chapman (1967) revealed that *kr1* located on the chromosome 5B, and *kr2* on chromosome 5A controlled crossability. Krowlow (1973) located *kr3* on chromosome 5D. Zeven (1987) summarized the crossabilities of some 1400 bread wheat varieties or lines. He indicated that most of the varieties or lines with high crossability percentage were landraces from China, Japan and Eastern Sibiria.

In 1950's, about 30,000 wheat landrace accessions were collected in China, and much attention has been given regarding their agronomic traits and disease resistance. From 1985 on, we have worked on the crossabilities of Chinese hexaploid wheats. The results revealed that one new gene *kr4* also controls the wheat-rye crossability, and was located on the chromosome 1A (Luo *et al*., 1989; Zheng *et al*., 1992). The present paper summarized the results of our investigation on the crossabilities of Chinese bread wheats with rye.

MATERIALS AND METHODS

A total of 864 landrace accessions of Chinese bread wheat (*Triticum aestivum* L.) was involved in the investigation. These landraces were collected in 1950's and conserved by the provincial academic organizations in China (Table 1). the wheat landraces were crossed with rye (*Secale cereale* L. cv. Zinling rye, used as the male tester). The emasculation and pollination techniques were the same as the previous paper (Luo *et al*., 1992). Thirty days later following pollination, the number of florets with and without seeds were recorded for each spike included in the experiment. The percentage of successful crosses over the total numbers of florets pollinated were used in a *t*-test, which was adopted to detect the crossability difference between a wheat landrace and the control (Chinese Spring).

RESULTS AND DISCUSSION

The tests on the crossability percentages have been carried out during 1985-1991. Eight hundred and sixty-four landraces of Chinese common wheat (*Triticum aestivum* L.), which were from Sichuan, Shaanxi, Henan, Gansu, Yunnan, Guizhou, Hunan, Shanxi, Hebei, and Tibet have been included in the investigation of wheat-rye crossability. For delimitating the differences among years, Chinese Spring was selected as a control. It is known that Chinese Spring possesses the genotype of *kr1kr1kr2kr2kr3kr3Kr4Kr4*. In seven continuous years, the percentages of seed set in crosses of Chinese Spring with

Table 1. The crossability types and their distribution in the Chinese bread wheat landraces

	Group 1		Group 2		Group 3		Group 4			
Original locality	No. of varieties	%	No. of varieties	%	No. of varieties	%	No. of varieties	%	Total	Source
Sichuan	65	36.72	62	35.03	34	19.21	16	9.09	177	Ms Zhou and Ms Jiang, Sichuan Academy of Agricultural Sciences
Shaanxi	19	26.39	29	40.28	17	23.61	7	9.72	72	Germplasm Laboratory, Shaanxi Academy of Agricultural Sciences
Henan	8	17.39	18	39.13	13	28.26	7	15.22	46	Prof. M.Z. Ren, Henan Academy of Agricultural Sciences
Gansu	16	33.33	16	35.42	14	27.08	2	4.17	48	Mr. W.G. Li, Gansu Academy of Agricultural Sciences
Yunnan	38	62.30	22	36.07	1	1.64	0	0.00	61	Mr. S.Y. Wu, Yunnan Academy of Agricultural Sciences
Guizhou	13	33.33	18	46.15	6	15.38	2	5.13	39	Mr. X.B. Fang, Guizhou Academy of Agricultural Sciences
Hunan	8	18.18	15	34.09	14	31.82	7	15.91	44	Mr. S.Q. Yun, Hunan Academy of Agricultural Sciences
Shanxi	13	19.40	36	53.73	13	19.40	5	7.46	67	Prof. H.C. Xu, Shanxi Academy of Agricultural Sciences
Hebei	8	28.57	12	42.86	4	14.29	4	14.29	28	Prof. F.R. Sun, Hebei Academy of Agricultural Sciences
Tibet	236	83.69	41	14.54	5	1.77	0	0.00	282	Collections in 1988, sponsored by IBPGR, FAO, UN.
Total	424	49.07	269	31.13	121	14.00	50	5.79	864	

rye were 82.1%, 74.0%, 80.8%, 78.2%, 73.6%, 71.9%, and 73.0%. The weighted average being 75.9%. There is no significant difference between the maximum (82.1%) and the minimum (71.9%) ($tvt_{0.05}$). As the landraces were tested separately in the different year(s), the crossability percentage of Chinese Spring in the year was applied in the *t*-test.

According to the results of *t*-tests and Lein's (1943) suggestion, the landraces were divided into four groups.

Group 1: The crossability percentages were lower than 5%, therefore being very difficult to cross with rye or non-crossable. There is no recessive *kr* allele in this group.

Group 2: The crossability percentages were 5% or higher, but significantly lower than that of Chinese Spring. There exists one or two pairs of recessive *kr* genes.

Group 3: having the similar crossabilities to Chinese Spring. This group possesses recessive *kr1*, *kr2* and *kr3* genes.

Group 4: showing much higher crossability percentages than Chinese Spring, and having the genotype of *kr1kr1kr2kr2kr3kr3kr4kr4*.

The high frequency of easily crossable materials occurred among the landraces investigated. Of the 864 landraces, 121 varieties showed similar crossability to Chinese Spring, 50 landraces had significantly higher crossability than Chinese Spring. 424 landraces were non-crossable or very difficult to cross with rye, 269 varieties were crossable with rye, but their crossabilities were much lower than that of Chinese Spring (Table 1).

From Table 1, 14% of landraces investigated had similar crossability to Chinese Spring (Group 3). The landraces belonging to this group frequently occurred in Hunan, Henan, Gansu, Shaanxi, Sichuan, Shanxi, Guizhou and Hebei, but rare in Yunnan and Tibet. Of the 864 landraces, 5.8% showed much higher crossability percentages than Chinese Spring (Group 4). The higher crossability landraces were from Hunan, Henan, and Hebei Provinces of China. This area appears to be the center of geographical distribution of recessive *kr4* gene. There was no distribution of recessive *kr4* gene in Yunnan and Tibet regions.

The results of this investigation revealed that Chinese wheat landraces are rich in high crossability resources. It is known that Chinese Spring, a strain of a landrace in Sichuan province of China, has been selected as a standard cultivar in the genetic study of wheat primarily for its easy crossability with rye. It is believed that the landraces with much higher crossability than Chinese Spring from China may make further contribution in the aspects of genetic studies and the practices of transferring alien genetic materials from some species of genera in Triticeae into wheat. As a representative of high crossability germplasm, "J-II" has been successfully used in the crosses of wheat with *Psathyrostachys huashanica* Keng and *Roegneria ciliaris* (Trin.) Nevski (Sun, 1992, Wang, personal communication).

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Use of Annual and Perennial Triticeae Species for Wheat Improvement.

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ABSTRACT

Constraints due to global biotic and abiotic stress continue to exist in wheat germplasm. Novel genetic diversity resides in several annual/perennial Triticeae species that can be introgressed into wheat through intergeneric hybridization, of which *Thinopyrum curvifolium* is the principle source as it addresses the emphasis here for achieving wheat derivatives resistant to *Helminthosporium* leaf blight (*Cochliobolus sativus*). Some additional sources like *Th. elongatum* (2n=2x=14) and *Secale cereale* are also mentioned. The interspecific hybridization strategy offers alien genetic introgression opportunities, for which the closely related *Triticum* species have a priority. Of these sources, the D genome *T. tauschii (Aegilops squarrosa)* accessions and some of the A genome species *(T. boeoticum, T. monococcum and T. urartu)* are being exploited.

INTRODUCTION

During the past two decades, significant emphasis has emerged on utilization of alien genetic variation for wheat improvement. Methodologies have evolved that elucidate the usage of alien species attaching priority as to their choice of utilization. The species choice is based heavily upon the genomic relationship between the alien source and wheat, complexity of the character to be transferred, and the polyploidy status of the contributing species. With the wide array of annual and perennial Triticeae species existant, such alien genetic incorporation procedures are categorized under intergeneric and interspecific hybridization. The former route is generally more cumbersome to exploit, and practical outputs tend to be long-term. The interspecific approach in contrast, provides a swift means of introgressing alien genes from closely related sources and yields quality products more simplistically. A blend of both approaches provides the opportunity of pyramiding a more diverse genetic pool better adapted to combat biotic- and abiotic-stress constraints as they may associate with durability of resistance. In this presentation, the focus in on development of wheat germplasm that expresses enhanced resistance to *Helminthosporium sativum; Cochliobolus sativus* Ito and Kuribay or *Helminthosporium* leaf blight; compared to cultivar BH 1146, which is globally recognized as a superior resistant cultivar. The disease is widespread in several wheat production countries such as Bangladesh, Nepal, Thailand, India, Uganda, Brazil, Bolivia and Paraguay. The yield losses can be alarming, and losses up to 83.0% may occur. In Mexico, a naturally infectious field screening hot site exists in Poza Rica where we have encountered losses up to 58%. This has provided us the crucial input necessary to advance our alien genetic introgression program whose details relative to *Helminthosporium* leaf blight are described further.

BREEDING

The Intergeneric Hybridization Approach

Screening of the alien Triticeae species initially identified an ideal resistant source in *Thinopyrum curvifolium* (2n=4x=29) which was hybridized to *Triticum aestivum* cv Chinese Spring. The F_1 hybrid, 2n=5x=35, was advanced by crossing onto it the wheat cultivars Glennson 81, then Alondra/Pavon and eventually selfed. These selfed derivatives were screened for resistance under the severely infected natural field conditions of Poza Rica, Mexico, leading to selection of elite lines with superior resistance to *Helminthosporium* leaf blight. Following three years of yield testing, stability has persisted for all the selected characteristics. The five best resistant lines were agronomically characterized for registration as genetic stocks (Table 1) and were distributed to breeding programs. All five lines represent better *C. sativus* resistance than other wheat germplasm available in CIMMYT based upon evaluations for leaf/node damage at the milk and dough stage of development, as well as symptoms on spikes and mature grains (Table 2). Yield tests further demonstrated superiority of these lines as compared to susceptible and the existant resistant check (like BH 1146). The *Th. curvifolium* derived germplasm now figures in up to 89.8% of the 1994 selections made by

Table 1. Agronomic characteristics of *Cochliobolus sativus* resistant spring bread wheat germplasms grown at Poza Rica, Mexico, during the 1990-91 and 1991-92 field crop cycles.

Germplasm	Grain yield	Days to physiol. maturity	Plant height	1000-Grain weight	Test weight
	kg ha^{-1}		cm	g	kg hl^{-1}
Line -295-1	1997	102	87	29.4	73.4
Line -295-2	1564	106	88	27.4	73.9
Line -295-3	1431	108	87	25.4	74.2
Line -295-4	1580	105	88	26.4	69.5
Line -295-5	1461	110	91	25.3	72.4
BH 1146 (Res. check)	982	100	85	27.1	71.7
Ciano 79 (Susc. check)	166	103	63	16.7	38.8

Pedigree of lines 1 to 5: Chinese Spring/ *Th. curvifolium*//Glennson 81/3/Alondra/Pavon

Days from emergence

Table 2. Disease reactions of five spring wheat germplasm lines to *Cochliobolus sativus* at Poza Rica, Mexico during the 1990-91 field crop cycle.

Germplasm	Leaves † a	Leaves † b	Spike ‡ (1-9)	Grain § (1-5)
Line 295-1	93	94	2	2
Line 295-2	92	93	2	2
Line 295-3	93	93	2	2
Line 295-4	92	92	2	2
Line 295-5	92	94	3	2
BH 1146 (Resistant)	93	95	6	3
Ciano 79 (Susceptible)	99	99	9	5

† Two-digit scoring system: first digit = height of infection; 5 = up to mid-plant, and 9 = up to flag leaf; second digit = disease severity on infected leaves; 1 = low and 9 = total leaf destroyed; a = score at early milk stage, b = score at soft dough stage.

‡ 1 = low infection and 9 = high infection.

§ 1 = low grain infection and 5 = severely infected.

our wheat breeding program in Poza Rica. All lines have the euploid complement of 2n=6x=42 chromosomes and are satisfactory combiners with other wheat cultivars. Cytogenetic, biochemical, and molecular analyses have not enabled the detection of alien introgression from *Th. curvifolium*. However, through limited initial use of the A600 probe (courtesy CSIRO, Canberra, Australia) presence of alien DNA was apparent. This needs further validation.

Helminthosporium spot blight resistance was also observed in *Th. elongatum*, *Th. scirpeum*, *Th. intermedium*, *Leymus racemosus*, *Th. bessarabicum* and *Secale cereale*. Screening data supporting the resistance of *Th. elongatum* is evidenced from the field performance of its 2n-8x=56 chromosome amphiploid (Table 3) compared to a susceptible wheat cultivar Goshawk "S". Being a diploid, *Th. elongatum* is the next priority source being exploited other than *S. cereale*. In all such intergeneric hybrid based alien transfers, an infusion of the introgression manipulation methodology during initial stages of the program is preferable (Kimber, 1993), of which use of the *ph* loci is one approach (Mujeeb-Kazi et al., 1993).

The Interspecific Hybridization Approach

The interspecific route offers a rapid means of introgressing novel diversity from the closely related wild grasses because of their genomic proximity to the A. B and D genomes of *T. aestivum*. Several sources are being utilized, with the most extensive being that of the several

Table 3. Disease reactions of *Thinopyrum elongatum* based germplasm to *Cochliobolus sativus* at Poza Rica, Mexico, during the 1992-93 field crop cycle.

Test material	Leaves † a	Leaves † b	Spike ‡ (1-9)	Grain § (1-5)
Th. elongatum/GH"S"	92	92	2	2
CS/*Th. elongatum*	92	93	3	x
8A 206	92	93	3	3
GH"S"	94	96	7	4
CS	94	97	7	x
Cno 79 (Susceptible)	99	99	9	3
BH 1146 (Resistant)	93	95	6	3

† Two-digit scoring system: first digit = height of infection; 5 = up to mid-plant, and 9 = up to flag leaf; second digit = disease severity on infected leaves; 1 = low and 9 = total leaf destroyed; a = score at early milk stage, b = score at soft dough stage.

‡ 1 = low infection and 9 = high infection.

§ 1 - log grain infection and 5 = severely infected

Table 4. Disease reactions of AABBDD synthetic hexaploids and AAAABB hexaploids to *Cochliobolus sativus* during the Poza Rica, Mexico 1992-1993 field-crop cycle.

Germplasm	Leaves † a	Leaves † b	Spike ‡ (1-9)	Grain § (1-5)
GAN	96	96	7	5
GAN/*T. tauschii* (236)	92	92	3	x
DOY_1	95	97	7	5
DOY_1/*T. tauschii* (447)	92	92	3	x
DOY_1/*T. tauschii* (510)	92	92	3	x
SCOOP_1	97	97	8	5
----/3/*T. monococcum* (98)	92	92	3	x
----/3/*T. monococcum* (118)	92	93	3	x

† Two-digit scoring system: first digit = height of infection; 5 = up to mid-plant, and 9 = up to flag leaf; second digit = disease severity on infected leaves; 1 = low and 9 = total leaf destroyed; a = score at early milk stage, b = score at soft dough stage.

‡ 1 = low infection and 9 = high infection.

§ 1 = low grain infection and 5 = severely infected.

accessions of *T. tauschii (Aegilops squarrosa)* via the synthetic hexaploid bridge as a crossing step to *T. aestivum*. In all these aspects of genetic improvement, the durum and bread wheat cultivars are susceptible. Hence, when field resistance is observed either in the synthetic hexaploid or

this dual approach since the *T. tauschii* accessions field screening failed to provide conclusive data. In the same context the A genome species are utilized by producing the AAAABB hexaploids, which are screened for resistance and crossed further to their respective durum parents for relevant improvement of durums. The A genome accession, after it has been identified as resistant in the AAAABB hexaploid is next used in direct crosses to susceptible *T. aestivum* cultivars. Data from DD genome synthetics and A genome hexaploids for the *Helminthosporium* leaf blight screening is presented in Table 4. The three durum cultivars; GAN, DOY_1 and SCOOP_1 express a high degree of susceptibility as observed on leaves, spikes, and mature grains. The resistance of the AABBDD and AAAABB germplasm was highly expressive except for grain finish scores that were not recorded because of late maturity of these derivatives.

Alien genetic diversity from annual/perennial Triticeae species has significantly contributed to improvement of bread wheat germplasm and avenues now exist to further enhance the diversity of durum wheats through A genome exchanges. Though not yet exploited, D genome transfers to the A genome need further research inputs, and this may further enhance diversity for durums. In general, it appears that adequate genomic variations exist, which if pyramided into cultivated wheats could ensure a considerable level to resistance to *Cochliobolus sativus*. The transfer and diagnostic methodologies will contribute to such an outcome.

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Plant Germplasm Resources

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ABSTRACT

Landraces and wild relatives of crops from centers of diversity have been rich sources of resistance to new pathogens, insect pests, and other stresses as well as for traits to improve food and fiber quality, animal feed, and industrial products. Because very few crops grown in the U.S. are native, plant introductions are vital to our agriculture. The National Plant Germplasm System (NPGS) was established to acquire, preserve, and distribute plant genetic resources from around the world so that scientists have immediate access to these source materials. The active collection is maintained and distributed by 19 national germplasm repositories. The base collection is preserved at -18°C at the National Seed Storage Laboratory. The NPGS's genetic resources are made freely available to all *bona fide* users for the benefit of humankind. Recent international agreements such as the Biodiversity Convention will impact acquisition and exchange of germplasm, but the NPGS goal is to maintain the germplasm exchange critical to feeding the increasing world population in the future.

INTRODUCTION

Landraces and wild relatives of crops from centers of diversity have been rich sources of resistance to new pathogens, insect pests, and other stresses as well as for traits to improve food and fiber quality, animal feed, and industrial products. This valuable genetic diversity has resulted from evolutionary processes including mutation, recombination, natural selection, migration, and genetic drift in many ecological niches. Human intervention has produced both positive and negative effects on diversity.

No country has all of the plant genetic resources required to develop and maintain a high level of agricultural productivity. The U.S. has an extremely limited number of native species of economic importance including some grasses, sunflower, cranberry, blueberry, strawberry, pecan, and a few other species. As with many countries, our exceptionally productive agricultural systems were founded on introduced plant genetic resources.

Immigrants from Europe and Asia brought seed with them. Prior to that, native North Americans had introduced maize, beans, squash and other crops from Central and South America. In 1819 American consuls overseas were asked to collect seeds of useful plants. The U.S. Patent Commissioner administered the introduction of plants from 1836 to 1862. The continuing need to acquire and introduce plant germplasm into the U.S. was one of the reasons for establishing the U.S. Department of Agriculture (USDA). The Organic Act, of 1862, establishing the Department of Agriculture, directed the first Commissioner of Agriculture, Isaac Newton, "to collect, as he may be able, new and valuable seeds and plants; to test, by cultivation the value of such of them as may require such tests; to propagate such as may be worthy of propagation, and to distribute them among agriculturists." In 1898, the Seed and Plant Introduction Section, which later became the Plant Introduction Office, was established to manage plant explorations and introductions.

Before the late 1940's, introductions were sent directly to interested scientists without any requirement that they be maintained. Adequate preservation methodologies and facilities were not available, and many accessions were lost.

Landraces and wild relatives are useful sources of genetic diversity to meet plant breeders' needs. But, as farmers in centers of diversity switch to new stress tolerant, higher yielding cultivars, these valuable sources of useful genes will be lost forever unless they have been collected and preserved *ex situ* in gene banks.

EX SITU CONSERVATION STRATEGIES

Ex situ collections of germplasm can be maintained as 1) living and growing organisms and 2) living but quiescent organisms (Eberhart et al., 1995). Examples of living and growing collections include field and screen house collections, botanical gardens, and cell and tissue cultures.

In the living but quiescent collections, organisms are stored in a state of "suspended animation." Examples are seeds and cryopreserved tissues and cultures in gene banks. Only orthodox seeds (those which are tolerant to desiccation) and dormant vegetative buds from apple are currently stored in quiescent collections, but the technology is developing rapidly that will permit the preservation of most forms of plant germplasm.

Technological demands are minimal in living and growing collections. However, these collections are expensive in terms of labor and space. Most importantly, living and growing collections may be susceptible to frosts, droughts, diseases, insects, and other disasters. Collections of living but quiescent organisms and tissues provide a low risk backup to the living and growing collections. Once in storage, preserved organisms require minimal space and labor, and this permits the preservation of many collections.

Technologies for preserving *Ex Situ* Collections

The basic principle of preserving quiescent biological tissues is to limit biochemical changes that are caused by either metabolism or the stochastic processes of aging. For many biological materials, the procedures used to limit chemical reactions (dehydrating and/or freezing) are lethal. Thus, these preservation procedures cannot be used in certain quiescent *ex situ* collections. There are many different types of tissues than can be used as propagules. Seeds and pollen are propagules that are sexually derived from plants, whereas propagules such as vegetative buds, shoot tips, somatic embryos, cell suspensions, and root tissues are asexually derived. For purposes of conserving genetic diversity, the choice of propagule depends on the ease in which it can be preserved and whether particular gene combinations are desired.

For seeds, we distinguish between 'orthodox' and 'recalcitrant' types. Orthodox seeds are easily stored, while recalcitrant seeds are more difficult to store. Fortunately, many crops important to U.S. agriculture form orthodox seeds. Triticeae cereal and grass seeds have good longevity in storage (Harrington, 1972; Priestley et al., 1985), and some have been reported to survive more than 100 years (Roos, 1986). However, a number of crop species (e.g., wild rice, citrus, avocado, mango, cacao, coffee) and several tree species (e.g., oak, maple, buckeye) produce recalcitrant seeds. The basic distinction between orthodox and recalcitrant seeds lies in their relative ability to survive desiccation.

Preservation of Orthodox Seeds

The technologies for preserving orthodox seeds are well understood for the most part. Seeds should be dried and stored at a low temperature (Justice and Bass, 1978). Research by Justice and Bass (1978), Bass (1980), and Bass and Stanwood (1978) showed that reducing the storage temperature from 5°C to sub-zero temperatures increased seed longevity from less than 10 years for some species to several decades for most species.

The ultra-low temperature of liquid nitrogen used in cryogenic storage should extend seed longevity (Stanwood, 1980, 1985). After 10 years of cryogenic storage, no major differences in viability were observed between onion seeds stored at -18°C and liquid nitrogen temperatures (Stanwood and Sowa, 1995). However, major differences were observed between the sub-zero temperatures and 5°C. The protocols for cryogenic storage of orthodox seeds were established by Stanwood and Bass (1981). Seeds are stored in the vapor phase above liquid nitrogen (approximately -160°C). The choice between using conventional storage at -18°C or storage at liquid nitrogen temperatures depends on whether 1) the accession shows damage during initial exposure to liquid nitrogen, 2) the species produces large seeds (annual operating cost per sample in liquid nitrogen is at least three times higher than conventional storage at -18°C), and 3) the longevity characteristics of the species.

Although seed drying extends longevity, there are limits to the beneficial effects; and the optimum moisture content varies with the chemical composition of the seed (Vertucci and Roos, 1990, 1993; Vertucci et al., 1994; Ellis et al., 1989, 1990). Drying seeds beyond a critical moisture content can result in accelerated deterioration at above zero temperatures. Using basic thermodynamic principles, scientists at the NSSL (Vertucci and Roos, 1990, 1993; Vertucci et al., 1994) have established that, contrary to the viability equations (Ellis and Roberts, 1980; Ellis et al., 1989), the effects of storage temperature and water content of seeds are not independent. Consequently, the optimum water content for seed storage varies both with the seed species and with the temperature of storage. Clearly, there are insufficient funds to determine the specific optimum moisture content for each of the 8,000 species represented in the NPGS collection. However, the thermodynamic principles used by Vertucci and Roos (1990, 1993) and Vertucci et al. (1994) can be used to predict optimum moisture levels for all orthodox seeds at all storage temperatures. Based on the finding that 25% RH provides the optimum moisture level for storage at 25°C for all orthodox seeds studied, the optimum water content at any other storage temperature can be calculated. This procedure has eliminated the requirement of determining moisture contents for each accession and saves approximately two hours of processing time for each seed sample.

Preservation of Orthodox Pollen

Pollen from many plant species can be preserved using the same principles that are used for orthodox seeds (Connor and Towill, 1993). Preservation of pollen produced from long-lived perennial plants is especially

useful for the plant breeder. Pollen storage requires little space and labor. Like orthodox seeds, preservation of pollen in living but quiescent collections can serve as a backup for living and growing *ex situ* collections (Towill, 1985; Connor and Towill, 1993).

Preservation of Desiccation-Sensitive Propagules

Unlike most biological tissues, orthodox embryos and pollen tolerate severe dehydration; this ability makes them amenable to storage in quiescent collections. Desiccation-sensitive tissues cannot be easily stored at sub-freezing temperatures because the water that is necessary for their survival freezes with lethal consequences. A number of methods by which tissues can be exposed to sub-freezing temperatures without lethal ice formation have been developed. These methods involve optimizing the water content and then cooling tissues to the desired temperature at an appropriate rate. Two methods of handling recalcitrant seed have given results varying from excellent (80%) (Vertucci et al., 1991; Wesley-Smith et al., 1992; Vertucci et al., 1993) to mediocre (30-50%) (Wesley-Smith et al., 1993). Survival rates depend largely on the species and its developmental status. The first method is applicable to those tissues that can survive water contents as low as 0.3 g H_2O/g dw (30% seed moisture) or water potentials as low as -15 MPa. In this method, the moisture content and temperature are optimized so that both desiccation and freezing damages are avoided (Vertucci, 1989; Vertucci et al., 1991, 1995). The critical moisture content for desiccation damage increases as temperature is reduced (Vertucci et al., 1995). Thus, the window of survivable moisture levels narrows as the storage temperature declines (Vertucci et al., 1991, 1993, 1995). This method is presently being adopted for long term storage of recalcitrant seeds of wild rice (*Zizania palustris*)(Vertucci et al., 1995).

The second method for preserving recalcitrant seeds is used for embryos which are extremely sensitive to dehydration and cannot survive water contents lower than about 0.6 g H_2O/g dw (60% seed moisture). These materials must be preserved in the "vitrified" state (Wesley-Smith et al., 1992).

Similar cryopreservation procedures have been used with other propagules with variable success rates. Survival rates of 0 to 80% after exposure to liquid nitrogen can be obtained for vegetative buds of apple (Towill, 1990; Forsline et al., 1993) and apical shoot tips of sweet potato (Towill and Jarrett, 1992), survival being largely dependent on genetic constitution and developmental stage.

In vitrified samples with high moisture contents, lethal ice crystals do not form even though samples are stored at sub-freezing temperatures (Fahy et al., 1984). Ice is prevented because the samples are treated with cryoprotectants and then cooled at such a fast rate that ice crystals do not have time to form. The solution in these samples becomes a glass. There are several steps required for successful cryopreservation through vitrification (Towill, 1990). First, a stable glass must be created. This is usually accomplished by loading cells with protectants, and then adjusting the water content of cells to optimal levels which limit desiccation damage but encourage glass formation. The sample must then be cooled appropriately, and this usually means at extremely fast rates.

The protectants that are used in cryopreservation have two purposes: 1) to prevent cell constituents from denaturation during the desiccation phase and 2) to stabilize the glass. Research shows that many plant systems naturally accumulate these protectants during particular developmental stages. For example, during fall, winter-hardy woody tissues acclimate and become more tolerant to sub-freezing temperatures. Also, during maturation, orthodox seeds accumulate massive quantities of sugars and proteins believed to be protectants against various stresses. Scientists at the NSSL and elsewhere are studying the mechanisms of protection with the objective of incorporating these chemicals into tissues that do not accumulate them naturally (Towill and Jarrett, 1992). Non-natural protectants such as dimethyl-sulfoxide (DMSO) and ethylene glycol are also commonly used.

Vitrified samples must be stored at temperatures where the glass cannot "melt." This necessitates storage at very low temperatures, either directly in liquid nitrogen (-196°C) or in the vapor above liquid nitrogen (about -160°C)(Fahy et al., 1984). Thawing cryopreserved samples is critical also and is usually done rapidly to avoid formation of ice. When samples are retrieved from storage, they are grown in culture and then transplanted or grafted onto existing stocks. The success of a certain cryopreservation treatment is evaluated by the proportion of propagules that develop into growing plants.

THE NATIONAL PLANT GERMPLASM SYSTEM

The Research and Marketing Act of 1946 (Public Law 733) authorized the creation of four Regional Plant Introduction Stations (Ames, Iowa; Pullman, Washington; Geneva, New York; Griffin, Georgia) with the mission to acquire, maintain, evaluate, and distribute germplasm to scientists to be used for crop improvement. The National Small Grains Collection, now in Aberdeen, Idaho, began in 1894 as a breeder's collection in Beltsville, Maryland. The Inter-Regional Potato Introduction Station, Sturgeon Bay, Wisconsin, was established in 1947. National Clonal Germplasm Repositories were established in the mid-1980s to provide more systematic maintenance of vegetatively propagated germplasm. These repositories grow and maintain the active collections and distribute samples to scientists worldwide. The National Seed Storage Laboratory (NSSL), Fort Collins, Colorado was dedicated in 1958 as a long-term storage facility to preserve the base collection for backup of the active collections.

These units have been integrated into a National Plant Germplasm System (NPGS) (ARS Information Service, 1990; Shands et al., 1989). The NPGS is a network of cooperating institutions, agencies, and research units in the Federal, State, and private sectors. The mission of the NPGS is: "To effectively collect, document, preserve, evaluate, enhance, and distribute plant genetic resources for continued improvement in the quality and production of economic crops important to U.S. and world agriculture. This is achieved through a coordinated effort by the U.S. Department of Agriculture in cooperation with other public and private U.S. and international organizations. Plant genetic resources in the NPGS are made freely available to all *bona fide* users for the benefit of humankind."

In addition to the active and base collections in NPGS, plant breeders maintain working collections of plant materials used in their programs. As cultivars, parental lines, and elite germplasm are developed, released, and registered, these are entered in the NPGS active and base collections.

In the National Plant Germplasm System, the four Regional Plant Introduction Stations, the National Clonal Germplasm Repositories, the Inter-regional Potato Introduction Station, the National Small Grain Collection, specific crop collections, and the Woody Landscape Collection of the National Arboretum each functions, and is accepted, as a national plant germplasm repository even though some are partially supported by regional and inter-regional funds. The more than 440,000 accessions maintained in the NPGS active collections have been divided among these 19 repositories.

These repositories cooperate and participate in a coordinated national program of acquiring and exchanging foreign and domestic plant germplasm potentially valuable for agricultural, horticultural, medicinal, industrial and environmental uses. The new acquisitions must be increased, characterized, and preserved as part of the active collection. Each repository conducts a systematic evaluation program to obtain specific information on disease and insect resistance, nutritional quality, agronomic and physiological attributes, and other traits of interest. Information on the collection and characterization (passport data) and evaluation data are entered in the Germplasm Resources Information Network (GRIN). When requested, samples are distributed to scientists worldwide at no cost for use in crop improvement and basic research. Research relating to improved methods of collection, regeneration, propagation, preservation, evaluation, and distribution is conducted, and the results are published.

The National Germplasm Resources Laboratory (NGRL) located at the Beltsville Agricultural Research Center (BARC), Beltsville, MD, is responsible for a number of activities that support the entire NPGS.

The Plant Introduction Office (PIO) coordinates the acquisition and exchange of plant germplasm; documents passport data and descriptive information for newly acquired material and assigns unique Plant Introduction (PI) numbers; publishes an annual USDA Plant Inventory of newly received accessions; and serves as a liaison on quarantine matters. Plant germplasm for the NPGS is acquired through exchanges, exploration (domestic and foreign), special projects and agreements, gifts, and travelers. In addition to introduced germplasm, all released plant materials (cultivars, germplasm releases, parental lines, and genetic stocks) that are registered by the Crop Science Society of America are assigned PI numbers and the seed is deposited in the appropriate active collection and the NSSL by the originator.

The Plant Exploration Office (PEO) works with germplasm curators, Crop Advisory Committees (CAC), state universities and others to assess the genetic diversity in germplasm collections currently held by the NPGS and others as compared to total genetic diversity that may exist in nature. This assessment is used to develop long-range strategies for increasing the genetic diversity of U.S. collections. Based on these strategies, gaps in current germplasm collections are identified and communicated to the appropriate CAC or to other crop specialists for their concurrence. Priorities for exploration are influenced by several factors such as the completeness of the U.S. collection, the need for specific traits of agricultural significance, the threat of immediate loss of old landraces and wild relatives in centers of diversity because of agricultural changes or urban development, and political factors affecting future availability of germplasm.

The Germplasm Resources Information Network (GRIN) is the official database of the NPGS and is maintained on a computer in the National Agricultural Library at Beltsville, Maryland. The functions of the GRIN database for the NPGS are to: 1) act as a repository of all information on NPGS plant germplasm, 2) unify the NPGS with regard to data standards and coordinate the movement of germplasm, 3) allow users of the germplasm fast access to the most current data available, 4) facilitate and track the distribution of germplasm, and 5) provide to germplasm maintenance sites a system of inventory management that automatically signals the need for germplasm regeneration.

Data in GRIN are available to any plant scientist or researcher worldwide, either through direct connection to the database or through contact with the curator for the active collection of the crop of interest. GRIN contains data on taxonomy, origin, evaluation and characterization for plant germplasm preserved in the NPGS. All

movements and distributions of germplasm within the NPGS and foreign countries are recorded in GRIN.

All plant germplasm entering the NPGS from outside the U.S. must comply with federal quarantine regulations, which are designed to facilitate the exchange of plant germplasm while limiting/ preventing the movement of pathogens. Regulations are written, interpreted, and enforced by APHIS. Scientists cooperate to import plant germplasm free of pests. Accessions of certain crops must be grown under quarantine at designated sites under APHIS inspection, including greenhouses at specified locations and the ARS St. Croix research station, before they can enter the NPGS active and base collections.

The NGRL facilitates the activities of Crop Advisory Committees. The public and private scientists on these committees represent the germplasm user community for a particular crop or a group of crops. These committees provide crop-specific expert guidance on germplasm needs, collection gaps, descriptors, documentation, regeneration, evaluation, and research goals to various components of the NPGS.

Although the ARS components of the NPGS are administered by the Area Director for the geographic location of that component, the Associate Deputy Administrator for Genetic Resources and the National Program Leader for Plant Germplasm on the National Program Staff provide leadership for the NPGS and coordinate activities. They also provide administrative support to the various advisory boards and committees for plant genetic resources.

Plant germplasm collections in NPGS include older and current crop cultivars, elite breeding lines, landraces of crops that have emerged over millennia through selection by farmers, wild and weedy plants related to cultivated crops, and genetic stocks maintained for research.

The active collections of *Hordeum, Secale, Triticum, Aegilops, X Triticosecale, Avena*, and *Oryza* are maintained and distributed by staff of the National Small Grains Collection (NSGC), Aberdeen, Idaho. More than 132,000 samples of seed were distributed during 1993, including about 14,000 to U.S. scientists, 10,000 to foreign scientists, and 78,000 to cooperators for germplasm evaluation. More than 1.4 million evaluation records representing 152 descriptors are available on GRIN for the small grains collections. Regenerations are grown in field and greenhouse nurseries at Aberdeen, Idaho; Maricopa, Arizona; and Stuttgart, Arkansas (rice). The 3,011 new accessions added in 1993 included barley from China and Nepal, various small grains from Russia and Georgia, *Aegilops* from Turkey, Israel, and Syria, and wheat from Turkey.

The active collections of Triticeae species other than small grains are maintained and distributed by staff at the Regional Plant Introduction Station, Pullman, Washington. During 1993, 448 new accessions were added; and 1154 samples of various Triticeae grasses were distributed. Regenerations are completed at the Pullman and Central Ferry farms. The NPGS holdings of Triticeae species are shown in Table 1.

As accessions propagated by seeds are regenerated or increased at the repositories, seed samples are divided with part staying in the local active collection and the other part deposited in the NSSL base collection. The principal mission of NSSL is to preserve the base collection of the NPGS and to conduct research to develop new and improved technologies for the preservation of seed and other plant propagules. The goal of NSSL is long-term preservation of back-up samples of all accessions maintained in active collections at national plant germplasm repositories. The NSSL facility was expanded fourfold and modernized in 1992. The new storage vaults have the capacity to store and protect more than one million samples.

Seed samples received at NSSL are dried, counted, tested for viability and placed in moisture-resistant containers in sub-zero cold vaults (-18°C) or stored above liquid nitrogen (-160°C) in cryotanks. Samples are monitored periodically for viability, and sub-standard samples are regenerated by the appropriate repository.

Minimizing genetic change during *ex situ* preservation is paramount to retain as much genetic variation as possible for future use (Crossa et al., 1994). For seed, a key first step to minimize genetic change is to preserve the initial regenerated sample in the base collection. This regeneration should be done with an appropriate number of plants with the required pollen control under optimum growing conditions to produce high quality seed. Careful processing and drying are required to maintain high viability. Storage of dry, high quality seed at sub-zero temperatures can extend viability for many years before a second regeneration of the base collection is necessary. When continuing demand on the active collection occurs, seed from the base collection should be used for every second or third regeneration.

Plant germplasm preservation research at NSSL focuses on the development of new and improved technologies for the long-term preservation of all forms of plant germplasm. This research is expected to increase: 1) the number of species that can be stored at NSSL, 2) the longevity of the various accessions, and 3) the efficiency of viability testing of accessions. Longer storage periods and reduced number of field and/or greenhouse regeneration cycles will result in lower costs and greater genetic integrity of the germplasm. Research of the Plant Germplasm Preservation Research Unit at the NSSL is addressing also questions on the nature of seed aging under dry conditions and low temperatures, how the rate of deterioration can be predicted and monitored efficiently, and how the effects of aging can be reversed. Research scientists at NSSL work closely with all components of NPGS.

Table 1. National Plant Germplasm System Holding of Triticeae*

Species	Common Name	Numbers of Accessions
Aegilops spp.		3672
Agropyron cristatum	Fairway c crested wheatgrass	298
Agropyron desertorum	Standard crested wheatgrass	107
Agropyron fragile	Siberian crested wheatgrass	83
Agropyron spp.		167
Amblyopyrum muticum		12
Elymus canadensis	Canada wildrye	45
Elymus caninus	Bearded couch	43
Elymus dahuricus	Dahurian wildrye	168
Elymus elymoides	Squirreltail	10
Elymus glaucus	Blue wildrye	44
Elymus lanceolatus subsp. *lanceolatus*	Thickspike wheatgrass	32
Elymus lanceolatus subsp. *wawawaiensis*	Snake river wheatgrass	40
Elymus sibiricus		147
Elymus trachycaulus subsp. subsecundus	Beaded wheatgrass	33
Elymus trachycaulus subsp. *trachycaulus*	Slender wheatgrass	4
Elymus trachycaulus subsp. *violaceus*	Violet wheatgrass	6
Elymus villosus	Hairy wildrye	2
Elymus viginicus	Virginia wildrye	40
Elymus spp.		1102
Elytrigia repens	Couchgrass. Quackgrass	149
Elytrigia spp.		464
Eremopyrum spp.		35
Heteranthelium spp.		38
Hordelymus europaeus		7
Hordeum vulgare subsp. *vulgare*	Barley	26013
Hordeum spp.		2084
Leymus angustus	Altai wildrye	218
Leymus arenarius	Beach wildrye	24
Leymus cinereus	Great Bain wildrye	78
Leymus condensatus	Giant wildrye	2
Leymus millis	Beach wildrye	7
Leymus racemosus	Mamoth wildrye	24
Leymus spp.		145
Pascopyrum smithii	Western wheatgrass	50
Psathyrostachys juncea	Russian wildrye	165
Psathyrostachys spp.		15
Pseudoroegneria spicata	Nluebunch wheatgrass	137
Pseudoroegneria sppl		101
Secale cereale subsp. *cerale*	Rye	1806
Secale spp.		125
Taeniatherum spp.	Medusa's-head rye	27
Thinopyrum ponticum (Elytrigia elongata)	Tall wheatgrass	74
Thinopyrum intermedia (Elytrigia inter.)	Intermediate wheatgrass	156
Triticum aestivum	Common Wheat	34603
Triticum dicoccoides		1670
Triticum dicocconemmer		533
Triticum durum	durum wheat	6832
Triticum monococcum	einkorn wheat	235
Triticum spelta	spelts	1179
Triticum timopheevii		51
Triticum timopheevii var. *araraticum*		268
Triticum turgidum		460
Triticum spp.		2058
Total		85907

*spp. is a grouping of species not listed separately, or accessions not yet classifed.

The NPGS maintains one of the largest *ex situ* collections of plant genetic resources in the world. A detailed report of the NPGS history, policies, and architecture is given in *Plant Breeding Reviews* (ed. by J. Janick, 1989). Since 1898, about 575,000 accessions with real or potential economic importance to U.S. agriculture have been acquired through the Plant Introduction Office. Many of these are among the more than 440,000 accessions, representing over 8,000 plant species, that are now preserved in the NPGS.

The NPGS has been described as a "user-driven system." Between 1986 and 1992, the NPGS distributed an average of 175,400 samples each year to U.S. public scientists (67%), U.S. private industry scientists (12%), foreign public scientists (9%), foreign private industry scientists (10%), and international centers and USAID (2%).

CORE SUBSETS

When a scientist determines that there is inadequate genetic variation in available germplasm for a desired attribute, new accessions are needed that will provide the highest probability of identifying useful source materials with minimum screening. Sometimes this can be achieved by obtaining accessions from an area where the problem has been endemic for many years; e.g., low soil pH. A list of candidate accessions can often be generated when appropriate information is in the database.

In other cases, especially for new pathogen strains or insect biotypes, searching database information is of little or no value. When the scientist must search within the crop collection for the desired trait, an initial screening of a smaller, diverse subset may reduce time and costs. The idea of developing such a subset was proposed by Frankel (1984) and further developed by Brown (1989a,b, 1995). They suggest that "A core collection consists of a limited set of accessions derived from an existing germplasm collection, chosen to represent the genetic spectrum of the whole collection. The core should include as much as possible of its genetic diversity. The remaining accessions in the collection are called the reserve collection." The core subset is suggested to be about 10% of the crop collection, but may vary from 5% for very large collections to 50% or more for very small collections, with about 3,000 suggested as a maximum number.

Brown (1989a) recommended stratified sampling methods when establishing core collections. Grouping begins with taxonomic affinity (e.g., species, subspecies, cytological races). Accessions within each taxon can be then assigned to strata based on ecogeographic zones and genetic characteristics (e.g., ploidy level, photoperiod response, races, etc.). Groups such as races of maize (based primarily on ear morphology) may be preferable to country of origin for defining groups because geopolitical boundaries often are incongruent with ecogeographic niches. In other crops, country of origin (or region of adjacent countries) may be the only available means for developing preliminary groups.

Development of a useful core subset may involve the following steps: 1) assembling and reviewing passport data and other information to be used in establishing non-overlapping groups, 2) assigning accessions to appropriate groups, 3) choosing accessions for the preliminary core subset from each group, and 4) collecting data on phenotypic and genetic traits for accessions in the preliminary core and using multivariate analytical methods to construct clusters and dendrograms to elucidate systematic and statistical genetic relations for further refinement of the core subset.

When funding is available to characterize and statistically analyze the entire crop collection for several descriptors, steps 2, 3, and 4 can be conducted simultaneously. Assigning heavier weights to genetic markers and highly heritable phenotypic traits may improve clustering. Groups generated as clusters from statistical analyses of the data will usually be the most robust. If only a few descriptors were analyzed initially, additional descriptors may be measured for the preliminary core, and then step 4 repeated with data from all available descriptors. When financial resources are limiting or very large numbers of accessions must be characterized, steps 2, 3, and 4 will need to be completed sequentially.

Proportional sampling within each group may provide a more representative sample of the total genetic diversity in the core subset than would a completely random sampling from the crop collection. Once the number needed from each group has been determined, accessions for the core subset are usually chosen randomly within each group. However, some curators are choosing accessions with more desirable agronomic traits within each group.

Clusters generated by multivariate analyses may provide a better understanding of patterns of genetic divergence and diversity and will often identify ecogeographic regions that have not been adequately sampled, especially when the origin of each accession in the core is plotted geographically. This information may be valuable in planning future acquisitions.

The core collection concept has gained wide acceptance and core collections are being developed in many countries (Hodgkin et al., 1995; Knüpffer and van Hintum, 1995). The NPGS is developing a core subset for each of the major crop collections (Erskine and Muehlbauer, 1991; Holbrook et al., 1993; Diwan et al., 1994). The core subset will then be used for more extensive characterization and evaluation. The reserve subset will be maintained as an important part of the NPGS base and active collections.

The core subset is expected to remain dynamic with addition, deletion, and substitution of accessions as additional pertinent information becomes available and as new accessions are acquired. Nevertheless, with time, changes to the core should decrease in frequency and magnitude.

INTELLECTUAL PROPERTY RIGHTS

The U.S. Plant Variety Protection Act (PVPA) requires that a sample of each protected cultivar be stored at the National Seed Storage Laboratory. These voucher samples are not to be distributed. However, the owner has the option to provide a second sample to the NPGS base and active collections of NPGS which provides easy access by users for research purposes. Patented plant materials are not accepted by the NPGS. Accessions in the NPGS base and active collections are not eligible for PVP or for patents.

INTERNATIONAL COOPERATION AND COORDINATION

The need to preserve, exchange, and utilize plant genetic resources is recognized worldwide. Even countries with great genetic diversity in certain crops are heavily dependent on many crops introduced from other areas. Because the U.S. has had to import nearly all of its crop germplasm, the NPGS maintains a very comprehensive germplasm collection from around the world. The NPGS has assisted several countries in recovering germplasm of their key crops, which had been lost for various reasons. Many countries now have genetic resource preservation programs with an associated gene bank. IPGRI indicates that the number of gene banks worldwide holding *ex situ* collections is 1060 (personal communication). Several of these, in addition to NSSL, were part of the former IBPGR network of designated base collections (Table 2). The NPGS maintains a close working relation with many of these programs and freely exchanges germplasm.

The International Maize and Wheat Improvement Center (CIMMYT) is developing the International Wheat Information System that will integrate and make available data from three major components: the Wheat Management System; the Wheat Germplasm Bank System; and the Wheat Data Management System. The software will have general application to self-pollinated crops. This system may serve as a model and become part of a CGIAR systemwide database improvement project coordinated by IPGRI that will facilitate the consolidation and exchange of information and genetic resources.

The development and adoption of the Convention on Biological Diversity, however, will alter procedures and policies for germplasm exchange. The objectives of the Convention (as stated in Article 1) are "the conservation of biological diversity, the sustainable utilization of its components, and the fair and equitable sharing of the benefits arising out of the utilization of genetic resources by appropriate access to genetic resources, and by appropriate transfer of relevant technologies, taking into account all rights over those resources and to those technologies, and by appropriate funding". Article 15, Access to Genetic Resources, reaffirms the sovereign rights of States over their natural resources. It also states that States shall endeavor to create conditions to facilitate access to genetic resources and not to impose restrictions which run counter to the objectives of this Convention. However, access shall be on mutually agreed terms. The Convention provides for sharing benefits derived from shared genetic resources with the country of origin, or the

Table 2. Genebanks with Base Collections of Triticeae Species*

Institution	Species	Number of Accessions
CAAS (China)	*Triticum*	*10,427*
CIMMYT (Mexico)	*Triticum*	73,794
CNR (Italy)	*Triticum*	32,000
ICARDA (Syria)	*Triticum, Hordeum, Triticale*	50,255
NGB (Sweden)	*Hordeum, Secale*	14,410
NIAR (Japan)	*Hordeum*	6,236
PGI (Japan)	*Aegilops*	6,774
PGR (Canada)	*Hordeum*	2,200
BG/PAS (Poland)	*Secale*	1,320
PGRC/E (Ethiopia)	*Hordeum*	12,648
VIR (Russia)	*Triticum*	73,082

*Most of these were part of the former IBPGR network of designated base collections (Number of accessions were provided by IPGRI).

country providing such resources where they have been acquired in accordance with the Convention. *Ex situ* collections located outside of the country of origin that were acquired prior to the entry into force of the Convention (December, 29, 1993) do not fall under the terms of the Convention.

In order to comply with the Convention, it is expected that material transfer agreements (MTAs) will be required to accept and to distribute accessions acquired after December 29, 1993. The form of these MTAs for the NPGS is still under development. Possible requirements of a recipient might be as follows: 1) to report to both the donor and NPGS any evaluation results, 2) to acknowledge the germplasm contribution (indicating source country) in publications and variety releases, and 3) to negotiate a license with the donor in the event derived products are developed that have potential commercial value. Although NPGS will need to notify the source nation when an accession subject to a MTA is distributed, the responsibility for monitoring commercial developments by the recipient is expected to remain with the source country, since NPGS has no charter or funding to be a collector or agent.

SUMMARY

Because very few crops grown in the U.S. are native, plant introductions have been vital to our agriculture. The development of a comprehensive NPGS for *ex situ* preservation of plant genetic resources obtained from around the world was necessary to provide scientists with source materials for their programs.

Technologies required to preserve genetic resource propagules in *ex situ* collections in a living but quiescent form are being developed and refined. In the past decade, there have been major technological advances which permits living organisms to be preserved in "suspended animation." This technology will enable us to store our valuable genetic resources safely and efficiently. The more than 440,000 accessions maintained by the NPGS include local landrace collections, improved cultivars, wild crop relatives, and genetic stocks. The active collection is maintained and distributed by nineteen national plant germplasm repositories. The base collections for seed crops are preserved at sub-zero temperatures at the National Seed Storage Laboratory, Fort Collins, Colorado. Plant genetic resources of the NPGS are made freely available to all *bona fide* users for the benefit of humankind. Between 1986 and 1992, an average of 175,400 samples per year were distributed worldwide by NPGS.

It is important that genetic changes during *ex situ* preservation are minimized. The procedures now used by the NPGS for orthodox seeds include regenerating a high quality initial sample for the base collection, carefully drying and storing this base collection sample at sub-zero temperatures, and using seed from the base collection sample for regenerating every second or third generation for the active collection. Improved technologies and new facilities help insure that these valuable resources will be available in future years with minimum genetic shifts.

Core subsets consisting of about 10% of each crop collection are being developed to represent most of the genetic diversity of each crop species and its relatives. The NPGS is identifying and characterizing a core subset of each major crop to facilitate the use of plant germplasm resources in crop improvement and to improve efficiencies of breeders and active collection curators.

Recent international agreements such as the Convention on Biological Diversity will impact acquisition and exchange of germplasm, but the NPGS's goal is to maintain the free exchange that is needed to continue to increase agricultural productivity to be able to feed the increasing world population in the future. Not only have public and private scientists used introduced germplasm from the NPGS and other sources to produce stress tolerant and high yielding varieties and hybrids, but also farmers have used these improved products to increase yields and lower production costs so that the average U.S. family now spends less than 12% of its income for food.

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Forage Species in Xinjiang Northern Natural Grasslands: Grasses

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ABSTRACT

Forage germplasm resources, their distributions, potential agronomic characteristics and genetic aspects of the perennial grasses originating in Xinjiang northern regions are discussed. Grass diversity in the natural grasslands is immense for the range of environments to which it is endogenously adapted. Among the perennial species in the mountain area are summer growing and winter-dormant ecotypes, whereas in the plain outland summer-fast-growing, summer-dormant and winter dormant ecotypes occur. Flowering uniformity within the grass tribe provides the chance for gene exchange. The genetic variation for growth rate, tiller development, regrowth and yield between species and within a species has provided plant breeder with abundant material for pasture improvement both in the native and introduced grasses. Because of overgrazing, reclaiming, destroyed woodlands and hedgerows the threat to the loss of genetic diversity in the natural grasslands must be a matter for our concern.

INTRODUCTION

There are 70 genera and nearly 300 grass species in northern Xinjiang natural grasslands (1,2), one fourth of which are present in all of the pastures and primarily provide support for grazing animals and herbivores. They belong to the Mediterranean and other Eurasian temperate species. A feature of these species is that each of them is encountered endogenously over a vast range of environments, and the individual members of a species represent a considerable genetic resource that is adapted to many environments. The northern Xinjiang regions including Yili, Tachin, Altai, Bltala, Changji and Urumqi districts from the north of Tiansan to the south of the Altai mountains are composed of 206.7 million hectare of pastures. Water and temperature are the two main factors limiting growth and development of local vegetation. Temperature is similar to drought-desert climates. However, Tiansan mountains east-west stretched over in south provide a natural defense for the area and bar the way of Atlantic and Antarctic wet currents form the western gap. This results in more rainfall and partly eases the influence of the typically continental climates. In addition, within the regions topography rises and falls, providing more rainfall which could reach to more than 300-350 mm with an average temperature 5°C in the mountains, but can also be less than 250 mm with 2-7°C in the plains or basins. Therefore, although the area has drought-desert climates the geological backgrounds which create the local climate with water and temperature, especially in the mountains, provide the temperate grasses an ideal environment for growth. Due to their environmental condition the Xinjiang natural pastures are among the world's richest in greater diversity. On the basis of such a condition and vegetation, there are traditionally unique grazing regimes of four-seasonal round and bia-seasonal round in Xinjiang natural grasslands for use. Areas of greatest diversity have inevitably been the central places grazed by animals and herbivores. It was found that among the perennial grasses in the mountains summer-growing and winter-dormant pastures occur, whereas, in the plain low-land summer-fast-growing, summer-dormant and winter-dormant ecotypes occur. But the annual species occupy and survive the hot-summer and cold-winter by fast growth and seed dormancy.

GERMPLASM

Agronomic characteristics and adaption to the environment

Research on agronomic characteristics including plant development, forage yield, forage quality, and other concerned factors were selected in order to evaluate the potential for use as pasture species.

Plant development

The plant development of the pasture species is one aspect of successful survival and adaptation to the indigenous environment, and causes major differences in

plant yields. The Xinjiang environment is characterized by long cold winter and hot dry summer. According to field observations the perennial grasses such as *Dactylis*, *Bromus*, *Poa*, *Agrostis*, *Elymus*, *Phleum*, *Elytrigia* and *Alopecurus* grow in areas that receive more that 350 mm precipitation. These grasses germinate at the end of April. As temperature increases their growth rate slows down. Their growth is rapid by the middle of June and reaches its peak by the end of July. By the end of the July the plants flower and set seed. Growth of most grasses at higher elevations is completed by the middle of September. Although some species such as *Agrostis gigantea* can extend their growth to the end of October. The perennial grasses of the genera *Achnatherum*, *Leymus*, *Agropyron*, *Aeluropus* and *Calamagrostis*, which grow in the lowland plain, complete their development early because rainfall is limited and maximum summer temperature often exceed 40°C. For these species their seed is formed and summer-dormancy occurs before the end of July or the beginning of August. If sufficient water becomes available they can initiate growth in autumn. Annual species typically have short stature and complete their growth and development by the end of July and winter over as seeds. This pattern of growth in annual species is controlled by the seasonal distribution of rainfall and changes in temperature. The perennial grasses escape the influence of grazing by tillering intensively and allowing a greater opportunity for flowering and subsequent seed production.

The seasonal yields and variation

The yield is a multiple index measuring the assimilating efficiency to minerals, water, sunlight and CO_2 in their environments by the plant itself. It is controlled not only by their inherited process but also outer environmental conditions. As usual the yields of perennial grasses in the mountain area appear in a skewed curve with single peak neither between or within ones, but as the environments are varying yields are different due to growing height, tiller development and regrowth rate (Table 1). Based on the present data genotype differences within and between species is evident.

The variation observed strongly supports a genotype (species) environment interaction. Forage availability of annual species is seasonal, resulting from growth and development each year. Therefore, the yield differences, both between and within a species, is due to the nutrient utility in their environments and disparities exiting in the height and growth rate. However, within present and traditional grazing patterns, there is relatively high selective pressure on the less grazing tolerant species. This pressure will soon cause a species composition change from highly desirable forage grasses to those much less palatable species (*Achnatherum splendens* and *A. inebrians*). The short- and long-term planning should include education on grazing management and the improvement of the degraded grassland pasture with improving cultivars of grasses and legumes breed for grazing and salinity species.

Flowering response and adaption to the environment

The perennial temperate grasses are cross-pollinating, self-pollinating and sexual in their reproductive characteristics. Cross-pollination among perennial temperate grasses is predominately wind driven. When pollen exchange between the different individuals is accomplished by the wind, their genetic materials would have presumably been exchanged. Evans *et al*. (1964) reported on the uniformity among members within the grass tribes on their response to environmental factors in inducing reproduction (3). According to the side study, the flowering period of the grasses in mountain areas is mainly from July to August except in extreme alpine systems, where flowering may be in June. However, flowering period in the plain low lands, where it is hot and dry is from June to July. their is a slight variation within species with regard to flowering data based on different environments with latter maturing species related to an increasing latitude. This flowering uniformity will insure the constant gene exchange within a species with limited exchange between different species. Based on the present data, it appears that flowering uniformity, frequency of gene exchange and genetic variation would exert an influence on species in the process of adaption and evolution. The variation observed in weight/one thousand seeds of *Dactylis glomerata*, *Bromus inermis* and *Agrostis gigantea* (see Table 1) would support the presenting genetic diversity for seed weight within the above grass species.

Table 1. The height, tiller yield and weight/thousand of grass species.*

Species	Accession #	Origin	Height cm	Tillers	Yield ha	Weight/ 1000
Dactylis glomerata	XJG02-1-1	Altai	113.25	5.6	7.24	0.8706
	XJG02-1-3	Yili	113.63	13.9	5.34	0.4542
	XJG02-1-5	Tachen	124.38	7.3	9.25	0.8558
Bromus inermis	XJG01-1-1	Yili	122.75	12.9	8.24	2.8037
	XJG01-1-4	Altai	131.25	12.6	9.91	2.6595
	XJG01-1-5	Tachen	113.75	9.3	5.23	4.1428
Agrostis gigantea	XJG05-1-1	Yili	78.38	20.0	2.88	0.1074
	XJG02-2-9	Altai	82.25	12.6	6.68	0.1092
	XJG05-5-6	Tachen	84.65	9.3	4.95	0.1220
Psathyrostachys juncea	XJG09-2-1	Altai	123.4	39.0	12.39	2.1170
	XJG09-2-2	Tachen	129.4	80.1	12.89	3.1853
Leymus angustus	XJG10-2-1	Tachen	136.3	55.9	8.31	3.5738
	XJG10-2-2	Yami	122.0	44.2	4.31	4.1234

*The data is from the station of the south mountains in Urumqi.

Morphological and biological deterrent

The economic benefit of animal grazing on forage species is dependent upon their morphological characteristics. Grasses that have been selected under heavy grazing pressure tolerate the defoliation, or have evolved avoidance mechanism, that makes it unpalatable. Possible examples within the Xinjiang grazing pastures include *Stipa* species which have a very long awn on the seed, and *Achnatherum inebrians*, which contains alkaloids at toxic level and if grazed may be fatal to animals. However, the majority of grass species can tolerate treading and grazing of animals and maintain a lush level of tillers, regrowth and rhizome development. The forage quality of grass species declines and becomes coarse due to a silica gel that increases under a dry climate. However, the crude protein and digestible carbohydrates remain remarkable high 8 to 42% respectively, during seed set. Thus, they provide a highly nutritious and palatable forage to animals, which constitutes the major source of forage for grazing animals within the Xinjiang natural grassland husbandry.

Genetic aspects

Grasses generally have a large degree of genetic variability with species and many species are closely related. Many species have arisen from hybridization and further hybridization. *Dactylis glomerata* and *Poa pratensis* are two examples where extensive hybridization has occurred. Natural grasslands of Xinjiang cover a wide range of environments, and changes in temperature and rainfall occur within a short distance in the steep mountainous terrain found in Xinjiang. This allows considerable opportunity for gene exchange within a species or even between species because of flowering uniformity and possible cross pollination. This situation creates conditions for heterozygosity, mutation and polyploids. Under species environments, co-adapted gene complex have evolved over a long period of time by selection. During this selection process the perriality and tiller development are far more favorable in the survivability and development of this new species ecotype. Due to a high level of environmental variation observed within the Xinjiang natural grasslands, there is an abundance of different ecotypes. Such raw genetic stock have provided an abundant source of materials for forage breeders, for the improvement of natural pastures and establishment of artificial pastures. One must conclude that grasses have are well adapted to a wide range of environments and that they exhibit considerable genetic variability for many characteristics. Unfortunately, the grasses of Xinjiang have not been studied in detail.

Genetic conservation

In comparison with cereal crops, forage germplasm comprises a large number of families, genera, and species. The forage species of Xinjiang have co-evoluted with indigenous herbivores and domesticated grazing animals through a long period of time. Although man's participation in this process has been relatively recent, the effects have been disastrous. The clearing of woodland, hedgerows and especially over-grazing has disrupted the original balance among species. This situation is not the same as that for cereals with the loss of landraces and their replacement by a single cultivar. The natural pasture area for hay production in Xinjiang grasslands has been reduced from 26.7 million hectares in the 1960's to 15. Three million hectares in 1990's, and forage yields have been reduced by 20 to 30%. Degenerated and deserted pastures are increasing by about 66.7 thousand hectares each year and is causing an ecological crisis. The genetic diversity of Xinjiang grasslands is seriously threatened. This genetic diversity is very important to our environment and food production and is being lost unnoticed. The effect of the loss of this germplasm is difficult to precisely evaluate, but will certainly have an impact on further generations. Because permanent pastures continue to represent a large proportion of the land area that comprises the forage diversity of Xinjiang, forage germplasm from these areas must be urgently collected and preserved.

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Diversity of Trypsin Inhibitors in Cultural and Wild Barley

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ABSRACT

Diversity of trypsin inhibitors was studied in 35 spring barley cultivars (*Hordeum vulgare*), 21 samples of *H. spontaneum*, and 3 samples of *H. agriocrithon*. Six variants of trypsin inhibitor spectra were identified by native electrophoresis method followed by specific development of activity. Four variants were found in both cultivated and wild barley, and the other two were revealed only in *H. spontaneum*. Trypsin inhibitor activities (TIA) and soluble protein contents were determined in four cultivars with different variants of trypsin inhibitors. It was shown that TIA differed in the cultivars studied and did not correlate with soluble protein contents.

INTRODUCTION

Trypsin inhibitors found in endosperm of cereals are of great interest for genetics and breeding because they play important role in protection of grain against microorganisms and insect pests [1]. They are also significant for protein utilization by monogastric animals [2]. That is why studying diversity of trypsin inhibitors and variations of their activities in different cultivars is the subject of research programs. Polymorphism of barley trypsin inhibitors was previously described by Salcedo *et al.* [3] and Moralejo *et al.* [4]. They identified three allelic variants of trypsin inhibitors (BTI-CMe1, -CMe2, and -CMe3) and demonstrated that the last two consisted of several components (BTI-CMe2.1, -CMe2.2, -CMe2.3 and BTI-CMe3.1, -CMe3.2 accordingly). TIA of these individual components were shown to be different. This communication describes new variants of endospermal trypsin inhibitors revealed in cultural and wild barley and presents the results of determining total TIA in different barley cultivars with respect to their soluble protein contents.

MATERIALS AND METHODS

Study of 35 *Hordeum vulgare* cultivars (1993 crop) as well as 21 *H. spontaneum* and 3 *H. agriocrithon* samples (1992 crop) was carried out using seeds provided by Dr. A.A. Pomortsev (Russia) and Prof. E. Nevo (Israel). Trypsin inhibitors were extracted from individual embryoless grains by 0.1 M Na-acetate, ph 4.9 (4 v/w) at 4°C during a night and separated by electrophoresis in 6% polyacrylamide gel in tris-Na-EDTA-borate system (ph 8.3) according to [5]. After electrophoresis proteins were transferred from a gel to a gelatine layer of Micrat film for 10 minutes and zones of trypsin inhibitors were developed by the method [6]. The film was dried, put on 1% agarose gel containing 0.05 M tris-HCl, ph 7.8, 0.1 M NaCl, trypsin 250 ng/ml, and incubated with it at 37°C for 60 minutes. Bands of undigested gelatine on the film corresponded to trypsin inhibitors.

Isoelectric focusing in 4% polyacrylamide gel containing 4-9 Servalytes and 9 M urea was performed for determining Isoelectric points (pI) of trypsin inhibitors from cv. Nutans 970 [7]. Proteins were extracted from embryoless ground grains by Na-acetate; supernatant was desalted by gel-filtration on BioGel P6-DG (Bio-Rad) column (12 cm) and concentrated on Minicon B-15 (Bio-Rad) concentrator. The pH gradient of the gel was measured by determining pH values in water extracts from ' cm sections of the gel. Proteins were stained by Coomassie R-250, and bands of trypsin inhibitors were revealed by replication to Micrat film as described before.

Four cultivars with different variants of trypsin inhibitors were chosen for analysis of their TIA and soluble protein contents. Embryoless grains of each cultivar were crushed with Cyclotec 1093 Sample Mill (Tecator). Proteins were extracted with 3 w/v 0.1 M Na-acetate, pH 4.9, at 4°C during a night (three replications for each cultivar), and TIA were determined by method [8] with BAPA (N alpha-benzoyl-DL-arginine-p-nitroanalide HCl, Serva) as substrate. One unit of inhibitor (U) was defined as the amount of inhibitor that could inhibit 1 mg of trypsin (TPCK treated, Serva). Protein contents were determined with Bio-Rad (Breadford) reagent in the same extracts. Bovine serum albumin (Serva) was used as standard for protein calibration. Systat Version 5.0 was used for statistical calculations of the obtained results. Least significant difference (LSD_{05}) was estimated as described [9].

RESULTS AND DISCUSSION

Study of different samples of cultivated and wild barley by one-dimensional native electrophoresis with subsequent development of trypsin inhibitor bands allowed us to reveal six variants of spectra. Four of them were specific both for cultivated and wild barley, and the other two were found only in *H. spontaneum* (Fig. 1, Table 1). In the studied cultivars the most frequent variant was D, and variants A and C were relatively rare. All three samples of *H. agriocrithon* had the same variant of trypsin inhibitor (Table 1).

As may be seen from Fig. 1, three variants of trypsin inhibitors (C, D, and E) are close to each other by their electrophoretic mobilities (patterns 1-3 and 7-9), the fourth one (A) is considerably different (patterns 4-5), and the remaining two have intermediate mobilities (patterns 6, 10). It should be noted that it was difficult to determine correspondence of our variants of trypsin inhibitors revealed by one-dimensional electrophoresis with the known trypsin inhibitors classified by Moralejo [4] based on the results of two-dimensional electrophoresis. These difficulties were connected with differences in sets of varieties studied and with resolution capabilities of the methods used. So we preliminarily designated our variants as A-E according to their electrophoretic mobilities (Fig. 1). One variant of *H. spontaneum* (Fig. 1, pattern 10) consisted of two components. However, because the component with higher activity against trypsin had the same mobility as B-variant, we designated it as B'. A-variant of trypsin inhibitor seems to be interesting because of its extremely fast mobility in alkaline electrophoresis. So we further characterized it by isoelectric focusing.

The results of IEF presented in Fig. 2 demonstrated that A-variant of trypsin inhibitor (cv. Nutans 970) consisted of four components with pI 6.2, 6.1, 5.8, and 5.6 as well as two minor components with pI 5.9 and 5.7, thus, all the components of this variant have acid pI.

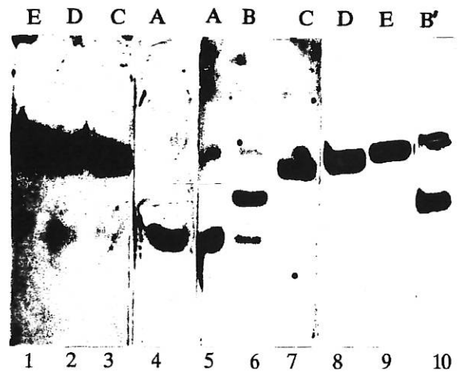

Fig. 1. Diversity of trypsin inhibitors in cultural (1-4) and wild (5-10) barley.

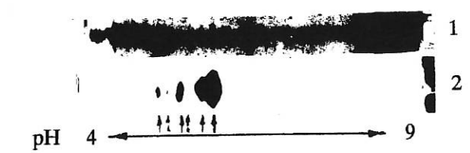

Fig. 2. Bands of proteins (1) and trypsin inhibitors (2) of cv. Nutans 970 revealed by isoelectric focusing.

Table 2. Trypsin inhibitor activities and soluble protein contents in four barley cultivars.

Variety	TIA (U/g of flour)	Soluble protein Content (mg/g of flour)
Pirkka	0.94	9.97
Kashticky	0.83	9.97
Nutans 558	0.37	10.07
Nutans 970	0.83	11.23
LSD_{05}	0.14	2.75

The presence of trypsin inhibitors with acid pI in endosperm of barley cultivars Emir and Proctor was previously demonstrated by Bruhn and Djurtoft [10]. However, in these cultivars trypsin inhibitors with pI 5.8-6.2 were only additional for the main trypsin inhibitor with alkaline pI and are characterized by weak trypsin inhibitors activity (1/3 of the total TIA). In cv. Nutans 970 bands of trypsin inhibitors with alkaline pI were absent. Our preliminary studies allows us to assume that additional bands of trypsin inhibitors with acid pI increase or appear in cvs. Emir and Proctor during storage or accelerated aging of barley grains (Ladogina, unpublished). However, further investigations are needed for clarifying the nature of these changes in trypsin inhibitor spectra.

The next aim of our work was to study TIA and soluble protein contents in four barley cultivars (Pirkka, Kashticky, Nutans 558, and Nutans 970) with different variants of trypsin inhibitors (E, D, C, and A variants respectively). Data of the analysis presented in Table 2 demonstrate that variations in protein contents were not significant in all cultivars studied ($F = 1.0114$, $P\ 0.436$). However, variations in TIA were highly significant ($F = 65.19$, $P\ 0.000$).

Within the restricted set of the examined barley cultivars we could not reveal correlations between soluble protein contents and TIA. Data, similar to ours, were obtained by Tanner and Reinbergs [11] for wheat and rye: in their study TIA was independent of the level of soluble and total protein content.

In our study cv. Nutans 558 differed from the other cultivars by extremely low TIA (approximately two-fold). The set of the studied cultivars is insufficient for analysis of reasons of such decrease of TIA in cv. Nutans 558, and additional investigations are needed. However, it may be possible to use this cultivar for obtaining barley forms with low TIA.

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Breeding Potential of Exotic Barley Germplasm

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Abstract

Utilization of exotic germplasm offers an approach to broaden genetic variability in breeding populations. This study was conducted in order to 1) compare germplasm of exotic origin with adapted Swedish barleys with respect to genetic differences and 2) to evaluate first cycles of pre-breeding i.e. agronomic traits in complex exotic x adapted crosses. Allozyme studies showed the following Nei's gene diversities among parents: 0.13 (adapted parents), 0.16 (landraces) and 0.25 (*H. spontaneum*). Cluster analysis based both on allozyme and agronomic data indicated that parental groups were genetically divergent. Earliness, straw length, number of ears per plant and thousand kernel weight (TKW) were studied. The best sources for earliness were adapted parents and landraces. Mean straw length was greatest in *H. spontaneum* lines. Number of ears per plant was quite similar in all groups.The highest TKW was among landraces and adapted parents. Hybrids from the complex crossing programme exceeded parents in earliness and TKW. An index composed from the four traits showed the most favorable frequency distributions for adapted parents and hybrids. Both genetic and agronomic studies indicate that new variation from exotic germplasm may be introduced into barley breeding material. In addition, through recombination, agronomically valuable genotypes can be obtained and they can be utilized in long-term breeding programes.

INTRODUCTION

Genetic variation serves as the basis which plant breeders depend upon to develop improved cultivars. Adequate genetic variation must be available in breeding stocks in order for plant breeders to make further improvements in crops. The most important sources of genetic variation are breeders' own breeding populations. For genotypic diversity not already in the breeding program elite, adapted germplasm from comparable programs within the same ecogeographical region can be chosen to facilitate their ease of incorporation and utilization. Only when sufficient variation is not available from these sources, do breeders turn to gene banks or seek for variation in exotic material (Baenziger & Peterson 1991).

The difficulties involved in introducing new genetic variation into breeding programmes coupled with wariness on the part of breeders have led to concern about the genetic similarity of modern cultivars. As a consequence of this, cultivars of today are genetically vulnerable (Ford-Lloyd & Jackson 1986) i.e. they are incapable to fight against pests, pathogens or environmental conditions due to large number of genetically identical individuals in a cultivar (Wilkes 1989). In addition, genetic similarity may lead to slower gain in breeding. Finally, in the future there could be difficulties meeting the new demands of our changing agricultural environment, if we do not diversify the genetic base of our crops.

One way to solve the problem of genetic erosion or at least reduce the rate of erosion while still producing cultivars that are commercially competitive, is to establish genetically diverse breeding populations. Usually, some cycles of pre-breeding are needed before unimproved germplasm can be introgressed into a breeding population. Pre-breeding involves the transfer of certain characteristics from exotic material into breeding material that is more similar to the improved cultivars currently in use. The end products of pre-breeding are usually deficient in certain desirable characters; however, they are attractive to plant breeders due to their greater potential for direct utilization in a breeding programe than the original unadapted exotic sources (Wynne & Halward 1989).

Utilization of exotic germplasm has been reported earlier, among others, in two temperate cereals, oats (Lawrence & Frey 1973, 1975, Frey et al.1984) and barley (Vega & Frey 1980, Rogers 1982, Lehmann & Bothmer 1988). These studies support the idea that useful genes affecting quantitative as well as qualitative traits can be obtained from exotic germplasm. In their reviews on utilization of exotic germplasm Frey et al. (1984), Bramel-Cox & Cox (1988) and Cox (1991) emphasize the importance of evaluation of exotic germplasm for its utility.

Table 1. Parent lines used to develop the experimental barley population

Name/Origin Accession n:o		2/6-row	Naked/ covered	Seed colour black/light	*Hordeum* ssp. *vulgare/spontaneum*
Aisling	Sweden	2	c	l	ssp. *vulgare*
Alfa	Sweden	2	c	l	ssp. *vulgare*
Ariel	Sweden	2	c	l	ssp. *vulgare*
Ellinor	Sweden	2	c	l	ssp. *vulgare*
Golf	Sweden	2	c	l	ssp. *vulgare*
Hulda	Sweden	2	c	l	ssp. *vulgare*
Ida	Sweden	2	c	l	ssp. *vulgare*
Jet	Ethiopia	2	n	b	ssp. *vulgare*
Kara	Sweden	2	c	l	ssp. *vulgare*
Korinna	Sweden	2	c	l	ssp. *vulgare*
Magda	Sweden	2	c	l	ssp. *vulgare*
Milka	Sweden	2	c	l	ssp. *vulgare*
Nancy	Sweden	2	c	l	ssp. *vulgare*
Shirley	Sweden	2	c	l	ssp. *vulgare*
Sv 87368	Sweden	2	c	l	ssp. *vulgare*
Sv 89412	Sweden	2	c	l	ssp. *vulgare*
Sv 89425	Sweden	2	c	l	ssp. *vulgare*
Sv 87443	Sweden	2	c	l	ssp. *vulgare*
Sv 892052	Sweden	2	c	l	ssp. *vulgare*
Sv 892368	Sweden	2	c	l	ssp. *vulgare*
Sv 892508	Sweden	2	c	l	ssp. *vulgare*
Sv 892596	Sweden	2	c	l	ssp. *vulgare*
Sv 898133	Sweden	2	c	l	ssp. *vulgare*
Sv 898219	Sweden	2	c	l	ssp. *vulgare*
H 4035	Pakistan	6	n	l	ssp. *vulgare*
H 4086	Pakistan	6	n	l	ssp. *vulgare*
H 4166	China	2	c	l	ssp. *vulgare*
H 4175	China	6	c	l	ssp. *vulgare*
H 4241	China	6	c	l	ssp. *vulgare*
H 4246	China	6	c	l	ssp. *vulgare*
H 4248	China	6	n	l	ssp. *vulgare*
H 7405	China	2	c	l	ssp. *vulgare*
H 7614	China	6	c	l	ssp. *vulgare*
H 3038	Syria	2	c	l	ssp. *spontaneum*
H 3042	Syria	2	c	l	ssp. *spontaneum*
H 3057	Jordan	2	c	l	ssp. *spontaneum*
H 3059	Jordan	2	c	l	ssp. *spontaneum*
H 3064	Jordan	2	c	l	ssp. *spontaneum*

In addition, comparisons of divergence between wild and cultivated populations with variation within the cultivated gene pool are needed to make utilization of wild germplasm more efficient. Hence, the objectives of this study were 1) to compare germplasm of exotic origin (unadapted landraces and wild barley) with adapted Swedish barleys with respect to genetic differences and agronomic performance and 2) to evaluate first cycles of pre-breeding, that is to say, agronomic traits in complex exotic x adapted crosses.

MATERIALS AND METHODS

Plant material

The experimental population was developed by intermating 40 barley lines selected for phenotypic diversity and resistance to various barley diseases. The material included 25 spring barley varieties and lines adapted to Swedish conditions and 15 exotic lines. The latter comprised 10 cultivated landraces and 5 accessions of wild barley, *Hordeum vulgare* ssp. *spontaneum* (hereafter called *H. spontaneum*) (Table 1). From 1 to 7 individuals of each accession were used as parents in each crossing generation. The plants within accessions were chosen at random. The parents were intercrossed pairwise so that an exotic parent was always crossed with an adapted one. As a result 20 2-way hybrids were obtained from the first crossing generation. These hybrids were further intercrossed in a half-diallel design and from this 190 double cross hybrids were produced. In the third generation, the hybrids from the previous crossing generation were intercrossed pairwise and 95 hybrid lines were achieved. These highly heterozygous 8-way hybrids, which contained from 25 to 50 % exotic germplasm, were used in the glasshouse experiment.

Allozyme studies

Allozyme variation of 6-phosphogluconate dehydrogenase (6PGD), malate dehydrogenase (MDH), aconitate hydratase (ACO), esterase (EST), NADH dehydrogenase (NDH) and glucosephosphate isomerase (GPI) at 11 loci was assayed to characterize genetic diversity in the parental material. The methods of horizontal starch gel electrophoresis, including details of sample preparations and staining methods have been described in detail earlier by Veteläinen (1994).

Glasshouse experiment

The experiment was conducted in a randomized block design in a glasshouse. Because it was not known whether the hybrids were of spring or winter type, all the hybrids as well as the *H. spontaneum* seeds were vernalized at + 4 °C for 16 days prior to sowing. The vernalization medium was 0.8 % water-agar together with calcium-sulfate (0.01 %) (Ahokas 1982). Ten seeds from each of the parental and hybrid line were sown in separate pots. The experiment was divided into ten blocks and each block was divided into two groups. Group A included all the parental lines and group B the 8-way hybrids. This experimental arrangement was made to minimize interplant competition for light. Nitrogen fertilizer was added when the third plant leaf had emerged. An 18-hour photoperiod was used in the glasshouse with a day/night temperature 18 °/14° C . These light and temperature conditions were designed to imitate Nordic conditions during the growing period.

Traits

Four different agronomic traits were measured from each plant. Heading date was recorded as days from planting to the date when the first head was emerged. Straw length (cm) was measured from the tallest tiller. Number of ears per plant was counted at maturity. Thousand kernel weight (TKW) was measured in grams. An index (scale 4-15) from four components was constructed as follows:

$$INDEX = i_{ear.em} + i_{no/ears} + i_{straw} + i_{TKW}$$

Each trait was divided into four classes (Table 2). The class including the top lines scored 4, while class with the lowest values scored 1 for each index component. Early heading plants with short straw and high TKW were considered most favorable (score 4). However, a moderate number of synchronous emerging ears per plant were considered best in this study. Therefore, the lowest and the highest class were treated similarly in the case of number of ears per plant, when calculating the index. Phenotypic classes were used for cluster analysis (Table 2.).

Statistical analyses

The General Linear Models (GLM) procedure of the Statistical Analysis System (SAS Inst. 1990) was used for the analyses of variance, which was carried out separately for parental material and hybrids. Differences found by the analysis of variance between the means of hybrids and different parental groups were further analysed by Tukey's test. To analyse electrophoretic and agronomic class data, the NTSYS-pc statistical package was used (Rohlf 1993). The statistical method used for electrophoretic data took into account the presence or absence of each allozyme band as differential feature. A total of 28 bands were considered for the statistical comparisons. First, a similarity matrix was formed by calculating Dice's (1945) similarity coefficient for each of the pair of parental lines. Then, the matrix was submitted to average linkage cluster analysis (UPGMA) to produce a dendrogram. Correspondingly, to analyse agronomic class data , simple matching coefficient (SM) (Sokal & Michener 1958) was calculated in order to produce a dendrogram. The formula was SM=m/n, where m is number of matches in class and n total sample size.

To compare different parent groups, Nei's (1975) measure of gene diversity was calculated for each parental group (adapted parents, landraces, *H. spontaneum*). The formula used was $H = 1 - p_{ij}2/m$, where p_{ij} is the frequency of the *ith* allele at the *jth* locus and m is the total number of loci examined.

RESULTS AND DISCUSSION

Genetic diversity and cluster analyses

Of the 11 allozyme loci, 9 (82 %) showed polymorphism among the 40 parental lines. Altogether, 28 alleles were found, of which 9 were found exclusively in exotic parents and 4 only in *H. spontaneum*. The maximum number of alleles at a given locus was four. Gene diversities within parental populations were 0.13 (adapted parents), 0.16 (landraces) and 0.25 (*H. spontaneum*), which indicates that wild barleys were genetically the most variable parent group.

Associations among the parental lines of the experimental population revealed by UPGMA cluster analyses based on electrophoretic and agronomic data are presented in Fig. 1. and 2., respectively. The parental material was divided into four main clusters when using electrophoretic data. The first cluster included Swedish varieties and lines with one exception, which was the Chinese 6-rowed landrace H 7614. The second main cluster included landraces and two Swedish breeding lines Sv 892368 and Sv 89412. The latter line included two different genotypes a and b (Fig. 1). The difference was found in one single EST-locus. The occurrence of these Swedish lines within the cluster of Asiatic landraces may be caused by the primitive landrace, *Hordeum distichum* cv.

Table 2. Phenotypic classes and score values for index

Trait	Phenotypic class	Score for index
Ear emergence, days	30-55	4
	56-80	3
	81-105	2
	106-120	1
Number of ears per plant	1-5	1
	6-10	3
	11-15	2
	15<	1
Straw length, cm	60-80	4
	81-100	3
	101-120	2
	120-135	1
TKW, g	10-28	1
	29-38	2
	39-48	3
	49-65	4

Laevigatum, which has been used as a resistance source to barley mildew in the establishment of these two Swedish lines. The third cluster included all *H. spontaneum* lines, except one from Jordan, which was genetically most distant from all the other parental lines. Overall results from the cluster analysis conformed with the country of origin (Table 1) within the landrace and *H. spontaneum* clusters. The cluster analysis based on agronomic data (Fig. 2) reveals that unadapted material is not only genetically, but also agronomically different from adapted material. The distinction of landraces from *H. spontaneum* lines is not so pronounced in this case, possibly suggesting that these two groups are adapted to similar environmental conditions. Thus, overall results of diversity and cluster analyses revealed that adapted parents, landraces and *H. spontaneum* were genetically divergent and that exotic germplasm could be utilized as a source of new genetic variation.

Agronomic traits in parents and hybrids

All genetic variation is not necessarily useful for breeding purposes and genes to be utilized should either contribute directly, or in combination with other previously evaluated breeding material (Smith & Duvick 1989). Therefore, the next step was to evaluate four easily measurable agronomic traits in order to detect possible additional desirable characters in parental material. Furthermore, the parents were compared with the hybrids in terms of agronomic performance.

The results from analysis of variance are shown in Table 3. Summary statistics with Tukey's test for different parental groups and hybrids are presented in Table 4. The earliest heading parent group was landraces followed by the adapted parents.

The *H. spontaneum* lines were considerably later. Hybrids were remarkably earlier than all the parent groups suggesting that utilization of *H. spontaneum* in this extent (12.5 % of parental material) did not affect earliness negatively.

Straw length had the lowest mean among adapted parents while *H. spontaneum* had the highest. This is in agreement with an earlier study (Jaradat 1989) that showed that one of the most important traits distinguishing *H. vulgare* from *H. spontaneum* is plant height. However, the mean was lower among hybrids than among landraces and *H. spontaneum* lines. Thus, in this respect, exotic germplasm was inferior to adapted one, but affected the performance of the hybrids only moderately.

To acquire an estimate of the yielding capacity of the parental lines, two yield components were measured. Both TKW and number of ears per plant have been shown earlier (Puri et al. 1982, Benbelkacem et al. 1984) to be positively correlated with yield. In an earlier study (Rogers 1982), *H. spontaneum* grain yields were found to be extremely low. Yet, when crossed with adapted cultivars, transgressive high-yielding segregates were found in their progeny. In this study, TKW means were almost similar among landraces and adapted parents, but lower in *H. spontaneum*. The hybrid mean exceeded all the parental means in this trait. The second yield component, number of ears per plant, was quite similar in all the parent groups, although slightly higher in adapted than in exotic parents. Thus, there are some indications that exotic material included genes which would not be seriously detrimental to yielding capacity.

The analysis of agronomic traits in the parents shows that exotic germplasm is not necessarily inferior to adapted, when measuring individual traits. However, agronomic performance is a sum of several traits and

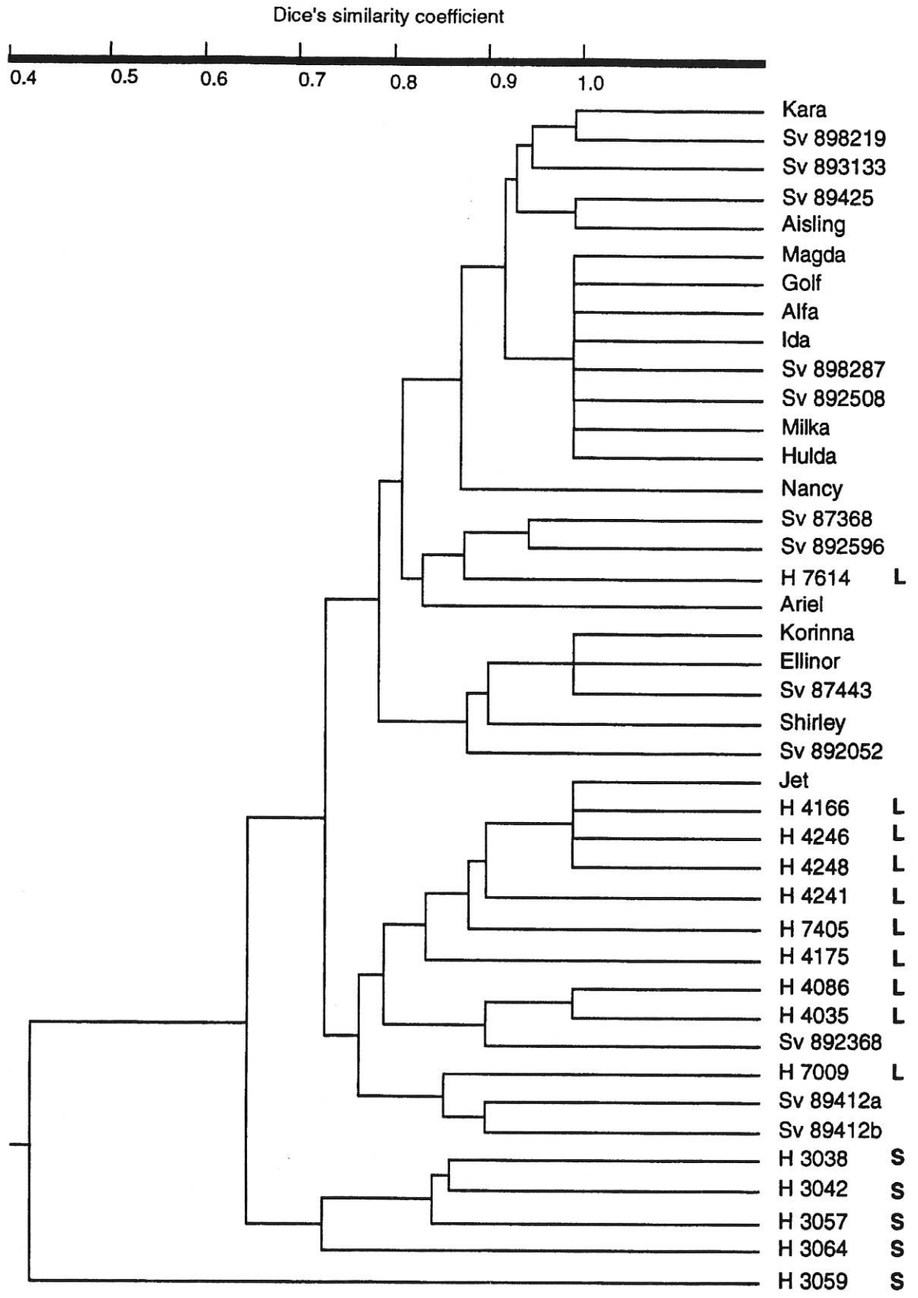

Fig. 1. Dendrogram of 40 parent lines revealed by UPGMA cluster analysis based on electrophoretic data, L = landrace, S = *Hordeum spontaneum.*

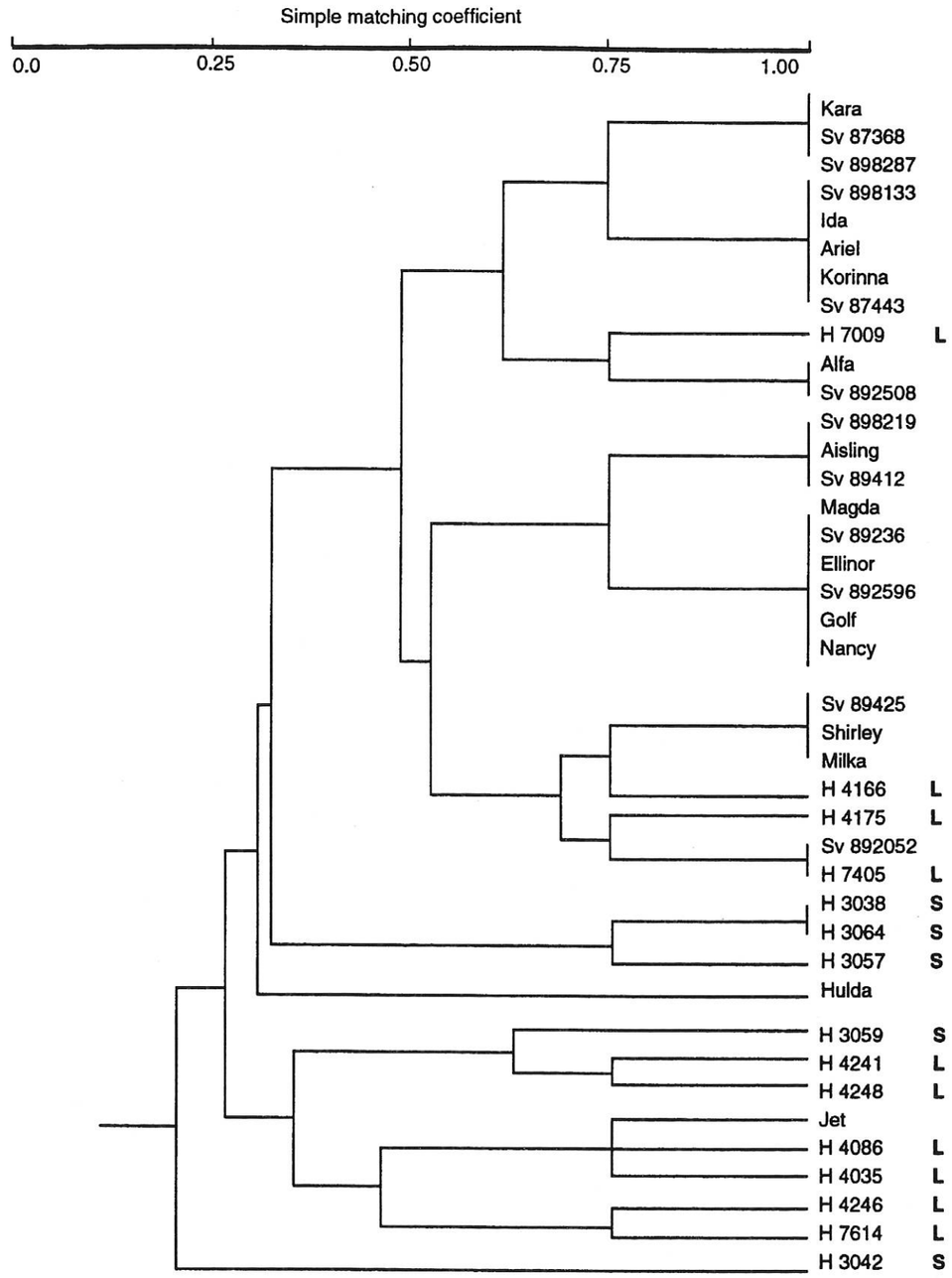

Fig. 2. Dendrogram of 40 parent lines revealed by UPGMA cluster analysis based on agronomic data, **L** = landraces, **S** = *Hordeum spontaneum*.

therefore an index was calculated for each line. The frequency distribution (Fig. 3) for the index shows that adapted parents exceed exotic germplasm in overall performance. Yet, it is apparent that landraces may possess a desirable combination of traits, for example, early plants with short straw and high TKW. Twenty per cent of the landraces studied were in the classes with the highest indices. Contrarily, all *H. spontaneum* lines had a very poor combination of agronomic traits. Around 50 % of the hybrids had indices 13 or 14 but only 20 % of the adapted parents fell into the highest classes.

These studies suggest that new genetic variation from exotic sources can be introduced into barley breeding material. In addition, through recombination agronomically valuable genotypes can be achieved and utilized in long-term breeding.

Table 3. Analyses of variance of parent and hybrid lines of four traits

Source of variation	df	MS			
		Ear emergence	No ears/ Plant	Straw length	1000 kernel weight
Blocks					
- A (parent lines)	9	85.1	27.1 **	109.9	150.1 *
- B (8-way hybrids)	9	225.2	20.4 **	566.7 **	184.8 **
Parent lines	39	2150.6 **	48.2 **	3280.8 **	281.4 **
- between groups	2	24669.7 **	398.6 **	40427.5 **	988.6 **
- adapted	24	303.7 **	21.4 **	463.8 **	247.2 **
- landraces	9	1866.8 **	54.5 **	3476.1 **	234.6 **
- spontaneum	4	2610.7 **	19.9	1170.1 **	238.4 **
8-way hybrid lines	94	547.2 **	15.6 **	703.8 **	228.2 **

* significant at the 5 % level
** significant at the 1 % level

Table 4. Summary statistics and Tukey$_{FS}$ test for 4 traits measured on parent and 8-way hybrid lines (C.V= coefficient of variation)

Group	Ear emergence (days)				No ears/plant				Straw length (cm)				1000 kernel weight (g)			
	min	max	C.V	mean=)	min	max	C.V	mean	min	max	C.V	mean	min	max	C.V	mean
Adapted (N=25)	42.1	72.5	10.2	56.3B	9.0	15.6	12.2	12.2A	66.6	96.0	8.7	80.1C	32.0	49.3	12.6	40.3B
Landraces (N=10)	34.8	82.7	26.5	51.5C	4.7	12.9	26.5	8.8C	71.9	130.1	19.2	97.1B	33.0	47.8	11.9	40.5B
Spont. (N=5)	65.1	115.3	19.6	91.8A	8.7	13.0	13.9	11.0B	102.7	134.6	10.2	124.2A	26.6	40.7	16.0	32.5C
Hybrids (N=95)	31.1	70.1	15.6	48.1C	8.2	14.3	11.6	10.8B	73.6	119.2	9.1	93.4B	39.5	62.8	9.9	49.2A

=) Values within the same column followed by the same letter are not significantly different from each other at the 5 % probability level according to Tukey$_{FS}$ test

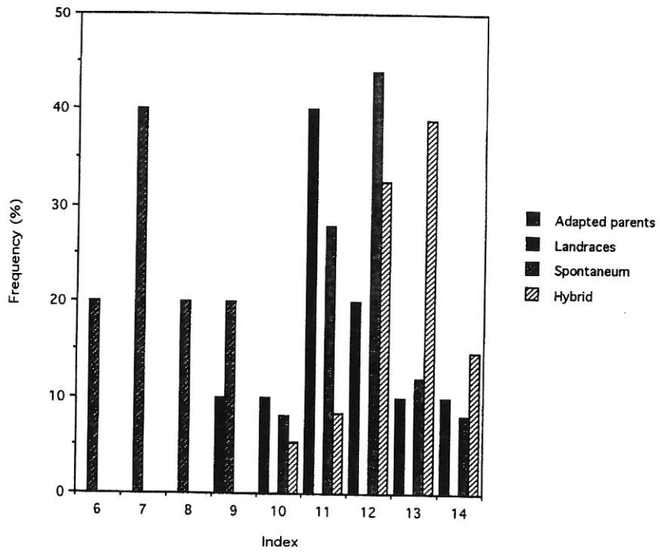

Fig. 3. Frequency distribution for index combined from four traits measured in parents and hybrids.

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Evaluation and Utilization of Biodiversity in Triticeae for Wheat Improvement

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ABSTRACT

To adapt new varieties to a wide spectrum of environments breeders and farmers have emphasized the need for broadening the current narrow genetic base of modern varieties of important cereal crops such as wheat and barley. In response to this need, several thousand samples of indigenously cultivated Triticeae species and their wild relatives have been collected from the centers of diversity. However, gene bank collections are of little use if they are not evaluated and the information disseminated widely. Evaluation is essentially the link between conservation and use. Some of the collected material has been evaluated at the International Center for Agricultural Research in the Dry Areas (ICARDA) in Syria. In the past cereal breeders were averse to using germplasm that after years of work yielded uncertain results. However, in recent years they have begun to successfully utilize non-conventional germplasm (wild/alien and obsolete forms) in their crossing blocks. The substantial progress at ICARDA in the evaluation and utilization of Triticeae germplasm for crop improvement in the low rainfall areas of West Asia and North Africa is described.

INTRODUCTION

As a result of considerable rise in collection and conservation activity of Triticeae genetic resources during the last two decades a very large quantity of accessions of wild, cultivated and obsolete (primitive) forms have been assembled at various genetic resources conservation centers around the world. However, genetic resources merely kept safely in storage can be of little value to plant breeders for utilization in their crop improvement programs unless these are evaluated and the resulting information made available through communication and exchange. Evaluation is essentially the link between conservation and use.

A population structure of a species is defined as the totality of ecological and genetical relationships among individual members which may co-evolve as a result of gene exchange but may also diverge under localized forces of evolutionary change (Jain, 1975). Landraces and obsolete cultivars are as a rule products of several years of crop evolution and it is vital to preserve their genetic composition during and after evaluation. Instances have been reported where polymorphic cereal populations have undergone radical changes in their genetic composition in one growing cycle (Shevchuk, 1973). However, in the case of samples collected from village markets or those which are subjected to biased sampling methods in the farmer's field, it is sufficient to safeguard and maintain their genes and not necessarily their gene frequencies within populations (Porceddu and Damania, 1992).

It is now generally agreed that the gene bank curator must be regarded as being responsible for characterization of new incoming material whereas the evaluation of the same should be the task of a germplasm scientist, other than a breeder, attached to a crop improvement program (Jana, 1987). In fact, Frankel (1987) categorically states that genetic resources have been utilized without elaborate characterization but never without evaluation mostly by, or in close interaction with plant breeders. The aspects of evaluation and documentation of cereal genetic resources has been reviewed recently by Damania (1990).

EVALUATION OF GENETIC RESOURCES COLLECTIONS

Cultivated genetic resources which survive evaluation for biotic and abiotic stresses are normally suitable as potential crossing material for one or two specific traits, hence it would be useful as a donor of these traits rather than as lines for release as commercial cultivars.

Many wheat evaluation studies have used ranking as a method of describing results. This ranking may change from one site to another for some quantitative characters such as plant height and days to heading (Damania, 1983). Such unstable characters cannot be adequately described when studied at a single location. Thus the concept of multi-location testing has gained importance in evaluation projects in recent years.

However, there are traits such as resistance to diseases and tolerances to certain types of soils (such as saline) for which variability can only be observed at particular sites where the incidence of that particular stress is the greatest, the so called "hot spots". For example, for screening against resistance to *Septoria tritici* (leaf blotch) ICARDA uses a humid and relatively high rainfall site located near Lattakia on the Mediterranean coast in Syria. Experiments on tolerance to salinity and drought are conducted at a site on the shores of salt lake Jabboul in northern Syria. Jana *et al.* (1983) first used this site to evaluate 3000 durum wheat accessions from various countries and out of these only ten lines were found to be highly tolerant to combined stresses of salinity and drought. In recent years screening for salt tolerance is being carried out with more accurate results with the use of hydroponics or sand culture because past experience has shown that soil salinity in a field is highly variable (Damania *et al.* 1994).

A total of 662 accessions of twenty-four *Aegilops* spp. were planted at Tel Hadya, the principal experimental station of ICARDA. In the subsequent three seasons, which were highly variable for temperatures and precipitation, a number of these accessions were dropped from the study due to their poor tolerance to one or more biotic and abiotic stresses prevalent at that site. Accessions with poor viability and growth vigor were also eliminated. Hence, in the subsequent season only 206 elite accessions were isolated as more or less pure lines selections. The number of species were reduced to just twelve with only 4 species being dominant among these as the most tolerant and hence useful for providing donor genes for wheat improvement in wide crossing programs (Table 1).

Unlike *Aegilops* spp., the wild progenitors of wheat belonging to the genus *Triticum* are commonly sympatric with their cultivated forms. They differ in phenotype and adaptation but remain sufficiently related genetically to cross and produce fertile hybrids with exchange of genes particularly in the direction of the cultivated forms. The ecological environment of growth for the purposes of preliminary evaluation should be made as identical as possible to that of the original habitat of the germplasm. However, this is not always possible in a collection where samples originate from all corners of the areas of their distribution. Therefore, no single evaluation location can be entirely suitable for all accessions or Triticeae species. Darlington (1969) states that barley and emmer wheat (*Triticum dicoccum*) originated in Syria. ICARDA is fortunate in being located within the center of diversity for cultivated and wild Triticeae and, as such, is as near to an ideal site for evaluation as can possibly be found (Srivastava and Damania, 1989). Evaluation carried out at near ideal sites minimize the effect of natural selection on the accessions' genetic make-up and also ensures an adequate harvest of seed quantity for distribution or further evaluation.

Recombinant DNA technology has a great potential for elucidating the biochemical and molecular bases of the complex processes underlying agronomically interesting traits and also for making otherwise unattainable changes in plant genotypes. However, for monocotyledon species such as wheat, practical achievements are not expected in the immediate future. On the other hand, chromosome engineering, i.e. sexual transfer of chromosomal segments between related Triticeae species through manipulation of the homoeologous pairing process allows successful introduction of useful genes of alien origin into cultivated wheat due to the availability of molecular techniques as analytical and selection tools.

Table 1. List of species of *Aegilops* which were tolerant to frost, drought and heat stress over four seasons at Tel Hadya.

Species	Ploidy	Genome	No. of lines
Aegilops biuncialis	4X	UM	50
Aegilops caudata	2X	C	8
Aegilops columnaris	4X	UM	27
Aegilops kotschyi	4X	US	1
Aegilops ovata	4X	UM	53
Aegilops peregrina	4X	US	4
Aegilops speltoides	2X	S	2
Aegilops squarrosa	2X	D	2
Aegilops triaristata	6X	UMUn	6
Aegilops triuncialis	4X	UC	39
Aegilops umbellulata	2X	U	5
Aegilops vavilovii	6X	DMS	8
		Total	205

UTILIZATION OF GENETIC RESOURCES COLLECTIONS

For the purpose of utilization systematic analysis and description of samples is useful in distinguishing between populations, identifying duplicates, as well as providing information on the extent of variation for desirable traits within a given genetic resources collection. It is axiomatic that the more evaluation information on a collection is available the greater the chances of its rational utilization. Collection site information is extremely important. For instance, at ICARDA newly received germplasm which is described as having a short maturity period and collected from heat stress prone areas receives immediate attention of the breeders as these traits are essential for evading periods of drought and high temperatures during grain filling in the dry areas of West Asia.

There are three ways in which obsolete forms and wild relatives of our cultivated cereal crops can be utilized (Frankel, 1970): i) Introductions for direct use as crops, ii) introductions which can confer particular traits to the adapted cultivars such as, disease resistance, protein content, etc. (this type of utilization is the most prominent way in which obsolete forms and wild relatives of Triticeae have been utilized), and iii) introductions to increase yield *per se*, irrespective of the effect of physical or biotic stresses present in the environment. The extent of evaluation and initial usage among the three categories of germplasms are almost proportional to the degree of their utilization. However, wild Triticeae species, especially those from the secondary gene pool, remain one of the least collected, conserved and exploited categories of germplasm.

Varietal improvement and the incorporation of yield stability in the improved cultivars for the low rainfall areas through the use of landraces has been impressive in wheat. For example, Duwayri *et al*. (1987) crossed Stork, a semi-dwarf high yielding durum wheat cultivar under optimum conditions with Haurani, the local well adapted durum landrace in Jordan and Syria which produces reasonable yields under stress conditions. A number of lines which resulted from these crosses appear promising in low as well as moderate rainfall zones of West Asia and North Africa (WANA).

It becomes obvious that if greater use of obsolete and wild Triticeae material has to be made it is essential to remove (or at least suppress) the close linkage between desirable traits and unfavorable alles. This may be done through transporting the germplasm to areas similar to the native habitats where evaluation and selection can be carried out under favorable conditions of soil, photo-periods and temperatures. For wild species, particularly the putative progenitors, either a naturally introgressed population or an artificially directed back-crossing program would improve their chances of inclusion in a breeding program. This preparatory activity is often referred to as germplasm enhancement or pre-breeding (Chang, 1985).

Sears (1956) gave a good example of pre-breeding efforts involving a wild relative of wheat. In that early report *Aegilops umbellulata* was initially crossed with *Triticum dicoccoides* to produce an amphidiploid progeny. This was crossed with a *T. aestivum* cultivar but the F_1 was male sterile and had to be back-crossed to *aestivum* twice. The progenies of this back-cross were tested for leaf-rust resistance which was present in the wild species. A resistant plant was isolated carrying 21 bivalents. This plant was then crossed with Chinese Spring to produce Transfer which was widely used in North America as a leaf-rust resistant cultivar. Since then, other wild species of wheat have been utilized by Canadian and U.S. breeders as gene sources for improving winter-hardiness, short stature and cytoplasmic male sterility in wheat (Stalker, 1980).

The utilization of wild relatives has also yielded promising results in producing lines of wheat with disease resistance as well as tolerance to drought and salinity. An experiment to assess tolerance to artificially created salinity and its effect on morphological traits in some lines of *Triticum boeoticum* and *Triticum dicoccoides* was carried out at ICARDA using sand culture techniques in a controlled environment with eight replicates. In general, *T. dicoccoides* was found to be more tolerant to salinity than *T. boeoticum* (Damania *et al*. 1994).

The real bottle neck in the utilization of wild and obsolete/rare (primitive) forms in wheat crop improvement has been the lack of genetically pure lines with stabilized desirable characters incorporated therein. Breeders are averse to utilizing germplasm which may retard progress on their improved lines and/or that which may require years of back crossing to eliminate undesirable traits which are very often inherited when wild or obsolete/rare (primitive) material is used. For example, in a simple *Triticum durum* x *T. dicoccoides* cross characters such as, brittle rachis, glume hairiness, profuse unsynchronized tillering, hybrid necrosis, grass clumping and loose crown persist in subsequent generations but rapid progress can be made by making a top cross of this material with durum wheat.

To alleviate this problem an extensive program of pre-breeding was established. Crosses between wild progenitors (mainly *Triticum dicoccoides*) and durum wheat, and between durum wheat and obsolete forms with disease resistance (such as *T. dicoccum*), were made during 1989-90 in order to develop genetic stocks with stable desirable characters which the breeders could use directly in their crossing programs. Also, Haurani was crossed with *T. dicoccoides* using the latter as the male parent because lower fertility has been reported when *T. dicoccoides* was used as a female parent (M. Tahir, pers. comm.). Selections were made in 1991-92 season and subsequent seasons and the first segregates were tested in 1992-93 for inheritance of desirable traits with encouraging results.

The *T. durum* x *T. dicoccoides* cross also transfers certain disease resistance, high protein content as well as improved yield to the cultivated form. The 1000-kernel weight of the durum varieties used as female parents was much higher than that of the *T. dicoccoides*. Nevertheless, in selected progenies high 1000-kernel weight from the female parent and high protein content from the male is retained (Srivastava and Damania, 1989).

A number of obsolete forms such as, *T. polonicum*, *T. turgidum* and *T. carthlicum* (which are tolerant to drought and possess resistant to yellow rust) were also crossed with accessions of *T. dicoccum* to improve gene combinations in the latter. Progenies of these crosses were planted in plastic house and their characteristics studied. The number of crosses and seeds obtained are given in Table 2.

The utilization strategies for Triticeae genetic resources at ICARDA are as follows: a) large-scale screening for tolerance to biotic and abiotic stresses; b) evaluation of the extent of variability within the species for agronomic traits; c) selection of a small number of accessions with stable desirable traits to initiate a crossing program with cultivated wheat; and d) evaluation of a number of early generation progenies with non-brittle rachis. At present the early generations of the hybrid material are being grown at several sites representing the actual crop growing environments in the WANA region.

Table 2.List of crosses between cultivated, wild and obsolete species of wheat and number of seeds obtained after harvest.

Female parent		Male parent		No. of seeds
T. durum Haurani	x	*T. dicoccoides*	600340	1
T. durum Haurani	x	*T. dicoccoides*	600548	1
T. durum Haurani	x	*T. dicoccoides*	600474	1
T. durum Haurani	x	*T. dicoccoides*	600455	13
T. durum Haurani	x	*T. dicoccoides*	600874	3
T. durum Cham 1	x	*T. dicoccoides*	600340	8
T. durum Cham 1	x	*T. dicoccoides*	600340	2
T. durum Cham 1	x	*T. dicoccoides*	600415	6
T. durum Cham 1	x	*T. dicoccoides*	600415	2
T. durum Cham 1	x	*T. dicoccoides*	600392	2
T. durum Cham 1	x	*T. dicoccoides*	600392	12
T. durum Cham 1	x	*T. dicoccoides*	600392	12
T. durum Cham 1	x	*T. dicoccoides*	600435	1
T. dicoccum 600780		x *T. turgidum*	09805	5
T. dicoccum 600768		x *T. turgidum*	09805	6
T. dicoccum 600770		x *T. turanicum*	22276	5
T. dicoccum 600768		x *T. dicoccum*	14253	3
T. dicoccum 600765		x *T. polonicum*	12197	1
T. dicoccum 600765		x *T. dicoccum*	14215	1
T. dicoccum 600774		x *T. dicoccum*	14215	7
T. dicoccum 600767		x *T. turanicum*	12276	3
T. dicoccum 600767		x *T. turanicum*	12276	6
T. polonicum 12194		x *T. dicoccum*	600771	5
T. polonicum 12194		x *T. turgidum*	136071	3
T. spelta 08861		x *T. compactum*	37367	2

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Attempts to Produce Alien Addition Lines in *Triticum durum*

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ABSTRACT

Aneuploid stocks in durum wheat are few, and alien additions are particularly rare. The present contribution describes the results of a program aimed at the addition of alien chromosomes to tetraploid wheat. *Aegilops caudata*, *Ae. longissima* and *Dasypyrum villosum* were used as chromosome donors. The crossing program involved the production of amphidiploids with the wheat cytoplasm and the recurrent crossing with durum wheat pollen. 15 monosomic addition (2n= 29) plants were obtained and partly selfed and/or backcrossed to wheat. Two disomic addition (2n= 30) plants, possessing two different *Ae. caudata* chromosomes, were obtained; unfortunately they were very weak and highly sterile, thus preventing their further propagation. The difficulties experienced during the development of the crossing program are discussed in detail.

INTRODUCTION

In Italy, and especially in the Southern regions, *Triticum durum*, macaroni wheat, is a cereal crop of relevant interest. In addition to a long lasting cultural tradition, the cultivation of this crop is maintained because of its importance in the pasta making industry. In the latest years some legislation is being developed which introduces a "premium" for pasta types made out of Italian durum wheat varieties; as a consequence there is a renewed breeders interest in improving this species.

Moreover, there is a growing consumer's interest in "natural" products, that is in food produced from plants grown without any chemical treatment. Consequently there is a need for plant types particularly fitted to be "biologically" grown. Several interesting traits can be found in wild species which could be introduced in improved varieties fitted for the above aims, such as genes coding for storage proteins able to improve the technological quality of the semolina or genes conferring resistance to biotic or abiotic stress.

Most of the outstanding cytogenetic work in wheat has been carried out on *T. aestivum*. In durum wheat, conversely, aneuploid stocks are few, possibly because of two reasons: minor economic importance of this crop as compared to common wheat, and more intrinsic technical difficulties due to a lower level of genetic tolerance to the aneuploid condition.

Nevertheless, in the cv. 'Langdon', some aneuploid series have been developed, namely double-ditelosomics, dimonotelosomics, D-genome disomic substitutions, and some other well balanced aneuploids, such as intervarietal substitutions (Joppa 1993); in the cv. 'Senatore Cappelli' also primary trisomics were established (Blanco *et al.* 1982). Reports of alien disomic or monosomic additions are very few and concern some 2n= 29 aneuploid lines carrying chromosomes from *Dasypyrum villosum* (Blanco *et al.* 1987).

In 1988 at the Germplasm Institute of Bari we started a project aimed to produce monosomic or, better, disomic alien additions of *Aegilops* or *Dasypyrum* chromosomes to *Triticum durum*. The present contribution reports on the results obtained from this project.

MATERIALS AND METHODS

All the *Triticum* and *Aegilops* accessions used in the present study are part of the collection held at the Germplasm Institute. 2n= 42 amphidiploids involving *Triticum durum*, *Aegilops caudata*, *Ae. longissima* and *Dasypyrum villosum*, respectively, with wheat cytoplasm obtained earlier. Five different wheat accessions were used as the female parent in the production of the amphidiploids and as recurrent male parent in later crosses. A list of the parent material is given in Table 1.

BC_2 seeds were scored for chromosome number: root tips from plantlets showing 2n=29 were banded in order to try cytologically to identify the extra chromosome. 2n= 29 BC_2 plants were selfed in order to try the obtention of disomic additions or crossed with the wheat parent in order to maintain them.

Table 1. Origin of the amphidiploids used in the present study: female parent was *durum* wheat (TTnn)

Amphidiploid	Origin
GXX04	TT01 x *Ae. caudata*
GXX05	TT05 x *D. villosum*
GXX06	TT48 x *Ae. longissima*
GXX07	TT48 x *Ae. longissima*
GXX09	TT01 x *Ae. caudata*
GXX12	TT01 x *Ae. longissima*
GXX13	TT24 x *Ae. caudata*
GXX15	TT08 x *Ae. caudata*

TT01: cv 'Senatore Cappelli', TT05: landrace 'Bidi', TT07: landrace 'Sciacca', TT48: landrace 'Capinera', TT24: cv 'Langdon'

Crosses were made in the field in different years; spikes of the female parent were hand emasculated and pollinated. Chromosome counts were made on the root tips of the hybrid seeds, after pretreatment with ice-water for 18-24 hours and overnight fixation in Farmer's fluid, using the Feulgen squash method. Chromosome banding was performed using the technique of Giraldez *et al.* (1979).

RESULTS AND DISCUSSION

We decided to follow a crossing scheme starting from some 2n= 42 amphidiploids (*T. durum* x alien diploid species) which had been previously obtained in crossing programs. The main advantage of this choice was essentially the reproducibility of the results, since it was possible to restart the backcrossing program at any stage, which would not be possible starting with hybrids (amphihaploids). Moreover the amphidiploids have better female fertility than amphihaploids and consequently the BC_1 seed set is higher. Amphidiploids with the wheat cytoplasm were chosen in order to avoid cytoplasmic influence on gametogenesis. The female gametes of amphihaploids show high levels of meiotic non reduction, thus yielding gametes which are genetically indistinguishable from those of the relative amphidiploid.

In Table 2 the data from the backcrossing (BC_2) program are reported on the basis of the amphidiploids involved. 509 BC_2 seeds were obtained out of 5155 pollinated spikelets on BC_1 plants (0.099 seeds/spikelet); the low seed set depends on the reduced fertility of the 2n= 35 BC_1 plants. The backcrosses of some amphidiploids, were much more fertile than the average, and particularly high values were observed in BC_2 from XX 05 (0.363 seeds/spikelet) and XX 15 (0.381 seeds/spikelet); also backcrosses of XX 09 showed high fertility (0.171 seeds/spikelet) although not as high as the previous hybrids. These amphidiploids involved *D. villosum* (XX 05) and *Ae. caudata* (XX 09 and XX 15). Most of our work concentrated on these BC_2 seeds. The backcrosses of other amphidiploids set fewer seeds.

Table 2. Seed set of each amphidiploid when backcrossed to wheat

Amphidiploid	Seeds	Spikelets	Ratio
XX04	31	965	0.03
XX05	182	501	0.36
XX06	1	357	0.0
XX07	63	1652	0.04
XX09	104	607	0.17
XX12	0	24	0.0
XX13	59	868	0.07
XX15	69	181	0.38

Table 3. Chromosome constitution of BC_2 plants

2n =	Plants
28	342
29	15
30	20
31	23
32	27
33	4
34	12
35	31

The big difference observed in seed set in the backcrosses of different amphidiploids might be the influence of the alien genome in the amphidiploids alone or the interaction resulting from the genetic combiation in the hybrid. In fact, although possessing the same genome, the accessions used in the crossing program were rather genetically different: TT 01 was the old Italian cv 'Senatore Cappelli' (in XX 09); TT 08 was an old Sicilian landrace (in XX 15); in the program we also used an accession from Ethiopia which strongly differed from the others (TT 48). The influence of the genotypes on the crossability in interspecific hybridization had been already evidenced during the course of the crossing program (Pignone and Cifarelli 1990).

The BC_2 seeds were analysed for chromosome numbers; out of 509 seeds 37 failed to germinate; other few plants were too weak and did not reach maturity. Most of the analysed seeds (342) showed an euploid chromosome complement. Only 15 seeds showed a 2n= 29 genotype; in all these plants the added chromosome belonged to *Ae. caudata*. The results of chromosome number analysis are shown in Table 3.

No 2n= 29 BC_2 plants with *D. villosum* addition could be detected. On the basis of previous results (Blanco *et al.* 1987), we expected to obtain some *D. villosum* additions; possibly this failure depends on the different wheat and *Dasypyrum* genotypes used in our crossing program.

The 2n= 29 plants derived from the same *Ae. caudata* line and from two different wheat accessions, TT 01 and TT 08, the most successful accession in producing addition lines was TT 08. Nine out of 15 plantlets had its genetic background.

The chromosomes of these plants were stained with the C-banding technique in order to identify the added chromosome. It is interesting to note that from a cytological point of view the additions only showed 4 different chromosomes in similar relative proportions. Plants showing the same added chromosome also possessed very similar morphological traits, thus confirming the cytological assessment.

The maintenance of these addition lines was tried following two paths: selfing or crossing with the wheat parent.

In general 2n= 29 BC_2 plants were not fully vigorous: tillering was poor, culms developed late and spikes were rather small. Anthers were shrivelled and did not deliver much pollen. Selfing was, therefore, quite difficult, and often self fecundation was manually assisted, breaking mature anthers within the same flower. Nevertheless 157 selfed seeds were obtained. Only 6 of them showed 2n= 29 chromosomes (4%), and 2 were 2n= 30 (1%). Few plants showed some rearrangement of the added chromosome which appeared as a telo or isochromosome (Figure 1). The number of seeds obtained is low, inferring any evaluation of the transmission rate of the added chromosome and, moreover, data from additions involving different chromosomes are grouped; nevertheless it appears that this value is rather low and possibly even lower than the one observed in *D. villosum* additions (Blanco *et al.* 1987).

Cytological examination allowed us to determine that two different disomic additions had been obtained; 2n= 30 chromosome plants were extremely weak, had a chlorotic aspect and were very late. They produced few small spikes which were completely male sterile, showing undeveloped anthers. Fecundation with wheat pollen was also attempted without success.

Backcrossing of the 2n= 29 plants was attempted in order to maintain the obtained additions. The seed set was poor, since most of the seeds resulted 2n= 28 tetraploids (Figure 2). Only three 2n= 29 true addition plants were recovered while in other cases the added chromosome appeared rearranged. In four plants the extra chromosome had a C-banding pattern different from the donor chromosomes, thus indicating a more complicated rearrangement than centromere misdivision alone.

The low level of fertility of 2n= 29 plants poses a big limitation to the maintenance of these cytogenetic stocks. It is likely that hybrids with different genetic combinations might have a more favourable transmission. In fact, some unreported data might indicate that changing the wheat parent accession at each generation improves the proportion of healthy aneuploid plants; this method has the disadvantage of introducing undesired variation in the wheat genome which could spoil any use of the aneuploid stocks. This restriction also poses a reservation for the utilization of this aneuploid material, because under these conditions any possible use is hampered by the possibility of loosing the lines.

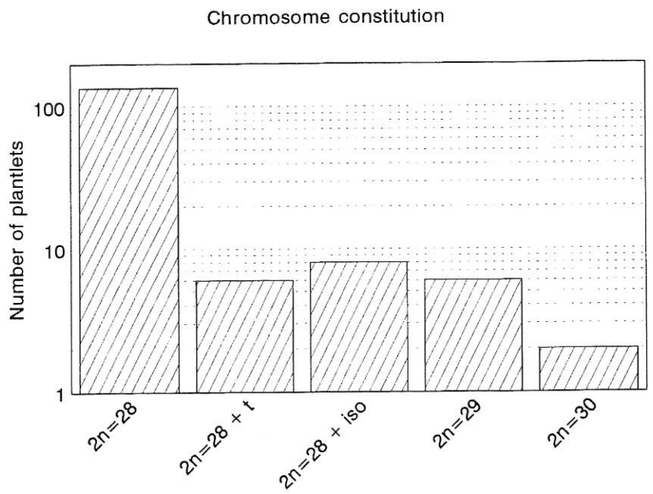

Figure 1. Seed set of selfed 2n= 29 plants

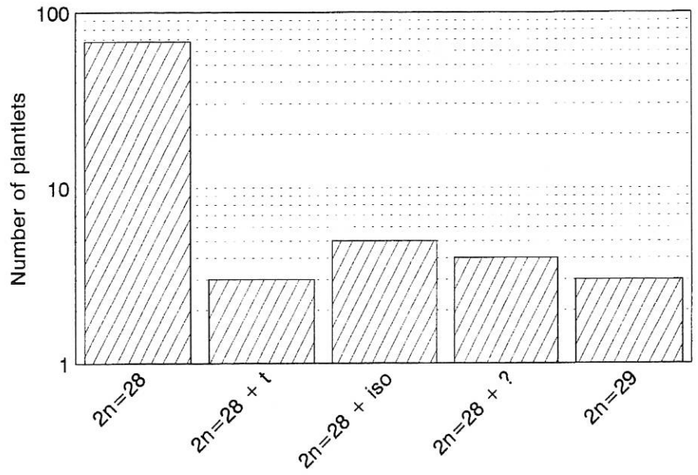

Figure 2. Seed set of 2n= 29 plants crossed to the wheat parent

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Geographical Distribution, Ecology and Diversity of *Triticum urartu* Populations in Jordan, Lebanon and Syria

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INTRODUCTION

Wild diploid wheat *Triticum urartu* Tumanian ex Gandilyan was discovered in 1930 in Armenia by Tumanian and scientifically described in 1972 by Gandilyan (Gandilyan 1972). Subsequently, *urartu* wheat has been identified as a wild progenitor of cultivated durum and bread wheats and a donor of their A genome chromosomes (Chapman et al. 1976, Dvoák 1993). The high chromosome homology makes transfer of genes from diploid wheats to cultivated tetraploid and hexaploid wheats feasible (Kerber and Dyck 1973, The 1973, McIntosh et al. 1984, Valkoun et al. 1986) and *T. urartu* may, therefore, be a valuable source of genes in wheat breeding programs. However, a better knowledge of geographical distribution, natural habitat and diversity among and within populations is needed to explore the full potential of this wild wheat.

It was originally believed that the species is endemic to Armenia, later it was also found in Iran, Iraq, Lebanon and Turkey (Johnson 1975, Dorofeev 1979, p.37). Its presence in Syria was first reported by Rifaie et al. (1981). In spite of these discoveries, the number of *T. urartu* germplasm samples from the southwestern part of the Fertile Crescent was very limited. Consequently, the International Center for Agricultural Research in the Dry Areas (ICARDA) in collaboration with national agricultural research systems (NARS) of Jordan, Lebanon and Syria conducted a number of exploration and collection trips, which have substantially increased the number of gene bank accessions and brought new data on the geographical distribution, ecology and present status of *T. urartu* populations in their natural habitat.

This information, complemented by genetic diversity analyses based on agro-morphological descriptors and

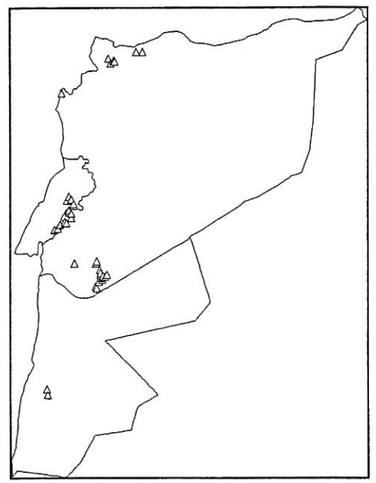

Figure 1. Geographical distribution of *T. urartu* in Lebanon, Jordan and Syria.

gliadin electrophoresis data, will be used to identify sites for *in situ* conservation in the region of origin.

MATERIALS AND METHODS

Exploration and collection trips, which focused not only on *T. urartu* but also on other wild *Triticum* species, were conducted in Jordan (1991), Lebanon (1993), and Syria (1991, 1992 and 1993) in cooperation with the respective NARS to regions for which the presence of wild wheats had previously been reported, or where climatic and pedological data suggested favorable conditions for wild wheats. On site, populations were mostly sampled as bulks but single plant samples were collected from some large stands.

The single-plant progenies were grown in the field at the main ICARDA experimental research station in Tel Hadya, 30 km south of Aleppo, northern Syria, during the 1992/1993 growing season, and evaluated for a number of discriptors. Multivariate statistical analysis was employed to assess among-population diversity. SPSS/PC software was employed for data processing. Within-population genetic diversity was assessed from gliadin polymorphism, which was detected by means of Al-lactate polyacrylamide gel electrophoresis (Al-PAGE).

RESULTS AND DISCUSSION

Geographical distribution and habitat

The exploration trips showed that *T. urartu* is a typical element of the flora of the southwestern part of the Fertile Crescent, where the species is more common than other diploid wild wheat, *Triticum boeoticum* Boiss. emend. E. Schiem. The geographical distribution in this region of *urartu* wheat is presented in Fig. 1.

In general, its distribution overlaps with that of *Triticum dicoccoides* (Körn. ex Asch. & Graebn.) Schweinf. However, it is rare in Jordan and in the lowlands of the Hauran plain in southern Syria. On the other hand, *urartu* wheat is better adapted to stressful environments than *T. dicoccoides* and can be found in drier parts of the Beka'a valley in Lebanon and low-rainfall sites in northern Syria, close to the border with Turkey. It is also able to resist the harsh environment of higher mountains and occurs at altitudes above 1800 m asl in the Lebanon and Anti-Lebanon mountains. In these high sites it was accompanied by wild barley, *Hordeum spontaneum* L., but no other *Triticum* or *Aegilops* species were present. The better adaptation to a long period of low winter temperatures in the high mountains may be related to a difference in vernalization response between *urartu* and *dicoccoides* wheats (unpublished results).

Of the total of 46 *T. urartu* populations presently identified in Jordan, Lebanon and Syria, 15 populations were allopatric, 30 populations were sympatric to *T. dicoccoides* and only seven to *T. boeoticum* populations (Table 1). The latter species is absent in Jordan and rare in Lebanon and Syria, where it is restricted to higher rainfall areas. This corroborates a wheat evolution theory in which the diploid *urartu* wheat was a progenitor of the tetraploid wild emmer, *T. dicoccoides*.

Table 2 shows a preference of the species for volcanic soils derived from a basaltic parent rock. The highest frequency of sites are in the altitude range of 1000-1500 m asl, and a quarter of the populations are above 1500 m asl. This documents the good adaptation of *urartu* wheat to a mountain environment. Lowland sites are almost entirely restricted to northern Syria, except for one site in southern Syria, found in the middle of the Hauran plain.

Table 1: Sympatric occurence of *T. urartu* with other wild wheats in the Near East.

Country	Allopatric	Sympatric			
		TB*	TD	TB & TD	Total
Jordan	2	-	-	-	2
Lebanon	3	1	3	1	8
Syria	10	1	21	4	36
	15	1	24	5	46

* TB = *T. boeoticum*, TD = *T. dicoccoides*

Table 2: *Triticum urartu* population frequency distribution by habitat characteristics

Country	Parent rock			Altitude (m asl)			Rainfall (mm)		
	BA*	LI	AL	<1000	1000-1500	>1500	350	350-450	>450
Jordan	1	1	-	-	-	2	2	-	-
Lebanon	-	5	3	-	7	1	-	4	4
Syria	26	10	-	8	20	8	20	11	5

* BA = basalt, LI = limestone; AL = alluvium

Table 3: *Triticum urartu* population frequency distribution by area occupied.

Country	Area occupied (m^2)			
	< 100	100-1000	>1000	Total
Jordan	-	-	2	2
Lebanon	-	3	5	8
Syria	17	9	6	32

Almost half of the collection sites belong to the low-rainfall category with annual rainfall below 350 mm. This, as well as 30 per cent of sites in the category of 350-450 mm, indicate a good adjustment to drier habitats.

Characterization of *T. urartu* populations

Population size

Population size as expressed by the area occupied varies from a few square meters to several hectares (Table 3) and depends essentially on the size of each area of suitable habitat available, since *T. urartu* can easily compete in favorable habitats with other species, mostly annual grasses and legumes. The largest populations were found in the Jebel Druz (Jebel Al-Arab) in southern Syria where stony grazing areas and a long-term fallow are scattered among small ruminants during the cereal-growing season. In additions, these populations are sometimes interconnected by stony field borders inhabited by *T. urartu* and *T. dicoccoides* and this may result in a network of one- and two-dimensional populations of several square kilometers in size.

Weediness

Populations of *urartu* wheat in Syria and the Lebanese mountains are not very weedy and, usually, do not grow inside cereal fields. However, in the central Beka'a valley in Lebanon a truly weedy population was found growing in a barley field. The *urartu* population was able to compete successfully with cultivated barley in that dry site.

Diversity among populations

The geographical pattern of population diversity was studied in seven Syrian populations and two subpopulations from Jordan by means of discriminant analysis using the following six descriptors: time to heading, time to maturity, spike length, awn length, number of spikelets per spike and plant height. A plot of the first two canonical discriminant functions and the subsequent hierarchical cluster analysis based on group centroids of the three significant canonical functions separated three major groups: (i) populations from southern Syria, which were early and short-awned; (11) Jordanian germplasm - early and long-awned; and (iii) populations from northern Syria - late and short-awned.

Within-population diversity

Genetic diversity within populations was estimated from gliadin polymorphism data. Gliadin storage proteins are extremely variable and may represent a multiple gene family (Payne 1987). In other diploid wheats, the wild *T. boeoticum* and cultivated *T. monococcum* L., gliadin polymorphism is controlled by two independently inherited loci, *Gli-A1* and *Gli-A2*, located on chromosomes 1A and 6A, respectively (Metakovsky and Baboev 1992a, 1992b). The first 'upper' locus usually controls the synthesis of ω- and γ-gliadin, whereas the other 'lower' locus codes for α- and β-gliadin. Our electrophoregrams of gliadins in *T. urartu* indicated occasional recombination between all α-gliadin bands recombined as a block with gliadin of the lower electrophoretic mobility, mostly ω- and γ-gliadin. Consequently, variants in the α-gliadin 'lower' block were considered to represent diversity in the *Gli-A2* locus and those in the 'upper' block were supposed to correspond to the *Gli-A1* locus. Mean Nei heterozygosity index values (H_e) were calculated for 28 populations as an average of the two loci data. The results are summarized in Table 4.

The most diverse populations (H_e 0.70) of *T. urartu* were found in the Jebel Druz (Jebel Al-Arab) in southern Syria, and in the northern part of the Jebel Sema'an in Aleppo province, northern Syria. The Nei heterozygosity index values are very high and similar to those reported for hordein in a population of wild barley, *H. spontaneum* K.

Table 4: *Triticum urartu* population frequency distribution by genetic diversity categories.

Country	Nei heterozygosity index (H_e)			
	<0.35	0.35-0.70	>0.70	Total
Jordan	1	1	-	2
Lebanon	5	3	-	8
Syria	8	3	7	18
Total	14	7	7	28

Koch (Nevo et al. 1983). In general, the values are much higher than genetic diversity data for wild diploid wheat populations assessed by allozyme polymorphism (Smith-Huerta et al. 1989).

In situ conservation

Since adequate sampling and maintenance in *ex situ* collections of the original genetic diversity of the highly diverse populations of *T. urartu* is an almost impossible task, we suggest *in situ* conservation as a complementary conservation strategy for the most diverse populations in distinct ecoregions. At present, projects for *in situ* conservation of wild *Triticum* spp. are being prepared in collaboration with national programs of Jordan, Lebanon and Syria, as well as with the International Plant Genetic Resources Institute (IPGRI).

CONCLUSIONS

The present study indicates that *T. urartu*: (1) is relatively frequent in the southwestern part of the Near East Fertile Crescent; (2) occupies a wide range of habitats and it is better adapted to stressful environments than *T. dicoccoides*, with which it is mostly sympatric; (3) shows preference for soils of volcanic (basalt) origin; (4) is differentiated into distinct ecotypes; (5) displays high genetic diversity in populations in northern and southern Syria; (6) may provide useful genes to wheat breeding programs for stress tolerance, and (7) should be conserved in the original habitat in addition to *ex situ* conservation in gene banks to maintain its high genetic diversity under the dynamic natural environment.

Acknowledgments - We are grateful for the contribution of national programs in germplasm collecting and information gathering, namely: The Jordan University of Science and Technology (JUST), Irbid, and the National Council for Agricultural Research and Technology Transfer (NCARTT), Amman, Jordan; The Agricultural Research Institute (ARI), Tel Amara, Lebanon; and The Agricultural Research Center (ARC), Douma, Syria. Thanks also to Prof. J. Giles Waines, University of California, Riverside, U.S.A., for assistance in manuscript preparation and editing.

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Genetic Effects of Alien Cytoplasms on Heat Tolerance in Wheat

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ABSTRACT

Heat tolerance of five spring wheat cultivars (lines), i.e., 352-35, NPFP, 881, Chinese Spring and Siete Cerros 66, and their fifty alloplasmic lines were tested using electrolyte leakage method to investigate genetic effects of alien cytoplasms of *Triticum* and *Aegilops* on heat tolerance in common wheat. Results indicated that: (1) significant variations in heat tolerance exist between nuclear donor genotypes and their alloplasmic lines, and between alloplasmic lines of same nuclear genotype for all of the five cultivars. Alloplasmic lines can be more heat tolerant or susceptible to high temperature stress than nuclear donor cultivars, suggesting that alien cytoplasms affect heat tolerance significantly, and there are genetic variations of cytoplasms among species of *Triticum* and *Aegilops* in heat tolerance; (2) Effects of one species cytoplasm on heat tolerance is quite different in different nuclear genotype backgrounds, which suggested that there exists interaction between alien cytoplasms and nuclear genotype; (3) Cytoplasms of *Ae. longissima* and *Ae. crassa* enhance heat tolerance in most of the nuclear genotype backgrounds. It is concluded that genetic variability exists among the alien cytoplasms in heat tolerance, and can be useful in genetic improvement of the trait in wheat.

INTRODUCTION

Temperatures of 18 to 22°C are considered optimal for wheat growth and development. High temperature over 30°C is quite frequent in most of the wheat production areas, and produce adverse effects on yield and quality (Wardlaw *et al.* 1989; Shpiler and Blum, 1986; Blumenthal *et al.* 1990; Randall *et al.*, 1990). High temperature stress is one of the limiting factors for wheat production.

Electrolyte leakage or membrane thermostability (MT) has been found to be a good indicator of heat tolerance in crop plants (Blum, 1988). It has been reported that MT is correlated with field performance of wheat under heat stress (Shanahan *et al.*, 1990; Saadalla *et al.*, 1990). Shanahan *et al.* (1990) demonstrated that the MT in spring wheat is correlated with grain yield and test weight under heat stress conditions, and concluded that the MT test can be an useful screening procedure for selecting spring wheat genotypes that tolerate high temperature stress. Saadalla *et al.* (1990) also found correlation of MT with grain yield and quality in winter wheat under heat stress conditions.

Information on genetic control of heat tolerance are important for the genetic improvement of the trait. However, there have been few studies regarding these respects in wheat (Porter *et al.*, 1989; Moffatt, 1990). Recently, Sun and Quick (1991) reported that homologues 3 and 4 are associated with heat tolerance in tetraploid wheat. In diallel crosses, involving 10 wheat cultivars of different heat tolerance measured by chlorophyll fluorescence, Moffatt *et al.* (1990) found that significant maternal as well as reciprocal effects exist while general combining ability effects are also significant, suggesting cytoplasmic differences, and cytoplasmic and nuclear interaction in heat tolerance. Similar results were reported by Porter *et al.* (1989).

Objectives of this study were to characterize genetic effects of alien cytoplasms of *Triticum* and *Aegilops* species on heat tolerance in common wheat by using alloplasmic lines of same nuclear genotypes.

MATERIALS AND METHODS

Five spring wheat cultivars (lines), i.e., 352-35, NPFP, 881, Chinese Spring (CS) and Siete Cerros 66 and their fifty alloplasmic lines, which include cytoplasms of 8 *Triticum* species, 11 *Aegilops* species and one *Haynaldia* species, were tested for heat tolerance. Seeds of these cultivars and alloplasmic lines were kindly provided by Professor Xu Nai Yu, Department of Biology, Wuhan University, and Professor Wu Yu Wen, Institute of Genetics, Chinese Academy of Sciences. TAM107 and Chinese Spring, extremely heat tolerant and extremely susceptible, respectively, were included in each test as standard cultivars since they were used as routine control in our previous tests.

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higher than genetic diversity data for
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	nucleus donor genotypes							
	NPFP		Siete Cerros 66		352-25		CS	
	df	MS	df	MS	df	MS	df	MS
	2	52.48	2	111.26	2	2.04	2	11.12
**	10	536.75**	12	259.77**	7	1000.14**	10	107.23**
	20	22.85	24	13.84	14	21.85	20	14.82

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RESULTS AND S

Significant differences exist among the five nucleus donor genotypes in heat tolerance measured by MT. Analysis of variance (Table 1) showed that there were highly significant difference in heat tolerance between each of these five genotypes and its alloplasmic lines, and among alloplasmic lines of same nuclear genotypes as well. RI values of all of the alloplasmic lines for each of the five nucleus genotypes were presented in Figures 1, 2, 3, 4, and 5.

Accession 881 is as extremely heat susceptible as Chinese Spring, with RI value of 82.07%. When its cytoplasm was substituted by those of *Ae. crassa, Ae. juvenalis, Ae. cylindrica, and Ae. longissima*, heat tolerances were significantly improved, with the values of RI reduced by 21.55%, 32.67%, 39.70% and 42.13%, respectively. It is worth to note that heat tolerance of alloplasmic line (*Ae. longissima*) 881 reaches the level of extremely heat tolerant cultivar TAM107, even though 881 itself is quite heat susceptible. Other alloplasmic lines show no significant differences in heat tolerance as compared with 881 itself (Fig.1).

NPFP is also heat susceptible, but more tolerant than CS. When its cytoplasm was substituted by that of *T.dicoccum*, heat tolerance was reduced, with RI value increased by 8.57%. However, when its cytoplasm was substituted by those of *Ae. speltoides, Ae. squarrosa* and *Ae. longissima*, the heat tolerance were significantly improved, with RI values reduced by 8.37%, 28.41% and 28.68%, respectively. The latter two alloplasmic lines became as tolerant as TAM 107. Other alloplasmic lines showed no differences in heat tolerance with NPFP itself (Fig. 2).

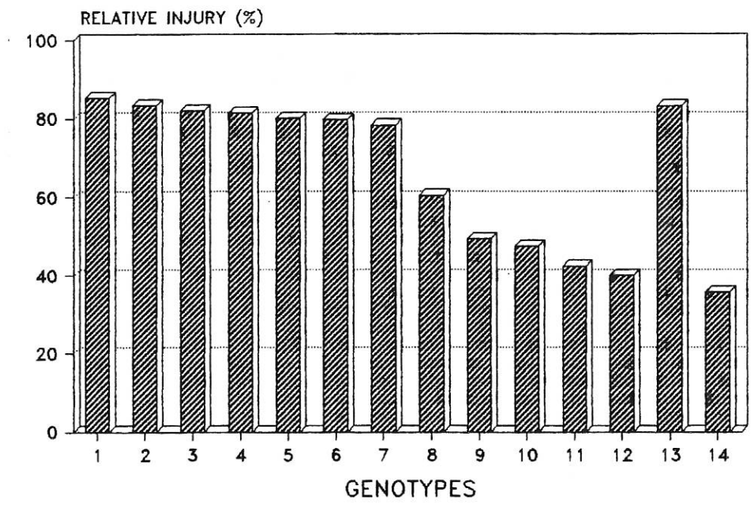

Figure 1. Variation in heat tolerance of nuclear donor genotype 881 and its alloplasmic lines (species in bracket representing cytoplasm donor), as compared with heat tolerant susceptible standard genotypes. 1. (*Ae ventricosa*) 881; 2. (*Ae. squarrosa*) 881; 3. 881; 4. (*T. dicoccoides*) 881; 5. (*T. dicoccum*) 881; 6. (*Ae variabilis*) 881; 7. (*Ae. speltoides*) 881; 8. (*Ae crassa* 4x) 881; 9. (Ae. juvenalis) 881; 10. (CS) 881; 11. (*Ae. cylindrica*) 881; 12. (*Ae. longissima*) 881; 13. CS; 14. TAM107.

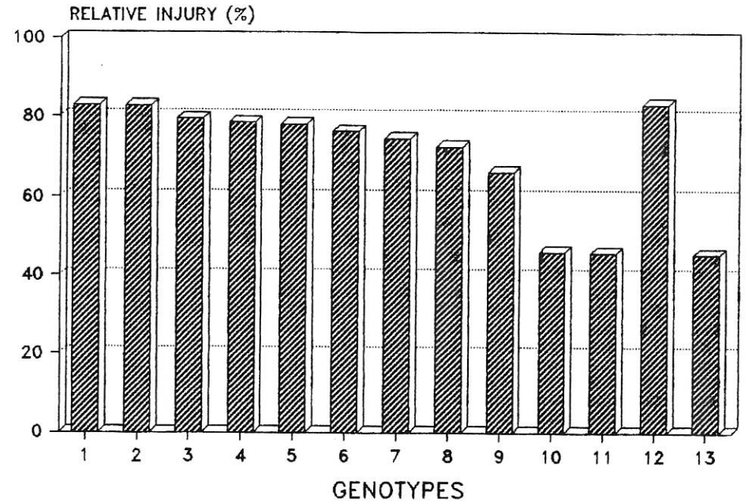

Figure 2. Variation in heat tolerance of nuclear donor genotype NPFP and its alloplasmic lines (species in bracket representing cytoplasm donor), as compared with heat tolerant and susceptible standard genotypes. 1. (*T. dicoccum*) NPFP; 2. (CS) NPFP; 3. (*Ae juvenalis*) NPFP; 4. (*Ae. ventricosa*) NPFP; 5. (*Ae. variabilis*) NPFP; 6. (*Ae. cylindrica*) NPFP; 7. NPFP; 8. (*Ae. crassa* 4x) NPFP; 9. (*Ae. speltoides*) NPFP; 10. (*Ae. squarrosa*) NPFP; 11. (*Ae. longissima*) NPFP; 12. CS; 13. TAM107.

Siete Cerros 66 is a relatively heat tolerant cultivar, but not so tolerant as TAM107 (Fig. 3), its RI value was 10% higher than TAM107. It was found that all of the alloplasmic lines, except the one with cytoplasm of *Ae. longissima* which showed no significant change in RI value were decreased in heat tolerance, with RI values increased by 6.84% to 28.27%. Seven of them became as susceptible as CS (Fig. 3).

Accession 352-35 is also relatively heat tolerant; its RI value was more than 10% higher than TAM107 (Fig. 4). Two alloplasmic lines with cytoplasms of *Ae. juvenalis* and *Ae. speltoides* showed no significant change in heat tolerance, while others reduced significantly in heat tolerance with RI values increased by 12.36% to 35.77%. Six of them became as susceptible as CS (Fig. 4).

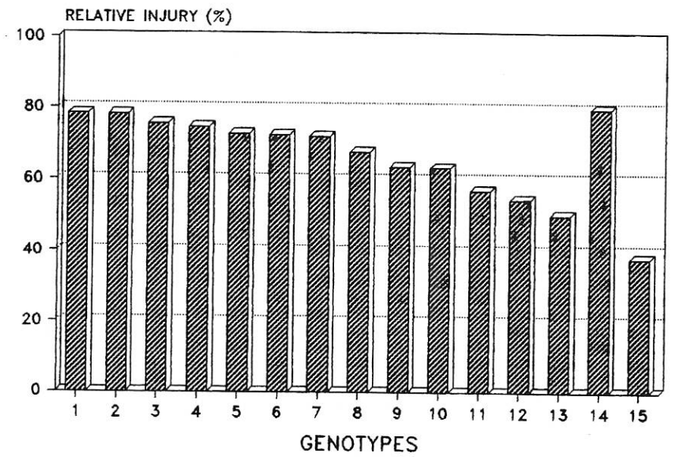

Figure 3. Variation in heat tolerance of nuclear donor genotype Siete Cerros 66 (SC) and its alloplasmic lines (species in bracket representing cytoplasm donor), as compared with heat tolerant and susceptible standard genotypes. 1. (*T. persicum* var. *fuliginosum*) SC; 2. (*T. turanicum* var. *notabile*) SC; 3. (*T. spelta* var. *vavilovii*) SC; 4. (*T. dicoccum* var. *pseudomacrotherum*) SC; 5. (*Ae. cylindrica*) SC; 6. (*Haynaldia cylindrica* (Albena)) SC; 10. (*Ae. kotschyi*) SC; 11. (*Ae. ventricosa*) SC; 12. (*Ae. longissima*) SC; 13. SC; 14. CS; 15. TAM107.

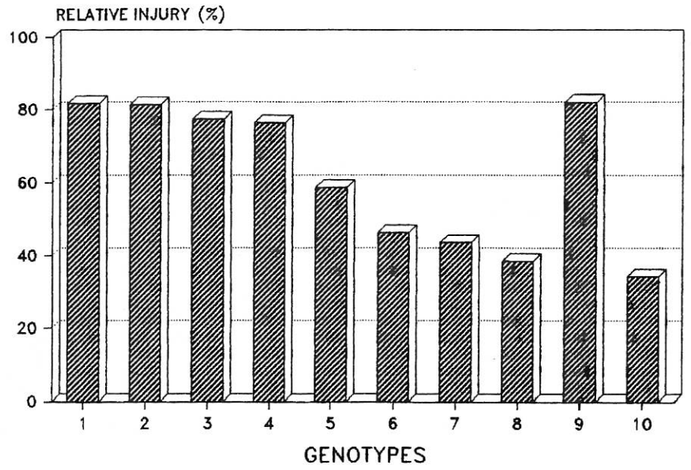

Figure 4. Variation in heat tolerance of nuclear donor genotype 352-35 and its alloplasmic lines (species in bracket representing cytoplasm donor), as compared with heat tolerant and susceptible standard genotypes. 1. (*T. dicoccoides)* 352-35; 2. *(CS)* 352-35; *3. (Ae. ventricosa) 352-35; 4. (T. dicoccum*) 352-35; 5. (*Ae. squarrosa*) 352-35; 6. 352-35; 7. (*Ae. juvenalis*) 352-35; 8. (*Ae. speltoides*) 352-35; 9. CS; 10. TAM107.

It was found that there were six alloplasmic lines of CS showing improvement in heat tolerance, as compared with CS itself, with RI values reduced by 7.93% to 16.39%. Only when its cytoplasm was substituted by those of *Ae. kotschyi*, *Ae. ventricosa* and *Ae. triuncialis*, there was no significant changes in heat tolerance (Fig. 5).

DISCUSSION

Both Porter *et al.* (1989) and Moffatt *et al.* (1990) reported in their diallel crosses of wheat cultivars that there exist significant maternal as well as reciprocal effects, suggesting that cytoplasms as well as cytoplasmic and nuclear interactions were associated with genetic control of heat tolerance. In this study, we found that heat tolerance was quite different between wheat cultivars and their alloplasmic lines as well as among alloplasmic lines of same nuclear genotypes, suggesting that genetic variations in heat tolerance existed among cytoplasms of *Triticum* and

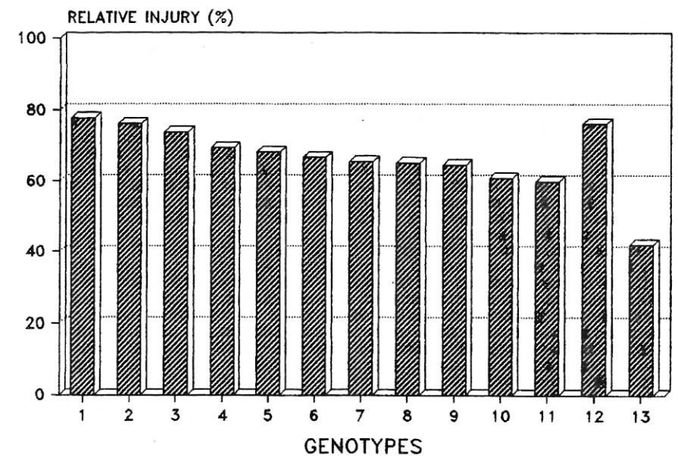

Figure 5. Variation in heat tolerance of nuclear donor genotype Chinese Spring (CS) and its alloplasmic lines (species in bracket representing cytoplasm donor), as compared with heat tolerant and susceptible standard genotypes. 1. (*Ae. kotschyi)* CS; 2. CS; *3. (Ae. ventricosa)* CS; *4. (Ae. triancialis*) CS; 5. (*Ae. sharonensis)* CS; *6. (Ae. crassa* 4x*)* CS; *7. (T. dicoccum*) CS; 8. (*Ae. longissima*) CS; 9. (*Ae. speltoides*) CS; 10. (*Ae. cylindrica*) CS; 11. (*Ae. squarrosa*) CS; 12. CS; 13.

Aegilops species, and alien cytoplasms affected heat tolerance in wheat. On the other hand, one alien cytoplasm may have contrasting effects on heat tolerance in different nuclear genotypes, which indicates that there are cytoplasmic and nuclear interaction. For example, cytoplasm of *Ae. squarrosa* increased heat tolerance of NPFP and CS, but reduced heat tolerance of 352-35 and Siete Cerros 66, and exerted no significant effect in 881 nuclear genotype background. The cytoplasm of *Ae. ventricosa* reduced heat tolerance of 352-35 and Siete Cerros 66 but had no effect on NPFP, 881 and CS. It was worthy to note that cytoplasm of *Ae. longissima* tended to increase heat tolerance in three (NPFP, 882, CS) out of the four cultivars while it had no effect only in one case (Siete Cerros 66). Cytoplasm of *Ae. crassa* increased heat tolerance in two (881 and CS) of the three cultivars, but had no effect in one cultivar (NPFP). Therefore, we propose that alien cytoplasms may be useful to enhance genetic variability in heat tolerance of wheat.

TTC reduction was used by Porter *et al.* (1989), and chlorophyll fluorescence used by Moffatt *et al.* (1990) for heat tolerance test. TTC reduction measures cell viability after high temperature stress, and chlorophyll fluorescence measures thermostability of thylakoid membrane and electron transport system. Heat tolerance tested by both above methods show cytoplasmic effect. We also found in a diallel that heat tolerance tested by electrolyte leakage show significant reciprocal effect as well (unpublished). These suggested that cell viability after heat stress, thermostability of thylakoid membrane as well as membrane thermostability, as an inheritable trait, were all related with cytoplasmic factors. Since mitochondria and chloroplast are main carrier of cytoplasmic genomes, we may assume that genetic differences of heat tolerance in cytoplasms of *Triticum* and *Aegilops* species are related to genomes of these organelles.

In fact, Kihara (1951) noticed that cytoplasmic genome, like nuclear genome, showed genetic variability. Based on endonuclease restriction fragment patterns of chloroplast DNA and effects of alien cytoplasms on morphology (including fertility) of wheat, Tsunewaki *et al.* (1988) grouped cytoplasms of 36 species of *Triticum* and *Aegilops* into 16 types. Genetic effects of alien cytoplasms on growth and development, grain quality, disease and pest resistances, physiological characters and ability to induce callus of wheat have been extensively studied (Zhang Yan *et al.* 1990). Some combination of cytoplasm and nuclear genotype showed heterosis. We report here for the first time that alien cytoplasms affect heat tolerance of wheat as well, and that some alloplasmic lines show nucleus-cytoplasmic heterosis in heat tolerance. Alien cytoplasms may find their uses in enhancing genetic variability in heat tolerance of wheat.

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Evidence for Resistance to Root Lesion Nematode (*Pratylenchus neglectus*) in Wheat

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ABSTRACT

Pratylenchus neglectus is widespread throughout the South Australian cereal belt, invading wheat as well as other crops grown in rotation with wheat. The multiplication rate of *P. neglectus* was assessed in the seven wheat-rye (Chinese Spring-Imperial) addition line, and a number of wheat varieties which had shown low nematode numbers in roots in a preliminary test or were tolerant to *P. thornei*. Local wheat varieties were included as susceptible checks. Individual plants were sown in pots, inoculated with larvae after five days and transferred to a controlled temperature waterbath. After seven or eight weeks, the nematodes were extracted from roots by misting for five days and counted. Significant differences (p) were found between varieties in the number of nematodes per plant. All addition lines contained a low number of nematodes both per plant and per gram of root. Abacus (triticale), Persia 20 and Virest (wheats) and the 7R addition line showed a significantly (p) lower number of nematodes per plant compared to the check varieties. The lines with low number of nematodes per plant may be useful sources of resistance to incorporate into a breeding program.

INTRODUCTION

There are many reports worldwide indicating that root lesion nematodes (*Pratylenchus* spp.) cause significant losses in crop production. The nematodes attack many leguminous crops and cereals, including wheat and barley. The symptoms caused by the nematode include root lesions (Vanstone, 1991) and reduced root and shoot growth (Dropkin, 1989; Farsi *et al*., 1993a). The presence of three species of *Pratylenchus (P. neglectus, P. thornei* and *P. zeae*) has been reported in roots of Australian wheat crops (Colbran and McCulloch, 1965; Vanstone, 1991) and up to a 20% yield reduction due to *P. neglectus* as been recorded (Taheri *et al*., 1994). To reduce the nematode population in infested fields, and hence yield loss, a useful approach is to introduce resistant wheat varieties.

In South Australia, cereal cyst nematode (*Heterodera avenae*) has been a serious problem for decades (Davidson and Se, 1930). A resistance gene was detected in a wheat variety introduced from Afghanistan (O'Brien and Fisher, 1974) and transferred to local cultivated wheat varieties making resistant varieties available to farmers (Brown and Young, 1982). As no Australian wheat variety resistant to *P. neglectus* has been found, a search for sources of resistance to *P. neglectus* in related species and imported material has been undertaken. In previous experiments, the triticale variety Abacus and some wheat varieties, Persia 20 (from Iran) and Virest (from Italy), showed a lower number of nematodes per plant. The experiment reported here was conducted to confirm the resistance of these sources, to test the resistance of other exotic material, and to investigate the possibility of locating the responsible gene or genes on rye chromosomes by using wheat-rye (Imperial) addition lines.

MATERIALS AND METHODS

The genetic material examined included two susceptible local commercial wheat variety checks (Spear and Molineux), lines reported to be tolerant to *P. thornei* by Dr. J.P. Thompson of the Queensland Wheat Research Institute, some imported wheat varieties (Virest, Persia 20, Surak-I-Bahari and Iraq 48), canola as a less susceptible species (Vanstone *et al*., 1993a), a 1B/1R substitution line and seven Chinese Spring wheat-Imperial rye addition lines (Table 1).

Since the transmission of addition chromosomes to the next generation is about 70% depending on the type of addition line (P.A.E. Ellis, pers. comm.), root tips were first checked for the presence of the rye chromosome by staining root tip cells by the Fulgen method and examining chromosomes under a light microscope.

Table 1: Material investigated for resistance to *P. neglectus*.

Name of Line/Variety	Pedigree/Source	Reason for inclusion
Spear	Sabre/MEC3//Insignia	Susceptible local check variety
Molineux	((Pitic 62*Festiguary) *Warigal#)	Susceptible local check variety
Virest	AUS 11894	Resistant to *P. neglectus* in previous experiment
Persia 20	AUS 5205	Resistant to *P. neglectus* in previous experiment
SUN 290 B	4*Potam//Cook/Sr24	Tolerant to *P. thornei*[a]
Iran 28357	AUS 10938	Tolerant to *P. thornei*[a]
Surak-I-Bahari	AUS 7869	Tolerant to *P. thornei*[a]
USDA CI 9040	AUS 7639	Tolerant to *P. thornei*[a]
Iraq 48	AUS 4930	Tolerant to *P. thornei*[a]
Abacus (triticale)	CIMMYT, Mexico	confirming previous results (resistant to *P. neglectus*)
1B/1R(Cs[b]-Imperial substitution line)	496/86 pl 1[c]	Locating resistance genes
1R (Cs-Imperial rye)[d]	365/91 pl 1[c]	Locating resistance genes
2R (Cs-Imperial rye)[d]	372/91 pl 3[c]	Locating resistance genes
3R (Cs-Imperial rye)[d]	366/91 pl 1[c]	Locating resistance genes
4R (Cs-Imperial rye)[d]	363/91 pl 1[c]	Locating resistance genes
5R (Cs-Imperial rye)[d]	26/92 pl 1[c]	Locating resistance genes
6R (Cs-Imperial rye)[d]	366/91 pl 3[c]	Locating resistance genes
7R (Cs-Imperial rye)[d]	374/91 pl 2[c]	Locating resistance genes
Canola (*Brassica napus*, variety Barossa)		Less susceptible to *P. neglectus* and *P. thornei*

a - Detected by Dr J.P. Thompson, Queensland Wheat Research Institute (QWRI).
b - Chinese Spring, hexaploid wheat variety.
c - Maintenance numbers for lines kindly supplied by Dr. K.W. Shepherd, Waite Agricultural Research Institute.
D - Addition line.

A red, sandy loam soil collected from a farming property at Palmer (65 km east of Adelaide) was steam pasteurized for 30 minutes at 70° C. Sterilized plastic pots without any drainage holes were filled with 650g of soil. Seeds were surface sterilized, pre-germinated and sown one to a pot in a completely randomized design of 19 entries with eight replications.

After five days, each pot was inoculated with about 350 larvae (mixed stages) and 200 eggs of aseptic *P. neglectus* obtained from carrot cultures (V.A. Vanstone, pers. comm.). Pots were left in the waterbath at 22±1° C in an evaporatively cooled glasshouse, and watered with distilled water whenever necessary.

Four replicates were harvested after seven and the remaining four after eight weeks, when soil was washed from the roots under running tap water. Roots were chopped into 1 cm lengths and misted for five days to extract the nematodes (Southey, 1986). Nematodes were counted and the roots were dried and weighed.

Prior to analyses of variance, the data were transformed to Ln (x+200) (Proctor and Marks, 1974) to render variances independent of the means.

RESULTS AND DISCUSSION

The differences between entries both in number of nematodes per plant and in number of nematodes per gram of dry root were statistically significant (p). The entries could be classified into two groups for the number

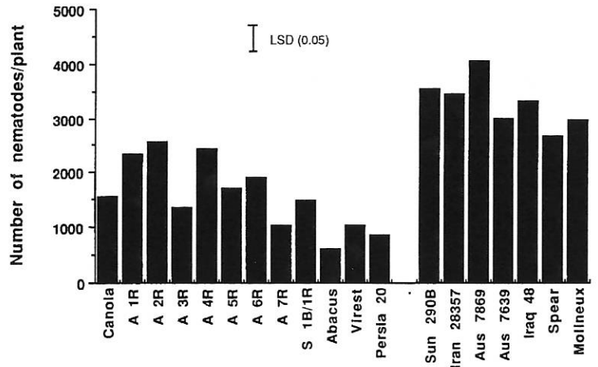

Figure 1. Number of nematodes per plant in 19 entries grown for 53 or 60 days in a waterbath at 22±1°C and inoculated with 550 *P. neglectus* (350 larvae + 200 eggs). Each value is the mean of eight replicates.

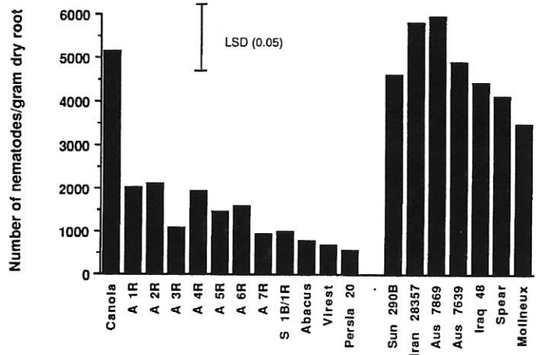

Figure 2. Number of nematodes per gram dry root in 19 entries grown for 53 or 60 days in a waterbath at 22±1°C and inoculated with 550 *P. neglectus* (350 larvae + 200 eggs). Each value is the mean of eight replicates.

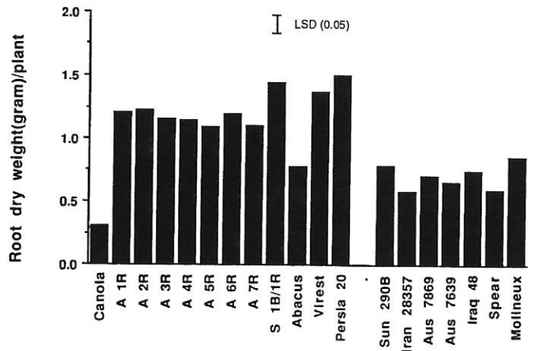

Figure 3. Mean dry root weight of 19 entries grown for 53 or 60 days in a waterbath at 22±1°C and inoculated with 550 *P. neglectus* (350 larvae + 200 eggs). Each value is the mean of eight replicates.

of nematodes per plant or per gram of root (Figs. 1 and 2). The first group, including all addition lines, the substitution line (1B/1R), Abacus triticale, Persia 20 and Virest wheats, was significantly different (p) from the second group including wheat checks and other wheat varieties.

The differences between entries for root dry matter were also statistically significant (p) (Fig. 3). Canola, which was included as a less susceptible species, had the lowest root dry matter (Fig. 3) and thus was associated with a high number of nematodes per gram, whereas, with 1481 nematodes per plant, canola was as low as the addition lines and significantly different from the wheat check Molineux (Fig. 1). Persia 20 had the highest amount of root dry matter, while its number of nematodes per gram of dry root was the lowest of all the genotypes.

Among wheat varieties other than Persia 20 and Virest, only Spear was significantly different from AUS 7869 in terms of the lowest and Surak (AUS 7869), with 4050 nematodes per plant (about five times that of Abacus), the highest number of nematodes per plant (Fig 1). Persia 20 and Virest occupied the second and fourth positions, respectively, following Abacus, but the difference between them was not significant. Among addition lines, 7R showed the lowest number of nematodes per plant and it was significantly different (p) from the 1R, 2R, and 4R addition lines.

Although there are various methods of controlling root lesion nematodes, including rotation with non-host crops (Bolton and Waele, 1989; Vanstone, 1993a), cultivation (Thompson *et al.*, 1981), solarization (Vito *et al.*, 1991), fertilizer application (Vanstone *et al.*, 1993b) and nematicide application (Farsi *et al.*, 1993b), the most economical method of nematode control is the use of resistant varieties. These varieties, if available, would allow farmers to produce high-yielding crops without a substantial increase in nematode numbers which could damage subsequent crops.

For screening to detect resistant varieties, the number of nematodes per gram of dry root has been suggested by most investigators (e.g. Denis *et al.*, 1989); Marull *et al.*, 1990). The number of nematodes per gram of dry root is a function of root growth, while resistance describes the reduction in nematode multiplication imposed by the plant. Faster growing roots will always appear more resistant when judged on the number of nematodes per gram of dry root (e.g. Persia 20 in Fig. 2), even though this character may have no influence on the multiplication of nematodes and hence on resistance. In terms of the number of nematodes per plant, canola was as resistant as the addition lines but, in the number of nematodes per gram because of low root dry weight, it would be regarded as being susceptible. As the economic damage by nematodes is predominantly related to the size of the population invading the seedlings of a subsequent crop, the number of nematodes per plant is a more appropriate estimate of resistance for an agricultural crop than the number per gram of root (J.M. Fisher, pers. comm.).

A low number of nematodes per plant was also counted for Abacus, Persia 20 and Virest in previous experiments and this suggests the existence of a resistance gene or genes in Persia 20 and Virest from Iran and Italy, respectively. To confirm this result, further experiments are being conducted to study feeding, moulting and reproduction of the nematode inside the plant roots.

Although the 7R addition line had the lowest number of nematodes per plant among the addition lines, its difference with 3R was not significant, and since all addition lines were significantly different from the second group of wheats (Fig. 1), it is not possible to locate the resistance gene or genes on a specific chromosome of rye.

Abacus, consisting of rye and tetraploid wheat (durum) genomes, could be used either as a rotational crop or as a donor parent to incorporate the *P. neglectus* resistance gene (or genes) into commercial local wheat varieties. In South Australia, a gene found in triticale T 701-4-6, as been shown to have a higher level of resistance to cereal cyst nematode than that of the one found in the wheat AUS 10894, originating from Afghanistan (Asiedu, *et al.*, (1990). There have been attempts to transfer the gene from triticale T 701-4-6 into commercial wheat varieties in South Australia, but the linkage of undesirable genes with the resistance gene has made this difficult to achieve (Dundas *et al.*, 1987). Translocation of resistance genes from triticale to wheat may be possible, but requires further investigation. Fortunately, there appears to be sufficient resistance to *P. neglectus* in wheats such as Persia 20 and Virest, so such translocations will not be necessary in this project.

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Resources and Utilization of Triticeae in Xinjiang, China

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ABSTRACT

Because of its special geographic and topographic features, Xinjiang has a rich diversity of germplasm resources of Triticeae species. This paper introduces the historical collections and research work on Triticeae species in Xinjiang and describes the distribution of 80 species and 14 subspecies of the tribe Triticeae which are represented in 10 genera and 5 subtribes. Also the utilization of the main Triticeae species that are valuable for crop improvement and forage production are summarized.

INTRODUCTION

Xinjiang is located in the northwest part of China and is one of the most remote areas of Eurasia. It has a total land area of 1.6 million km^2 and is surrounded by high mountain ranges and plateaus. The climate is extremely dry and belongs to the temperate desert zone. Because of the high mountains in Xinjiang, the vertical vegetation zones include desert, grassland, forest, subalpine, alpine meadows and glacier, which occur in sequence from the lowlands to the high mountains. Because their climates are cold and moist, the high mountains develop many big rivers which flow to expansive desert basins. These rivers allow the formation of forests along the rivers and oasis, meadows and swamps in desert areas. A diversity of ecosystems and rich plant germplasm are well developed in Xinjiang (J.P. Zhong, 1984). Xinjiang is the richest area in China for Triticeae germplasm not only because it has many species but also those species have many superior agronomic characteristics such as high yield, early maturity and many outstanding resistances. Xinjiang is a natural gene pool for wheat improvement and grass breeding.

As early as last century, many Russian scientists began exploring the vegetation of Xinjiang area. *Psathyrostachys languinsa* (Trin) Nevski was discovered. After 1950, L.A. Udaczen and Z.F. Miguschova discovered *Triticum petropavlovskyi* Udez. et Migusch. The earliest large-scale survey and study of the plant germplasm resources of Xinjiang was done by the teachers and students of Xinjiang August First Agricultural College (XAFAC). During 1953 and 1954, the crop landraces with about 10,000 accessions were widely collected, and Triticeae accessions comprised a major part of the collection, such as the discovery of *Roegneria confusa* var. *breviaristata* Keng. In 1956, a comprehensive survey team organized by the Chinese Academy of Sciences (CAS) investigated and studied the water, soil, biological resources, agronomy and animal husbandry in Xinjiang and collected large numbers of plant specimens. Several authoritative books, such as *Xinjiang Agricultures, Vegetations and their Utilization in Xinjiang*, were published, which summarized the collected species of Triticeae. Since that initial survey, the Xinjiang Desert Biology and Soils Institute of CAS (such as a new record of *Aegilops tauschii* Cosson), Xinjiang University and XAFAC collected many plant specimens during their research programs and published their surveys and research results. This included the series of books, *Key for Xinjiang Plants*, published by XAFAC in 1982. A total of 66 species and 12 subspecies representing 10 genera of the Triticeae tribe were recorded in those books. During 1981 and 1983, a survey team organized by XAFAC and joined by the Institute of Crop Variety Resources in the Chinese Academy of Agricultural Science (CAAS), Laboratory of Crop Variety Resources in the Xinjiang Academy of Agricultural Science (XAAS), and Sichuan Agricultural University thoroughly investigated the wild relatives of crop species in Xinjiang. In 1984 a special issue describing this work was published, in which 73 species and 15 subspecies representing 10 genera of Triticeae were reported (Survey Team for Xinjiang Native Relatives of Crops, 1984). All the seeds were stored in XAFC, XAAS and CAAS. During 1979 and 1985, survey teams for Xinjiang rangeland resources under the direction of the Xinjiang Animal Husbandry Bureau joined XAFC and Shihezi Agricultural

College to investigate and study the rangelands and forage resources of Xinjiang. The books, *The Flora Records of Main Grass Crops in Xinjiang* and *Name List of Xinjiang Plant Species on Rangelands*, were published. In the first book the morphological characters, distributions, ecological characteristics, nutrient value and feeding value of 33 Triticeae species were described. During 1986 and 1990, XAAS again collected and surveyed the native relatives of grain crops in Xinjiang, which was an international project sponsored by IBPGR. A total of 513 accessions representing 59 species and 4 subspecies of Triticeae were collected (J.Q. Lu, 1991) *Kengyilia gobicola* Yen et Baum were discovered and a new genus of *Kengyilia* was established, as well as a new record of *Leymus aemulans* (Nevski) Tzvelev. During 1986 and 1993, the Department of Grassland Science in XAFAC and the Grassland Research Institute in the Xinjiang Academy of Animal Science conducted research projects to collect, evaluate and store Xinjiang's forage germplasm. Some of the collected seeds were Triticeae species (Project Group, 1992; B.J. Li, 1991).

A. Germplasm Resources of Triticeae Species in Xinjiang

According to the previous studies and our current research results, there are 80 species and 14 subspecies (varieties) representing 10 genera and 5 subtribes of Triticeae in Xinjiang (N.R. Cui, 1987).

Subtribe: Triticinae Trin. ex Griseb. in Ledeb. Fl. Ross. 4:324, 1852.

Annual, biennial or perennial herbs with rhizomes; one spikelet at each spike node; most of terminal spikelets are normally fertile. Other spikelets range on both sides of inflorescence axis imbricately. This Subtribe has *Triticum L., Aegilops L., Secale L., Elytrigia* Desv. genera.

Triticum L.: This genus has about 20 species, which are distributed in the western part of Asia and southern Europe. In Xinjiang there are 3 cultivated species, i.e., *T. petropavlovskyi, T. aestivum L. and T. turgidum L.*

Aegilops L.: This genus has about 30 species, which are mostly distributed in the Mediterranean Region and central Asia. Only one species is found in Xinjiang, i.e. *A. tauschii* Coss., which is distributed in the grassland zone in Yili District.

Secale L.: This genus has about 5 species, but only one species is found in Xinjiang, i.e. *S. cereale* L., which was previously cultivated and has become a weed in crop fields. This species does not occur in natural vegetation.

Elytrigia Desv.: This genus has about 40 species, which are distributed in the cold-temperate zone of the western hemisphere. There are 3 species and 2 subspecies in Xinjiang, which mainly grow in shrub grasslands, forestry meadow and meadows in desert areas. They include *E. aegilopoides* (Drob.) N.R. Cui, *E. ferganensis* (Drob.) Nevski., *E. repens* (L.) Desv. ex Nevski, *E. repens* subsp. *elongatiformis* (Drob.) Tzvel. and *E. repens* subsp. *longearistata* N.R. Cui.

Subtribe: *Agropyrinae* N.R. Cui in J. of August 1st Agri. College, 10(4):3, 1987

Annual or perennial herbs without rhizomes, forming dense bunches; one spikelet occurs at each node of the spike axis. The Terminal spikelets are sterile or deteriorated. Other spikelets normally range on the both sides of the spike axis, comblike. Glumes and lemmas are pressed flat bilaterally; significant carina occurs on the back of glume and lemma. The Subtribe includes *Agropyron* Gaertner and *Eremopyrum* Jaub. et Spach.

Agropyron Gaertner: This genus has about 15 species, which are mostly distributed throughout grasslands in Eurasia. Five species are present in Xinjiang, which are widely distributed on grasslands in Xinjiang from desert-grassland, grassland to alpine and cold grassland within a range of altitudes from 600 to 4000 m. They include *A. cristatum* (L) Beauv., *A. mongolicum* Keng, *A. desertorum* (Fisch. ex Linc(Schult., *A. fragile* (Roth) Cand. and *A. pectinatum* (M. Bieb.) Beauv.

Eremopyrum Jaub. et Spach: This genus has about 8 species, which are distributed in the temperate-cold zone of Eurasia. Four species are found in Xinjiang and grow on semi-deserts and play an important role as ephemeral plants in early spring. Their life cycle is about 6 to 7 weeks. Those species include *E. bonaepartis* (Spreng.) Nevski, *E. distans* (C. Koch.) Nevski, *E. orientale* (L.) Jaub. et Spach and *E. triticeum* (Gaertn.) Nevski.

Subtribe: *Elyminae* Bews

Perennial herbs with short rhizomes or not, forming dense bunches; one or two spikelets on each node of the spike axis. The terminal spikelets are normally fertile and other spikelets range on the both sides of spike axis or tend to the one side imbricately. Only one genus in this subtribe is found in Xinjiang.

Elymus L.: This genus has about 160 species which are distributed in the temperate-cold zone in the northern hemisphere. In Xinjiang there are 41 species and 7 varieties or subspecies which grow in the mountain meadow grasslands, forest meadows, alpine and subalpine meadows and cold alpine grasslands. They are *E. atratus* (Nevski) Hand.-Mazz., *E. breviaristatus* (Keng) Keng f., *E. cylindricus* Franch, *E. dahuricus* Turcz., *E. excelsus* Tzvel.,*E. nutans* Griseb., *E. sibiricus* L., *E. tangutorum* (Nevski) Hand.-Mazz., *E. abolinii* (Drob.) Tzvel., *E. abolinii* var. *divaricatus* (Nevski) D.F. Cui, *E. abolinii* var. *plurifloridus* D.F. Cui, *E. alaschanicus* (Keng) S.L. Chen, *E. alatavicus* (Drob.) A. Löve, *E. altaicus* D.F. Cui (D.F. Cui, 1990), *E. altissimus* (Keng) A. Löve, *E. aristiglumis* (Keng) S.L. Chen, *E. batalinii* (Krassn.) A. Löve, *E. breviglumis* (Keng) A. Löve, *E. caninus* (L.) L., *E. confusus* subsp. *breviaristatus* (Keng) D.F. Cui, *E. sclerus* A. Löve, *E. fedtschenkoi* Tzvel, *E. glaberrimus* (Keng) S.L. Chen, *E. glaberrimus* var. *breviaristus* S.L. Chen ex D.F. Cui, *E. gmellinii* (Ldb.) Tzvel., *E. kengii* Tzvelev, *E. humilis* (Keng) S.L. Chen, *E. tsukushiensis* Honda, *E. tsukushiensis* subsp. *macerimus* Keng, *E. kashgaricus* D.F. Cui (D.F. Cui, 1990), *E. kokonorics* A. Löve, *E. komarovii* (Nevski) Tzvel., *E. kronokensis* (Keng) Tzvel., *E. magnicaespes* D.F. Cui (D.F. Cui 1990), *E. mutabili* (Drob.) Tzvel., *E. mutabili* var. *nemoralis* S.L. Chen ex D.F. Cui, *E. pseudonutans* A. Löve, E. platyphyllus (Keng) A. Löve *E. praecaespitosus* (Nevski) Tzvel., *E. schrenkianus* (Fisch. et Mey.) Tzvel., *E. sinkiangensis* D.F. Cui (D.F. Cui, 1990), *E. sylvaticus* (Keng et S.L. Chen) S.L. Chen, *E. toroldianus* (Oliv.) G. Singh, *E. czilikensis* (Drob.) Tzvelev, *E. tschimganicus* (Drob.) Tzvel., *E. tschimganicus* var. *glabrispiculus* D.F. Cui, *E. nevskii* Tzvelev, and *E. viridulus* (Keng) S.L. Chen.

Subtribe: *Hordeinae* Dum in Observ. Gram. Belg.: 91,1823

Annual or perennial herbs, forming dense bunches; normally three spikelets grow on each node of the spike axis. Each spikelet has 1-2 (3) flowers; except for the cultivated species in the genus *Hordeum*, the spike axis of all other species has rachis nodes that break off one by one. This subtribe has two genera, *Hordeum* L. and *Psathyrostachys* Nevski.

Hordeum L.: This genus has about 20 species distributed in the temperate zone. There are 7 species and 2 subspecies in Xinjiang, which mainly grow in meadows, river valleys, mountain grasslands and cold-alpine grasslands where the moisture is better. They are *H. bogdanii* Wilensky, *H. brevisublatum* (Trin.) Link., *H. nevskianum* Bowd., *H. roshevitzii* Howden., *H. turkestanicum* Nevski, *H. violaceum* Boiss. et Huet., *H. vulgare* L., *H. vulgare* var. *aegiceras* (Nees ex Royle) Aitch, and *H. vulgare* var. *nudum* (Ard. ex Schult.) Hook. f.

Psathyrostachys Nevski: This genus has about 10 species mainly distributed in Central Asia. There are 3 species and 1 subspecies in Xinjiang, which grow on the desert-grassland and grassland, i.e. *P. juncea* (Fisch.) Nevski, *P. juncea* subsp. *hyalatha* (Rupr) Tzvel., *P. kronenbergii* Nevski, and *P. lanuginosa* (Trin.) Nevski.

Subtribe: *Leyminae* N.R. Cui in J. of August 1st Agri. College, 10(4):4,1987

Perennial herbs with rhizomes; 2 or more spikelets normally grow on each node of spike axis; lemmas and glumes range on the spike axis alternately, thus making the back of lemmas nude, which is caused by the rotating of the spikelet axis. This subtribe only has one genus.

Leymus Hochst.: This genus has about 30 species distributed in the temperate-cold zone of the northern hemisphere. Most species originate from the center of Asia. In Xinjiang there are 12 species and 2 subspecies growing in mountain grasslands, deserts, salinized lowland meadows in the cold-alpine grassland zone and sandy soil. They are *L. angustus* (Trin.) Pilg.. *L. chinesis* (Trin.) Tzvel., *L. karelinii* (Turcz.) Tzvel., *L. multicaulis* (Kar. et Kir.) Tzvel., *L. multicaulis* subsp. *petraeus* (Nevski) N.R. Cui, *L. tianschanicus* (Drob.) Tzvel., *L. ovatus* (Trin.) Tzvel., *L. paboanus* (Claus) Pilg., *L. pishanica* S.L. Lu et Y.H. Wu (S.L. Lu and Y.H. Wu, 1992), *L. racemosus* (Lam.) Tzvel., *L ramosus* (Trin.) Tzvel., *L. ruoqiangensis* S.L. Lu et Y.H. Wu (S.L. Lu and Y.H. Wu, 1992), *L. secalinus* (Georgi) Tzvel., and *L secalinus* subsp. *pubercens* (O. Fedtsch.) Tzevel.

B. The features and utilization of the Triticeae germplasm in Xinjiang

1. The Xinjiang Region has a large diversity of Triticeae species with a total number of 80 species with 14 subspecies (varieties) representing 10 genera. Of them, 10 species and 5 subspecies (varieties) are endemic species or new records in Xinjiang. Because Xinjiang was an important crossing point of the ancient Silk Road, the wheat landraces were exchanged frequently. During 1958-1977, based on collections from the western part of Tarim Basin, 7 species and 56 varieties of *Triticum* taxa were discovered, and there were many natural hybrids (Z.L. Wang, 1989). The species and the genera of Triticeae in Xinjiang account for 77.3% and 90.0% of those in China, respectively. Therefore, Xinjiang is perhaps the most important gene pool for Triticeae grasses within China.

2. Large amounts of plant biodiversity can be found within Xinjiang's, meadows, grasslands, desert grasslands, alpine-cold grasslands, salinized low meadows, and deserts. Some species are adapted to over-grazed pastures, while others are distributed on different rangelands, and have other characteristics such as drought resistance, winter-hardiness, salt-resistance and grazing tolerance (F. Xiao *et al*., 1992a, 1992b; J.L. Li, 1990).

3. The species have various life-forms, such a hemicryptophyte, geocryptophyte, annual and biennial, perennial, and ephemeral plants.

4. There is wide variability among plants within a population for plant height and weight, stem lignification, type and size of spike, flowers per spike suggesting considerable opportunity for selection. G.L. Sun *et al*. (1990) observed the chromosomes of 48 perennial accessions in Triticeae that were collected in Xinjiang and found that there were different chromosome levels occurring within some species in *Roegneria, Leymus* and *Hordeum*. The karyotypes of selected species in *Elymus* (Y.H. Liu, 1985) and two species in *Eremopyrum* (G.X. Yan *et al*., 1991) were studied.

5. The genes for resistance to diseases and insects have been identified in many species. L.Y. Li (1990) inoculated 42 accessions of Triticeae grasses collected in Xinjiang with a dominant strain of *Erysiphe graminis* f. sp. *tritici* from Sichuan Province. The results showed that 5

species from hexaploid wheat were all susceptible, and the wildrye collected from Xinjiang and the Tritacales from Rwanda were immune. All species were resistant, except for *Aegilops tauschii*, *Elymus dahuricus*, *Leymus secorlinus*, *Roegneria fedtschenkoi* and *R. longiglumis*. It is clear that there is a rich gene pool for resistance to powdery mildew in species of Triticeae in Xinjiang. A total of 40 accessions of *Triticum aestivum* collected in Xinjiang were inoculated with composite cultures AR, 411, BM, and 121 of *Erysiphe graminis* f sp. *tritici*), yellow rust (*Puccinia striiformis*) and leaf rust (*P. recondita* f. sp. *tritici*). Seven accessions, (six species *Agropyron pectinatum*, *Elymus tangutorum*, *Elytrigia repens* subsp. *elongiformis*, *Eremopyrum triticeum*, *E. tsukushiensis* and *Secale cereale*) proved to be highly resistant to all three diseases (L.Y. Li it al., 1992), D. Ma and J.J. Lu (1994) conducted an evaluation of aphid resistance under cages in 123 accessions of 41 species of Triticeae collected in Xinjiang. The results showed that *Leymus secalinus*, *Psathyrostachys juncea* and *Leymus karelinii* have high resistance or are nearly immune to two kinds of wheat aphids. Thirteen accessions of *Elytrigia repens* and *Hordeum bogdanii* are susceptible to both wheat aphids. According to observations of leaf-structure by a scanning electron microscope, leaves from resistant plants had thick epidermal cell walls, and hard epidermal hairs, compressed vein cells, small air cavity and thin vessels in the xylem compared with susceptible plants. There were no significant differences between resistant and susceptible plants in total sugars, reducing sugar and crude protein contents. However, there were significant differences in fat and cellulose contents. The fat and cellulose contents are probably involved in aphid resistance.

C. The development and utilization of Triticeae germplasm

To improve disease resistance and grain yield of wheat through wide hybridization between wheat and native *Elytrigia repens* was conducted in Xinjiang as early as in 1950's. In 1962, a spring wheat cultivar, 'Jiefang No. 2',which was a hybrid between 'Tacheng Black Head' wheat cultivar and a native wheatgrass, was released by Yanji Agricultural Science Institute. However, few wheat cultivars have been developed by wide-crossing with the wild relatives of wheat.

Elymus sibiricus is largely used as a forage in Xinjiang. During 1980 and 1988, it was aerial-seeded as a main seed for improving mountain meadows with a total area of 16,133 ha. In the second year, its average hay yield reached 6.3 t/ha. However, because its forage quality was poor and the forage yield decreased quickly after the third year, *Elymus sibiricus* has been gradually replaced with smooth bromegrass in Xinjiang (Xinjiang Animal Husbandry Bureau, 1989). Z.M. Yang it al. (1992a, 1992b) conducted six regional variety tests and two production tests for four smooth bromegrasses varieties with *Elymus sibiricus* cultivars as checks at five different ecological locations from the plains to mountain meadows during 1988 and 1990 in northern Xinjiang. The results indicated that the yields of *E. sibiricus* were significantly lower than the smooth bromegrass and its forage quality and persistence was poor. 'Qitai' smooth bromegrass cultivar is adapted to large areas in the northern Xinjiang from plain deserts with an altitude of 670 m to the middle mountain area with an altitude of 1900 m. 'Qitai' had good productivity and the highest varietal stability among four smooth bromegrasses including cv. 'Lincoln'. The strategy of replacing *E. sibiricus* with 'Qitai' for mountain meadow improvement was proven correct.

Wheatgrasses are the most important grasses on semi-arid rangelands. They have high drought resistance and provide good quality forage and high seed yield. S.J. Song (1992) tested 34 varieties and accessions of *Agropyron* and *Psathyrostachys* without irrigation at Urumqi, where annual precipitation was 230 mm. During three years of observation, the average hay yield of *Agropyron* accessions varied from 3.87 to 9.40 t/ha per year. Yields of *Psathyrostachys* accessions varied from 4.71 to 7.00 t/ha per year. Slender wheatgrass provided the highest yield of 9.40 t/ha. *Psathyrostachys juncea* ranked second with a yield of 7.00 t/ha. Accessions that were suitable for early spring grazing included *Agropyron pectinatum*, 'Fairway' crested wheatgrass, native *A. mongolicum* and native *Psathyrostachys juncea*. They provided high yields in early spring. Concerning yield and yield distribution in growing seasons, slender wheatgrass, *A. mongolicum* from Inner-Mongolia, native *P. juncea* and *P. juncea* from Hebei province were regarded as grasses for spring-autumn pastures. As a result, wheatgrasses play an important role in spring and autumn pastures in Xinjiang.

Psathyrostachys juncea is another important grass in semi-arid pastures in Xinjiang. It has high drought resistance, grazing tolerance and salt tolerance. 'Ziniquan', a registered cultivar in Xinjiang, produced 3 to 4.5 t/ha of hay per year. The highest yield reached was 7.2 t/ha during the 4th and 5th growing season. So far, the cultivated area is small (J.L. Li, 1990). When *P. juncea* was mixed with fescue, alfalfa and *Kochia prostrata* to establish mixed pastures succession gradually produces a stable mixture of *P. juncea* and alfalfa. The results suggested that *P. juncea* could be used in mixture with alfalfa on spring-autumn pastures having an annual precipitation of 295 mm to 469 mm in Xinjiang (J.L. Li *et al.*, 1991).

Hordeum bogdanii is a prospective grass on plain lowland meadows because it has a high salt tolerance. The relative seed germination rate was 81% on saline soil compred to 74% when germinated on straight salt, which was lower than smooth bromegrass among the tested materials. Its relative seedling emergence ranked first with 70.0%, and the relative seedling survival rate was 100%. Besides this, *H. bogdanii* has drought and cold resistance. It yields 8.87 t/ha during the second growing season under irrigation. This grass is one of the most promising grasses for improving saline pastures in Xinjiang (F. Xiao *et al.*, 1992a, 1992b).

Leymus racemosus is resistant to drought, salt, insects and diseases and has a long spike and produces large quantaties of seed. J.H. Qi *et al.* (1993, unpublished) isolated the total DNA from *L. racemosus* and broke DNA into defined lengths. They transferred the DNA into wheat through wheat pollen tubes. Among 8,900 transferred offsprings, one plant had big spikes. The length of the biggest spike reached 16 cm, which was nearly double the length of the check. The flowers were still fertile and the seeds were big and full. Although this DNA transfer method is still controversial, it should be emphasized that some superior variations have been obtained in wheat by this method.

Based upon recent research, it is clear that Triticeae germplasm from Xinjiang represents a considerable gene pool in the world for wheat improvement and grass breeding. The collection and evaluation of Triticeae species in Xinjiang and their use in crop breeding is very important.

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Ecogeographic Regions and Related Triticeae Distribution of China

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INTRODUCTION

China is situated in the southeastern part of Eurasia Continent with a vast territory. As a result of the difference in heat capacity and precipitation, the country is divided into five climate zones, viz. frigid-temperate, temperate, warm temperate, subtropic, and tropic zone. Owing to the geographical position of the land and its bordering ocean, the southeastern monsoon and southwestern monsoon from the Pacific Ocean and the Indian Ocean, respectively, are the main sources of rainfall in China. Thus, it is moist in the east and south, dry in the west and north. Between the moist and dry regions is the transitional semiarid region. The longitudinal zonaline of vegetation distribution from the southeast to northwest is also clear. Forest zone, steppe zone and semidesert zone appear in order. According to heat capacity of different zones and level zonaline of vegetation distribution, the country can be divided into eight climate-vegetation regions. The 8 regions clearly reflect the ecogeographic conditions of China. The various genera and species of Triticeae are distributed in different ecogeographic regions (Fig. 1).

I. Frigid-temperate coniferous forest region

It lies in the southmost part of the Eurasian coniferous forest region which is located in the north part of the Eurasian Continent, at the west of 127° 20' E and north of 49° 20' N. This is the northmost vegetation region of China. General altitude is 700-1000m about 1400m of the highest peaks, and with broad valleys. The temperature of this region is lowest area in China, with an annual mean temperature of -2°C to 5.5°C. Its winter is long and very cold, but more sunny and less snowing. The temperature of nine months is lower than 10°C. Its summer is relatively short and warm. The frost free period in a year is about 80-100 days. The annual precipitation is 350-550mm. The florestic composition is mainly temperate Asiatic and Arctic-alpine component, and poor in species. There are few species of Triticeae in this region, only 3 genera and 7 species. (Table 1).

II. Temperate coniferous and broad-leaf mixed forest region

This region is between Eurasian coniferous forest and warm temperate deciduous broad-leaf forest regions. It lies at 40° 15' -50° 20' N and 126° - 35° 30' E. The topography is plain and hilly. Its climate is influenced by monsoon of ocean, which cause more rainfall and longer growth period of plant. Free frost season lasts about 100-180 days. Annual precipitation is 500-800-1000mm. Main florestic component is temperate Asiatic and East Asiatic (China-Japan) composition. Species of the tribe Triticeae belonging to 6 genera and 16 species (Table 1), are widely distributed.

III. Warm temperate deciduous broad-leaf forest region

This region is located at 32° 300' - 42° 30' N, and 103° 30'-124° 10' E. The topography is broad in the east and narrow in the west, as a triangle, from east to west with an altitude is 100-1500m. Its annual mean temperature is 9°C to 14°C, and from south toward north increase successively. Frost free season is 180-240 days. the annual precipitation is 500-900mm. The mainly florestic composition is China-Japan component of east Asiatic component and warm Asiatic component. It is fairly more species of Triticeae in warm temperate deciduous broad-leaf region. There are 8 genera, composed of 31 species in this region (Table 1).

IV. Subtropical evergreen broad-leaf forest region

It is the largest region in China. The whole area covers nearly one-fourth of the area of China. It is located at 20° -34° N, 98° 10' - 124° E. The topography types are complicated and various. There are plain, basin, hilly, plateau, and mountain. General elevation above sea level is from 200m to more than 3000m. Its annual mean temperature is 14°C to 22°C. Frost free period is 240-350 days. The annual rainfall is 750-2000mm. Zonality vegetation is evergreen broad-leaf forest, evergreen-deciduous broad-leaf mixed forest monsoon evergreen broad-leaf forest. The mainly florestic composition is China-Japan and China-Himalaya florestic components of East Asia. In subtropic evergreen broad-leaf forest region, there are more than 15,000 species of higher plants, but the plants of tribe Triticeae are poorly represented in this region, with only 3 genera and 9 species. Some species, such as *Elymus tangutorum* and *E. nutans* are found at an altitude above 1000m (Table 1).

V. tropical monsoon rainforest and rainforest region

It is located at 4° -24° N and 85° -123° E. The topography is hilly and alluvial plains. The altitude is lower than 500m in the east, with an altitude at 400-1000m in the center. The basin among mountains and montane area are in the west part with an altitude at 300-500m, 1500-2500m, respectively. The annual mean temperature is 22°C to 26.5°C. The annual precipitation is 1000-3000mm. Zonal vegetation is monsoon rainforest

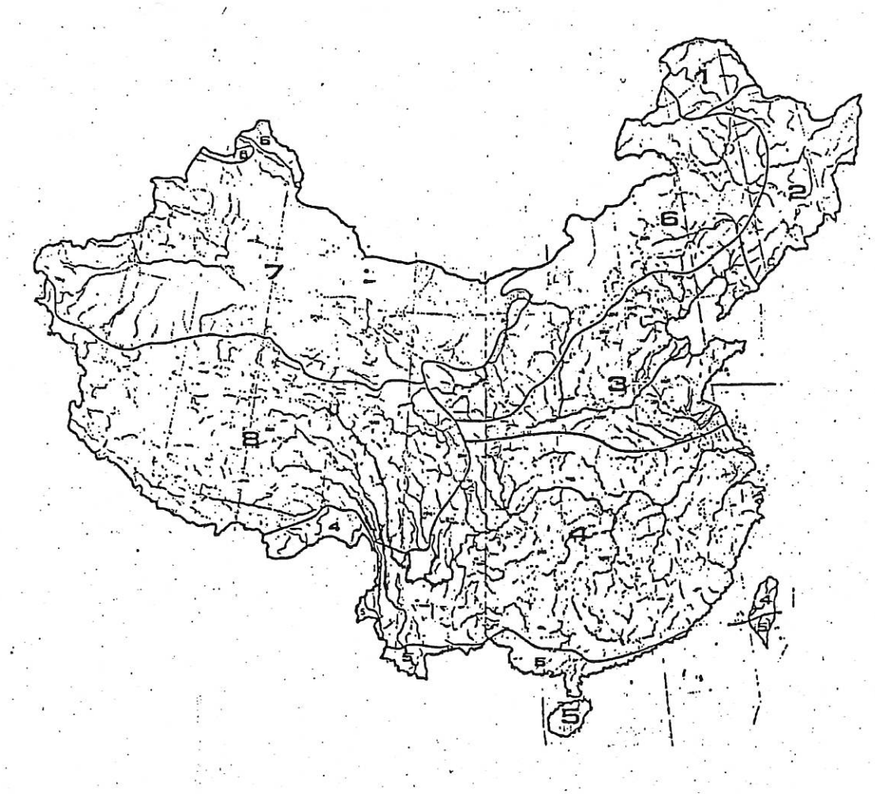

Figure 1. A map of eco-geographic regions of China
1. Frigid-temperate coniferous forest region
2. Temperate coniferous and broad-leaf mixed forest region
3. Warm temperate diciduous broad-leaf forest region
4. Subtropical evergreen broad-leaf forest region
6. Warm steppe region
7. Warm semidesert region
8. Zing-Zhang (Zinghai-Xizang) plateau frigid vegetation region (after Editorial Board of vegetation of China)

and rainforest, The mainly florestic composition is tropical southeast Asia component. We did not find any plants of the Triticeae in the zonal vegetation. It is in the south part of East Himalaya Mts. of southeast Tibet. From 3000-4800m, including a coniferous forest zone and an alpine meadow zone, we can find two genera *(Roegneria* and *Elymus)* and 10 species (Table 1).

VI. Warm steppe region

Warm steppe region of China is an important component Eurasia Steppe. This region of China is located at 18° -46° N and 83° - 128° E. The annual mean temperature is -3°C to 8°C. The frost-free period lasts 100-170 days. Yearly rainfall is 150-450 (550)mm which is concentrated in summer. Zonality vegetation is warm steppe. The main florestic composition is arid Asia component, Old World temperate component. This warm steppe region is rich in genera and species of Triticeae, consisting of 7 genera and 35 species (Table 1).

VII. Warm semidesert region

The warm semidesert region is located in the east part of "Asia-Africa semidesert Region", west of 108° E and north of 36° N. It covers nearly one-fifth of China. General topography features are high mountains together with basins. The climate of this region is controlled by the Mongolia-Siberia anticyclone. The annual mean temperature is 4°C to 12°C. Frost free period lasts 140-210 days. The annual mean precipitation is 100-150mm, except Junggar and high mountains which have more rainfall. Zonal vegetation is warm semidesert. Main florestic composition is center of Asia component, Central Asia component and dry Asia component. Genera and species of Triticeae are also rich in this warm semidesert region, represented by 12 genera and 51 species (Table 1).

VIII. Qing-Zang (Qinghai-Xizang) Plateau frigid vegetation region

Qing-Zang Plateau is located at 28° -37° N and 75° -103° E. Topography is high in the northwest and low in the southwest. Mountains with an altitude is 3500-5000m, the highest peaks are at an altitude above 500-6000 (7000) m. The climate of Qing-Zang Plateau. The air circulation is influenced by two basic currents, viz. summer half year by Indian tropical ocean monsoon-southwest monsoon, and winter half year controlled by westerly circulation. The southwest monsoon also had effect to the east part in this region. The annual mean temperature is lower than 0°C owing to high elevation. The topography is a major influence factor on rainfall. The southeast part of this region, has an annual mean precipitation of 500-1000mm. The annual mean precipitation is (100) 250-350 (550)mm in the center. The northwest part has a yearly rainfall of 50-60mm.

The main florestic composition is East Asia (China-Himalaya) component, the center of Asia component and Qing-Zang component. The main vegetation types in this region are frigid shrubs, frigid meadow and frigid steppe. The topography is a major influence. The vertical zonals are well-developed and complicated. There were 7 genera and 50 species of Triticeae found in the Qing-Zang Plateau.

As mentioned above, the eight regions indicate the ecogeographic conditions of China clearly. The warm steppe, warm semidesert and Qing-Zang Plateau frigid vegetation regions are rich in Triticeae, but the later is rich only in species of *Roegneria* and *Kengyilia.*

Table 1. The distribution of wild resources of *Triticeae* in China

Species	Ecographic region*							
	I	II	III	IV	V	VI	VII	VIII
Aegilops tauchii			+					+
Agropyron cristatum	+	+				+	+	+
A. desertorum						+	+	+
A. desertorum var. *pilosiusculum*		+					+	+
A. michinoi							+	
A. mongolicum			+				+	
Elymus atratus								+
E. breviaristatus								+
E. caninus								+
E. cylindricus		+	+				+	+
E. dahuricus	+	+	+				+	
E. magnicaespitosus								+
E. mutabilis								+
E. nutans	+	+	+	+		+		+
E. sibiricus	+	+				+	+	+
E. submuticus								+
E. tangutorum			+	+				+
E. villifer			+					+
Elytrigia repens		+					+	+
Eremopyrum bonaepartis								+
E. distans								+
E. orientale								+
E. triticeum								+
Hordeum bogdanii		+				+	+	+
H. brevisubulatum								
ssp *brevisubulatum*	+	+	+			+	+	+
ssp *nevskianum*								+
ssp *turkestanicum*							+	+
H. jubatum		+						
H. roshevitzii		+				+	+	+
Hystrix duthiei			+					+
H. komarovii			+					
Kengyilia gobicola								+
K. grandiglumis								+
K. habahenensis							+	
K. hirsuta					+			+
K. kokonorica								+
K. laxiflora								+
K. longiglumis							+	+
K. melanthera								+
K. mutica								+
K. nana								+
K. pamirica								+
K. rigidula								+
K. stenachyra								+
K. tahelacana								+
K. thoroldiana								+
K. zhoashuensis								+
Leymus amaelans								+
L. angustus							+	+
L. chinensis		+	+				+	+
L. multicaulis							+	+
L. ovatus								+
L. paboanus								+
L. pseudoracemosus								+
L. racemosus								+
L. secalinus	+				+		+	+

Table 1. The distribution of wild resources of *Triticeae* in China

Species	Ecographic region*							
	I	II	III	IV	V	VI	VII	VIII
L. tianshanicus								+
Psathyrostachys huashanica				+				
P. juncea							+	+
P. kronenburgii								+
P. lanuginosa							+	
P. stoloniformis								+
Pseudoroegneria cognata								+
Roegneria abolinii var *divaricata*								+
R. alashanica							+	+
R. aliena				+				
R. altissima								+
R. amurensis		+					+	
R. anthosachnoides						+		+
R. aristiglumis								+
R. barbicalla				+				
R. breviglumis								+
R. brevipes								+
R. cacuminus								+
R. calcicola						+		
R. ciliaris	+	+	+					+
R. ciliaris var *japonensis*			+					
R. confusa var *breviaristata*							+	+
R. curvata							+	+
R. dolichanthera						+		
R. dura								+
R. foliosa							+	
R. glaberrima							+	+
R. glaucifolia					+			+
R. gmelinii	+	+	+				+	+
R. grandis			+					
R. hondai			+				+	
R. intramongolica							+	
R. jacquemontii					+			+
R. komarovii							+	
R. leiantha								+
R. leiotropic						+		
R. longiaristata ssp *canaliculata*					+			+
R. minor				+				
R. multiculmis				+				
R. nakai			+	+			+	
R. nutans					+			+
R. parvigluma					+			+
R. pendulina	+	+	+					+
R. platyphylla								+
R. puberula					+			
R. pubicaulis								+
R. pulanensis								+
R. purpurescens								+
R. retroflexus								+
R. scabridula							+	
R. shrendiana var *schrenkiana*							+	+
var *pamirica*					+			+
R. shangdongensis				+	+			
R. serotina				+				
R. sinica								+
R. stricta			+			+		+
R. sylvatica								+
R. tianshanica								+
R. tschimganica					+			+
R. tsukushiensis var *transiens*			+		+		+	

Table 1. The distribution of wild resources of *Triticeae* in China

Species	Ecographic region*							
	I	II	III	IV	V	VI	VII	VIII
R. tibetica								+
R. varia								+
R. viridula								+
R. ugamica								+
R. yangii								+
Secale segatale								+

I. Frigid-temperate coniferous forest region.
11. Temperate coniferous and broad-leaf mixed forest region.
III. Warm temperate deciduous broad-leaf forest region.
IV. Subtropical evergreen broad-leaf forest region.
V. Tropical monsoon rainforest and rainforest region.
VI. Warm steppe region.
VII. Warm semidesert region.
VIII. Qing-Zang Plateau frigid vegetation region.

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Characterization of Wheat-*Aegilops umbellulata* Recombinant Lines by *in situ* Hybridization

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Abstract

Novel proteins, including the high molecular weight (HMW) glutenin subunits, could improve breadmaking quality of wheat. *Aegilops umbellulata* Zuck (2n=2x=14) is a useful reservoir of variation for introduction of genes for the protein improvement. In particular, the Glu-U1 gene codes for a very high HMW glutenin subunit not found in any bread wheats (Law and Payne, 1983). The production of translocations and recombinant lines allows the introduction of small alien chromosome segments through manipulation of homeoelogous chromosome pairing (Gale and Miller, 1987; Islam and Faradi, 1988). We applied non-radioactive *in situ* hybridization to characterize wheat-*Ae. umbellulata* recombinant lines. *In situ* hybridization is a powerful technique for the identification and physical location of alien material in wheat (Schwarzacher *et al.*, 1992) as well as for the physical mapping of specific genes and repetitive sequences. Plant seeds were screened by SDS-PAGE to ensure that only those carrying the HMW glutenin subunits from *Ae. umbellulata* were analysed. Genomic *in situ* hybridization allowed the identification of the recombinant chromosome and the physical mapping of the translocation breakpoints. At the same time, we determined the number of copies and the physical locations and relative order of the rRNA (NORs) and 5S-Rna genes, using the specific probes pTa71 and pTa794, respectively.

INTRODUCTION

The production of hexaploid bread wheat *Triticum aestivum* L. emend Thell, has been increasing through the years. Today wheat is the major cereal growing all over the world. Wheat is widely used for baking bread and breeders are interested in improving the bread-making quality of this cereal.

The commonly used genetic strategies include a group of methods aiming at a main goal: to improve the quality of cultivated wheats using the genetic resources of wild related species. In fact, the wild relatives of wheat offer a reserve of genetic material for the introduction of novel genes coding for disease and resistance, endosperm proteins, high photosynthetic rate, salt tolerance and protein content improvement (Knott, 1961; Austin *et al.*, 1984; Foster *et al.*, 1987).

Special plant breeding program have been developed in order to allow the transfer of wild genes into a wheat background (Law, 1981). The interspecific and intergeneric hybridization followed by the production of synthetic amphiploids and backcrossing, is a necessary step towards the introduction of alien genes into wheat. It is possible to transfer whole or parts of genomes from wild plants into cultivated wheat (reviewed by Gale and Miller, 1987).

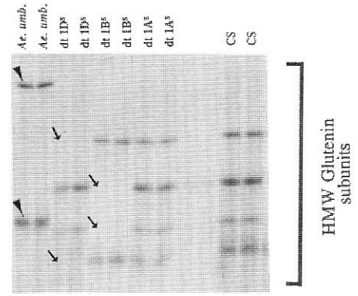
The successful transfer of single genes from alien species requires that the genes or chromosome segments carrying them can be incorporated into a wheat chromosome and expressed as it was in the alien species. Novel genes have been transferred through induced homoeologous pairing and recombination between wheat and alien chromosomes by either suppressing or removing the activity of the Ph1 gene.

One example of a wheat wild relative that can provide valuable genes and so be used for its improvement, in respect to bread-making quality, is *Ae. umbellulata* Zhuk. (2n=2x=14).

DISCUSSION

Endosperm Proteins

The endosperm storage proteins -glutenins and gliadins- play a very important role in bread-making quality,



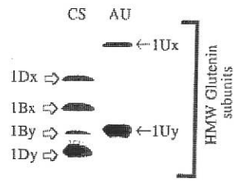


Figure 1. Electrophoretic pattern of the total endosperm proteins from Chinese Spring euploid (CS), Chinese Spring aneuploids from group 1 (nullisomic-tetrasomic and ditelosomic lines), *Ae. umbellulata* and Chinese Spring 1U chromosome substitution lines, fractionated by 10% SDS-PAGE. The figure shows only the high molecular weight (HMW) glutenin subunits and arrows indicate the chromosomes responsible for each protein bands.

the high molecular weight (HMW) glutenins subunits being the most important (Payne *et al.*, 1987). The genes coding for these proteins are normally designated as Glu-1 and Gli-1, respectively. It is known that Glu genes of several species related to wheat are located on chromosomes homoeologous to the wheat group 1 chromosomes, thus chromosome 1U in *Ae. umbellulata*. The analysis of the total endosperm proteins by 10% SDS-PAGE (Sodium DodecylSulphate-Polyacrylamide Gel Electrophoresis) allows the separation and identification of the HMW glutenin subunits and other protein. The electrophoretic banding pattern in *Ae. umbellulata* is less complex than in hexaploid wheat showing only two bands for the HMW subunits, which are classified according to their mobility, 1Ux (the larger with less mobility) and 1Uy (the smaller HMW glutenin subunit). Compared with Chinese Spring, the *Ae. umbellulata* HMW glutenin 1Ux band is heavier (i.e. shows less mobility) than any of the hexaploid wheat bands. In wheat cultivar Chinese Spring, four HMW glutenin subunits were identified (Payne *et al.*, 1981; Payne and Lawrence, 1983): two of these are coded by chromosome 1D (1Dx and 1Dy) and the other two are coded by chromosome 1B (1Bx and 1By) (Figure 1).

To introduce into wheat the novel proteins from *Ae. umbellulata*, chromosome 1U has been substituted for chromosome 1A producing a marked improvement in bread-making quality, although also introducing numerous less desirable traits. Another gene or perhaps gene family located on chromosome 1U of *Ae. umbellulata* is Gli-U1, coding for gliadin proteins. This chromosome has been substituted for the chromosomes 1B and 1D although in these cases no improvement to bread-making quality was observed. It seems, therefore, that the improved quality of wheat is most likely to be due to Glu-U1 gene(s) (Harris, 1983).

Study of Recombinant Lines:

Alien Marker Genes and Genomic in situ Hybridization

Islam-Faradi (1988) undertook a series of crosses to transfer the Glu-U1 from *Ae. umbellulata* into *Triticum aestivum* cv. Chinese Spring. The recombination between homoeologous chromosomes was induced through manipulation of the 5B chromosome dosage. Chinese Spring (CS) with chromosome 1U substituted for either chromosomes 1A and 1B was crossed with CS nullisomic 5B-tetrasomic 5D (N5B-T5D). About 3251 recombinant seeds were obtained in the end of the breeding program. Some recombinant lines were characterized for a number of alien gene markers using different electrophoretic techniques in order to establish the extent of the recombination. The recombinant lines were characterized based on the presence or absence of alien marker genes (Glu-U1, Tri-U1, NOR-U1, 5S-Rrna, Gpi-U1 and Gli-U1) as well as the cytological analysis of the hybrids between these lines and a number of telocentric lines.

The methods for detecting alien chromosomes or chromosome segments need to be efficient and applicable

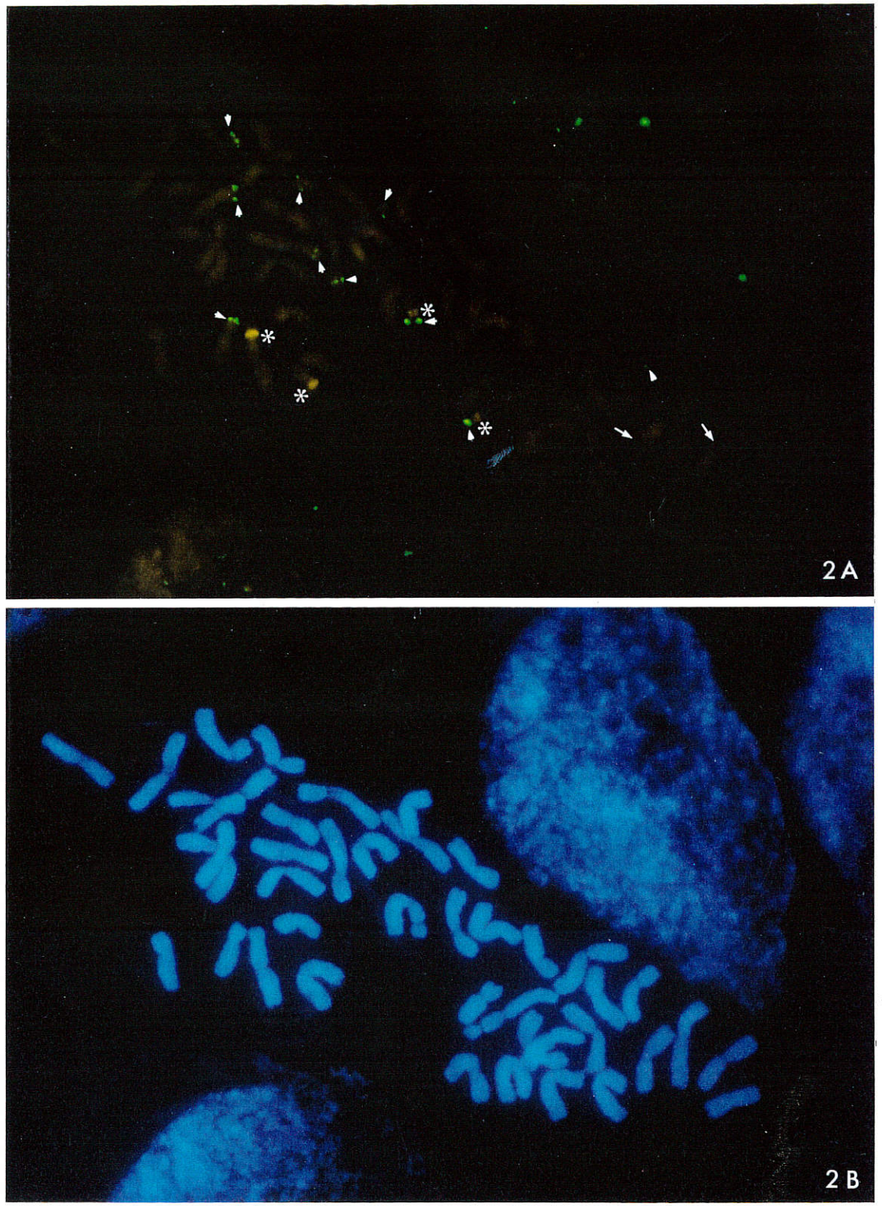


Figure 2: Multicolor *in situ* hybridization on a wheat *Ae. umbellulata* disomic recombinant line (A56), using simultaneously the total genomic DNA from *Ae. umbellulata* (labelled with Biotin-11 dUTP), pTa71 (labelled with Fluored-14-dUTP) and pTa794 (labelled with Digoxigenin-11-dUTP). (A) We can identify the pair of recombinant chromosomes (the arrows indicates the centromeric breakpoints), the four wheat major NORs (stars) and ten wheat 5S-rRNA genes (arrow heads). (B) DAPI stained chromosomes.

to many situations. Markers like the useful genes themselves, plant morphology, isozymes, RFLP analysis and meiotic studies are often informative and very valuable.

Recently a new and powerful technique has been applied to identify alien material in wheat. Non-radioactive DNA:DNA *in situ* hybridization of labelled nucleic acid probes to chromosomal DNA spreads permits the identification and physical localization of a single copy nucleic-acid sequence (Ambros *et al*., 1986), chromosome segments (Le *et al*., 1989; Heslop-Harrison *et al*., 1990; Schwarzacher *et al*., 1992), chromosomes (Lichter *et al*., 1990) and whole genomes (Schwarzacher *et al*., 1989; Leitch *et al*., 1990) in all cell cycle stages.

Hybridization with labelled total genomic DNA can be used to distinguish chromosomes or chromosome segments originating from different genomes in hybrids or recombinant lines (Heslop-Harrison *et al*., 1988; Schwarzacher *et al*., 1989, 1992), and is now known as genomic *in situ* hybridization (GISH). Differentiation between two related genomes can be improved when the labelled genomic probe is hybridized in the presence of an excess concentration of unlabelled total genomic DNA from one of the genomes (=blocking DNA). This way only the sequences specific for the target are available for hybridization (Anamthawat-Jónsson *et al*., 1990).

Schwarzacher *et al*. (1992) used genomic *in situ* hybridization to identify alien chromosomes and whole chromosome arms in wheat. They showed that this method is widely applicable in various wheat-alien recombinant lines and is a fast, accurate and sensitive technique, likely to be very useful both for cytogenetic analysis and plant breeding. In fact, it is possible to have simultaneous information on the number, size and morphology of the alien chromosome.

In situ hybridization to wheat - *Ae. umbellulata* recombinant lines

Multiple target *in situ* hybridization is made possible since several nucleic acid probes can be labelled with different non-radioactive haptens which are then visualized by independent detection systems (Hopman *et. al*., 1986; Cremer *et al*., 1988).

The main steps for *in situ* hybridization involve making the chromosome preparation on a slide and the labelling of nucleic acid sequence as the probe. Both probe and preparation are then denatured to make all nucleic acids single stranded and, under controlled conditions, the probe is allowed to hybridize with its complementary single stranded sequence in the preparation forming a new labelled double stranded molecule. The hybridization sites are detected and visualized depending on the type of label attached to the probe.

In this investigation we applied the multiple target *in situ* hybridization, using as probes the total genomic DNA from *Ae. umbellulata* together with specific cloned DNA sequences (pTa71, pTa794, pSc119.2) not just to map physically the translocation breakpoints but also to identify the wheat chromosome involved in the initial translocation (pSc119.2) and at the same time map the number of copies and the location of the rDNA (pTa71) and 5S-rRNA (pTa794) genes.

The translocation breakpoints were identified in ten recombinant lines using the total genomic DNA from *Ae. umbellulata* (150 ng/slide) in the presence of blocking DNA from Chinese Spring (35 x the probe concentration). To map physicaly the 5S rRNA and the rDNA genes, the probes pTa794 and pTa71 were added to the probe mixture (50-100 ng/slide) (Figure 2).

In other experiments, we used both total genomic DNA from *Ae. umbellulata* and the repetitive DNA sequence, pSc119.2, as the probe . The hybridization pattern of this tandemly repeated sequence allowed the identification of different wheat chromosomes involved in the translocation with the chromosome 1U from *Ae. umbellulata*.

Characterization of Wheat - *Ae. umbellulata* recombinant lines

Since the recombinant lines were obtained by manipulation of the homoeologous pairing control system, multiple translocations may have taken place amongst homoeologous wheat chromosomes. In fact, the use of the pSc119.2 clone as a second probe showed that the hybridization pattern of this sequence may no longer correspond to the one proposed for Chinese Spring (Mukai *et al*., 1993).

We were able to detect breakpoints not only at centromeres but also along chromosome arms. The recombinant lines investigated had been previously characterized by isozyme and pairing analysis at meiosis (Islam and Faradi, 1988). The results from this investigation show significant differences to the proposed chromosome structure.

Genomic *in situ* hybridization does not involve screening and characterization of markers and gives unique information about the sizes of alien chromosome segment that are transferred during plant breeding programs. When combined with isozyme and RFLP markers, the method enables detailed physical characterization of the organization of genes and their regulatory sequences along chromosomes. By knowing the physical location of breakpoints and hence of genes we can design further crossing experiments to optimize and direct the transfer of very small desirable chromosome segments into a wheat background.

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Geographic Distribution of Alleles for Est-5, Gliadin, α- and β-Amylase in *Triticum tauschii*

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ABSTRACT

Analyses for seed esterase-5, gliadin, α- and β-amylase diversity were carried out on 38 accessions of *Triticum tauschii* from the Middle East, the Former USSR, and the provinces Xinjiang, Shaanxi, and Henan of China. Results indicated that (1) the allele polymorphism for these loci was greatest in the Middle East accessions and decreased in accessions from the former USSR, to Xinjiang, to inland provinces of China; (2) the degree of allele polymorphisms was gliadin >EST-5 >α-amylase-2 >α-amylase-1> β-amylase-1; and (3) the seven accessions collected from the inland provinces of China were uniform for each of the loci except EST-5, which showed some minor differences. Except for gliadin, all spectra of the seven Chinese accessions were also observed in some accessions from Xinjiang, the former USSR, and the Middle East accessions. We concluded that the Middle East is the center of origin for *T. tauschii* and that its dispersal route to China was most likely via the former USSR and Xinjiang.

INTRODUCTION

Triticum tauschii (Cosson) Schmalh (*Aegilops squarrosa* L.), donor species of the D genome of *Triticum aestivum* L., mainly grows in Turkey, Syria, Iraq, Iran, Caucasus, Turkey, Armenia, Pamir-Alai, Tianshan, and Afghanistan (Lubbers et al., 1991). It may be the most suitable among the progenitor species for direct gene introgression, because its genome (D) is homologous with the D genome of *T. aestivum* (Riley and Chapman, 1960). Additionally, *T. tauschii* has greater useful genetic variability than the other two genome donors (Gill and Raupp, 1987). On the one hand, a number of accessions of *T. tauschii* have been collected in China, CIMMYT and America (Gill and Raupp, 1987; William and Mujeeb-kazi, 1993), which has built up a large gene pool for wheat improvement; on the other hand, transfer of target gene from *T. tauschii* to wheat cultivars needs more time and labor than gene introgression between cultivars. It's essential to avoid duplication of the same genotype in gene transfer programs. A core collection (Brown et al., 1987) of *T. tauschii* would be desirable. Another interesting question is the origin of *T. tauschii* growing in inland provinces of China. In this paper we 1) analyze the distribution of isozyme and storage protein diversity across the geographic ranges of selected accessions; 2) classify these accessions into groups based on isozyme variation; and 3) suggest strategies for efficient use of these accessions.

MATERIALS AND METHODS

Thirty eight accessions of *T. tauschii* with distinct collection sites in 128 collections were chosen as experimental materials (Table 1). Chinese Spring is used as the check.

Isozyme isoelectric focusing (IEF) analysis

Two gel sizes (24 cm x 16 cm, 24 cm x 12 cm) were prepared in IEF analysis of isozymes. The composition of gels is listed in Table 2. Different size gels and different isolytes were used for various enzymes (Liu, 1991).

(1) Seed esterase (EST-5)

Gel size 24 x 16; Isolytes and rate: 3.5-10 : 5-7 = 1:1. Anolyte: 1 M H_3PO_4 or 0.04 M L-glutamic acid; Catholyte 0.1 M NaOH. Sample preparation: The endosperms of two seeds of *T. tauschii* were crushed and put into an eppendorf tube and then extracted with 200 μl 20% sucrose solution over 1hr at room temperature and finally centrifuged.

Staining: 75 mg α-naphthylacetate + 75 mg β-naphthylacetate + 100 mg Fast blue RR salt + 7.5 ml acetone + 200 ml 80 mM phosphate buffer.

(2) β-amylase-1 (β-Amy-1)

Gel size 24 x 16; Isolytes and rate: 4.2-4.9 : 4.5-5.4 : 4-6 = 1:1:1. Anolyte: 0.01 M L-glutamic acid; Catholyte:

Table 1. The native sites of 38 accessions of *Triticum tauschii*.

Sample No.	Accession No.	Collection sites	Collection time
C1	Y302	Yanglin, Shaanxi	1989
C2	Y303	Luoyang, Henan	1989
C3	Y304	Anyang, Henan	1990
C4	Y305	Xinxiang, Henan	1990
C5	Y306	Qixian, Henan	1990
C6	Y307	Jiaozuo, Henan	1990
C7	Y308	Jiaozuo, Henan	1990
C8	Y309	Yili, Xinjiang	1982
C9	Y92	Yili, Xinjiang	1982
C10	Y93	Yili, Xinjiang	1982
C11	Y95	Yili, Xinjiang	1982
C12	Y96	Yili, Xinjiang	1982
C13	Y97	Yili, Xinjiang	1982
C14	Y98	Yili, Xinjiang	1982
C15	Y99	Yili, Xinjiang	1982
C16	Y121	Former USSR	?
C17	Y124	Former USSR	?
C18	Y127	Former USSR	?
C19	Y128	Former USSR	?
C20	Y218	Former USSR	?
C21	Ae37	Former USSR	?
C22	Ae39	Former USSR	?
C23	Y287	Armenia, Former USSR	?
C24	Y294	Azebajan, Former USSR	?
C25	Y296	Azebajan, Former USSR	?
C26	Y295	Iran(near Baku of Former USSR)	?
C27	Y314	Iran	?
C28	Y169	Iran	?
C29	Y170	Iran	?
C30	Y173	Iran	?
C31	Ae38	Iran	?
C32	Y220	Iran	?
C33	Y219	Iran	?
C34	Y57	Middle East	1983
C35	Y122	Afghanistan	1983
C36	Y176	Afghanistan	1983
C37	Y168	Pakistan	?
C38	Y172	Pakistan	?

0.1 M NaOH. Sample preparation: Extract enzyme with 200 µl 20% sucrose solution containing 0.01M DTT from crushed endosperms of two seeds for about 1hr, then the mixture was centrifuged. Staining: Incubate the focused gel in 200 ml 2% boiled flour solution for 7 min at room temperature, then pour out the flour solution and add a solution of 3 ml Stock* + 1 ml Acetic acid + 96 ml distilled water.

(Stock: 1.6 g I + 4.9 g KI + 250 ml H_2O).

(3) α-Amy-1 and α-Amy-2

Gel size 24 x 12; Isolyte and rate: α-amy-1 5-7:6-8=1:2; α-amy-2 3-5 : 4.2-4.9 : 5-6 = 1:3:1. Sample preparation: Residue of two seed having germinated 5-7 days are extracted with 150 µl distilled water for 1-2 hrs. extracted solution was incubated in a 70 °C water bath for 20 min for inhibition of β-amylase activity. The solution was centrifuged. The rest of steps were the same as that for β-Amy-1.

Acid-polyacrylamide gel electrophoresis of gliadin

Two seeds of *T. tauschii* were crushed with pliers and transferred to a 1 ml eppendorf tube, to which 0.4 ml extraction solution (25% 2-chloroethanol containing 0.05% methyl green) and 0.4 ml 16% glycerin were added. The contents of the tube were thoroughly mixed over 2 hrs and centrifuged. The supernatant was used for electrophoresis. Electrophoresis was run at 500 V and 35 mA. Procedure for gel preparation (T=12%, C=4%),

Table 2. Gel compositions and rate of the two different size gels (Liu, 1991).

Chemicals	Gel size	
	24 x 16	24 x 12
Mixture solution of acrylamide and N.N'-methylene-bis-acrylamide(30%+1%)	1.5ml	1.0ml
Glycerol(16%)	9.75ml	6.5ml
Isolytes	0.66ml	0.44ml
TEMED	15µl	10µl
APS(1.5%)	0.39ml	0.28ml
Total	12.3ml	8.22ml

fixation and staining was the same as that described by Draper (1987).

Data analysis

For each locus class of α-Amy-1, α-Amy-2, β-Amy-1 and Est-5, Jaccard's distances were estimated between every pair among the 38 accessions using the following formula

$$JD(i, j) = 1 - M/[(T_i + T_j)-M]$$

M is the number of band matches between the accessions i and j, T_i is the total number of bands in the accession i, and T_j is the total number of bands in the accession j (Phillips et al, 1993). Mean Jaccard's distance (JD) of the four enzymic locus was calculated by JD(i,j) = 1/4Σ JD(i,j). Value of JD(i,j) estimates the difference between the accessions i and j. Relative reliability of JD was checked against the finger print of collection (A-PAGE patterns of seed gliadin).

RESULTS AND DISCUSSION

1. Est-5

Because of the selfing nature of *T. tauschii*, genotype fixation can be reached in a shorter time after a new mutant occurrs, leading to a genotype frequency equivalent to the phenotype frequency. Est-5 is controlled by chromosome 3D (Ainsworth et al., 1984; McIntosh et al., 1993). Three genotypes were found in seven accessions from inland of China (Fig. 1 lanes 1-7). Four genotypes were found in eight accessions collected from Xinjiang (Fig. 1, lane 8-15). All of the three genotypes of inland China existed in the collections from Xinjiang. In the ten accessions from former USSR (Fig.1, lanes 16-25), four genotypes were found, of which three had similar patterns in the collections from China. In the thirteen accessions (Fig. 1, lane 26-38) from the Middle East countries, six genotypes and one accession of null at Est-5 were found. Most of these genotypes appeared in collections from the former USSR. Of particular interest is the observation that one genotype of China (Sample 4 & 10) was found in the

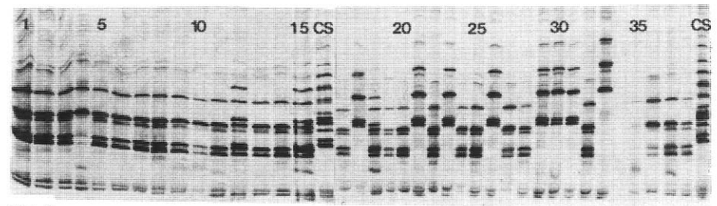
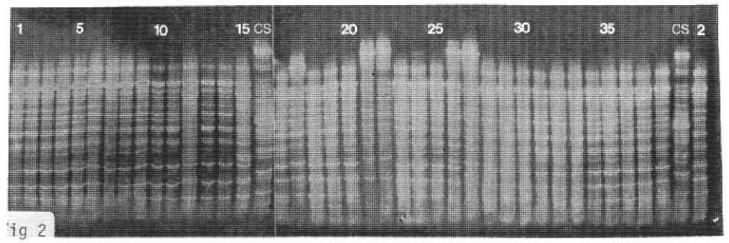


Fig 1



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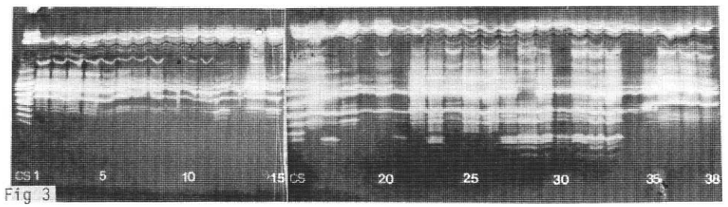

Fig 3

Fig. 1 IEF spectra of Est-5 in 38 accessions of *Triticum tauschii*.

Fig. 2 IEF spectra of β-Amy-1 in 38 accessions of *Triticum tauschii*.

Fig. 3 IEF spectra of α-Amy-1 in 38 accessions of *Triticum tauschii*.

Middle East (Sample 36) but not in the former USSR. This might be caused by either not enough collections were assessed or genetic erosion occurred in the former USSR. One prominent feature is that the nearer the collection site to the Middle East, the higher the polymorphism is (Fig. 1).

2. β-Amy-1

The locus controlling β-Amy-1 should be located on 4D of T. tauschii (Ainsworth et al., 1987; Liu, 1991; McIntosh et al, 1993). Allele polymorphism is poor on this locus (Fig. 2). Only five genotypes were detected in the 38 accessions. There are one in Shaanxi and Henan, two in Xinjiang, three in the former USSR, and two in the Middle East. One genotype is common throught the four areas (Fig. 2, lanes 1-11, 13, 14, 16-20,23, 24, 25, 26, 28-38).

3. α-Amy-1

Genes controlling α-Amy-1 were located on chromosomes of the homoeologous group 6 in common wheat and *T. tauschii* (Ainsworth et al., 1987; McIntosh et al., 1993). Allele polymorphism is higher than that of β-Amy-1 (Fig. 3). Seven accessions from inland of China were of one common genotype (Fig. 3, lanes 1-7), which also appeared in collections from Xinjiang (Fig. 3, lane 14) and the Middle East countries (Fig. 3, lanes 36, 37, 38).

Another principle genotype (Fig. 3, lanes 27, 28, 29, 31, 32, 33) appeared in collections of the former USSR (Fig. 3, lanes 17, 23) and did not occur in collections from China. Again, the evidence that *T. tauschii* was disposed from the Middle East countries to the former USSR, then to China is obvious.

4. α-Amy-2

Loci responsible for this isozyme have been located on the homoeologous group 7 (McIntosh et al., 1993). Allelic diversity for this locus matches that for α-Amy-1 (Fig. 4). All of the seven accessions from Chinese inland shared the same genotype which was only found in collections of the Middle East. A prominent genotype was shared by a number of collections from different areas except those from Shaanxi and Henan (Fig. 4, lanes 9, 10, 12, 13, 17, 19, 20, 22, 24, 25, 26, 27, 31, 36, 37, 38). This strongly demonstrates the genetic relationships among *T. tauschii* populations occurring in these areas.

The isozymes should not be singly used for systematical studies. It is more appropriate to create a composite index to measure the difference or similarity of each pair of accessions. For each locus class, Jaccards' distance were estimated between all pairs of the accessions tested. Average linkage clustering tree(Fig. 5) was produced based on mean JDs using UPGMA (Rohlf, 1993).

Fig 4

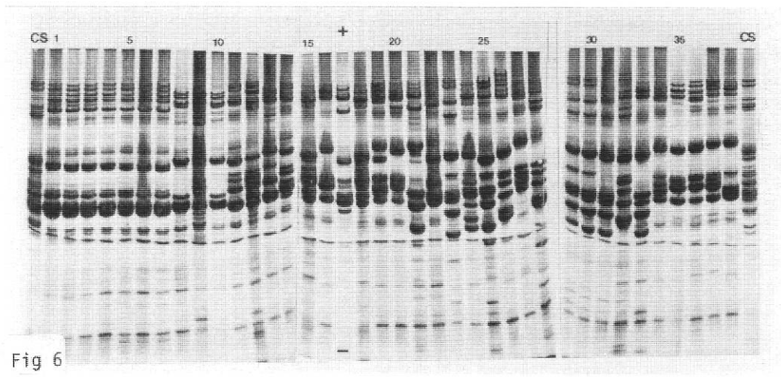

Fig 6

Fig. 4 IEF spectra of α-Amy-2 in 38 accessions of *Triticum tauschii.*

Fig. 6 A-PAGE spectra of gliadin in 38 accessions of *Triticum tauschii.*

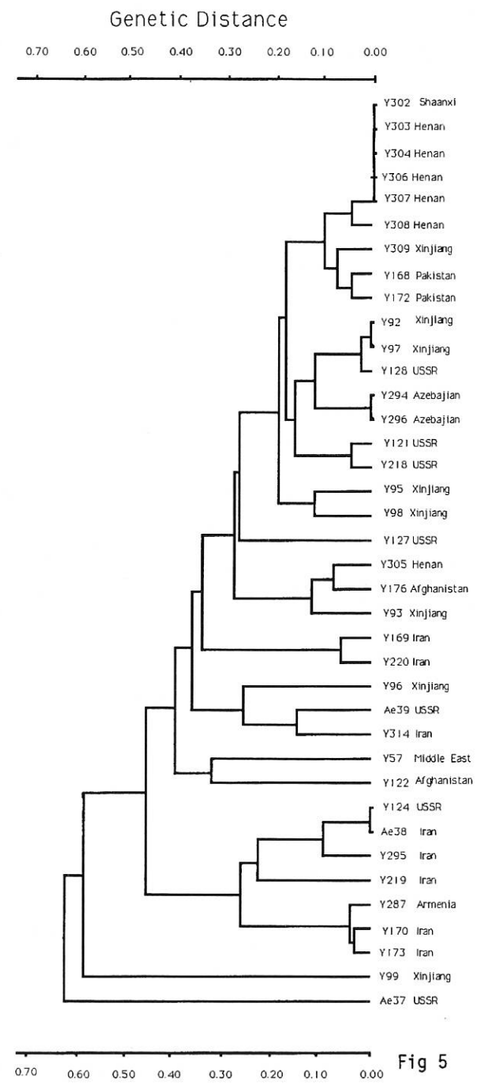

Fig 5

Fig. 5 Average linkage clustering tree for 38 accessions of *Triticum tauschii.*

5. Gliadin

A-PAGE spectra of gliadin of wheat have been commercially regarded as fingerprints of cultivars. Gliadin was controlled by multigene families on short chromosome arms of the homoeolgous groups 1 and 6 (Bietz, 1987; McIntosh et al., 1993). Because of the evolutionary relationship between *T. tauschii* and *T. aestivum*, A-PAGE spectra of gliadin could also be used to detect difference or identity of *T. tauschii* collections. A-PAGE spectra of the 38 *T. tauschii* accessions (Fig. 6) indicated that: 1) spectra polymorphism of gliadin was descending i.e., that was the Middle East the former USSR Xinjiang inland of China; 2) the seven accessions from Shaanxi and Henan shared a common spectra (lanes 1-7), Y309 (lane 8) and Y93 (lane 10) of Xinjiang were almost identical, Y287 (lane 23) from Armenia, Y170 and Y173 (lanes 29, 30) from Iran also shared an identical spectra.

Using enzymatic and nonenzymatic proteins, numerous investigations have focused on studying enzyme efficiency, genetic variability, gene flow, hybridization, species delineation, and phylogenetic relationships (Bietz, 1987; McIntyre, 1988; Phillips et al, 1993; Murphy et al, 1990; Sun and Corke, 1992; William and Mujeeb-Kazi, 1992). Phylogenetic relationships among accessions collected from the four different areas are clearly identified by spectra of Est-5, α-Amy-1, α-Amy-2, and β-Amy-1, especially by the former three kinds of isozymes. Probably because of the unimportant physiological function of gliadin for species survival and its multigene inheritance, most of its mutations were neutral and thus accumulable. This makes A-PAGE pattern of gliadin a finger-prints for cultivars and collections (Bietz, 1987; Draper, 1987; Zhang et al, 1994). On the other hand, A-PAGE spectra of gliadin may be too sensitive to be used in phylogenetic research between populations of a species. This view could be substantiated by direct comparison of gliadin spectra and isozyme spectra. It is also indirectly shown by the mean JD value from Est-5, α- and β-amylase, and A-PAGE spectra of gliadin of accessions. There were cases in which the average JD value was very low between a pair of accessions but their gliadin spectra were obviously different. In contrast, if two accessions had an identical spectra of gliadin, the mean JD value between them was always low (Fig.5, Fig. 6).

Chinese scientists have questioned the origin of *T. tauschii* in Shaanxi and Henan for many years. This paper clearly demonstrates that they are mainly uniform and most probably transmigrated from the Middle East to the inland of China via the Silk Road. They are closely related with Y309 (Xinjiang), Y168, Y172, and Y176 (all three from the Middle East) (Fig.5). Because *T. tauschii* usually grow as a weed in wheat fields of Shaanxi and Henan, it was spread in this area most probably by both birds and humans.

For transfer of desirable genes from alien species to cultived crops, it is important to avoid duplication of the same donors. The collections were divided into clusters based on genetic distance index from isozyme (Fig. 5). This makes it possible to select appropriate accessions to form a core collection. For example, the accessions from the inland of China (except Y305) may be used as one germplasm resource in gene transfer programs. One remaining question to be answered is the correlation between biochemical locus polymorphism and morphological diversity. Research is being carried out to shed insights on that question.

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Variation in Structure of Starch Granule-Bound Starch Synthase (*Wx* Protein) in Diploid, Polyploid Wheats and *Aegilops*

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ABSTRACT

The SDS-PAGE and the determination of N-terminal amino acid sequences of the waxy proteins in cereals were carried out. The similarity of the waxy proteins in cereals was high. The motif of VFVGAEMAP near the N-terminal appeared in common among wheats, *Aegilops*, rye, barley, rice and corn. Based on the analysis of the primary structure, the waxy proteins could be divided into two classes, one including wheats, *Aegilops* species, rye and barley, the other including rice and corn. In polyploid wheats, the waxy gene in each genome was expressed co-dominantly. The primary structure of the waxy protein in each genome in wheats was different. There occurred some point mutations in the waxy gene during the processes and after the establishment of polyploid wheats, proposed by cytogenetical studies.

INTRODUCTION

In cereal endosperm, the type of starch such as normal or glutinous is determined by the waxy gene, which is governed by the Mendelian segregation. The waxy gene encodes the NDP-glucose-starch glucosyltransterase which synthesizes amylose. This enzyme is a starch granule-bound type and also called the waxy protein. In the present study, we conducted the SDS-PAGE, determined the sequence of N-terminal amino acids of the waxy proteins in cereals, and compared the similarity of primary structure of waxy proteins in wheats and *Aegilops* species.

MATERIALS AND METHODS

Seeds used were *Triticum monococcum* KU105 (genomes AA), *T. durum* (cv. Stewart and var. *hordeiforme*, AABB)), *T. aestivum* (cv. Chinese Spring, cv. Norin 61, cv, Shirodaruma, and nullisomic-tetrasomic Chinese Spring, AABBDD), *Aegilops speltoides* (SS), *Ae. squarrosa* (DD), *Secale cereale* cv. Prolific (RR), *Hordeum vulgare* cv. Satsuki-nijo (HH), *Oryza sativa* (Japonica cv. Norin 19 (normal), cv. Iwai-mochi (glutinous) and Indica In108 (normal), AA), *O. glaberrima* WO440 (A^gA^g) and *Zea mays* cv. Koshu.

Starch granules were prepared from mature seeds according to Echt and Shwartz (1981). SDS-PAGE of the proteins bound to starch granules were performed according to Taira et al. (1991). The waxy proteins were detected by Western immunoblotting using the antiserum against the 59.5KD protein of *T. monococcum* which reacted with the antiserum against the waxy protein of normal rice (Taira et al., 1991). The determination of an amino acid sequence from the N-terminus of waxy proteins was conducted using Applied Biosystems 473A.

RESULTS AND DISCUSSION

The SDS-PAGE showed that the waxy proteins detected in this study had the molecular weights ranging from 58.1KD to 60.3KD. The glutinous rice had no band. All the waxy proteins extracted from wheats, *Aegilops* species, rye, barley, rice and corn reacted with the antiserum. To the antiserum against the waxy protein from *T. monococcum*, the reaction of waxy proteins was strong in wheats, *Aegilops* species, rye and barley, but weak in rice and corn.

When compared with the waxy protein of *T. monococcum*, the amino acid sequences from N-terminus to the residue of waxy proteins from *Ae. speltoides*, *Ae. squarrosa* and rye were identical. The homology was 94% in barley, 83% in rice and 72% in corn (Fig. 1). In barley and rice, the 5th residue was deleted. In rice, the 4th and

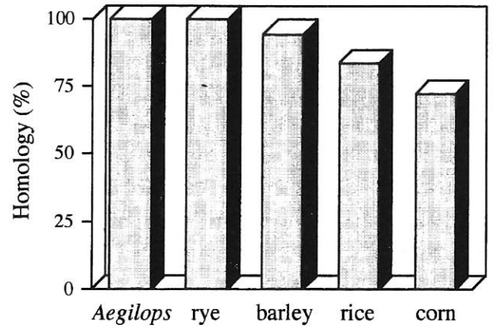

Fig. 1. Homology of the *Wx* proteins in the region from N-terminus to the 18th residue when compared with the amino acid sequence of *T. monococcum*.

the 9th residues were alanine and valine instead of serine and leucine, respectively. The waxy proteins in two ecotypes (Japonica and Indica) of *O. sativa* and *O. glaberrima* showed an identical sequence, that is, ATGAGMNVVFVGAEMAP. In corn (data in corn from Klösgen et al., 1986), the 2nd residue (threonine) was replaced by serine, the 3rd and 5th residues were deleted and the 4th and the 9th residues were the same as those of rice. All the waxy proteins from the cereals analyzed in this study had an identical motif of VFVGAEMAP from the 10th to the residue. Based on the amino acid sequences, the waxy proteins could be divided into two classes, one including wheats, *Aegilops* species, rye and barley, the other rice and corn.

In wheats and its ancestor species, SDS-PAGE of the waxy proteins showed that diploid species had one band and tetraploid and hexaploid did two bands (Fig. 2A). In

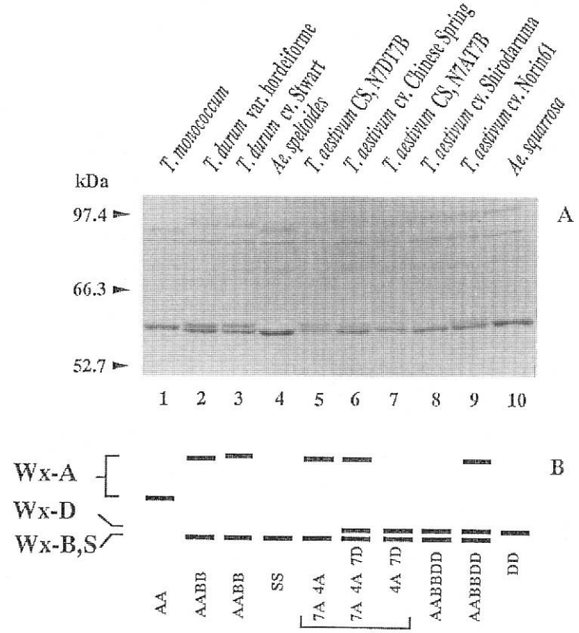

Fig. 2. Proteins separated by electrophoresis, which were bound to starch granules of endosperm in diploid, tetraploid and hexaploid wheats and *Aegilops* species.
A. SDS-PAGE pattern of proteins. **B.** A diagramatic representation of *Wx* proteins with molecular weight of ca. 60kDa.

tetraploid, the two bands had the same appearance, but in hexaploid, the lower band was more intense. Using nullisomic-tetrasomic Chinese Spring, the clearly separated two bands appeared in N7DT7B, whereas only one band was obtained in N7AT7B. On the other hand, the comparison with the band position of *Ae. speltoides* (genomes SS) and *Ae. squarrosa* (DD) on a gel showed that the lower band included two waxy proteins such as those encoded by the genomes of B and D. Figure 2B presented the relationship between the waxy proteins. We temporarily named the waxy proteins, that is *Wx*-A (encoded by the gene located on A genome), *Wx*-B (encoded by the gene located on B genome), *Wx*-S (encoded by the gene located on S genome) and *Wx*-D (encoded by the gene located on D genome). There was a difference of molecular weight between *Wx*-As, and *Wx*-A from *T. monococcum*, which had a smaller molecular weight for *Wx*-A than from the tetraploid and hexaploid wheats. The *Wx*-B and the *Wx*-S were identical. The *Wx*-D was slightly larger than the *Wx*-B and *Wx*-S. It is reported that the waxy genes were located on the homoeologous group 7 chromosome. However, since the gene located on chromosome 7B was translocated to chromosome 4A (Chao et al. 1989), the N7DT7B Chinese Spring showed two bands, *Wx*-A and *Wx*-B, whereas the N7AT7B exhibited two bands (visibly one band), *Wx*-B and *Wx*-D.

The determination of amino acid sequence from N-terminus revealed that *Wx*-A, *Wx*-B, *Wx*-S and *Wx*-D, except the *Wx*-B of hexaploid wheat, had exactly the same motif in the region from N-terminus to the residue. In *Wx*-B of hexaploid, the 5th residue, glycine, was substituted by alanine. Since hexaploid wheat was established by adding D genome to AB genomes (tetraploid wheat), the substitution in the 5th residue suggests that a point mutation occurred after the establishment of hexaploid wheat.

The partial digestion of waxy proteins by V8 protease provided a different band pattern on an SDS-PAGE gel. The bands showing a different molecular weight in the vicinity of 17KD were sequenced. The amino acid sequences of *Wx*-As of three species were identical in the region from N-terminus to the 20th residue, started with isoleucine. In *Wx*-B and D, valine in the 2nd residue was substituted by leucine. Additionally, valine in the 7th residue and lysine in the 16th residue were replaced by isoleucine and Wx-S. If *Ae. speltoides* is the donor of B genome in tetraploid wheats, some point mutations must have taken place during or after the establishment of tetraploid wheats.

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Isoenzyme Data on the Diploid Progenitors of Allotetraploid *Elymus* Species.

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ABSTRACT

Variation of alcohol dehydrogenase (ADH), aspartate aminotransferase (AAT), esterase (EST), and superoxide dismutase (SOD) isoenzymes has been studied by PAGE in five diploid *Pseudoroegneria* species *(aegilopoides, libanotica, inermis, spicata, and stipifolia)* and in several perennial H-genome *Hordeum* species *(brevisubulatum s.l., bogdanii, roshevitzii, jubatum, secalinum)* in comparison with those in allotetraploid *Elymus* species. The *Pseudoroegneria* and *Hordeum* species revealed a clear divergence by allozymes of AAT-B and SOD-B. The American and Eurasian SH genome *Elymus* allotetraploids are characterized by fixed heterozygosities of these isoenzymes with codominant expression of homoeozymes corresponding to allozymes of *Pseudoroegneria* and *Hordeum* species. The Asian SY genome *Elymus* species are mostly characterized by fixed heterozygosity of SOD-B with homoeozymes inherent to *Pseudoroegneria* species, suggesting that the *Elymus* S and Y genomes might have come from different *Pseudoroegneria* species. AAT-A and ADH-A displayed homologous variation with common morphs for *Elymus, Hordeum* and *Pseudoroegneria* species. Eurasian SH genome species are characterized by fixed heterozygosity of EST-A with one homoeozyme found in some *Pseudoroegneria* species, but the second homoeozyme not found among the *Hordeum* species.

INTRODUCTION

It has been found that most *Elymus (*Triticeae, Poaceae*)* species display fixed heterozygosity at several isoenzyme loci as characteristic of allopolyploid species with codominant expression of divergent homoeoallelic isoenzymes of composite genomes (Jaaska 1992). Cytogenetic studies of artificial intergeneric hybrids have provided evidence that *Elymus* species are allopolyploids with genomes S, H, P and Y derived from *Pseudoroegneria* (Nevski) A. Löve (genome S), *Hordeum* L. sect. *Stenostachys* Nevski (genome H), *Agropyron* Gaertn. s. str. (genome P), and unknown (genome Y) diploids (Dewey 1980, Jensen 1989, 1990, Lu and Bothmer 1990, a.o.). The goal of the present study was to investigate isoenzyme variation among a set of *Pseudoroegneria* and *Hordeum* sect. *Stenostachys* species in order to assess their suitability as putative genome donors of allotetraploid *Elymus* species of SH and SY genome groups.

MATERIALS AND METHODS

The seed accessions were mostly received from the USDA ARS Living Collection of Perennial Grasses in Logan (Utah, USA). Some were collected by the author in nature. The list of species and the number of accessions analyzed are given in Table 1.

Enzyme extracts for isoenzyme analyses were prepared from the shoots of etiolated seedlings 6-10 days old, immediately subjected to electrophoresis in vertical polyacrylamide gel slabs and then stained in histochemical reaction mixtures as described in recent papers (Jaaska & Jaaska 1986, 1990). The isoenzyme nomenclature distinguishing genetically heterologous, homo-eologous and homologous isoenzymes, named heterozymes, homoeozymes and allozymes, respectively, is followed (Jaaska and Jaaska 1984). Heterozymes, i.e. isoenzymes encoded by separate gene loci of a diploid genome, are designated by capital letters followed by a number, designating basic allozymic and homoeozymic electromorphs in the order of their decreasing mobilities. Additional morphs, differing from the basic morphs in small mobility shifts, are further specified by adding letters f (fast) or s (slow).

RESULTS AND DISCUSSION

The data on the electrophoretic variability of aliphatic alcohol dehydrogenase (ADH), aspartate aminotransferase (AAT), esterase (EST), and superoxide dismutase (SOD) diagnostic heterozymes in a set of Eurasian *Hordeum* sect. *Stenostachys* and *Pseudoroegneria* species studied are presented in Table 1.

Among the Eurasian *Stenostachys* barleys, the outcrossing *H. brevisubulatum* s.l. revealed extensive

Table 1. Electrophoretic phenotypes of alcohol dehydrogenase (ADH), aspartate aminotransferase (AAT), esterase (EST), and superoxide dismutase (SOD) isoenzymes in *Hordeum* sect. *Stenostachys* and *Pseudoroegneria* species (r - a rare morph; f or s - fast or slow variants of the numeric, basic morph; fixed heterozygosity is designated as a fraction of codominant morphs; N - number of accessions analyzed)

Species	N	ADH-A	AAT-B	AAT-C	EST-A	SOD-B
Hordeum sect. *Stenostachys*						
H.brevisubulatum s.l.	12	2;4r	5;6r;3r 4r;4fr	4;3r 5r;2r	2;2f;2s 3;1;1f;1s	3
H. bogdanii	11	2	5	4	2	3
H. roshevitzii	2	2	5	4	2	3
H. jubatum	4	2/4	5/6	4	2	3
H. secalinum	4	2	5/6	4	2	3
Pseudoroegneria						
P. aegilopoides	3	4	4	4	2s	4;5;2
P. libanotica	3	4;2	4	4;3r;5r	2s;3s;1r	4
P. spicata s.l.	5	4;2;3r 4sr;1r	4;3sr	4;2r;5r	2s;3s;3r 4;4f;1s	4;5;2
P. stipifolia	3	4;2	4	4	2s;3;3s;4f	4

intrapopulational polymorphism of four heterozymes with 2-7 frequent or rare allozymes. In a sharp contrast, the two autogamous species, *H. bogdanii* and *H. roshevitzii*, were identical and monomorphic for allozymes also found in *H. brevisubulatum* and in the two alloploids, *H. jubatum* and *H. secalinum*. It is remarkable that the East-Asian *H. jubatum* and the West-European *H. secalinum* showed fixed heterozygosity of AAT-B with a common triplet phenotype, combining codominant homoeozymes B5 and B6 which were found in the Asian *H. brevisubulatum* as a frequent and a rare allozyme, respectively. *H. jubatum* had an additional fixed heterozygosity of ADH-A with a triplet phenotype, combining homoeozymes A2 and A4 which were observed in *H. brevisubulatum* as a frequent and a rare allozyme, respectively. Rare allozymes are specified as those which were encountered only in heterozygous phenotypes with frequent allozymes in single individuals.

EST-A was the most variable heterozyme in *H. brevisubulatum* with seven moderately frequent allozymes, segregating among individuals as homozygous one-banded and heterozygous triplet phenotypes of a dimeric enzyme. In a sharp contrast, the four other species proved monomorphic for EST-A2. *H. brevisubulatum* displayed polymorphism of AAT-B and AAT-C with one frequent allozyme and 3-4 rare morphs. The frequent allozymes of AAT-B and AAT-C of *H. brevisubulatum*, B5 and C4, were monomorphic in the other autogamous species studied.

Pseudoroegneria species displayed homologous polymorphism of all five heterozymes, with EST-A and AAT-B as the most and less variable, respectively. ADH-A4, AAT-B4, AAT-C4, EST-A2s, and SOD-B4 were the most frequent allozymes, common to all *Pseudoroegneria* species which differed from each other only in the occurrence and frequency of other, less frequent and rare allozymes.

It follows from the data in Table 1 that the *Hordeum* and *Pseudoroegneria* species display homologous variation of ADH-A and AAT-C with common allozymes A2, A4, C4, C2r, C3r, and C5r, while EST-A had some common and some divergent allozymes. The two genera differed distinctly in allozymes of SOD-B and revealed divergence by the most frequent allozyme of ADH-A, AAT-B and EST-A. SOD-B was monomorphic for B3 shared by all the five barley species, whereas B4 was most frequent for *Pseudoroegneria* species, followed by B5 and B2. *Pseudoroegneria* species shared AAT-B4, which was a rare morph in *H. brevisubulatum*, while the *Hordeum* species shared AAT-B5 as the most frequent and specific morph. The most characteristic for the *Stenostachys* barleys were ADH-A2 and EST-A2, whereas the *Pseudoroegneria* species shared ADH-A4 and EST-A2s as the most frequent morphs.

The data on the variation of the same five diagnostic heterozymes among a set of *Elymus* allotetraploids are selected for comparison from Jaaska (1992) in Table 2 with corrections for SOD-B. Comparison of the data in Tables 1 and 2 shows a good agreement with the results of cytogenetic studies on the involvement of *Hordeum* sect. *Stenostachys* and *Pseudoroegneria* species in the origin of *Elymus* allotetraploids and with their belonging to the SH- and SY-genome groups. Most convincing in this respect are the data for SOD-B. It may be seen that both Eurasian and North-American *Elymus* SH-genome allotetraploids share fixed heterozygosity of SOD-B with homoeozymes B3 and B5 which are allozymes characteristic of *Hordeum* (H-genome) and *Pseudoroegneria* (S-genome) species, respectively. The East-Asian *Elymus* allotetraploids of the SY-genome group share a different fixed heterozygosity of SOD-B with homoeozymes B4 and B5, which were both found in two *Pseudoroegneria* species. Previous data (Jaaska 1992) on the occurrence of SOD-B3/5 heterozygosity in some SY-genome species proved erroneous due to confusing B4 for B3 on a gel slab zymogram.

The EST-A data allowed the distinction of the Eurasian SH-genome *Elymus* species from the North American SH-genome species (Jaaska 1992, Table 2). Both shared various variants of EST-A3, but differed in the second

Table 2. Electrophoretic phenotypes of ADH-A, AAT-B, AAT-C, EST-A and SOD-B in tetraploid *Elymus* species (data from Jaaska 1992, corrected for SOD-B; designations unified with Table 1)

Species	N	ADH-A	AAT-B	AAT-C	EST-A	SOD-B
Eurasian SH-genome group						
E. sibiricus	7	2/4	4	4	5/3s	3/5
E. caninus	4	2	4/5	4	5/3s	3/5
E. mutabilis s.l.	4	2/4	5	4	5/3s	3/5
North-American SH-genome group						
E. canadensis	3	2/4	5	4	2/3s;3s	3/5
E. glaucus	3	2/4	4;4/5 5/6	4/3s 4s/3s	2/3;3;2	3/5
E. trachycaulus	3	2	4/5	4	2/3	3/5
East-Asian SY-genome group						
E. abolinii	6	4	4	4/3	2	4/5
E. ciliaris	5	4;4/3	4;4/3	4/3;4	2	4/5
E. gmelinii s.l.	4	4	4	4/3	2s/3	4/5
E. strictus	2	4/6	4	4/3	2s/3	4/5
E. barbicallus	1	4/6	4	4/3	2/3	4/5
West-Asian SY-genome group						
E. caucasicus	1	4/1	4	3	1	4
E. longearistatus	3	4	4	4/3	1	4
E. panormitanus	3	4	4	1	1	4

homoeozyme that was A2 in the North-American species and A5 in all Eurasian SH-species. From Table 1 it follows that EST-A3 variants are distributed in some *Pseudoroegneria* species and that EST-A2 is characteristic of the *Stenostachys* H-genome, but EST-A5 has not been found among the species and accessions analysed.

Many SH-genome species displayed fixed heterozygosity of ADH-A, combining homoeozymes A2 and A4 which were observed as the most frequent allozymes in *Hordeum* and *Pseudoroegneria* species, respectively. However, two SH-genome species proved homozygous for A2 and several SY-genome species homozygous for A4 (Table 2). The two SY-genome species, *E. strictus* and *E. barbicallus*, combine A4 common in *Pseudoroegneria* with a unique A6 absent in diploids.

Most SY-genome species were homozygous for AAT-B4 characteristic of *Pseudoroegneria* species. The SH-genome species either were heterozygous for AAT-B4/5 or were homozygous for B4 or B5, which were the most characteristic allozymes of *Pseudoroegneria* and *Hordeum* species, respectively. Homozygosity of all analysed accessions of *E. mutabilis* and *E. canadensis* for AAT-B5 characteristic of the H-genome indicates silencing of the S-genome homoeozyme B4 in these species. The intraspecific polymorphism of AAT-B and AAT-C observed in *E. glaucus* may reflect either polyphyletic (vs. polytopic) alloploid origin of this species from different S- and H-genome donors or mutational changes in the homoeoloci at the tetraploid level.

AAT-B and AAT-C differ distinctly in the extent of fixed heterozygosity among the East-Asian SY-genome species: predominant homozygosity of AAT-B for B4 characteristic of *Pseudoroegneria* is contrasted with heterozygosity of AAT-C for homoeozymes C4 and C3 which were recorded as a frequent and a rare allozyme in *P. libanoticum* and *H. brevisubulatum*. Two accessions of *E. ciliaris*, one from the Russian Far-East and the second of Japanese origin, proved exceptional in this respect having heterozygous AAT-B4/3 and homozygous AAT-C4. Two other *E. ciliaris* accessions of Chinese origin had AAT-B4 and AAT-C4/3 as most other SH-genome species. The observed intraspecific polymorphism of AAT-B and AAT-C in *E. ciliaris* may indicate either independent alloploid origin or homoeozyme silencing in these geographic regions.

The three *Elymus* species of West-Asian distribution, *E. caucasicus*, *E. longearistatus and E. panormitanus*, differ from the other *Elymus* allotetraploids in a low level of fixed heterozygosity, suggesting their possible autoploid origin from intra-specific hybrids or alloploid origin from closely related *Pseudoroegneria* species. Previously (Jaaska 1992), these species were placed in a separate group of unknown genome composition. Recent cytogenetic studies have shown (Jensen and Hatch 1988, Jensen and Wang 1991, Lu and Bothmer 1993) that the three species are allotetraploids sharing divergent SY-genomes. Homozygosity of ADH-A, AAT-B and SOD-B in these species for common allozymes of *Pseudoroegneria* species may indicate that S and Y genomes are closely related and could be derived from different *Pseudoroegneria* species. The data on the shared homozygosity for EST-A1 in the three species is especially remarkable in this respect in view of extensive polymorphism of EST-A in *Pseudroegneria* species and rarity of EST-A1. At the same time, the data on different AAT-C phenotypes in the three species indicate their independent origin from different diploid progenitors. Thus, the accessions of *E. longearistatus* shared fixed heterozygosity for AAT-C4/3, combining a frequent and rare allozymes of *Pseudoroegneria* species and resembling in this respect the East-Asian SY-genome species. The accessions of *E. panormitanus* of Turkish and Iraqi origin, however, proved homozygous for a unique AAT-C1 which was not encountered among the sample of *Pseudoroegneria* species and accessions studied. *E. caucasicus* was homozygous for AAT-C3, a rare allozyme of *P. libanotica*, and heterozygous for a unique phenotype of

ADH-A, combining a frequent and a rare allozyme of *Pseudoroegneria*.

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N-terminal Sequences of Gliadins of *Aegilops*: on the Origin of Polyploid Wheat Genomes

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ABSTRACT

By gel filtration and high-performance liquid chromatography the major components of gliadins were isolated from seeds of the diploid species *Aegilops squarrosa* and *Aegilops longissima*, the possible donors of D and B genomes of polyploid wheat. The proteins were characterized according to electrophoretic mobility in polyacrylamide gel in an acid Al-lactate system, as well as in a system containing SDS. The N-terminal amino acid sequences of the gliadins were established. Three types of N-terminal amino acid sequences were detected in the ω-gliadins of *Ae. squarrosa*: one of them, SRQ, was found in *Ae. longissima* and hexaploid wheat. It is supposed that this type of sequence is specific to the whole *Aegilops* genus. The obtained data are discussed in terms of the origin of polyploid wheat genomes.

INTRODUCTION

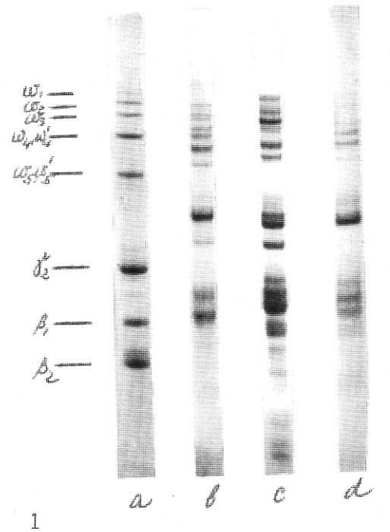
Polyploid wheats *Triticum turgidum* L. var. *durum* Dest and *Triticum aestivum* L. var. *aestivum* are allopolyploids with the genomic composition AABB and AABBDD, respectively. Numerous data support the fact that *Aegilops* species were the progenitors of the B and D genomes of polyploid wheats [1-3]. *Ae. bicornis, Ae. longissima, Ae. searsii* and *Ae. speltoides* are considered the presumed donors of the B genome [2] and *Ae. squarrosa* that of the D genome [4-7]. The D genome is very important; it confers high quality to hexaploid wheat grain and explains wide spread distribution of wheat as a major component of human and animal diet [8]. Gliadins are a group of polymorphic alcohol-soluble proteins, encoded in hexaploid wheat by gene families located on chromosomes of homoeologous groups 1 and 6. Although wheat gliadin structure has been studied intensively, the data concerning amino acid sequence of *Aegilops* species are limited. In this communication we report isolation and characterization of the gliadin N-terminal sequences of *Ae. squarrosa* and *Ae. longissima*.

MATERIALS AND METHODS

Samples of *Aegilops squarrosa* and *Aegilops longissima* (K-202, D-907, K-908, K-1297) from the collection of All-Russian Institute of Plant Industry were used for this study. Gliadins were extracted from flour as described [9]. Gliadin fractionation was performed by HPLC in an Acquapore RP-300 reversed-phase column (4.6 x 220) (Brownlee Labs, USA) with a precolumn (4.6 x 30). Proteins were eluted with an acetonitrile gradient (10-45%) at a rate 0.8 ml/min (Buffer A: 0.1% trifluoroacetic acid). Electrophoretic analysis of gliadins was conducted in PAAG in an Al-lactate buffer system at pH 3.1 [9]. The N-terminal amino acid sequencing was performed on a gas-phase protein and peptide sequenator (model 470 A) with automatic identification of PTG-amino acids on an PTG-analyzer (model 120 A) from Applied Biosystems (USA).

RESULTS AND DISCUSSION

Electrophoretic analysis of *Ae. squarrosa* gliadin components revealed 17 components in contrast to 25 in Bezostaya 1, used as standard (Fig. 1). It should be noted that no components were observed in α-zone of electrophoretic spectrum. This type of electrophoretic pattern is typical for the *tauschii* subspecies, while in *strangulata* subspecies 3 components are clearly distinguished in α-zone with one intensively stained component. Two-dimensional electrophoresis of *Ae. squarrosa* (data not shown) ω-gliadins revealed 2 groups of components with similar molecular masses but different charge. The first group includes components with molecular mass 63 kDa. The second group includes components with molecular mass 52 kDa. The gliadins of *Ae. longissima* can also be arbitrarily divided into four zones:



1

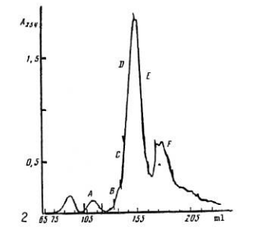
Fig 1. Electrophoretic separation of gliadins in 8% polyacrylamide gel (pH 3,1) a) *Ae. squarrosa,* b) *Ae. longissima* K-202, c) *Ae. longissima* K-908, d) *Ae. longissima* K-1297

Fig 2. Gel-filtration of alcohol-soluble proteins of *Ae. squarrosa* on a Sephacryl S-200 column

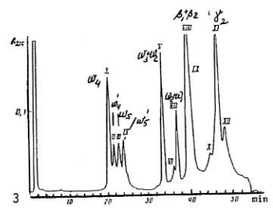
Fig 3. HPLC of alcohol-soluble proteins of *Ae. squarrosa* on a ProRPC 5/10 column (10 - 45%). The gradient of buffer B in buffer A is indicated in parentheses.

Fig 4. HPLC of alcohol-soluble proteins of *Ae. longissima* on an Acquapore RP-300 column with a linear acetonitrile gradient (10 - 45%). Fractions subjected to sequencing are marked by numbers: a) K-202; b) K-907; c) K-908; d) K-1297

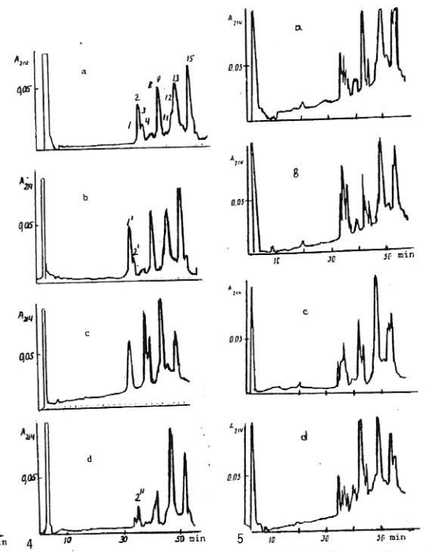
Fig 5. HPLC of a mixture of proteins of an alcohol extract of *Ae. longissima* and *Ae. squarrosa* on an Acquapore RP-300 column. Conditions of separation the same as in Fig. 2. a) *Ae. squarrosa* + K-202, b) the same + K-907, c) the same + K-908, d) the same + K-1297



2



3



4

5

α, β, γ, and ω (Fig. 1). Just as in *Ae. squarrosa*, in *Ae. longissima* there are virtually no gliadins in the α-zone of the spectrum. However, the ω-zone, in contrast to *Ae. squarrosa*, has substantially more components, with a number of proteins from four in *Ae. longissima* K-1297 up to nine in K-908. On the whole, in the gliadin fraction of *Ae. longissima* there are more electrophoretically distinguished components than in *Ae. squarrosa*, chiefly because of the proteins of the ω- and β-zones of the spectrum.

To fractionate alcohol-soluble proteins of *Aegilops squarrosa* we used gel-filtration on Sephacryl S-200. Chromatographic separation is presented in Fig. 2. Electrophoretic analysis showed that fractions A, B and C contained ω-gliadins, D-γ-gliadins and E-β-gliadins, F-α-gliadins and rapidly moving components of the alcohol extract. The fractions (A-D) were further separated by HPLC. HPLC separation in a reversed-phase column is shown in Fig. 3. Electrophoretic analysis demonstrated that fractions I-VII contained ω-gliadins, fractions VIII-IX corresponded to β-gliadins, while fractions X-XII corresponded to γ-gliadins. ω-gliadins were the first gliadin components eluted from the column and practically all of the components are isolated in pure form.

β-gliadins were eluted from the column in the form of one nonsymmetrical peak (denoted on the chromatogram

by peaks VIII and IX). For a better separation of this protein fraction, HPLC of the enriched fraction E, obtained by gel filtration, through Sephacryl S-200 was performed, using the "gentler" acetonitrile gradient. The main component of the γ-gliadins, as shown by electrophoretic analysis, was eluted from the column with reversed phase in a pure form, suitable for further investigation. The chromatographic patterns of gliadins of various samples of *Ae. longissima* are presented in Fig. 4. Chromatographic analysis, like electrophoresis, permitted us to detect considerable intraspecies heterogeneity of *Ae. longissima*. The high specificity of chromatographic profiles on reversed-phase columns permits us to use this method together with electrophoresis for the study of intraspecies variability of *Ae. longissima* and characterization of samples of different origin, which is already being used successfully for the identification of varieties and biotypes of durum and bread wheats [10, 11]. As with *Ae. squarrosa*, ω-gliadins of *Ae. longissima* are eluted from a reversed-phase column in the form of two groups of components: the first contains the ω-gliadins most mobile at pH 3,1, while the second contains the least mobile ones. To determine how similar the gliadins of *Ae. longissima* are in hydrophobic properties to the proteins of *Ae. squarrosa*, chromatography of a mixture of the proteins of both species was conducted (Fig. 5). An analysis of the chromatogram of mixtures of proteins provides evidence that some gliadins of *Ae. longissima* and *Ae. squarrosa* coincide in time of emergence from the column, while others differ. These data, together with the results of electrophoretic analysis, indicate that homologous gliadins of *Ae. longissima* and *Ae. squarrosa* differ somewhat in primary structure.

Gliadin fractions of *Aegilops* species obtained by HPLC chromatography were subjected to sequencing. The determination of the primary structure of gliadins is rather complicated. First of all, it is due to difficulties encountered in obtaining sufficient amounts of highly purified components. The use of HPLC permitted us to overcome this difficulty. The procedure for purification of individual components was substantially simplified. Moreover, the proteins isolated by HPLC give a low background upon sequencing, which facilitates the interpretation of the obtained results. Another problem in the sequencing of gliadins lies in the monotonous structure of these proteins, extremely rich in glutamine and proline. The combination of HPLC with the use of a highly sensitive gas phase sequencer allowed us to determine the N-terminal amino acid sequences of the major components of *Ae. squarrosa* and *Ae. longissima*.

As noted above, ω_1 and ω_2 of *Ae. squarrosa* could not be purified by reversed-phase HPLC, so during sequencing more than one amino acid was detected. However by comparing the obtained results with the known N-terminal sequences [12] we were able to distinguish the sequence beginning with ARQ. This type of sequence was also detected in *T. monococcum* [12]. Moreover, the investigated mixture probably contained a component with the sequence XXLPSPQQXX (were X is an unidentified amino acid). An analogous sequence was detected by Kasarda et al. [12] for the least mobile ω-gliadins of *Ae. squarrosa*. In addition to the amino acids that could be related to the known types of sequences, certain others, which did not fit into the structural variants described, were also detected. It was found that two ω-gliadins of *Ae. squarrosa*, ω_4 and ω_5, have the same N-terminal sequence, beginning with SRQ. This sequence is highly homologous to the N-terminal sequences of certain ω-gliadins of bread wheat [9- 12]. Several amino acid substitutions were observed, which might occur due to mutation of a single nucleotide in the codon. It is important to note that SRL-type of sequence in hexaploid wheats is encoded by chromosome 1B [11, 13]. Of the gliadins of *Ae. longissima* K-202, fractions 1-4, noted on the chromatogram (Fig. 2) were sequenced. Three of them (fractions 1, 3 and 4) coincided in mobility in the column with components I-IV of *Ae. squarrosa* (Fig. 5). In *Ae. squarrosa*, the proteins eluted in fractions II and IV have blocked N-terminal amino acids. In *Ae. longissima* K-202, the ω-gliadin of fraction 4 proved to be blocked. Components of fractions 1-3 had a virtually identical N-terminal sequence, beginning with SRQ. Only one substitution in position 7 was detected, where isoleucine is replaced by arginine in the component of fraction 3. Arginine has also been detected in position of the polypeptide chain in the ω-gliadin of *Ae. squarrosa*. Sequencing of fraction 1' and 2' of *Ae. longissima* K-908 showed that they also possesed the SRQ-type of N-terminal sequence and differ only in the amino acid in the forth position instead of leucine. The ω-gliadin of fraction 2'' of *Ae. longissima* K-1297 is likely to be blocked. It is noteworthy that this component coincides in position on the chromatogram with the ω-gliadin of *Ae. squarrosa* with and unblocked end.

Summarizing the results of the sequencing of ω-gliadins of *Aegilops*, we can conclude that the gliadins with the SRQ type of N-terminal sequence have been detected among the ω-fraction in all the samples of *Ae. longissima* analyzed, as well as in *Ae. squarrosa* and hexaploid wheats. Moreover, it has been established that certain ω-gliadins possess blocked N-terminal amino acids.

Sequencing of the major component of the γ-fraction of *Ae. squarrosa*, contained in fraction IX from the reversed-phase column, showed that it corresponds to the γ_2-sequence detected in *T. aestivum*. The β-fraction was sequenced both without separation into individual components and after separation by HPLC. In both cases only one type of N-terminal sequence, corresponding to the α-type of gliadins, was detected [14]. β-gliadin of *Ae. longissima* K-202 appeared identical to β-gliadin of *Ae. squarrosa* and bread wheats. Although data on the complete primary structure of ω-gliadins have not yet been obtained, in all probability, substantially more rigid limitations were imposed on the structure of gliadins of the

4 β type in the course of evolution than on the structure of ω-gliadins.

In summary, our analysis of the N-terminal amino acid sequences of *Ae. squarrosa* and *Ae. longissima* ω-gliadins permitted us to detect the SRL-type of sequence found in bread wheat, where it is encoded by chromosome 1B. This result is evidence in support of the hypothesis of possible involvement of *Ae. longissima* in the origin of the B genome of polyploid wheats, which is in accord with the data of other workers. The occurrence of the SRL-type sequence in *Ae. squarrosa*, the presumed donor of the D genome, indicates that this type of sequence is evidently characteristic of the entire genus *Aegilops*. It might have appeared simultaneously with the divergence of the genera *Triticum* and *Aegilops* and have entered the genus *Aegilops*, and with it been incorporated into the polyploid wheats. Further analysis of the N-terminal sequences of ω-gliadins of other *Aegilops*, potential donors of the B genome, will make it possible to confirm this hypothesis.

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Polymorphisms of Monomeric Prolamins in *Dasypyrum villosum* (L.) Candargy

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ABSTRACT

The prolamin storage proteins of caryopses from five populations taken from natural stands of *Dasypyrum villosum* in Italy were fractionated using polyacrylamide-gel electrophoresis at pH 3.1. A total of 80 bands of monomeric prolamins were grouped according to their comigration distance with the α-, β-, γ-, and ω-type prolamins (gliadins) of wheat. In each population all four groups of monomeric prolamins were present. Every group of bands of monomeric prolamins showed an asymmetrical continuous frequency distribution. Differentiation in modal frequency was observed for specific bands within and among the five populations. A high level of within population allelic polymorphism was detected for the three gene families at the Gli-V1, Gli-V2, and Gli-V3 loci which control the expression of the analyzed monomeric prplamins

INTRODUCTION

Dasypyrum villosum belongs to the secondary gene pool of wheat, and *D. villosum* x *Triticum* F_1 plants have been obtained with (Jan *et al.*, 1986) or without embryo culture (Tschermak, 1929; Strampelli, 1932; Sando, 1935; Stefani *et al.*, 1983; Bothmer and Classon, 1990). Addition lines of *D. villosum* chromosomes in a common wheat genomic background are available (Sears, 1953; Hyde, 1953; Blanco *et al.*, 1987). Therefore, *D. villosum* is a genetic resource for wheat improvement (Qualset *et al.*, 1993). Among the potentially useful traits that *D. villosum* genes can confer to wheat, those related to the amount and or composition of seed storage proteins and disease resistance are the best candidates. Among *D. villosum* populations the seed protein content ranges from 16.1 to 24.6% (Vapa *et al.*, 1993) and the same range of variability for protein content has been found within populations (Della Gatta *et al.*, 1984). The genetic basis of the majority of seed storage protein components is well known in wheat but not for *D. villosum*. The high-molecular-weight glutenins are controlled by a multiallelic locus (Glu-V1) on chromosome 1V of *D. villosum* (Zhong and Qualset, 1993; Montebove *et al.*, 1987; Blanco *et al.*, 1991). Apparently that locus is orthologous to the Glu-A1, Glu-B1, and Glu-D1 loci of wheat and the subunits coded by these loci (in particular those coded by Glu-B1) have molecular weights similar to those coded by Glu-V1.

Gliadin-like storage proteins are coded by genes on chromosomes 1V, 4V, and 6V (Montebove et al., 1987). Blanco *et al.* (1991) and Shewry *et al.* (1987, 1991) confimed the 4V and 6V locations. The loci coding for seed storage proteins located on chromosomes 1V and 6V are apparently orthologous to the Gli-1 and Gli-2 loci in wheat and are designated Gli-V1 and Gli-V2, respectively. The locus Gli-V3 on chromosome 4V has no equivalent to a locus on wheat chromosome group 4. Blanco *et al.* (1991) and Shewry *et al.* (1991) hypothesized that this locus may be the result of a translocation between chromosomes 4V and 6V. Subsequent divergence of amino acid sequences in the monomeric proteins coded by 4V and 6V gliadin loci has been hypothesized (Shewry *et al.*, 1991). As the storage proteins have a direct bearing on the rheological properties of flour dough, polymorphisms in *D. villosum* at these loci may provide useful alleles for modifying wheat end-use quality. Polymorphisms for high-molecular-weight glutenins have been reported by Zhong and Qualset (1993). Population variability in monomeric prolamins has not been studied; therefore, in this paper an analysis of polymorphisms occurring for protein monomers contained in the alcohol soluble fraction of *D. villosum* seed storage proteins is reported.

MATERIALS AND METHODS

Plant materials. Plants collected at five locations in Italy were used. The group of plants collected at a site were considered to represent a population. Since *D. villosum* is an outcrosser, the offspring of each collected plant is a half-sib (HS) family. The five sampled populations and the

number of families and half-sibs assayed in total from each population were: I-16a (10, 89), I-136 (8, 111), I-50 (5, 63), I-85 (7, 73), and I-120 (9, 119). The proportion of plants sampled from each population was about the same (30%); therefore, the variation in sample number was a reflection of a parallel variation in population size in the native stand. Populations are indicated according to latitude of the collection sites from north to south in a range of 800 km. Geographical coordinate and edaphic data on the collection site are reported in De Pace (1987).

Electrophoretic analysis

Monomeric alcohol-soluble storage proteins have been extracted and electrophoresed at pH 3.1 as described in Montebove *et al*. (1987). Each of the monomeric proteins appeared as bands in the electrophoregram visualized after immersion of the gel in Coomassie Brillant Blue R 250-ethanol solution. Each band (=monomeric prolamin) was numbered according to its migration distance relative to that of reference bands in the gliadin pattern of the bread wheat cultivar Marquis (see Fig. 1). The migration distance of the third anodal band and the last cathodal band in the Marquis electropherogram was used to mark the extremes of 80 equally spaced migration-length-units; the units were progressively numbered from 0 to 80 (Fig. 1). Each unit was considered as a location on the gel where the monomeric storage proteins in the *D. villosum* electrophoregrams of each analyzed individual can occur. This standardization was made on each gel which allowed an easy identification of homologous bands in the patterns of different individuals in the same or different gels.

Using the gliadin pattern of bread wheat cvs. Chinese Spring and Marquis as reference, the 80 migration-length-units have been assigned to four groups according to the occurrence in each group of bands attributed to the α, β, γ, and ω classes of wheat gliadins. Therefore, the *D. villosum* monomeric prolamins have been numbered according to this classification. Bands 0 to 11 were assigned to the ω class, bands 12 to 30 to the γ class, bands 31 to-58 to the β class, and bands 59 to 80 to the α class.

Data analysis

The frequency of the band occurring at the k^{th} position at each of the 80 standardized positions on the gel (= gel location) was calculated for each population. The relative frequency f_{ik} of band k in population i was defined as n_{ik}/n_i, where n_{ik} is the number of HS individuals showing the k^{th} band and n_i is the total number of HS individuals analyzed for the k^{th} band. Log-linear and general linear models applied to the relative frequencies showed significant between-population and population x gel location interaction variance preventing a between-population comparison over all gel locations. Therefore, the Walker-Duncan K-ratio test was applied to examine statistical differences in ik values in a pairwise comparison between populations at each k band position. This test does not require a significant F-test of population and population x gel location interaction variances as a prerequisite. Populations whose band frequencies f_{ik} did not differ statistically within a certain gel position were considered similar at that position. A similarity index I_{xy} over the 80 positions was calculated for each pair of populations. I_{xy} was defined as the fraction of positions within which two populations x and y did not statistically differ in band frequency and was estimated as $2M_{xy}/(M_x + M_y)$, where M_{xy} was the number of band positions for which populations x and y showed similar frequencies and M_x and M_y were the number of bands in each population. Dendrograms based on the dissimilarity index (=1-I) over all bands or within the α, β, γ, and ω classes of bands were constructed using the UPGMA clustering method and the NTSYS software package (Rohlf, 1987)

RESULTS AND DISCUSSION

The electrophoregrams of monomeric prolamins extracted from individuals of one HS family from each *D. villosum* population are presented in Fig. 1 as an example of the variability found for prolamin bands at each gel position. Variation (presence or absence of prolamin bands) was detected at each of the 80 locations. Each gliadin class was represented in the five analyzed populations (Table 1). The frequencies of the omega-class bands were highest among the four gliadin classes and contributed 12 to 17% of all of the bands in each population. The gel locations with few *D. villosum* monomeric prolamins are 28 to 33 and 48 to 58; the location of bands 28 to 33 correspond to the gel zone that separate ω + γ classes of prolamins from β class (Fig. 2). The positions of bands 48 to 58 separate β from α prolamins. The gel locations with the highest frequencies of *D. villosum* monomeric prolamins are those found between the reference bands 5 to 8, 9 to 12, 33 to 36, 45 to 48, and 65 to 68 (Table 2). These prolamins occur almost in the middle of the α, β, γ, and ω zones. However, the modal

Table 1. Proportion (%) of monomeric components (bands) in each prolamin class

Prolamin	Population				
Component	I-16a	I-136	I-50	I-85	I-120
ω	12.3	11.8	11.8	17.3	11.8
γ	41.8	40.7	40.9	38.8	39.8
β	23.4	21.7	20.8	21.6	24.6
α	22.5	25.8	26.5	22.3	23.8

Table 2. Groups of monomeric components (bands) that occurred at highest frequency (%) in each prolamin class.

Prolamin	Population									
	I-16a		I-136		I-50		I-85		I-120	
Component	Bands	%	Bands	%	Bands	%	Bands	%	Bands	%
ω	5-8	10.7	5-8	8.1	5-8	9.2	5-8	13.6	5-8	9.3
γ	9-12	9.5	13-16	11.0	13-16	9.4	13-16	10.7	9-12	8.1
β	33-36	5.9	37-40	6.7	37-40	5.7	33-36	7.1	33-36	8.4
	45-48	6.1	45-48	7.9	--	--	45-48	5.9	45-48	5.2
α	--	--	--	--	57-60	3.8	--	--	--	--
	65-68	8.3	69-72	7.6	65-68	7.8	65-68	6.5	65-68	5.9

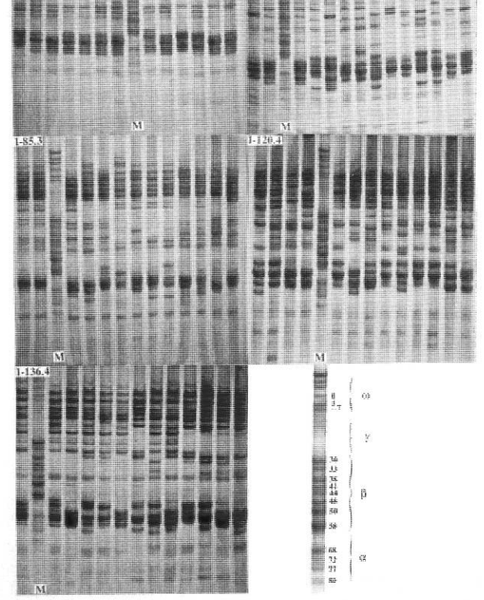

Figure 1. Electrophoretic patterns of monomeric prolaminsin half-sib families from five *D. villosum* collected in Italy. M is the reference bread wheat cultivar Marquis; band positions 0 to 80 and α, β, γ, and ω gliadin classes are indicated on the M reference lane (lower right).

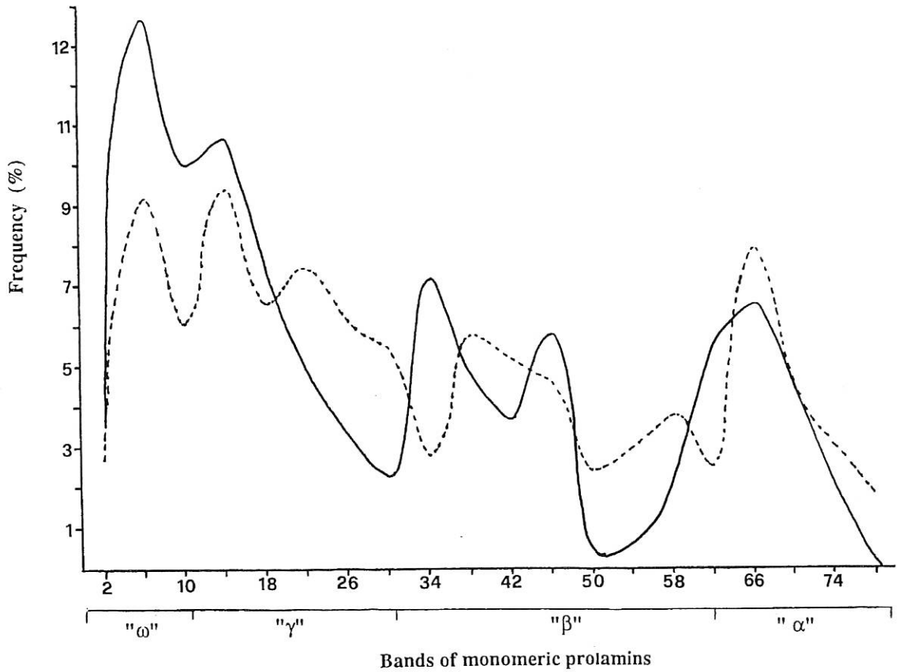

Figure 2. Comparison of band frequencies at positions 2 through 78 for populations I-50 (----) and I-85 (—), two populations showing highest coefficient of dissimilarity.

bands are different between some of the populations (Table 2).

The index of dissimilarity based on K-ratio test of frequency differences of the prolamin bands at each of the 80 positions or at the position of α, β, γ, and ω class zones were calculated for all possible paired comparisons between the five populations (data not shown). Dendrograms, based on the index of dissimilarity, (Fig. 3) showed that the most divergent populations for each class of monomeric prolamins were I-50 and I-85, while populations I-120 and I-16a were less divergent. Therefore, sampling of HS progenies from populations I-50 and I-85 would give the opportunity to detect almost all of the monomeric prolamins occurring in the five *D. villosum* populations.

The populations studied do not show a relationship between greater geographic distance between the collection sites and diversity in frequencies of bands of monomeric prolamins. In fact the opposite was observed.The dissimilar populations, I-50 and I-85, were nearest to each other and the most similar populations, I-120 and I-16a, were separated by the greatest distance among the five populations (De Pace, 1987). Micro-environmental factors rather than gross geographical diferences (such as latitudinal or longitudinal distances) may be more important in the development of diversity among populations. Population I-120 was collected in a site at 1000 m a.s.l. and was isolated from other *D. villosum* population stands. Population I-16a was collected in an inland hilly site at 300 m a.s.l. and was also isolated by distance from other *D. villosum* populations. On the other hand, population I-50 and I-85 were collected both at altitude of 10 to 50 m a.s.l. and by the seashore. Therefore, altitude may have played a role in driving some alleles to increase in frequency in populations I-120 and I-16a, and that frequency has been maintained by intrapopulation mating (outcrossing rate 0.8 as estimated by De Pace (1987)). Small population size and resulting genetic drift effects must also be considered as force in establishing allele frequencies at prolamin loci in *D. villosum*. Each of the 133 individuals analyzed in populations I-50 and I-85 appeared to have a unique pattern of monomeric prolamins.

Considering that the prolamin banding pattern in *D. villosum* is determined by alleles at three independent loci (Gli-V1, Gli-V2, and Gli-V3), it can reasonably be assumed that each prolamin band is coded by a component of a cluster of alleles belonging to a prolamin multigene family and that high polymorphism exists among as well as within clusters of genes. Since 68 of the 80 gel positions include at least one prolamin band, then each locus could encode for prolamin bands at an average of 68/3 = 23 gel positions. If individuals differed for only one band at one of those 23 gel

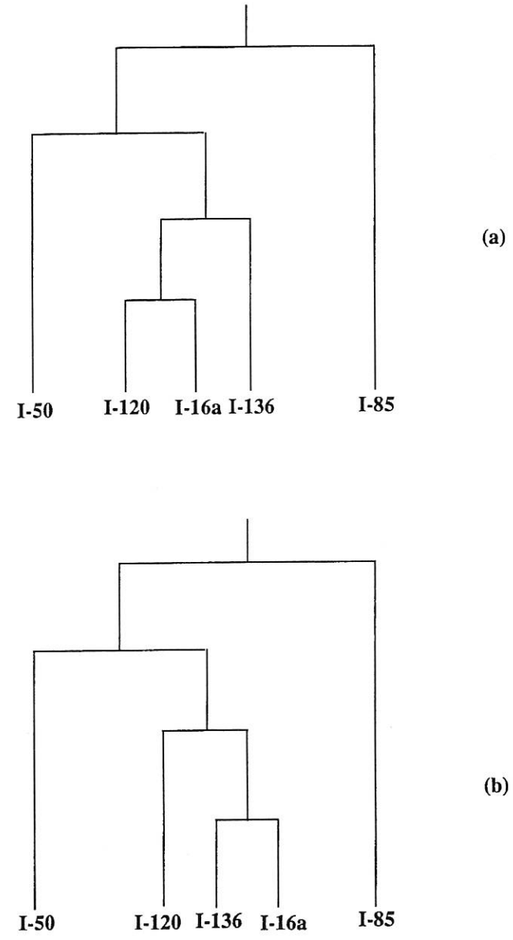

Figure 3. Dendrograms based on the dissimilarity index (a) over all monomeric components or on components within the ω and γ classes and (b) on monomeric components occurring in the α or β classes.

positions, there could be as many as 23 different alleles at one locus. From Fig. 1, this seems possible for the ω + γ, β, and α classes. On the basis of the observation that alleles at the Gli-V1 locus code for monomeric prolamins in the ω class and that Gli-V2 and Gli-V3 code for monomeric prolamins in the α and β classes (Blanco *et al.*, 1991: Shewry *et al.*, 1991), it is hypothesized that numerous alleles at each of the Gli-V1, Gli-V2, and Gli-V3 loci control the detected monomeric prolamin polymorphisms. Formal genetics of allelic diversity among the gliadin loci has not been done, but it is expected that the above conjecture would be borne out. For the high molecular weight glutenins subunits, Zhong and Qualset (1993) found 14 electrophoretically detectable alleles of the Glu-V1 locus in *D. villosum* populations from Italy and Yugoslavia. This is one of the highest number of alleles reported in diploid plants at a seed storage protein locus. Therefore, *D. villosum* is a plant species showing high polymorphism for seed storage protein components, and therefore is a valuable genetic resource to be explored or wheat seed storage protein improvement through alien gene transfer.

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The Presence of a Repeated DNA Sequence from *Triticum aestivum* in *Hordeum* species

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ABSTRACT

The rapid squash dot technique was used to analyze the distribution of a repeated DNA sequence from *Triticum aestivum* cloned in pTa1 among 54 accessions representing 21 *Hordeum* species. In general, pTa1 showed a strong cross-hybridization to the *H. vulgare* accessions. More variability was found in accessions of the closely related species *H. agriocrithon* and *H. spontaneum*. The wild American and Eurasian accessions displayed uniformity by a strong and very strong hybridization extent. Variability was found in *H. bulbosum* and *H. brachyantherum*. The wheat sequence was evident in 16 accessions representing 14 Hordeum species with a higher redundancy than in *T. aestivum*. A strong cross-hybridization signal was also found in 6 *Agropyron* species.

INTRODUCTION

The rapid evolution of certain repeated DNA sequences can cause a redundancy variation in plant genomes which can be useful for the inference of taxonomic relationships. This has been successfully shown for the genera *Cucurbita* (Grellet *et al.* 1986), *Brassica* (Martinez-Zapater *et al.* 1986), *Luzula* (Collet and Westerman 1987), *Nicotiana* (Speeckaert and Jacobs 1988), *Brassicaceae* (Hallden *et al.* 1987) and those in Triticeae (Bendich and McCarthy 1970; Flavell *et al.* 1977, 1979; Chakrabarti and Subrahmanyam 1985; Dvorak *et al.* 1988; Junghans and Hammer 1990). However, the presence of some repeated DNA sequences in related species was shown to be not in agreement with the classical taxonomy (Hutchinson and Lonsdale 1982, Jones and Flavell 1982, Lapitan *et al.* 1987, Gupta *et al.* 1989, Baldauf *et al.* 1992). Nevertheless, these sequences can also give some useful information. A repeated DNA sequence cloned from *Triticum aestivum* in pTa1 by Metzlaff *et al.* (1986) was present with a strong hybridization signal, in D genome containing *Triticum* and *Aegilops* species but also in *Agropyron*. Weak or middle cross-hybridization strengths were found in *Secale* and in *Triticum* and *Aegilops* species not containing the D genome. Surprisingly, pTa1 showed strong hybridization in *Hordeum vulgare* (Schubert *et al.* 1991). In the present paper we describe the distribution of pTa1 among 54 *Hordeum* and 6 *Agropyron* accessions using the rapid squash dot technique.

MATERIALS AND METHODS

The plant material investigated was obtained from the genebank collection of the Institute of Plant Genetics and Crop Plant Research Gatersleben, Germany (Table 1).

The repeated DNA sequence cloned in pTa1 from *Triticum aestivum* L. 'Chinese Spring' was isolated and characterized by Metzlaff *et al.* (1986). DNA hybridization was carried out according to the protocol of squash dot technique developed by Hutchinson *et al.* (1985) modified after Junghans and Metzlaff (1988). Root tips from germinated caryopses from at least two plants of every accession were squashed onto nitrocellulose filter. Hybridization of these filters with ^{32}P-labelled nicktranslated probe DNA was carried out at 65°C overnight according to Sambrook *et al.* (1989). After X-ray exposition the hybridization extent was evaluated according to a hybridization strength scale (0 = none, 1 = weak, 2 = moderate, 3 = strong, 4 = very strong hybridization) established by Schubert *et al.* (1990).

RESULTS AND DISCUSSION

Hybridization strengths of pTa1 containing a repeated DNA sequence of *T. aestivum* to *Hordeum* and *Triticinae* species are summarized in Table 1. Surprisingly, the wheat sequence displayed in 16 accessions representing 14

Table 1: Hybridization strengths of pTa1 to *Hordeum* and *Triticinae* species. *Hordeum* species outside the *H. vulgare*-complex were arranged on the basis of taxonomic aspects according to von Bothmer *et al.* (1982).

	Species		Accession no.	Origin	Hybridization strength[a] pTa1
Cultivated barley and closely related wild accessions					
1	*H. vulgare* L.s.l.				
	convar. *deficiens* . (Steud.) Mansf.	var. *deficiens* (Steud.) Koern. 'Abyssinian'	AHOR 3027	Ethiopia	3
2		var. *deficiens* (Steud.) Koern.	Schgt. 64	former USSR	3
3	convar. *distichon* Alef.s.l.	var. *medicum* Körn. 'Forezia'	AHOR 2500	France	3
4		var. *nutans* (Rode) Alef.'Defra'	AHOR 10288	Germany	2-3
5		var. *nutans* (Rode) Alef.	AHOR 1737	Crete	3
6		var. *nutans* (Rode) Alef.'Haisa'	Schgt. 56	Germany	2-3
7	convar. *intermedium* (Körn.) Mansf.	var. *atterbergii* Körn.	AHOR 2676	Ethiopia	3-4
8		var. *harlani* (Vav. *et al.*) Mansf.	AHOR 7211	Nepal	2
9	convar. *labile* (Schiem.) Mansf.	var. *nigripallidum-steudelii*	AHOR 3529	Ethiopia	3
10		var. *steudelii-nigripallidum*	Schgt. 66	Ethiopia	3
11	convar. *vulgare*	var. *densum* Ser.	AHOR 9950	Ethiopia	3
12		var. *dundarbeyi* Zhuk.	AHOR 2024	Japan	2
13		var. *himalayense* (Ritt.) Körn.	AHOR 5566	Ethiopia	3
14		var. *horsfordianum* Wittm. 'Spray'	AHOR 2762	USA	3
15		var. *coeleste* L.	AHOR 8819	Poland	3
16		var. *hybernum* Vib.	AHOR 1006	N. Greece	2-3
17		var. *hybernum* Vib.'Hohenfinower'	Schgt. 38	Germany	3
18		var. *subparallelum* (Orl.) Mansf.	AHOR 342	Japan	2-3
19		var. *trifurcatum* (Schlecht.) Wenderoth	Schgt. 48	Nepal	3
20	*H. agriocrithon* Aberg	var. *agriocrithon*	AHOR 9517	China	3
21			Schgt. 37	unknown	1-2
22		var. *dawoense* Aberg	AHOR 2268	Tibet	1-2
23		var. *paradoxon* Schiem.	AHOR 3907	unknown	2
24	*H. x lagunculiforme* (Bacht.) Bacht. ex Nikif.		AHOR 9600	Israel	3
25	*H. spontaneum* Koch x *H. vulgare* L.s.l. *convar.dist.* Alef. s.l.		AHOR 4869	Turkmenia	2
26	*H. spontaneum* Koch	var. *bactrianum* Vav.	AHOR 2705	Turkmenia	3
27		var. *ischnatherum* Coss.	AHOR 2680	Iran	0-1
28		var. *spontaneum*	AHOR 2681	Iran	2-3
29			Schgt. 54	unknown	1-2
30		var. *transcaspicum* Vav.	AHOR 2695	Turkmenia	2-3
Europe					
31	*H. bulbosum* L.4x		GRA 970		2
32	*H. bulbosum*		ssp. *bulbosum* GRA 667		0-1
33	ssp. *nodosum* (L.) Baum		GRA 599		4

Table 1: continued

	Species	Accession no.	Origin	Hybridization strength[a] pTa1
34	*H. murinum* L. ssp. *leporinum* (Link) Arcang.	GRA 601		2-3
35	ssp. *murinum*	GRA 888		3
America				
36	*H. chilense* Roem. et Schult.	GRA 972		4
37	*H. bogdanii* Wilensky	GRA 647		3-4
38	*H. flexuosum* Steud.	GRA 977		4
39	*H. intercedens* Nevski	GRA 979		3-4
40	*H. jubatum* L.	GRA 1140		4!
41	*H. lechleri* (Steud.) Schenck	GRA 981		4
42	*H. procerum* Nevski	GRA 985		4
43	*H. pusillum* Nutt.	GRA 987		3-4
44	*H. roshevitzii* Bowden	GRA 988		3
Eurasia				
45	*H. brevisubulatum* (Trin.) Link	GRA 1187		3
46		GRA 893		3
47	*H. marinum* Huds. ssp. *gussoneanum* (Parl.) Thell.	GRA 964		4
48	ssp. *marinum*	GRA 806		4
49		GRA 803		3-4
50	*H. secalinum* Schreb.	GRA 990		4
51	*H. violaceum* Boiss. et Hohenack.	GRA 603		3
North America				
52	*H. brachyantherum* Nevski 2x	GRA 966		0-1
53	*H. brachyantherum* Nevski 6x	GRA 968		4
South Africa				
54	*H. capense* Thunb.	GRA 971		3-4
55	*Agropyron aucheri* Boiss.	GRA 863		2-3
56	*Agropyron cristatum* (L.) P.B.	GRA 848		3
57	*Agropyron intermedium* (Host) P.B.	GRA 571		2-3
58	*Agropyron litorale* Dum.	GRA 868		3
59	*Agropyron trichophorum* (Link) Richter	GRA 838		3
60	*Agropyron ugamicum* Dobrov	GRA 874		3
61	*Avena sativa* L. var. *aurea* Körn.	AAVE 1889		0
62	*Secale cereale* L. ssp. *cereale* 'Petka'	HR 520		2
63	*Triticum aestivum* L. var. *lutinflatum* (Flaksb.) Msf. 'Chinese Spring'	ATRI 12922		3

[a] for mark description see 'Material and Methods'

58 55 33
36 50 54 37 46 51 56 59
63 61 62 31 52 44 43 42 41 38

55 41
50 54 37 46 56 63 58
63 61 62 33 31 44 43 42 38

49 6 7 19 2 17 10 - 57 63 61 62
48 53 39 37 40 45 27 21 34 32 35 29
13 1 14 26 30 28 25 24 23 22 20 47
63 61 62 15 9 12 5 16 18 3 4 11 8

Fig. 1: Squash dot hybridization of root tips from germinated seeds of *Hordeum* and *Agropyron* accessions with probe pTa1. For species identification compare position numbers with species numbers in table 1. The filters were hybridized with ^{32}P-labelled probe overnight at 65°C. They were washed twice for 15 min in 2xSSC / 0,1 % SDS solution at 65°C.

Hordeum species a higher redundancy (marks 3-4 to 4) than in *T. aestivum* (mark 3) (Fig. 1). The accessions of the *H. vulgare* complex showed in general homogeneity by middle and strong hybridization in spite of the wide geographic distribution and the large morphological differences between the accessions. The closely related wild races *H. agriocrithon* and *H. spontaneum* known to be partly included in *H. vulgare* evolution had marks from 0-1 to 3. This redundancy variability indicates a larger genetic variability in comparison to the *H. vulgare* accessions. The clearly strongest hybridization strength to the accessions investigated was found in *H. jubatum* (mark 4!). The morphological groups including wild species of America and Eurasia showed uniformity by strong and very strong hybridization signals. With regard to the American species this is in agreement with the supposed common origin. Only the diploid *H. brachyantherum* accession showed nearly no hybridization. Strong redundancy variability was also found in *H. bulbosum*.

The strong hybridization signal found by Schubert *et al.* (1991) in *Agropyron* was confirmed in the six *Agropyron* species analyzed. Anamthawat-Jonsson and Heslop-Harrison (1993) isolated a repeated DNA sequence from *H. chilense* which was also found in *H. vulgare* and *H. bulbosum*. A 47 bp stretch of this sequence showed 85% homology to pTa1. This explains the cross hybridization of pTa1 to *Hordeum* species. Several repeated DNA sequences were cloned from *H. vulgare* (Dennis *et al.* 1980; Ananiev *et al.* 1986; Salina *et al.* 1986; Junghans and Metzlaff 1988; Vershinin *et al.* 1990). Junghans and Hammer (1990) found six of these sequences with a very strong hybridization extent in all *H. vulgare* accessions investigated as well as in the closely related *H. agriogrithon* and *H. spontaneum* accessions. In contrast, the sequences showed a high variability in the wild barley accessions. Vershinin *et al.* (1990) also showed six separated *H. vulgare* sequences with the highest redundancy in *H. vulgare* itself in relation to six wild barley species. However, a highly repeated DNA sequence of rye cloned in pSc119 was absent in *H. vulgare*, *H. agriocrithon* and *H. spontaneum* but evident with a high variability in the other wild *Hordeum* species (Gupta *et al.* 1989).

Two subclones (H950 and Hch 1.3) of a family of dispersed repetitive sequences from *H. chilense* isolated by Hueros *et al.* (1993) were found separately in different species. H 950 cross hybridized to *H. murinum* and *H. brevisubulatum* but not to wheat. However, Hch 1.3 was absent in *H. murinum*, *H. brevisibulatum*, *H. marinum* and *H. vulgare* whereas it was present in American *Hordeum* species and also in wheat.

In general, the above-mentioned results obtained by the hybridization of separated repeated DNA sequences to related species confirmed the assumption of Jones and Flavell (1982) that in phylogenetic studies a group of repeated sequences should be used rather than a single clones.

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Molecular Relationships Between the Genera *Elymus* L. and *Triticum* L. Glutenins.

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ABSTRACT

The antigenic relationships between the glutenin of *Triticum aestivum* L. and major seed proteins of *Elymus* L. were studied by Western blotting using pooled antibodies against wheat glutenin as the probe. Along with high- and low-molecular-weight (HMW and LMW, respectively) glutenin-like proteins of *E. sibiricus* (SSHH genome) the probe reacted with two proteins (67 and 69 kDa), probably non-prolamins. Southern blotting of *Hind*III-digested total DNA from *Elymus* spp. with a synthetic oligonucleotide (a repeated motif from wheat HMW glutenin genes) revealed 3.2, 1.4 and 1.0 kb fragments in *E. trachycaulus* (SSHH genome) DNA and 1.2, 0.9, 0.8 kb in *E. pendulinus* (SSYY genome) DNA.

INTRODUCTION

Elymus L. is the most widely distributed genus among the Triticeae which includes about 150 perennial, polyploid species. These grasses possess numerous valuable qualities, such as wide adaptation range, salt tolerance, disease resistance, and good forage quality. This is why *Elymus* attracts considerable interest of cereal crop breeders [1]. No attempts to obtain fertile *Elymus*/wheat hybrids have yet been successful [2]. *Elymus*' genetic potential may prove attainable by means of genetic transformation and cellular engineering. The better we understand the relationships between the species, the more likely will it be that one can contribute to agricultural management.

The wheat *Glu*-1 locus containing the genes for HMW subunits of glutenin is known to be evolutionarily stable and is a useful marker for understanding chromosome relationships in the Triticeae [3]. For this reason we considered HMW glutenin a promising tool for detecting structural similarities in the *Elymus* genome. We found that some major seed proteins of *Elymus* were immunologically similar to glutenin subunits of common wheat. Besides, DNA sequence similarity of these plants was demonstrated by Southern blotting using a synthetic oligonucleotide (a repeated motif from wheat HMW glutenin genes) as a probe.

MATERIALS AND METHODS

Seeds of wheat (*Triticum aestivum* L., cvs. Chinese Spring and Cheyenne) were supplied by the Plant Breeding Institute, Germany. *Elymus sibiricus* (accession No. ALT.84.1), *E. trachycaulus* (VLA.86.2) and *E. pendulinus* (MES.86.8) were received from the collection of the Central Siberian Botanical Garden.

The fraction of total seed proteins similar to glutenin in solubility was prepared from individual grains and analyzed by SDS-PAGE as described in [4]. Wheat glutenin for immunization was prepared as described in [5]. Polyclonal rabbit antiserum against wheat glutenin was prepared according to [6]. Western blot analysis was carried out according to [6], except that diaminobenzidine was replaced with 4-chloro-1-naphthol. Total DNA of individual plants was isolated from young leaves according to [7]. *Hind*III, the Klenow fragment, and *lambda* phage DNA were used according to manufacturer's recommendations. The electrophoresis of *Hind*III-digested DNAs was performed in 0.7% agarose gel as described in [8]. DNA transfer to nitrocellulose, hybridization and autoradiography were performed using standard protocols [8]. The oligonucleotide probe was radioactive labeled with 32p-deoxynucleoside triphosphates by extending a 12-nucleotide specific primer on a 30-nt matrix as described in [8]. Both the matrix and the primer were synthesized by Dr. V.P. Kumarev.

RESULTS AND DISCUSSION

SDS-PAGE of Total Storage Proteins

SDS-PAGE (Fig.1A) shows the bands present in total storage protein fractions of *Triticum aestivum* L. (cv.

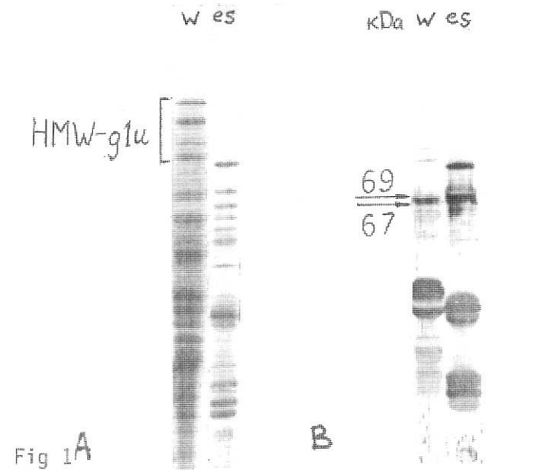

Fig 1

Fig. 1. Western blot analysis of cereal storage proteins using pooled antibodies raised against wheat glutenin (Panel B). Total seed proteins were isolated with SDS-ß-ME, fractionated by SDS-PAGE and transferred to nitrocellulose. Each sample was loaded twice to obtain two identical blots. One of these two blots (Panel C) was probed with the pre-immune serum (dilution 1:20). On the other blot the immunoreactive bands were visualized by incubation with horseradish peroxidase - A protein conjugate followed by reaction with 4-chloro-1-naphtol. The antiserum was diluted 1:60. Panel A depicts the protein levels and patterns as visualized by Coomassie brilliant R staining of the polyacrylamide gel. In all three panels the lanes are designated: w - wheat (*Triticum aestivum* L., cv. Chinese Spring) and es - *Elymus sibiricus*.

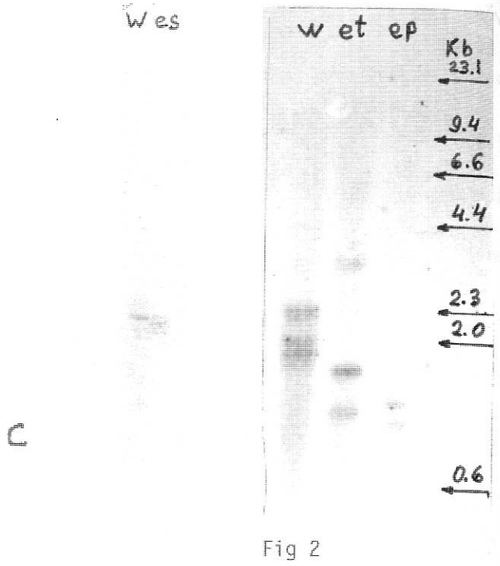

Fig 2

Fig. 2. Southern blot hybridization patterns of *Hind*III digests of DNA isolated from individual plants: Lane w - wheat (*Triticum aestivum* L. cv. Cheyenne), et - *Elymus trachycaulus*, ep - *Elymus pendulinus*. The blot was probed at 65°C with a radioactive-labeled synthetic oligonucleotide - a repetitive motif occurring in several wheat HMW-glutenin genes [15]. The electrophoresis was carried out in a 0.7% agarose gel. The fragment sizes were estimated using a molecular marker: *lambda* phage DNA digested with *Hind*III.

Chinese Spring) and *Elymus sibiricus* L. (Lanes w and es). Two groups of bands are present in the wheat protein fraction; the HMW, LMW subunits of glutenin are denoted according to [9,10] (Lane w). The components of the total *Elymus* protein fraction are shown in Lane es. In contrast to wheat only one HMW glutenin-like band is present in *Elymus sibiricus*.

Western blot analysis

Earlier works [11,12] have shown that some storage proteins of barley, wheat and rye are immunologically related. The storage proteins of *Elymus* spp. are being investigated, but their relationships to the proteins of other Triticeae still remain uncertain. Total storage proteins from several cereal grains were analyzed by SDS-PAGE (Fig. 1A) and Western blotting using antibodies raised against wheat glutenin (Fig. 1B). Control reactions of the immobilized protein fractions were carried out using pre-immune serum (Fig. 1C), which changed the hybridization pattern very slightly, if at all. It is noteworthy that pre-immune serum from some rabbits reacted with storage proteins. This effect may be ascribed to spontaneous immunization due to their cereal diet.

Triticum

The anti-glutenin serum reacted with all HMW and LMW subunits of glutenin and with two proteins of molecular weights 69 and 67 kDa (presumably non-prolamins). Proteins of the same mobility were also found in wheat roots, seedlings and embryos (not shown). Therefore we conclude that they are rather structural proteins than glutenins. This is in accordance with the suggestion that the wheat glutenin fraction contains some structural proteins [3, 13]. Wheat proteins with similar mobility have been regarded by Gupta et al. [10] as non-prolamins.

Gradual dilution of the antiserum showed that the binding strength decreased in the following order: 67-69-kDa proteins LMW-glutenins HMW-glutenins. This

fact is attributable to different immune responses caused by different accessibility of various antigenic determinants resulting from the spatial organization of glutenin.

Control reaction with threefold quantity of the pre-immune serum showed but a weak band in the LMW-glutenin area. Hence, non-specific reactions of the pre-immune serum could not cause any considerable distortions of the hybridization pattern

Elymus

The anti-glutenin serum reacted with one protein of *Elymus sibiricus* in the mobility region of the HMW subunits of wheat glutenin and with two groups of LMW glutenin-like proteins. Like in wheat, in addition to these signals two intense bands were found corresponding to proteins with molecular weights of 69 and 67 kDa respectively. The only HMW protein of *Elymus* showed stronger binding with the antiserum against wheat glutenin than the proteins of wheat itself. According to the above-mentioned hypothesis, this protein may be enriched with antigenic determinants related to those of the glutenin complex which are more accessible for the immune response. The serum dilution experiments showed equally high binding both with HMW and LMW glutenin-like proteins. Control reaction with the pre-immune serum revealed no significant bands. Therefore we conclude that *Elymus* may contain a locus functionally similar to the Glu-1 locus of common wheat.

DNA

Various *Elymus* species have been shown to contain two or three of five basic genomes, S, H, Y, P, and W in different combinations. The genome constitution of *Elymus sibiricus* is SSHH [1, 14]. It remained to be seen which of the *Elymus* genomes bore the Glu-1-like locus. So we undertook a Southern blot analysis of genomic DNAs of wheat and two *Elymus* species possessing different genomes: SSHH (*E. trachycaulus*) and SSYY (*E. pendulinus*). A 32p-labeled 30-nucleotide synthetic consensus fragment complementary to a repetitive nucleotide sequence motif

5'-GGGCAGCAACCAGGACAAGGGCAACAAGGT-3'

occurring within several HMW-glutenin genes [15] was chosen as the probe. As is seen in Fig. 2, total wheat DNA (cv. Cheyenne) digested with *Hind*III and hybridized with the probe gave rise to five fragments of sizes 2.5, 2.3, 1.8, 1.6, and 1.5kb. This is consistent with the previous investigations done with a cDNA probe [16]. Each of the two *Elymus* species studied showed three fragments: *E. pendulinus* - 1.2, 0.9, 0.8kb; *E. trachycaulus* - 3.2, 1.4, 1.0kb. The intensity of the band corresponding to the 1.4kb fragment of *E. trachycaulus* was higher than that of the neighboring bands. The presence of hybridizing fragments in the genomes of both *Elymus* species indicates that the locus similar to the wheat HMW-glutenin locus may be situated in the S genome or, perhaps, in both H and Y genomes simultaneously.

CONCLUSIONS

Biochemical, immunochemical and molecular data presented indicate that the genomes of two *Elymus* species studied contain a locus or loci both structurally and functionally similar to the Glu-1 locus of *Triticum aestivum* L.

Acknowledgments

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The *Elymus Trachycaulus* Complex in North America: More Questions than Answers

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ABSTRACT

Members of the *Elymus trachycaulus* complex of North America can be recognized morphologically by their combination of a) solitary spikelets, b) anthers less than 2.5 mm long, and c) cespitose growth. This circumscription is essentially pragmatic. In addition to *E. trachycaulus sensu stricto*, it includes such taxa as *Elymus alaskanus*, *E. macrourus*, *E. scribneri*, *E. sierrae*, *Agropyron latiglume*, and *A. subsecundum*. In North America, members of the complex extend from Alaska east to Newfoundland, south to Mexico through the Rocky Mountains, to the Missouri and Ohio Rivers in the central plains, and to southern Virginia in the Appalachian Mountains; the ecological range extends from coastal to alpine habitats and from dry hillsides to damp meadows. In Eurasia, members of the complex extend from Iceland and northern Europe to eastern Russia. Some species have a transberingian distribution, but the number of taxa thought to do so is highly dependent on the flora being consulted. So far as is known, all members of the complex are predominantly self-fertilizing allotetraploids based on the **St** and **H** genomes (Dewey 1963, 1966, 1967a, 1967b, 1968a, 1968b, 1969, 1975, 1976; Murry and Tai 1980).

INTRODUCTION

Authorities and synonyms are listed in the appendix. Names in *Elymus* have been used where available except when citing published works; in such cases, the authors' choice is followed. Genome designations are based on Wang et al. (1996); the **St** genome corresponds to the **S** genome of Love (1984) and Dewey (1984).

The primary purpose of this paper is to stimulate research on the many unanswered questions concerning the complex by reviewing existing knowledge-and ignorance. Improved understanding requires the completion of research studies specifically designed to answer the questions that exist concerning the bases of its variation. Some of the questions have been around since the beginning of this century; I hope that some will be addressed before the start of the next.

SYSTEMATICS

Relationships to non-North American taxa

The first question that needs to be addressed concerning the *E. trachycaulus* complex is its relationship to non-North American taxa. The similarity of *Elymus trachycaulus* itself to the Eurasian *E. caninus* has often been noted. Most North American taxonomists, including A.S. Hitchcock (1935, 1951) have treated them as separate species, as was recommended by Malte (1932), but C.L. Hitchcock (1969) treated them as conspecific subspecies. Malte (1932) reported that the spikelets of *A. caninum* diverge from the rachis at anthesis, returning to a loosely appressed position afterwards, whereas those of *A. trachycaulum* show almost no movement. His plants of *A. caninum* were grown from seed sent by the plant breeding station in Svalov, Sweden; he did not state how many plants he examined, nor how many populations they represented. His observations of *A. trachycaulum* were based on the progeny of nine morphologically diverse plants that he found on a vacant lot in Calgary, Alberta. The observations were made in the spring of 1914. I am not aware of any attempt to verify Malte's observations.

Malte also examined over 3,000 herbarium specimens. These came from one European herbarium, S, and four North American herbaria: CAN, GH, NY, and US (codes according to Holmgren et al. 1990). The most conspicuous morphological difference he found between *A. caninum* and *A. trachycaulum* was that the former had coarsely 3-veined glumes, whereas *A. trachycaulum* had glumes with 4-5 much less prominent veins. In addition, the glumes of *A. caninum* had a rather broad, scarious margin which formed a projecting edge just below the

apex, whereas in *A. trachycaulum* "the scarious margin is much narrower and in many cases obsolete". There were additional differences, but these he considered less significant. C.L. Hitchcock (1969), who collected extensively in the Pacific Northwest, stated that the difference in glume venation between the two taxa was not constant, and that the differences in glume texture and anther size supported their treatment as conspecific subspecies. Jozwik (1966) agreed with C.L. Hitchcock, but admitted that he had seen relatively few Old World plants. He noted that these were much more uniform in appearance than the North American plants.

The cytological data are limited. According to Dewey (1975), the chromosomes of *A. caninum* are structurally different from those of *A. trachycaulum*, *A. subsecundum*, and *A. dasystachyum* and, in addition, ". . . genomes of *A. trachycaulum* and probably other members of the 'slender wheatgrass complex' are structurally unique, in that they contain two sizable interchanges not found in other SSHH species" (Dewey 1975, p. 127). Dewey interpreted these data as supporting recognition of *A. caninum* and *A. trachycaulum* as distinct species. He did not elaborate on which taxa he included in the "slender wheatgrass complex".

The situation with respect to the northern members of the *E. trachycaulus* complex, i.e. the taxa Hulten (1968) treated as *A. macrourum* and *A. boreale* subspp. *boreale*, *alaskanum*, and *hyperarcticum*, is more complicated. All but *A. boreale* var. *alaskanum* were described first from Eurasia. Welsh's (1974) treatment is essentially identical to Hulten's, but Tsvelev's (1976) is substantially different. According to Tsvelev, *A. boreale* subsp. *hyperarcticum* is a synonym of *E. sajanensis* subsp. *hyperarcticus*; *A. boreale* subsp. *alaskanum* is close to, but not a synonym of, *E. sajanensis* subsp. *villosus*; and *A. boreale* subsp. *boreale* corresponds to *E. kronokensis* subsp. *subalpinus*. He recognized *E. macrourus* as occurring in both the Soviet Union and North America, but considered *A. sericeum*, which Hulten (1968) had treated as a synonym of *E. macrourus*, closer to *E. jacutensis* than *E. macrourus*. He also stated that *E. kronokensis* subsp. *kronokensis* (which differs in its leaf pubescence from subsp. *subalpinus*) occurs in arctic North America, whereas *E. sajanensis* subsp. *sajanensis* is restricted to Eurasia. Tsvelev (1976) also commented that *E. trachycaulus* becomes very close to *E. donianus* through subsp. *kamczadalorum* and subsp. *major*.

Love's (1984) treatment is similar to that of Hulten (1968) and Welsh (1974), except that he included the four taxa in *Elymus* rather than *Agropyron*. Because the name *E. borealis* had already been used (Scribner 1900), the three subspecies of *A. boreale* became subspecies of *E. alaskanus*. Love's interpretation of this species, however, also included *E. kronokensis*, *E. sajanensis*, and *A. latiglume*. Love treated *E. macrourus* and *E. jacutensis* as separate species, but differed from Tzvelev (1976) by including *A. sericeum* in *E. macrourus*.

Despite the number of different relationships that have been suggested between the *E. trachycaulus* complex and various Eurasian taxa, there have been few attempts at experimental evaluation of the alternative treatments. This is not an unusual situation; taxonomists are preparing floristic treatments are frequently forced to make nomenclatural decisions, which imply decisions about relationships, on the basis of minimal information. But several factors argue that, if any group merits coordinated circumboreal examination, the *Elymus trachycaulus* complex is it. The examination should include population-level studies of morphology, reproductive biology, and molecular variation within and between the two. The resulting data may not provide indisputable support for any particular taxonomic treatment, but they would provide a more factual basis on which to make decisions. Such studies could be conducted by cooperation and seed exchange between individuals at different institutions.

Variation within the Elymus trachycaulus complex

Even within North America, there are more questions than answers about the *E. trachycaulus* complex. The three morphological criteria listed in the introductory paragraph identify a group of plants in which there are some relatively distinct entities linked by an uncomfortably large number of morphologically intermediate plants. In the Triticeae, including the *E. trachycaulus* complex, the initial suspicion is that morphological intermediates are hybrids or hybrid derivatives. This suspicion arises from appreciation of the low barriers to hybridization within the tribe, and the observation that many of the intermediates occur in areas of sympatry. But morphological variation may reflect genetic differences, phenotypic response, hybridization, or any combination of these three factors. Unfortunately, almost all of the available data has been obtained as a by-product of cytogenetic studies.

Jozwik's (1966) study of *Agropyron trachycaulum*, *A. subsecundum*, *A. scribneri*, and *A. latiglume* in Sublette County, Wyoming, is exceptional. He studied the morphological variation within and between natural populations of the four taxa and found that: 1) Large variations in diagnostic characters were not associated directly with differences in habitat over the area in which a population was resident; 2) Populations with a great deal of morphological diversity were more likely to occur in disturbed areas; 3) Whenever one of the species was growing in an isolated area with no other members of the Hordeae [= Triticeae] present, there was little variation among members of the population (however, in instances where two species of the slender wheatgrass complex were growing side by side the general rule was that an array of intermediate types was present, and when one of the species was found growing with *Hordeum jubatum*, *Sitanion longifolium*, *Sitanion hystrix* var. *californicum*, *Elymus glaucus*, or *Agropyron scribneri*, a single sterile intermediate type was often found); and 4) Certain morphological strains of the slender wheatgrasses possessed distributions which coincided rather well with areas in which the distribution of one species of slender wheatgrass overlapped either with that of another or with that of one of the above-mentioned species outside the complex.

Jozwik's illustrations support the suspicion of many taxonomists that the taxonomic recalcitrance of the *E. trachycaulus* complex is partly attributable to its members' ability to form partially fertile hybrids with each other and with other members of the Triticeae. If the plants he collected at a site where *A. trachycaulum* was hybridizing with *H. jubatum* were mounted on separate herbarium sheets, they would be placed in at least two different taxa using any of identification keys currently available.

Jozwik germinated seed from some of the putative hybrids and compared the resulting plants with their parents. The offspring of one putative hybrid with long-awned lemmas included some plants with awn-tipped lemmas and some with long-awned lemmas. In one line, long-awned lemmas were associated with fairly long-awned glumes; in another they were associated with awn-tipped glumes. Jozwik (1966, p. 61) concluded that the unawned forms corresponding to A.S. Hitchcock's (1951) *Agropyron trachycaulum* and *A. latiglume* were basal species in the slender wheatgrass complex and that ". . . most, if not all, of the long-awned forms are derived by hybridization with *A. scribneri*, *E. glaucus*, *H. jubatum*, *S. longifolium*, *S. hystrix* var. *californicum* and perhaps other unidentified species. . . . Selection acts upon the progeny of these crosses and relatively stable long-awned races are the result." Earlier, Dewey (1963) had suggested that *A. subsecundum* var. *andinum* consisted, at least in part, of hybrids between *A. subsecundum* and *A. scribneri*.

Jozwik also grew seed from apparently non-hybrid plants in the greenhouse. In most cases, the offspring were very like their parents, as would be expected in predominately self-fertilizing taxa, but one long-awned plant gave rise to three long-awned plants and two awn-tipped plants. The parent might have been a hybrid, but selfed seed from the long-awned offspring were uniformly long-awned. Jozwik concluded that ". . . the great majority of slender wheatgrass plants are homozygous for most visually observable characters under natural conditions" (p.70), even though many of the plants may be descendants of a hybrid between different members of the *E. trachycaulus* complex or between a member of the complex and some other member of the Triticeae, including species of *Hordeum*. Malte (1932, p. 28) made a similar observation, based on "numerous experiments", but cited Kirk (1929) as saying that segregation may occasionally occur, indicating that some plants are heterozygous for one or more characters.

Artificial hybrids between members of the complex are generally described as being morphologically intermediate, but little, if any, additional information is given (but see Dewey 1976 for an exception). Somewhat surprisingly, the hybrids are often highly sterile, frequently more sterile than hybrids with more distantly related taxa (Table 1). Most hybridization studies have involved few populations, sometimes only one population (or seed accession) per parental species. Generalizing from such studies is dangerous, for individual plants differ considerably in their crossability. Of the 20 crosses Jozwik (1966) made between members of the complex, eight resulted in seed. Of the three crosses within *A. trachycaulum*, one yielded 10 germinable seeds, a 40% yield, but the other two gave none. Of the 13 crosses between the unawned *A. trachycaulum* and long-awned *A. subsecundum*, eight yielded no seed, but the other five yielded 7 (48%) germinable seeds, percentages Jozwik stated were close to those of the parents. Most, but not all, of the hybrids had awns of intermediate length. Two of his four crosses between *A. trachycaulum* and *A. latiglume* gave rise to viable seed, but the percentages were lower (4% and 8%, respectively) and only one seed grew to maturity.

Some of the variability ascribed to *E. trachycaulus* may reflect confusion of subsp. *subsecundus* with *E. glaucus*. M.L. Curto (on UTC 210327) noted that *Elymus glaucus*, which is supposed to have more than one spikelet per node, frequently develops spikes with only one spikelet per node when growing in the shade. These one-spikeleted plants, which are easy to confuse with *E. trachycaulus* subsp. *subsecundus*, are not occasional 'freaks'. Interestingly, along a road in northern Utah, it was the more exposed plants that developed only one spikelet per node (pers. obs.). Limited observations suggest that single-spikeleted *E. glaucus* differs from *E. trachycaulus* in having glumes with five relatively prominent veins that are scabrous throughout, leafier culms, lax blades, and awned lemmas, but I have found 'glaucus' glumes on plants that were otherwise more similar to *E. trachycaulus*. Even if the single-spikeleted specimens of *E. glaucus* are excluded from consideration, my observations tend to confirm Jozwik's (1966) conclusion that awned specimens of *E. trachycaulus* (specimens with awns more than 5 mm long) have different hybrid origins. Whether it will be possible to identify the parents of such hybrids morphologically is still to be determined. Part of the problem is that one has to be confident of the parentage of putative hybrids before determining the distinguishing characters of different hybrids. This means observing plants in the field. Plant breeders working with the complex could help by depositing voucher specimens of the parents and representative offspring in a recognized herbarium, one that regularly loans specimens to other institutions. I implore them to do so. It is singularly frustrating to know that a useful set of hybrids has been made, but to be unable to examine either the hybrids or their parents.

Examination of naturally occurring intermediates is

also essential. Field studies and morphological intermediacy may suggest the probable parentage, but it needs to be confirmed. Even non-intermediate plants should be examined. Dewey (1969, 1975) commented that it would be almost impossible to detect hybrids between *A. trachycaulum* and *A. dasystaychum* in the field. The same appears to be true of hybrids between *E. trachycaulus* and *Pseudoroegneria spicata*. Many plants along a forest trail west of Logan were identified as *E. trachycaulus,* but found to be sterile. The only other species of Triticeae present was *Pseudoroegneria spicata*. It was disconcerting to find that, even knowing that such plants were present, morphology was not a reliable predictor of which plants were sterile and which fertile.

Collins (1965) and Jozwik (1966) attempted to use biochemical markers (serology and flavonoids, respectively) to detect the origin of suspected hybrids, but with little success. Nucleic acid studies might prove more rewarding. Markers have been developed for distinguishing among the **St, H,** and **Y** genomes (Barkworth and Talbert, submitted), but no attempt has been made to determine whether **St** or **H** genomes from different species can be distinguished. If DNA markers could be found for the taxa involved, a whole range of biologically interesting questions could be addressed-but first the markers need to be found.

Despite the ability of members of the *E. trachycaulus* complex to form stable hybrids, the genetic basis of the characters used to distinguish its taxa is not known. It would be worth investigating. Godley (1949) discovered that, in *Elymus repens*, awn length, glaucousness, and rachis pubescence are each determined by a single gene, even though *E. repens* is a hexaploid. Awn and glume length are both used in delimiting taxa within the *E. trachycaulus* complex, but the genetic basis of these characters has not been determined.

There is also almost no information available on environmental plasticity in the *E. trachycaulus* complex, although C.L. Hitchcock (1969) stated "[*Elymus caninus sensu lato*] is a plastic species that is apparently especially susceptible to modification by soil and moisture conditions". Malte (1932) found that plants grown at the Central Experimental Farm, Ottawa, had more veins in the glumes than normal, 7 being a common number in the garden yet very rare in natural conditions. Jozwik (1966) observed that plants grown from seed under greenhouse conditions were most likely to differ from their parents in having more widely spaced spikelets and longer spikes. He found little modification of the diagnostic characters for the taxa he examined. Other studies of plasticity have focussed on agronomically, rather than taxonomically, significant characters.

Internal structure of the *E. trachycaulus* complex

Adopting an artificial circumscription of the *E. trachycaulus* complex does not resolve the problem of how many taxa should be recognized within the complex, nor at what level they should be recognized. Applying a treatment of the complex for the Manual of North American Grasses, it has seemed best to circumscribe taxa that are morphologically, ecologically, and geographically coherent, and to avoid making any new combinations until there are good data supporting the need for them. I am, however, the first to admit that there are good arguments for alternative treatments.

Elymus sierrae, *E. scribneri*, and *E. stebbinsii* are generally accepted as good species. The least controversial of these appears to be *E. sierrae*. Its morphological uniformity suggests that either it rarely hybridizes with other Triticeae, or its diagnostic characteristics are determined by a single gene or a gene combination that rarely segregates. In contrast to *E. sierrae*, *Elymus scribneri* appears to hybridize rather easily with other members of the complex that grow in the vicinity, usually *E. trachycaulus*, *E. alaskanus* subsp. *latiglumis*, and *E. elymoides*. Bowden (1965) and C.L. Hitchcock (1969) have suggested that *E. scribneri* is a hybrid derivative between *E. trachycaulus* or *E. alaskanus* and *E. elymoides*. This seems very likely, but there has been no experimental examination of the hypothesis. *Elymus stebbinsii*, a Californian endemic, is relatively easy to recognize by its combination of long anthers, acute, smooth glumes, and distant spikelets. *Elymus laevis* was supposed to be a long-awned version of *E. stebbinsii*, but the type specimen is an awned specimen of *E. trachycaulus*. There does appear to be an awned variant of *E. stebbinsii*, but it has no name at present.

It is the remainder of the complex that causes most problems. The northern phase, *E. alaskanus sensu* A. Love, tends to be shorter than typical *E. trachycaulus*, and to have shorter, more compact, purplish inflorescences. The diagnostic characters, however, are those associated with the glumes: in *E. alaskanus* the glumes are usually about 1/2 to 2/3 as long as the adjacent lemmas, thin, usually smooth and glabrous to lightly pubescent, oblanceolate, and have a broad hyaline margin that is wider on one side than the other and widest at the apex. The apex itself is rounded to obtuse, although the midvein often extends as a mucro. Under high magnification (20x), the edges of the apex often appear minutely toothed. Typical *Elymus trachycaulus*, in addition to being taller and having a longer inflorescence, has thicker glumes that are about as long as the adjacent lemmas and more than 3/4 the length of the spikelet. If there are hyaline margins on the sides, they are narrow and roughly equal in width and become narrower towards the tip. Even towards the apex, the edges of the glume are smooth.

As outlined, *E. alaskanus* and *E. trachycaulus* are morphologically distinct. Moreover, *E. alaskanus* is generally a more northern and higher elevation taxon than *E. trachycaulus*, but the large number of intermediate specimens suggests that gene exchange between the two encounters few obstacles. Of particular concern is subsp. *latiglumis*. This taxon, which often has glumes as long as those of *E. trachycaulus*, has frequently been treated as a variety of *E. trachycaulus*, probably partly because taxonomists are more familiar with the plants in the southern portion of the continent. Field observations suggest the possibility that, when grown at high elevations, *E. trachycaulus* tends to be shorter and to have shorter glumes with somewhat wider hyaline margins, i.e., to resemble subsp. *latiglumis*, but there have been no studies of the phenotypic plasticity in any member of the *E. trachycaulus* complex.

Elymus macrourus is another problematic taxon. A.S. Hitchcock (1915) described *Agropyron sericeum* from the Yukon, noting that it was a common entity in the Mackenzie valley. As noted earlier, Hulten (1968) concluded that Hitchcock's taxon was identical with *E. macrourus*, a species described from the upper reaches of the Angara River in Siberia. The similarity of specimens identified as *E. macrourus* to vigorous specimens of *E. alaskanus* leads me to suspect that they are ecotypes of that species, but I have no field experience, let alone experimental data, to support (or refute) this suggestion. Tsvelev's (1976) treatment makes it clear that such a study should involve plants from both sides of the Bering Strait.

Final comments

The vagaries of the *E. trachycaulus* complex are not compatible with the straightjacket of discrete, hierarchically arranged taxa required by the International Code of Botanical Nomenclature. This should not stop us from attempting to establish the bases of its complexity. Doing so will require completion of carefully designed experimental studies that draw on natural populations from throughout the range of the complex. If this paper stimulates such research, it will have succeeded in its purpose.

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Table 1. Synopsis of data from crosses between members of the *E. trachycaulus* complex and other Triticeae. names of species considered part of the complex are in **boldface**. Names used are those used in the original publications. The first parent is the female parents if crosses were only made in one direction or if the data for reciprocal crosses were presented separeately; if the direction of the cross was not clear, the parent in the complex is stated first.

Parent 1	Parent 2	Result	Citation
Agropyron caninum	*Agropyron spicatum*	5/34 florets set seed; 4 mature hybrids	Stebbins and Snyder 1956
Agropyron caninum	*Agropyron spicatum*	3/45 florets set seed; 0 mature hybrids	Stebbins and Snyder 1956
Agropyron dasystachym	***Agropyron trachycaulum***	12/21 seeds germinated	Dewey 1975
A. dasystachyum x trachycaulum	*Agropyron albicans*	3.2 seeds per spike; pollen 40-45%	Dewey 1975
A. dasystachyum x trachycaulum	***Agropyron subsecundum***	1.4 seeds per spike	Dewey 1975
A. dasystachyum x trachycaulum	***Agropyron caninum***	2.5 seeds per cross	Dewey 1975
A. dasystachyum x trachycaulum	*Agropyron dasystachym*	3.2 seeds per spike; pollen 5-75%	Dewey 1975
A. dasystachyum x trachycaulum	***Agropyron trachycaulum***	5.1 seeds per spike	Dewey 1975
Agropyron latiglume	***Agropyron trachycaulum***	1/59 florets set seed; 1 mature hybrid	Jozwik 1966
Agropyron latiglume	***Agropyron subsecundum***	0/16 florets set seed	Jozwik 1966
Agropyron parishii	*Elymus virginicus* var. *intermedius*	5/56 florets set seed; 2 mature hybrids	Stebbins and Snyder 1956
Agropyron parishii	*Agropyron spicatum*	3/98 florets set seed; 1 mature hybrid	Stebbins and Snyder 1956
Agropyron pauciflorum	*Elymus glaucus* var. *jepsonii*	2 mature hybrids	Stebbins et al. 1946
Agropyron pringlei	*Agropyron spicatum*	1 non-viable seed; hybrid no seed	Dewey 1976
Agropyron pringlei	***Agropyron scribneri***	16 seeds, all germinated; hybrid no seed	Dewey 1976
Agropyron pringlei	*Agropyron dasystachyum*	19 seeds, 12 germinated	Dewey 1976
Agropyron pringlei	***Agropyron violaceum***	3 seeds; hybrid no seed	Dewey 1976
Agropyron scribneri	*Elymus junceus*	Pollen sterile; no seed	Dewey 1967a
Agropyron scribneri	*Sitanion hystrix*	Pollen sterile; no seed	Dewey 1967b
Agropyron scribneri	*Hordeum brachyantherum*	8 seeds, none germinated	Dewey 1968a
Agropyron scribneri	***Agropyron latiglume***	3 seeds, all viable; hybrids sterile	Dewey 1968a
Agropyron sericeum	*Hordeum jubatum*	No seed; pollen sterile	Murry and Tai 1980
Agropyron spicatum	***Agropyron trachycaulum***	0/50 florets set seed	Stebbins and Snyder 1956
Agropyron subsecundum	***Agropyron trachycaulum***	34/221 florets set seed; 25 mature hybrids	Jozwik 1966
Agropyron trachycaulum	***Agropyron scribneri***	Sterile	Dewey 1963
Agropyron trachycaulum	***Agropron trachycaulum***	12/73 florets set seed; no mature hybrids	Jowik 1966
Agropyron trachycaulum	***Agropyron subsecundum***	23/98 florets set seed; 21 mature hybrids	Jozwik 1966
Agropyron trachycaulum	*Agropyron spicatum*	12/57 florets set seed; 10 mature hybrids	Stebbins and Snyder 1956
Elymus canadensis	***Agropyron subsecundum***	Sterile	Dewey 1977
Elymus canadensis	***Agropyron subsecundum***	5/12 florets set seed 8 seeds from open pollination of hybrid	Dewey 1966
Hordeum brachyantherum	***Agropyron scribneri***	9 seeds, 6 viable	Dewey 1968a

Appendix. Authorities and synonyms for names used in the text. The list of synonyms is not exhaustive. Synonyms in **boldface** also appear in the first column; for such names the authority is listed only in the first column.

Name Used	Synonyms
Agropyron boreale Turcz.	***Elymus alaskanus*** subsp. ***alaskanus***
var. *alaskanum* (Scribner & Merr.) Melderis	
var. *boreale*	***Elymus alaskanus*** subsp. ***borealis; Elymus kronokensis*** subsp. ***subalpinus***
var. *hyperarcticum* (Polunin) Melderis	***Elymus sajanensis*** subsp. ***hyperarcticus*** (Polunin) Tzvelev
Agropyron caninum (L.) P. beauv.	***Elymus caninus***
subsp. *caninum*	***Elymus caninus***
subsp. *majus* (Vasey) C.L. Hitchc.	***Elymus trachycaulus***
Agropyron dasystachym (Hooker) Scribner	***Elymus lanceolatus***
Agropyron latiglume (Scribner & J.G. Sm.) Rydb.	*A violaceum* (Hornem.) Lange;
	A. trachycaulum var. *latiglume* (Scribner & J.G. Sm.) Beetle;
	A. caninum var. *latiglume* (Scribner & J.G. Sm.) C.L. Hitchc.;
	Elymus alaskanus subsp. ***latiglumis***
Agropyron parishii Schribner & J.G. Sm.	***Elymus stebbinsii***
Agropyron pauciflоum (Schweinf.) Hitchc.	***Elymus trachycaulus***
Agropyron pringlei (Scribner & J.G. Sm.) Hitchc.	***Elymus sierrae***
Agropyron scribneri (Vasey) M.E. Jones	***Elymus scribneri***
Agropyron sericeum Hitchc.	***Elymus macrourus*** (but see text)
Agropyron spicatum (Pursh) Scribner & J.G. Sm.	***Peudoroegneria spicata***
Agropyron subsecundum (Link) Hitchc.	***Elymus trachycaulus*** subsp. ***subsecundus***
var. *andinum* (Scribner & J.G. Sm.) Hitchc.	***Elymus trachycaulus*** subsp. ***andinus***
Elymus alaskanus (Scribner & Merr.) A. Love	
subsp. *alaskanus*	
subsp. *borealis* (Turcz.) A. Love	***Elymus kronokensis*** subsp. ***subalpinus***
subsp. *hyperarcticus* (Polunin) A. Love & D. Love	***Elymus sajanensis*** subsp. ***hyperarcticus***
subsp. *latiglumis* (Scribner & J.G. Sm.) A. Love	

Appendix. Authorities and synonyms for names used in the text. The list of synonyms is not exhaustive. Synonyms in **boldface** also appear in the first column; for such names the authority is listed only in the first column.

Name Used	Synonyms
Elymus canadensis L.	
Elymus caninus (L.) L.	
Elymus donianus (F.B. Shite) A. Love & D. Love	
Elymus elymoides (Rafin.) Swezey	
Elymus glaucus Buckley	
Elymus jacutensis (Drobow) Tzvelev	
Elymus junceus Fischer	***Psathyrostachys juncea***
Elymus kronokensis (Kom.) Tzvelev	
subsp. *kronokensis*	
Elymus laevis Hoover	
Elymus lanceolatus (Scribner & J.G. Sm.) Gould	
Elymus macrourus (Turcz.) Tzvelev	
Elymus repens (L.) Gould	*Elytrigia repens* (L.) Nevski
Elymus sajanensis (Nevski) Tzvelev	
subsp. *hyperarcticus* (Polunin) Tzvelev	
subsp. *sajanensis*	
subsp. *villosus* (V.N. Vassil.) Tzvelev	
Elymus scribneri (Vasey) M.E. Jones	
Elymus sierrae Gould	
Slymus stebbinsii Gould	***Agropyron parishii***
Slymus trachycaulus (Link) Shinners	
subsp. *andinus* (Scribner & J.G. Sm.) A. Love & D. Love	
subsp. *subsecundus* (Link) Gould	[An artificial taxon]
Hordeum brachyantherum Nevsi	

Appendix. Authorities and synonyms for names used in the text. The list of synonyms is not exhaustive. Synonyms in **boldface** also appear in the first column; for such names the authority is listed only in the first column.

Name Used	Synonyms
Hordeum jubatum L.	
Psathyrostachys juncea (Fischer) Nevski	
Pseudoroegneria spicata (Pursh) A. Love	
Sitanion longifolium J.G. Sm.	
Sitanion hystrix (Nutt.) J.G. Sm.	***Elymus elymoides***
var. *californicum* F.D. Wilson	
var. *hystrix*	

Influence of Climatic Factors on the Distribution of Hordein Alleles in Barley

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ABSTRACT

Allele distribution of hordein-coding loci was studied in 226 spring barley cultivars grown in 25 natural agricultural provinces of the former USSR during the last 62 years. Significant relationships between climatic factors and distribution of Hrd A, Hrd B and Hrd F loci were established. It was shown that genetic structure of cultivar-populations is determined by the following climatic factors: annual rainfall, average temperature in July, accumulated effective temperature, and climate continentality.

INTRODUCTION

Results of hordein analysis in spring barley revealed polymorphism of Hrd A (Hor 1), Hrd B (Hor 2), and Hrd F (Hor 5) loci, as well as differences in frequencies and distribution of alleles of these loci (1). Earlier (2) we reported that the distribution and the frequencies of alleles of Hrd loci among cultivars from the European part of the USSR depend on such climatic factors, as annual rainfall and average temperature in July. This report presents the allele distribution from the entire crop are of spring barley in the former USSR.

Table 1. Natural agricultural provinces and their climatic characteristics in crop area of cultivated spring barley in the former USSR

A	B	Province	Zone	C	D	E	F	G
1	A	European	Middle taiga	600	16.5	1400	155	23
2	B	Baltic	South-taiga-forest	650	17.5	1900	118	33
3	C	Byelorussian		650	19.1	2400	148	30
4	D	Middle Russian		650	18.0	1900	159	35
5	E	West Siberian		450	17.5	1600	188	15
6	F	Amuro-Ussuriiskaya		850	19.9	2300	250	17
7	G	Ukrainian	Forest-steppe	550	19.4	2600	160	37
8	H	Middle Russian		500	19.3	2400	175	44
9	I	Predural 'skaya		500	19.2	2200	187	32
10	J	West Siberian		350	18.9	2000	203	23
11	K	Predaltaiskaya		600	18.4	1850	202	16
12	L	Ukrainian	Steppe	425	21.8	3100	168	26
13	M	Predkavkazskaya		600	21.7	3150	170	23
14	N	South Russian		425	21.1	2900	178	28
15	O	Zavolzhskaya		350	20.4	2500	195	14
16	P	East Siberian		275	19.7	2200	210	15
17	Q	Kazakh		400	18.9	1950	220	20
18	R	Predaltaiskaya		300	17.4	1700	265	14
19	S	South Ukrainian	Arid steppe	300	22.0	3350	160	15
20	T	Manychsko-Donskaya		300	21.5	3150	185	20
21	U	Zavolzhskaya		300	22.1	2850	210	12
22	V	Kazakh	Semidesert	300	20.5	2450	218	13
23	W	Prikaspiiskaya		250	22.5	2800	250	17
24	X	Central Kazakh		300	22.2	2400	240	10
25	Y	South Kazakh	Desert-steppe-foothills	300	23.1	3400	211	14

Heading legends: A = Number, B = Designation, C = Annual rainfall (mm), D = Average temperature in July (°C), E = Accumulated temperature >10°C, F = Index of climate continentality, and G = Number of varieties.

MATERIALS AND METHODS

Two hundred and twenty-six spring barley cultivars, grown in the USSR from 1929 to 1991 were studied. As territorial units, we used natural agricultural provinces (NAP, Tab.) included into the system of natural agricultural zones (Fig. 1). Natural agricultural zone is characterized by important biological properties of soil. Provinces differ from each other by fertility and hydrothermal regime of soil and by basic agroclimatic indices (5). 10-44 cultivars per each NAP were studied. Data from mountain crop areas of barley, i.e. the Caucasus, the Pamir, and the Urals, were not included. If one and the same cultivar was grown in several NAP, it was taken in consideration in each of these NAPs.

Hordein electrophoresis was performed in 12-14% starch gel in aluminum lactate buffer (pH 3.1), containing 3M urea according to Sozivov & Poperelya (3). From 40 to 100 individual grains were analyzed per each cultivar. Alleles of hordein-coding loci were designated according to a previously proposed nomenclature (4).

We analyzed 16 alleles of the Hrd A locus, 20 alleles of the Hrd B locus, and 4 alleles of the Hrd F locus; 13 rare alleles of A locus and 28 of B locus were united. As an index of genetic similarity, a multidimensional analysis was carried out (6). On the Basis of the obtained matrix of genetic distances, we performed linear multidimensional scaling as proposed by Kruskal (7), and clusters were isolated according to the method of Rao (8).

RESULTS

The homogeneity of the allelic composition of the populations in NAP was estimated by $x^2 = 279.7$, d.f. = 96, and P 0.0001, for the Hrd B locus $x^2 = 275.1$, d.f. = 72, and P 0.0001, and for the Hrd F locus $x^2 = 250.2$, d.f. = 48, and P 0.0001, respectively. The accumulated value of statistics was $x^2 = 805.1$, d.f. = 216, and P 0.0001.

The obtained data revealed significant heterogeneity of allelic frequencies for these three loci. On the basis of the analysis of allele frequencies in barley populations, we calculated indices of genetic distances. These indices characterize the similarity of allelic composition in the provinces compared. For the better display of data and their analysis, we performed linear multidimensional scaling and cluster analysis (8) based on the obtained matrix of genetic distances (Fig. 2).

The multidimensional scaling gave a significant linear relationship between the climatic characteristics of provinces and the first three factors calculated (in total 11 factors were examined). Figure 2 presents distribution of populations according to the first two principal components. The first factor explains 49.6% of the total variability, the second one 10.3%, and the third one 5.6%, respectively. The first three factors reflect a linear combination of the climatic characteristics of the provinces. The main portion of genetic variability of hordein loci in barley populations is determined by two climatic factors—rainfall and the average temperature in July (correlation coefficient is 0.900, P = 0.001). The second factor is effective temperatures and average temperature in July (correlation coefficient is 0.632, P = 0.004). The third factor is connected with the index of climate continentality and the average temperature in July (correlation coefficient is 0.546, P = 0.021). We estimated relationship between the values of the three first principal factors and frequencies of 42 alleles in barley populations. According to the problem of multiple comparisons, the accumulated value for the significance test is 0.0012 and critical value of the correlation coefficient is 0.609 (9). The results show that the first factor is significantly associated with 11 alleles (negatively with A2, A12, B8, F2 and positively with A13, A28, B35, B37, B38, B45, F3. For allele nomenclature see Pomortsev *et al*. (4)). The multiple correlation coefficient is 0.992. The second factor is significantly connected with 4 alleles (A8, A10, A23, B31, negatively). The multiple correlation coefficient is 0.915. The third principal factor is significantly associated with 3 alleles. However, in this case, the multiple correlation coefficient was not determined due to lack of data. Correlation of each allele is the following: A30 (-0.804), B36 (-0.861) and F0 (-0.861).

The obtained results show that the distribution and allele frequencies of Hrd A, B, F loci in barley cultivars significantly depend on different combinations of climatic factors. Such combinations of climatic factors as annual temperature, and climate continentality have the highest influence on the formation of genetic structure of populations.

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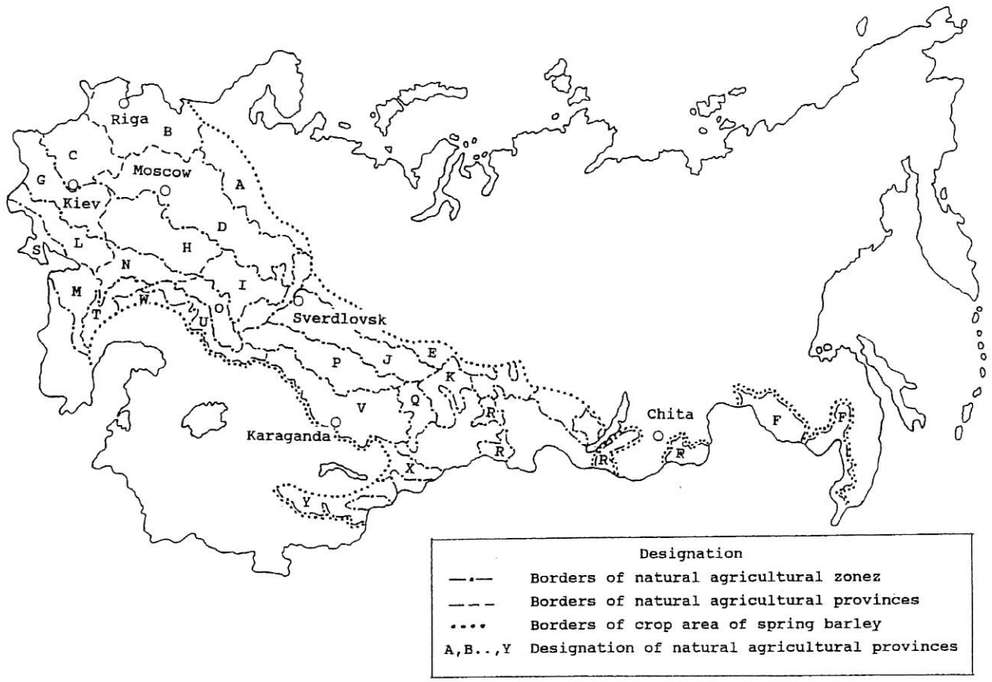

Fig.1. Scheme of natural agricultural provinces in the former USSR within the crop area of spring barley.

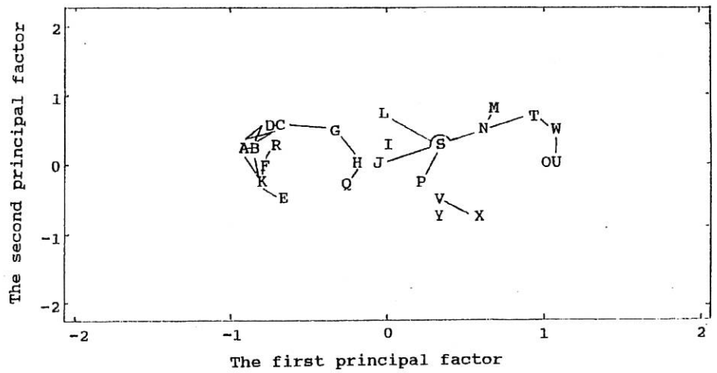

Fig.2. Plot of the first two principal factors of variety populations in natural agricultural provinces.

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Reinterpretation of Dispersal Strategies in *Triticum* L. and *Aegilops* L.

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ABSTRACT

Analyses of dispersal strategies in *Triticum* L. and *Aegilops* L., whether descriptive in nature or based on the concept of pivotal genomes, have proven inadequate in characterizing fully the adaptive radiation. *Aegilops* exhibits novel diaspore types whose variation is far greater than that found in other genera of the tribe Triticeae. Developmental links between the three principal diaspore types—wedge, synaptospermic, and barrel—provide an explanation for their origin and establish a pattern of relationship linking *Aegilops* to *Triticum* and to the other genera of the tribe. The dimorphic inflorescence of *Ae. speltoides* is the probable source of a multidirectional variation.

INTRODUCTION

The dispersal units of wild taxa in the wheat complex—*Triticum* L. *sensu* Dorofeev and Migushova (1979) and *Aegilops* L. *sensu* van Slageren (1994)—have traditionally been classified into four categories: (1) a wedge diaspore consisting of a spikelet subtended by a rachis internode (Fig. 1a); (2) a cylindrical diaspore consisting of an entire spike, strongly awned only on the apical spikelet, whose basal spikelet is subtended by a rachis internode (Fig. 1b); (3) a barrel diaspore consisting of a spikelet and the adjacent rachis internode (Fig. 1c); and (4) an umbrella diaspore consisting of an entire spike, ovate and multiawned, whose basal spikelet is subtended by a rachis internode (Fig. 1d). Each of these diaspore types is created by spontaneous disarticulation of the rachis. All four occur in *Aegilops*; only the wedge type is found in *Triticum*.

SYSTEMATICS

Adaptive radiation

Although there is a voluminous literature dealing with wheat evolution, comparative investigations of dispersal mechanisms are few in number. Eig (1929) was the first researcher to characterize the adaptive radiation found in *Aegilops*, noting that it covers "nearly the entire amplitude of variation" occurring in the tribe (Eig, p. 194). Zohary (1965) expanded Eig's descriptive system to include *Triticum*. He correlated dispersal strategy with polyploid speciation and in so doing, conformed his explanation of the pattern of adaptive radiation to the pivotal-genome theory proposed earlier by Zohary and Feldman (1962).

The pivotal-genome theory, which supports a genomic concept of the wheat complex (Kimber and Sears, 1987), divides *Triticum* and *Aegilops* into three large polyploid clusters. Each cluster represents a successful evolutionary group by virtue of dispersal and ecogeography (Zohary, 1965). At the head of each cluster is a diploid species whose genome represents the pivotal, unchanged genome of the member polyploid taxa. Its diaspore type characterizes the dispersal strategy for all taxa in the cluster. There are two polyploid clusters in *Aegilops*: the D-genome cluster headed by *Ae. tauschii* Coss. with a barrel diaspore and the U-genome cluster headed by *Ae. umbellulata* Zhuk. with the umbrella diaspore. In *Triticum*, the A-genome polyploid cluster, headed by *Tr. boeoticum* Boiss., has the wedge diaspore type. Although Zohary did not discuss *Tr. urartu* Gandil., this species, which has since been identified as the A-genome donor of tetraploid and hexaploid wheats (Chapman et al., 1976; Dvorák, 1976; Dvorák et al., 1992), should be designated, in place of *Tr. boeoticum*, as the head of this cluster.

Noticeably absent from this scheme is the cylindrical diaspore, which is typically associated with five diploid taxa (see below). Zohary designates this diaspore type as "unsuccessful" for two reasons: it is not associated with a pivotal diploid genome; and it has the characteristics of a cumbersome dispersal unit which is comparatively unfit relative to his concept of evolutionary success. Also missing from Zohary's discussion is a somewhat amorphous group containing the *Aegilops* taxa producing wedge diaspores [*Ae. speltoides* Tausch var. *ligustica* (Savign.) Fiori, *Ae.*

sharonensis Eig, *Ae. bicornis* (Forssk.) Jaub. & Spach, and *Amblyopyrum mutica* (Boiss.) Eig (=*Ae. mutica*)] the two taxa acknowledged by Zohary as transitional forms between the cylindrical- and umbrella-diaspore categories, *Ae. uniaristata* Vis. and *Ae. comosa* Sm. in Sibth. & Sm. var. *subventricosa* Boiss. (=*heldreichii*).

In the context of a genomic interpretation of the dispersal mechanism, these neglected taxa appear irrelevant. However, in work reported elsewhere (Morrison, 1994), I have established that this interpretation, particularly regarding the cylindrical diaspore, does not characterize fully the patterns of adaptive radiation. My disagreement with Zohary deals with the narrow perspective imposed by his adoption of the pivotal-genome theoretical framework by which he omits other measures of evolutionary fitness and fails to consider developmental relationships.

Significance of *Aegilops speltoides*

In my view, the key to understanding the novel dispersal strategies of the wheat complex can be found in the entire-spike diaspore, here designated synaptospermic diaspore using the terminology of Zohary (1937). It has been noted by several authors that both the cylindrical and umbrella diaspores will undergo an additional disarticulation on the ground to form secondary barrel diaspores (Eig, 1929; Schröder, 1931; Zohary, 1937; Frank, 1964; Morrison, 1994). This phenomenon implies that the primary barrel diaspore associated with *Ae. tauschii* is probably the outcome of a developmentally altered synaptospermic-diaspore strategy in which the original primary, basal-wedge disarticulation is suppressed. Moreover, the umbrella and cylindrical diaspores are both synaptospermic-diaspore types related by virtue of their mode of disarticulation and entire-spike structure. Given the probable linkage of the cylindrical, barrel, and umbrella diaspores, their developmental association with the wedge-diaspore type comes into question. In this regard, the dimorphic inflorescence structure of *Ae. speltoides* provides a plausible origin for the divergence of the wedge- and synaptospermic-diaspore strategies.

Although the taxonomic interpretation of *Ae. speltoides* inflorescence morphology suggests a clear separation between var. *speltoides* and var. *ligustica*, the two infraspecific taxa actually comprise a population of plants which differ only in dispersal strategy (Zohary and Imber, 1963). A closely linked block of Mendelian genes controls the dimorphic inflorescence trait; the wedge-diaspore type (*ligustica*) is dominant over the cylindrical-diaspore type (*speltoides*) (Sears, 1941; Zohary and Imber, 1963). Here, in one species, there is evidence of a simple genetic system which controls two distinct diaspore types.

In the cylindrical diaspore of *Ae. speltoides*, two forms of disarticulation occur: (1) the wedge break (diaspore with subtending rachis internode) which creates the primary, synaptospermic diaspore (Fig. 1b) and (2) a delayed, barrel break (diaspore with adjacent rachis internode) which creates the secondary, barrel diaspores (Fig. 2a). Assuming that the genetics of this system is adequate support for Eig's proposal of the wedge diaspore as the primitive type for the Tribe Triticeae, then the adaptive radiation in the wheat complex can be viewed to begin with *Ae. speltoides*. The wedge diaspore of var. *ligustica* (Fig. 1a) provides the link both to *Triticum* (the A-genome cluster *sensu* Zohary) and to the other genera of the tribe with the wedge-diaspore dispersal strategy. *Aegilops speltoides* var. *speltoides* serves as the starting point of a radiation of novel diaspore strategies found exclusively in *Aegilops* (see Fig. 2). It is also the link to the genus *Henrardia* C.E. Hubbard, which is the only other member of the Tribe Triticeae to exhibit the synaptospermic diaspore strategy (Morrison, 1994).

An alternative interpretation

Rachis disarticulation serves as the basis for characterizing the pattern of variation radiating from *Ae. speltoides* var. *speltoides*. The *speltoides* form undergoes a primary wedge disarticulation at the base to produce a cylindrical, synaptospermic diaspore and then undergoes a delayed barrel disarticulation when on the ground to produce a secondary barrel diaspore. This strategy is also found in *Ae. longissima* Schweinf. & Muschl., *Ae. searsii* Feldman & Kislev ex Hammer, *Ae. caudata* L., and *Ae. comosa* var. *comosa*.

Four different strategies have evolved from this original dispersal mechanism: (1) profuse awning, size reduction of the spike, and ovate spike shape (umbrella form) with a secondary barrel disarticulation in the terminal spikelet—*Ae. umbellulata* (Fig. 2b); (2) loss of the secondary barrel disarticulation—*Ae. uniaristata* (Fig. 2c) and *Ae. comosa* var. *subventricosa*; (3) primary barrel disarticulation due to a loss of the basal-wedge disarticulation—*Ae. tauschii* (Fig. 2d); (4) tough-rachis due to a loss of both modes of disarticulation—forms of *Ae. tauschii* (Fig. 2e), *Ae. comosa* var. *subventricosa*, and the polyploid *Ae. ventricosa* Tausch (Morrison, 1994). The tough-rachis forms are interesting because they possess a trait usually associated with human selection. Unlike the domesticated taxa such as *Tr. aestivum* L., these wild, tough-rachis taxa have very tough glumes which can be only broken with a force sufficient to destroy the integrity of the spike.

Clearly, non-wedge diaspores are an unusual dispersal strategy for the tribe. They indicate an evolutionary trend in *Aegilops* uniquely different from the trend characterizing domesticated *Triticum* taxa. This phenomenon provides justification for reevaluation of the genomic concept currently dominating the taxonomy of the wheats (Morrison, 1995). It also suggests that the genetic mechanisms underlying dispersal strategies hold a wealth of information which has yet to be exploited either for investigations of the adaptive radiation or for practical agricultural applications.

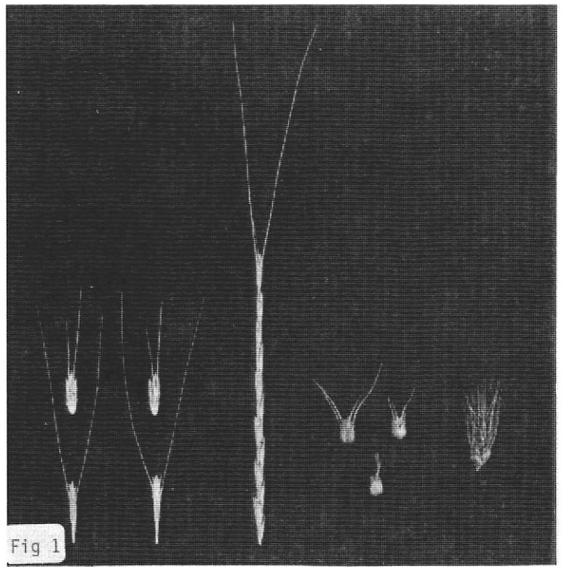

Figure 1 Diaspores of wild wheats according to the traditional interpretation: (a) wedge diaspore (*Tr. urartu*, top; *Ae. speltoides* var. *ligustica*, bottom); (b) cylindrical diaspore (*Ae. speltoides* var. *speltoides*); (c) barrel diaspore (*Ae. tauschii*); (d) umbrella diaspore (*Ae. umbellulata*).

Figure 2. Synaptospermic diaspore strategies: (a) cylindrical dispore with secondary barrel diaspores (*Ae. speltoides* var. *speltoides*); (b) umbrella diaspore with secondary barrel diaspore (*Ae. umbellulata*); (c) intact synaptospermic dispore with no secondary barrel diaspores (*Ae. uniaristata*); (d) primary barrel diaspores (*Ae tauschii*); (e) tough rachis with no disarticulation (*Ae. tauschii*).

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Systematics of the Triticeae: Problems and Progress

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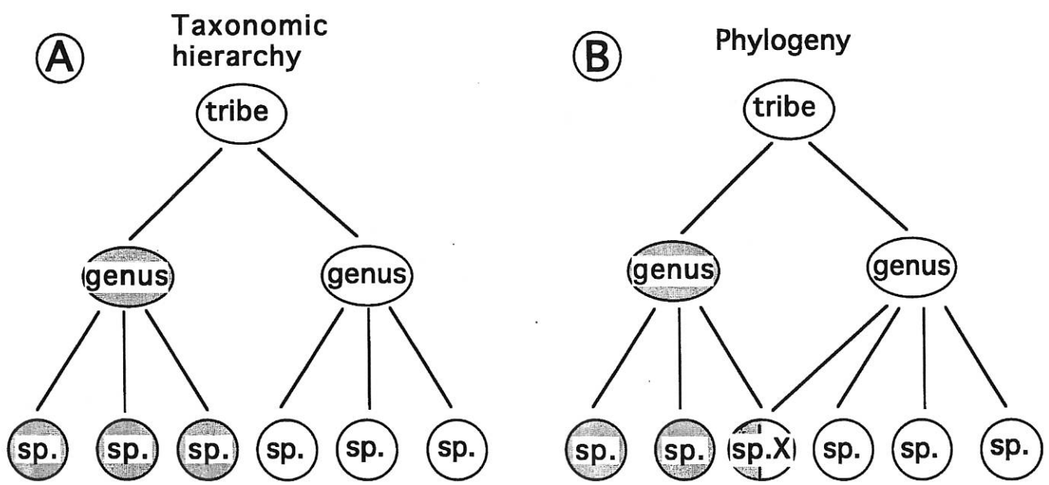
INTRODUCTION

Classifications are hierarchical structures. This means that as long as evolution can be diagrammed as a divergent tree, it can be easily converted to a classification. A hierarchical classification is simply an upside-down tree, as is obvious from Figure 1A.

A web cannot be transformed into a hierarchy without losing information. In Figure 1B, species X is connected to two genera. Either it can be placed with the shaded genus (on the left), in which case one loses the information that it contains part of the white genus, or it can be placed with the white genus, which ignores the information linking it to the shaded genus. Either way, there is always some information indicating that species X ended up with the wrong group. It is impossible to convert a web unambiguously into a hierarchy.

The analogy with the Triticeae is obvious. We know that the evolutionary history of the group, or even parts of

Figure 1. Comparison between a hierarchy (1A) and a web (1B). If a phylogeny is tree-like then it can be converted directly into a taxonomic hierarchy. If a phylogeny is web-like, then any conversion into a hierarchy involves loss of information.



the group, can be diagrammed as a web (Kellogg 1989; Kimber and Feldman 1987). The only way to convert this into a hierarchical classification is to break connections, which results in the loss of some information. The direction in which a connection is broken is also ambiguous - either way is correct, either way is wrong. There is evidence on both sides.

This is partly why the taxonomic history of the group is so complex (reviewed in Barkworth (1992); West et al. (1988)). If the history of the group is really web-like, then there are many ways to divide it up, and all are equally defensible.

But of course there *is* a history, even if it is complicated. Appreciable progress has been made since the last Triticeae symposium (three years ago; proceedings in *Hereditas* 116). This paper summarizes this progress briefly, and then concludes with some implications for taxonomy.

The sister group of the Triticeae

Several studies have addressed the circumscription of the supertribe *Triticanae*, the larger group that includes Triticeae, *Bromeae* and *Brachypodieae* (Macfarlane and Watson 1982). Recent data indicate that *Brachypodium* P. Beauv., is not closely related to the rest of the Triticeae at all. This is supported by multiple molecular data sets, including the internal transcribed spacer of the ribosomal RNA (ITS; Hsiao et al. 1994a), chloroplast DNA restriction sites (Davis and Soreng 1993; Kellogg 1992), 5S DNA spacer sequences (Kellogg and Appels, unpubl. data), sequences of the chloroplast genes *rps*4 (Nadot et al. 1994), and *ndh*F (Catalan, Olmstead and Kellogg, unpubl. data). A morphological study (Kellogg and Watson 1993) also supports this result, but the cladogram is so unresolved that by itself it is inconclusive. In contrast, all the foregoing studies find a close relationship of *Bromus* L. to the Triticeae. We can therefore conclude that *Bromus* is the appropriate outgroup for the tribe.

Diploid Triticeae - current knowledge

Several years ago I suggested that a research program on the Triticeae needed to focus first on the relationships among the diploid taxa (Kellogg 1989). The allopolyploids are known to combine more than one lineage and thus reflect a pattern of web-like (reticulate) evolution. The diploids, by contrast, may well exhibit divergent, hierarchical evolution. Our most powerful analytical tool at the moment, cladistic analysis, assumes that evolution is divergent, not web-like, and thus can only be used on diverging groups. The general approach, then, is to understand the relationships among diploids first, and then use that knowledge to reinterpret the relationships among the polyploids. Relationships among diploids have now been explored extensively, and the results are more intricate than expected.

In the 1989 paper, I published a cladogram for the diploids based on morphological data. If the same data are analyzed with newer algorithms (e.g. PAUP 3.1.1, Swofford 1993), many more equally parsimonious trees are found and the consensus tree is completely unresolved. In other words, the morphological data in that paper are completely uninformative on the relationships among the diploids. This problem has been rectified by Frederiksen and Seberg (1992 and in press, this symposium). Their tree is well-resolved, although still not well supported, despite a good detailed examination of many morphological characters.

There are many other data sets addressing relationships among the diploid taxa. Wang (1992) has published a phenogram summarizing chromosome pairing in the perennial diploids, Monte et al. (1993) have assessed relationships based on nuclear RFLPs, and McIntyre (1988) has studied relationships based on isozyme variation. The relationships described in these three papers do not agree, nor do they agree with the relationships indicated by morphology. These differences are not due simply to differences in the position of the root. The discrepancies are hard to evaluate, however. All three of these papers use only about half the genera in the tribe, so the differences may be due to serious sampling problems. There are also methodological differences in the studies - Wang (1992) is a phenetic analysis, McIntyre (1988) is cladistic, and Monte et al. (1993) is cladistic but the use of nuclear RFLPs for cladistic analysis poses serious problems of interpretation (Swofford and Olsen 1990).These data sets may be telling different stories about evolutionary history, or they may be all telling different parts of the same story; it is not possible to tell which.

Diploid Triticeae - recent sequence data on nuclear genes

Comparisons are easier for character-based (*sensu* Swofford and Olsen, 1990) data - sequences or restriction sites. There are now four molecular data sets for the diploids, three based on sequence data from nuclear gene families, and one on restriction site variation in the chloroplast, itself a sample of the underlying sequence. Two of these data sets include a complete sample of the diploid genera in the tribe, and the other two are near-complete. Thus sampling artifacts are unlikely. A detailed comparison of the nuclear gene trees is presented in Kellogg et al. (MS submitted). Their results are summarized briefly here.

There are two sets of sequences from two independent arrays of 5S RNA genes and their accompanying spacers (Kellogg and Appels, 1995). The two 5S arrays are located on the group 1 and group 5 chromosomes, and can be unambiguously distinguished by the length and sequence of the spacers. The array on the group 1 chromosome has shorter spacers than the group 5 chromosome array and will be designated the short-spacer array. Baum and Appels (1992), Reddy and Appels (1989) and Appels et al. (1992) have shown that the two loci evolve independently - there is little or no concerted evolution between them. They can thus be inferred to have separate evolutionary histories. The two 5S data sets between them include all but four genera of the monogenomic Triticeae - *Eremopyrum* (Ledeb.) Jaub. & Spach, *Heteranthelium* Hochst. ex Jaub. & Spach, *Peridictyon* Seberg, Fred. & Baden, and *Festucopsis* (C. E. Hubb.) Melderis. *Eremopyrum* includes four diploid species, *Festucopsis* two and the other two genera are monotypic. Some sampling problems thus remain for these data, but are, we believe, minimal.

The third sequence-based data set is for the internal transcribed spacers of the ribosomal RNA (ITS), produced by Hsiao et al. (1994b). Like the chloroplast data set, the ITS data cover all diploid genera in the tribe, and many of the diploid species. There are two ribosomal arrays in most Triticeae (Flavell and O'Dell 1976; Flavell and Smith 1974; Gill and Appels 1988; Miller et al. 1983), but only a single clear ITS sequence is ever produced in PCR amplifications. This implies that concerted evolution is operating across both ribosomal (NOR) arrays to homogenize them not only within an array but between arrays (as described by Arnheim, 1983; Schlötterer and Tautz, 1994). The ITS trees of Hsiao et al. (1994b) do not resolve relationships among all the groups, but do support a "Mediterranean clade", previously recognized by Sakamoto (1973) on biogeographic grounds. The Mediterranean clade includes *Triticum* L./*Aegilops* L., *Thinopyrum* A. Löve (s.l.), *Secale* L., *Henrardia* C. E. Hubb., *Crithopsis* Jaub. & Spach, *Taeniatherum* Nevski, and *Eremopyrum*. This group, however, only appears if transversions are weighted more heavily than transitions. Otherwise, the tree is largely unresolved.

The three nuclear gene trees do agree on the earliest branches of the cladogram. If they are rooted with *Bromus* as the outgroup, the next diverging branch(es) is a *Hordeum* L./*Critesion* Raf. clade (paraphyletic in some trees), and then *Psathyrostachys* Nevski. For the 5S long-spacer array, for which we lack a *Psathyrostachys* sequence, the next diverging branch is *Dasypyrum* (Coss. & Durieu) Durieu.

The three trees (like the chromosome pairing, nuclear RFLP, and isozyme studies cited above) also agree on the monophyly of all monogenomic groups except for the J/E genomes, and the monophyly of all species of *Aegilops* (*Triticum pro parte, sensu* Kimber and Feldman 1987). The species of *Aegilops* represent several genomic groups (and therefore were placed by Löve (1984) in several genera), but their close relationship has never been seriously questioned. The genomic similarity of members of *Pseudoroegneria* (Nevski) A. Löve, *Australopyrum* (Tzvelev) A. Löve and *Secale* is also well-established. These points of agreement thus do not offer much in the way of new insights into the tribe.

The trees differ in many ways:

1. The position of *Triticum monococcum* L., whether with the species of *Aegilops* or near the base of the tree.
2. The position of *Secale*, whether part of the "Mediterranean clade" or near the base of the tree.
3. The relationship of *Thinopyrum bessarabicum* (Savul & Rayss) A. Löve and *Lophopyrum elongatum* (Host) A. Löve, whether sister taxa, or unrelated.
4. The relationship of *Pseudoroegneria* and *Australopyrum*, whether sister taxa, or unrelated.
5. The relationship of *Crithopsis* and *Taeniatherum*, whether sister taxa, or unrelated.
6. The relationship of *Agropyron* and *Pseudoroegneria*, whether sister taxa or unrelated.

How compelling are these differences? They may reflect different evolutionary histories for different parts of the genome. Or they may simply be minor discrepancies created by random variations in the sequence data. We can rule out the possibility that the differences may be caused by unrecognized polymorphism in one or more of the data sets. Two to ten 5S DNA units were sequenced from individuals of each species; in most cases, the sequences from each species were more closely related to each other than to those of other species. For ITS sequences, two disparate accessions of each species were sequenced, and these were found to be identical. For chloroplast restriction site studies, most species were represented by two to 15 accessions; levels of polymorphism were very low, and involved only one phylogenetically informative restriction site. Thus the discrepancies are unlikely to be the result of sampling error.

How do we distinguish minor disagreements between gene trees from serious discrepancies that need some general explanation? This subject has received much attention in the literature (Adams 1972; Bull et al. 1993; Kluge 1989; Miyamoto 1985; Swofford 1991), and discussion continues. There is no generally accepted method. Kellogg et al. (MS submitted) used the following strategy to compare the three nuclear data sets:

1. Analyze all pairwise combinations of data sets.
2. Reduce each pair of data sets only to taxa in common.
3. Analyze each data set of the pair separately.

4. Assess support for each node; interpret nodes with bootstrap F or decay or = 2 as weakly supported and therefore ambiguous.

5. Determine significance of potential conflict using the incongruence length difference test (Mickevich and Farris 1981) with multiple randomizations (implemented independently by Farris et al. 1994 and Swofford 1995), and the Wilcoxon signed ranks test (Siegel 1956), applied to phylogenetic inference by Templeton (1983)

6. If conflict is poorly supported and not statistically significant , combine data sets.

7. If there is conflict, remove the taxon creating the conflict, and then combine the data.

Using this approach they found that the two 5S trees disagree strongly on the placement of *Triticum monococcum*. The short-spacer array (group 1 chromosomes) places *Triticum monococcum* in a monophyletic group with the species of *Aegilops*, whereas the long-spacer array (group 5 chromosomes) places it near the base of the tree and unrelated to *Aegilops*. Curiously, this latter placement is also supported by morphological data (Frederiksen and Seberg, 1992 and unpub.), isozyme (McIntyre 1988) and nuclear RFLP data (Monte et al. 1993), but not by chloroplast data (see below).

The conflict between the 5S gene trees indicates that in *Triticum monococcum* the two 5S arrays have different histories. It is possible that they are markers of entire chromosome arms, which would imply that portions of chromosome 1 or chromosome 5 in *Triticum monococcum* have a different origin from the rest of the genome.

Other than the position of *Triticum monococcum*, the two 5S gene trees agree with each other. If *Triticum monococcum* is omitted and the two data sets are combined they reinforce each other and produce one major clade including *Aegilops*, *Thinopyrum* (s.l.), *Crithopsis* and *Taeniatherum*. A second clade includes *Pseudoroegneria*, *Australopyrum* and *Agropyron*, and the base of the tree is a paraphyletic group consisting of *Henrardia*, *Secale*, *Dasypyrum*, and *Psathyrostachys*.

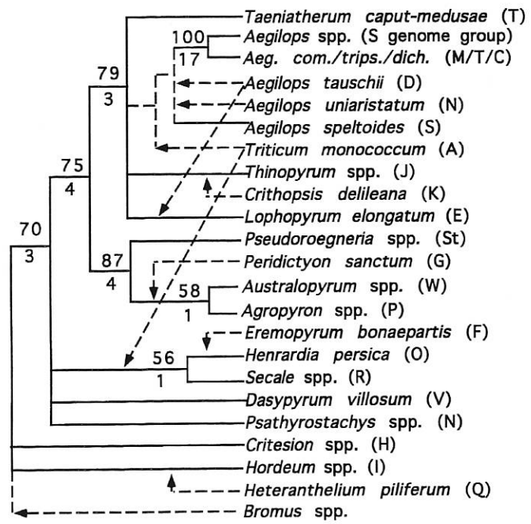


Figure 2. Phylogeny of the diploid Triticeae, using combined sequence data from two nuclear 5S DNA arrays and the nuclear ITS, redrawn from Kellogg et al. (MS submitted). Strict consensus of 108 trees, each with L = 488, CI = 0.711, RI = 0.571. Decay values are below the lines, percent of 100 bootstrap replications above. *Triticum monococcum* and *Aegilops tauschii* are drawn as reticulations because their gene trees indicate significantly different histories. Taxa connected with dotted lines were omitted from bootstrap and decay analyses because of extensive missing data. Tree was constrained by the topology indicated by solid lines, and dotted-line taxa placed accordingly.

Comparing either of the 5S trees with the ITS tree indicates many conflicting groupings. However, Kellogg et al. (MS submitted) have shown that most of the groups in the ITS tree are poorly supported. Small changes in weights of characters can produce significant changes in the tree. Therefore most of the conflict can be explained as lack of resolution in the ITS data. If transversions are weighted more and more heavily, then some conflict appears in the position of *Secale* and *Henrardia*. In the 5S data they are sister taxa and diverge just after *Psathyrostachys;* in the ITS data they appear as part of the "Mediterranean clade". It is not clear what causes this discrepancy, but, because it is aggravated rather than cured by weighting, we suspect it may reflect forces intrinsic to the ribosomal array rather than differences in evolutionary history.

Several taxa - *Heteranthelium, Dasypyrum, Crithopsis*, and *Secale* - have branches five to ten times as long as other branches in the ITS tree. Experiments with removing these one at a time from the ITS data set reveal that *Heteranthelium* and *Dasypyrum* can be placed in many different positions in the tree and thus lead to very poor support. *Crithopsis* has the same effect in reduced data sets, but not in the whole data set. This suggests that the pattern of homoplasy in the long branches reflects characters shared with several distantly related lineages. It is possible, but by no means proven by these data, that these taxa reflect hybrid ancestry followed by a complex process of gene conversion. Curiously, although *Secale* also has a long branch, its removal does not improve tree resolution. This implies that there may have been an increase in evolutionary rate on the branch leading to *Secale*, but that the long branch is not due to any unusual history.

Combining all three nuclear data sets gives the tree shown in Figure 2 (strict consensus of 108 trees, each with

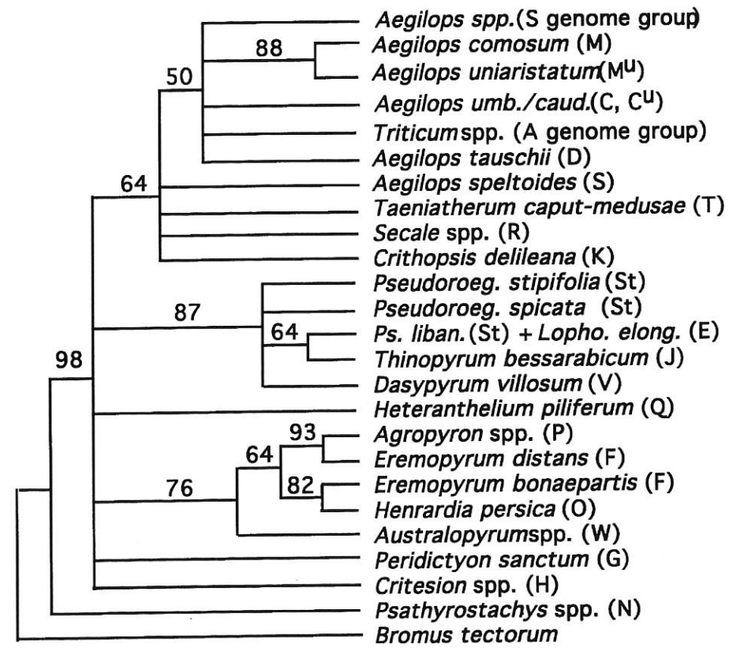


Figure 3. Phylogeny of the chloroplast genome of diploid Triticeae, redrawn from Mason-Gamer and Kellogg (1995). Numbers above branches are percentage of 100 bootstrap replicates. Strict consensus of 12 trees, each with L = 181, CI = 0.659, RI = 0.848.

L = 488, CI = 0.711; RI = 0.571). *Crithopsis delileana, Aegilops unaristatum, Peridictyon sanctum, Eremopyrum bonaepartis, Heteranthelium pilferum* and *Bromus* spp. were excluded because of missing data; their approximate positions are indicated by dotted lines. *Triticum monococcum* and *Aegilops tauschii* are shown as reticulations, indicating the different history of the 5S long-spacer array from either the ITS or 5S short-spacer array.

This is then our best hypothesis for evolution of the nuclear genes. There is strong evidence that the two 5S arrays in *Triticum monococcum* have different histories, and slightly weaker evidence that the 5S long-spacer array and the ITS of *Aegilops tauschii* have different origins. This may mean that chromosomes 1 and 5 have different histories.

Beyond this, *Aegilops* species form a clade with *Thinopyrum* species, and *Thinopyrum bessarabicum* and *Thinopyrum (Lophopyrum) elongatum* nuclear genes have distinct origins. *Pseudoroegneria, Agropyron* and *Australopyrum* form a group. *Henrardia* and *Secale* are early diverging branches, along with *Psathyrostachys*, and *Dasypyrum*. *Hordeum* and *Critesion* are paraphyletic and represent the earliest divergence.

Diploid Triticeae - recent chloroplast data

How does this compare with what we know of the chloroplast? Mason and Kellogg (in press) have analyzed restriction site variation in the chloroplasts of all diploid Triticeae and have produced a well-resolved tree (Figure 3). The tree, however, bears almost no resemblance to the nuclear gene tree. One of the best supported groups comprises *Pseudoroegneria, Thinopyrum/Lophopyrum*, and *Dasypyrum*. *Pseudoroegneria libanotica* and *Lophopyrum elongatum* are in fact identical for the restriction sites surveyed. There is no hint of polyphyly in *Thinopyrum* as indicated by the nuclear genes. There is no support (even among homoplasious characters) for a grouping of *Pseudoroegneria* with *Australopyrum* or *Agropyron*.

A second chloroplast group is *Triticum, Aegilops, Crithopsis, Secale*, and *Taeniatherum*. This bears some resemblance to the "Mediterranean clade" supported by the ITS data, but does not include *Thinopyrum* and *Lophopyrum*. Although the nuclear genes link *Henrardia* with *Secale*, the chloroplast genome places it with *Eremopyrum*.

The chloroplast genomes of *Australopyrum, Agropyron, Eremopyrum* and *Henrardia* are very similar The linkage of *Australopyrum* and *Agropyron* is well-supported in the nuclear gene tree as well, and this may be one of the few points of agreement between the two genomes.

The position of *Critesion/Hordeum* is near the base of the chloroplast tree, but they are not the earliest diverging branches as in the nuclear gene trees.

The explanation for the discrepancy between the chloroplast and nuclear gene trees is not clear; all possible explanations are *ad hoc* and rather unconvincing. It is tempting to suggest gene flow. However, this must have occurred sometime in the past because the genera are currently intersterile, as indicated by their distinct genomic designations. It is possible that the group is the result of a single rapid radiation, such that branching patterns near the base of the tree are impossible to recover; this should, however, give short branches at the base, but in many trees the basal branches are relatively long and well-supported.

Phylogenetic conclusions and future work

We have combined the data for the chloroplast and the nucleus, but it is not clear what the combination means. Depending on which data sets are included and how they are weighted, we can recover clades corresponding to the chloroplast tree, one or more of the nuclear trees, or combinations of both.

Despite a wealth of data, much more than in many other such groups, we have not recovered a single treelike history for the diploid Triticeae. So far, in fact, each piece of DNA investigated appears to have a distinct history. This would explain the discrepancies among the isozyme, RFLP and pairing trees mentioned above - each of these estimates relationships from many parts of the nuclear genome. If different parts of the genome have different histories, then phylogenies that sample the entire genome will be composites, and the ultimate resolution will reflect the different proportions of the genome sampled.

The next step is to determine, by constructing multiple data sets, what pieces of DNA we are tracking. By gene trees that mark different chromosomes or chromosome arms, we could test and extend the results of these analyses. For example, a gene tree for another gene on chromosome 1 should be similar to the 5S short-spacer tree. Similarly, a gene tree for a gene on chromosome 5 should be similar to the 5S long-spacers. Because each chromosome may have a different history in some taxa, particularly the annuals, genomic *in situ* hybridization could also point to more distantly related pieces of chromatin.

The morphological tree of Frederiksen and Seberg is quite different from any of the nuclear gene trees or the chloroplast tree. This is not surprising, in that morphology represents some complex integration of all genetic and epigenetic information. Ultimately, as we determine what the discrepancies among the gene trees are telling us, this may shed some light on the nature and mechanisms of morphological evolution.

Implications for classification

What does the foregoing tell us about classification in the Triticeae? We can now see that there is good evidence for almost any taxonomy. The case of *Triticum monococcum* is a good example. Data from the 5S array on chromosome 1 and from the chloroplast point unequivocally to placing *Triticum* and *Aegilops* in a single genus, which would then be called *Triticum*. But this overlooks the fact that the 5S array on chromosome 5 has a very different history. The chromosome 5 array justifies separating *Triticum* and *Aegilops* (as do isozyme and nuclear RFLP data). Either way, some information is lost. Either way, the decision is arbitrary.

Another example is the J and E genome situation; there has been much discussion about the pros and cons of combining them or keeping them separate, based on genome pairing data. Separating them is supported by the chromosome 1 5S array, keeping them together is supported by the chloroplast data, and the ITS and chromosome 5, 5S array are uninformative. Once again every answer is right, every answer is wrong.

This leads us to the unfortunate conclusion that there can be no stable classification of the Triticeae, because there are so many ways to turn the web into a hierarchy. Arguments over *Triticum* vs. *Aegilops*, *Thinopyrum* vs. *Lophopyrum* will continue because there is clear evidence on both sides.

But we can untangle the web. Even if we can never solve the classification question (because of the constraints of the Linnaean hierarchy), the phylogenetic question can be addressed. We can find out which parts of the genome have similar histories and which have different histories. This then opens all sorts of fascinating biological questions such as how the cytoplasm interacts with the nucleus, whether genetic change is really accelerated in *Secale*, how the complex gene-level histories result in the morphological pattern described by Frederiksen and Seberg (1992). In the long run this may be much more enlightening than arguing about whether two genera are the same or different.

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Uniformity of the α-Amylase Isozymes of *Aegilops cylindrica* Host. Introduced into North America: Comparison with Ancestral Eurasian Accessions

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ABSTRACT

We analyzed α-amylase isozymes of 27 North American and 426 Eurasian accessions to assess the diversity of *Aegilops cylindrica* Host. (jointed goatgrass). We found six characterized Eurasian accessions. However, 414 Eurasian accessions and all the North American accessions produced the same zymogram type. Our observations agree with the research showing uniformity of electrophoretic patterns of other seed proteins analyzed from material of *Ae. cylindrica*. It is difficult to establish the site of introduction of *Ae. cylindrica* based on the low diversity of α-amylase isozymes among US accessions.

INTRODUCTION

Aegilops cylindrica Host. (jointed goatgrass) is one of the most troublesome annual grass weeds in winter wheat-producing areas of the Pacific Northwest, Great Plains, and Southern Plains of the United States (Fig. 1). This grass weed is believed to have been introduced into North America as a contaminant in winter wheat grains brought from Eurasia. Since discovery of *Ae. cylindrica* in the USA early in the 20th century, expanding wheat

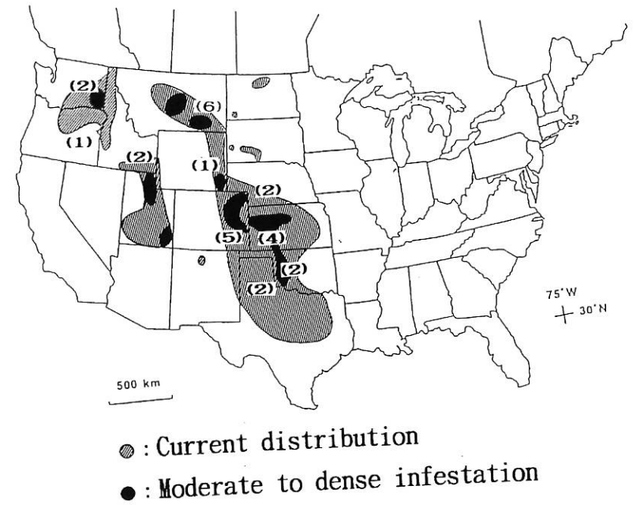

Fig. 1. Distribution and relative severity of *Ae. cylindrica* as a weed of winter wheat in the United States. Figures in parenthesis indicate number of accessions collected in each state. Data for distribution were taken from Donald & Ogg (1991).

acreage and production practices during the past quarter century have encouraged its spread and increase (Donald & Ogg, 1991).

Aegilops cylindrica is a tetraploid possessing the CCDD genome. The D genome of *Ae. cylindrica* was assumably derived from a diploid progenitor of wheat, *Ae. tauschii* Coss., and the C genome was from *Ae. markgrafii* (Greuter) Hammer on the evidence of genome analysis (Kihara, 1954), protein polymorphism (Johnson, 1967; Nakai, 1981) and cytoplasmic diversity (Maan, 1976). Several researchers have described the ecology and physiology of North American *Ae. cylindrica* such as seed germination (Morrow *et al.*, 1982; Donald & Zimdahl, 1987), vernalization requirements for flowering (Donald, 1984), gas exchange properties (Gealey, 1987, 1988, 1989), root and shoot phenology (Dotray & Young, 1993), and competitive relationships with winter wheat (Fleming *et al.*, 1988; Anderson, 1993).

This brief article is the first to report on the comparative diversity of α-amylase isozymes in North American and ancestral Eurasian of *Ae. cylindrica*. α-amylase isozymes are polymorphic, represent good genetic markers, and they are duplicated in polyploid wheat. The genetics of α-amylase have been valuable in wheat evolutionary studies (Nisikawa *et al.*, 1980, 1988, 1992). Nevo *et al.* (1993) also found a high level of diversity of α-amylase isozymes in the population of wild emmer wheat, *Triticum dicoccoides* Koern. ex Ausch. & Graebner, in Israel.

MATERIALS AND METHODS

Plant materials: Twenty-seven accessions of *Ae. cylindrica* were collected by weed scientists in the USA in fields of winter wheat or from roadsides near wheat fields located in the regions indicated on Figure 1. The ancestral Eurasian accessions (426 accessions) of *Ae. cylindrica* were provided by Drs. S. Ohta (Plant Germplasm Institute, Kyoto, Japan), H.E. Bockelman (USDA-ARS, Aberdeen, USA), A.B. Damania (ICARDA, Aleppo, Syria), M.C. MacKay (Australian Winter Cereal Collection, Tamworth, Australia) and I. Panayotov (Wheat and Sunflower Institute, Tolbuhin, Bulgaria).

Electrophoretic procedures: Sample solution of α-amylase were extracted with 1 ml of 0.05M Tris-HCl buffer (pH 7.0) from the endosperm of a single seedling six days after germination and incubated at 70° C for 15 min to inactivate the α-amylase. Electrophoresis was carried out by thin-layer (0.5mm) polyacrylamide gel isoelectrofocusing in a pH range of 4.0-8.0 by Pharmalite™ .

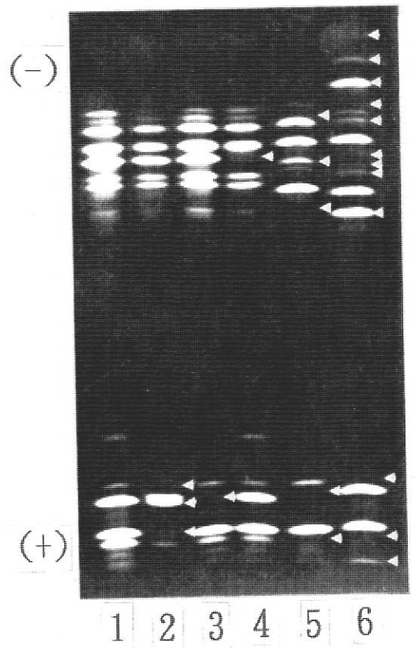

Fig. 2. α-amylase zymogram types. Arrows indicate the bands differing from the major type 1.

Table 1. Collection sites and accessions for zymogram types of α-amylase isozymes.

Collection site	Number of accessions	Zymogram type 1	2	3	4	5	6
North America	27	27	-	-	-	-	-
Bulgaria	38	37	-	-	-	1	-
Greece	1	-	-	-	1	-	-
Turkey	212	207	-	1	-	3	1
Georgia	7	7	-	-	-	-	-
Armenia	18	18	-	-	-	-	-
Syria	1	1	-	-	-	-	-
Iraq	1	1	-	-	-	-	-
Azerbaijan	6	5	1	-	-	-	-
Iran	52	50	-	-	-	1	1
Uzbekistan	1	1	-	-	-	-	-
Kazakstan	1	1	-	-	-	-	-
Afghanistan	9	9	-	-	-	-	-
ex USSR	20	18	2	-	-	-	-
ex Yugoslavia	16	16	-	-	-	-	-
Uncertain	26	26	-	-	-	-	-
total	453	441	3	1	1	5	2
(%)	(100)	(97.4)	(0.7)	(0.2)	(0.2)	(0.1)	(0.4)

RESULTS AND DISCUSSION

Variation pattern

We analyzed a great number of accessions, 27 from North America and 526 from Eurasia. Although there may be duplicated accessions due to reciprocal exchange of germplasm among the seed banks, we were unable to establish duplication of the accessions because of lack of information. We believe that the geographical variation has been fully sampled in Eurasian accessions. In Figure 1, we present the six zymogram types of the α-amylase isozyme. Twenty bands were identified from the zymograms. The arrows show the major differences among zymogram types. Although we found 6 types of variants (Fig. 2 and Table 1), 441 out of 453 accessions showed the same zymogram pattern (Table 1). The major type 1 of zymograms had 13 bands (8 bands in high and 5 in low pH range). We counted 12 accessions (5 accessions from Turkey, 2 from the Balkan Peninsula, 2 from Iran, 1 from Azerbaijan, and 2 from the former Soviet Union), which yielded different zymograms from the major zymogram 1 (Table 1). It contrasts with a high level of diversity found in tetraploid wheats with the AABB genomes (Nishikawa *et al.*, 1988, 1992; Nevo *et al.*, 1993).

These variants suggest that the Balkan Peninsula, Turkey, and Iran are the centers of diversity of *Ae. cylindrica*, though even here only little diversity of α-amylase isozymes can be found. Our observation of α-amylase isozymes agree with Johnson (1967), Nakai (1981), Jaaska (1981) and Masci *et al.* (1992), who observed uniformity of electrophoretic patterns of several proteins in *Ae. cylindrica*.

The sites of introduction

It is thought that there were multiple times and sites of introduction of *Ae. cylindrica* into North America. Johnston and Parker (1929) speculated that it was transported into Kansas in the late 19th century from the eastern Mediterranean, possibly with winter wheat cultivar 'Turkey' brought from Russia by Mennonite settlers. Mayfield (1927) added that it was also probably brought into Kansas in introductions of the cultivar 'Turkey' or cultivar 'Kharkov' made by the U.S. Department of Agriculture or by private seed firms and individuals during the early 1900s. A survey of current distributions and the relative severity of *Ae. cylindrica* as a weed in winter wheat fields has been summarized by weed scientists, who attended the 1988 jointed goatgrass workshop (Donald & Ogg, 1991). Densely infested areas may indicate the original sites of introduction of *Ae. cylindrica* into North America (Fig. 1), because *Ae. cylindrica* is more competitive than winter wheat as growing conditions become warmer and drier (Fleming *et al.*, 1988). Likewise, the development and soil water requirements of *Ae. cylindrica* were very similar to those of winter wheat (Anderson, 1993). Hence once invaded into the dry areas, *Ae. cylindrica* will survive and become a troublesome weed.

The evidence in this brief article pointed to a low population diversity for α-amylase isozymes in Eurasian accessions. The sampled populations of *Ae. cylindrica* in North America are only representative of populations located in or near wheat fields. They do not necessarily characterize populations of the species that have established in non-agricultural areas and have become part of the "wild" flora. In this respect, this research described preliminary rather than definitive genetic characterization of *Ae. cylindrica* populations in North America relative to the native Eurasian populations. It has been known that α-amylase are polymorphic in *Ae. tauschii* (Nishikawa *et al.*,

1980). It would be desirable to analyze α-amylase isozymes of diploid ancestor, *Ae. markgrafii*, because of no α-amylase data for *Ae. markgrafii*. They will be also useful to consider the low population diversity for α-amylase isozymes in the native Eurasian populations of *Ae. cylindrica*.

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The Genus *Elymus* L. in Asia. Taxonomy and Biosystematics with Special Reference to Genomic Relationships*

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ABSTRACT

Elymus is the largest genus in the tribe Triticeae Dumort. (Poaceae) and contains approximately 150 perennial and exclusively polyploid species occurring all over the world. Taxonomy of *Elymus* is extremely complex because of the huge morphological variation within and between species, the polyploid origin of the genus, and the frequent spontaneous hybridization between species, plus the wide divergence of classification concepts among taxonomists. Asia is an important center for the diversity of *Elymus*, which is reflected by: (i) more than half of the world's *Elymus* species originally occurs and abundant morphological variations are found in Asia; (ii) all different polyploidy levels and most genomic combinations known in this genus are reported from this region; and (iii) the important basic Y genome is essentially limited to Asia. This paper presents a historic review and current status of the genus *Elymus*. It also reports the number of *Elymus* species distributed in Asia, the general distribution and genomic constitution of the Asiatic *Elymus*. The perspective of the taxonomic treatment for *Elymus* is likewise discussed.

Introduction

Elymus L. is the largest genus in tribe Triticeae Dumort. (Poaceae) following the circumscriptions of Löve (1984), Dewey (1984), and Tzvelev (1989). Species in *Elymus* are widely distributed almost all over the world, occurring from the Arctic and temperate to subtropical regions. These species inhabit various ecological environments, e.g., grasslands, semidesert, mountain slopes and valleys, among bushes, and inside or along the edge of forests, and grow at altitudes from sea level up to over 5000 meters. A considerable morphological variation is found within and between *Elymus* species. The genus comprises only polyploid members which have originated from a few related genera in the Triticeae. Spontaneous interspecific and intergeneric hybridizations have taken place very frequently (Stebbins & Snyder 1956; Bowden 1965; Sakamoto 1966). All these factors together make taxonomic classification in this genus very difficult.

Apart from its biological interest for the study of evolution, the genus *Elymus* contains many species which are valuable forage crops or have a great potential for the improvement of cereal crops and forage grasses. A large number of research programs have been carried out on *Elymus* species worldwide, covering biosystematics, cytogenetics, ecology, molecular biology, and plant breeding aspects. This paper presents a brief review of the taxonomic classification of *Elymus* with special reference to its genomic relationships.

Historic review of the genus *Elymus*

Elymus was first described as a genus by Linnaeus (1753) based on the following six species, namely, *E. arenarius*, *E. sibiricus* (lectotype), *E. canadensis*, *E. virginicus*, *E. caput-medusae*, and *E. hystrix* (added in the addendum). He subsequently made some adjustments to his original treatment, such as transferring *Triticum caninus* L. to *Elymus* (*E. caninus* (L.) L.; Linnaeus 1755) and recognizing *Elymus europaeus* L. (Linnaeus 1767). Since then, *Elymus* has been considerably expanded from its original size containing only a few species to the one which now encompasses approximately 150 taxa (Löve 1984, Dewey 1984; Tzvelev 1989). According to the current generic assignment, three out of the eight species are not treated in *Elymus* any more, instead, they have been transferred, respectively, to *Leymus* [*L. arenarius* (L.) Hochst.], *Taeniatherum* [*T. caput-medusae* (L.) Nevski], and *Hordelymus* [*H. europaeus* (L.) Harz].

Table 1. Year of publication of generic synonymy (not italicized) of *Elymus* with the type taxon and its genomic constitution.

Genus	Author	Year	Type taxon
Elymus	Linnaeus	1753	*E. sibiricus* L.
Asperella	Humboldt	1790	A. hystrix Humb.
Hystrix	Moench	1794	H. patula (=*E. hystrix*)
Gymnostichum	Schreber	1810	G. hystrix (L.)
Zeocriton	Beauv.	1812	Z. hystrix (L.)
Sitanion	Rafinesque	1819	S. elymoides
Clinelymus	(Greseb.) Nevski	1832	C. sibiricus (L.) Nevski
Polyanthrix	Nees	1838	P. hystrix (Nutt.) Nees (= *E. elymoides*)
Roegneria	C. Koch	1848	R. caucasicus C. Koch
Anthosachne	Steudel	1854	A. scabra (R. Br.) Nevski
Stenostachys	Turczaninow	1862	S. naruroides Turcz.
Goulardia	Husnot	1896	G. canina (L.) Husnot
Terrella	Lunnell	1915	T. virginica (L.) Lunnell
Zeia	Lunnell	1915	Z. canina (L.) Lunnell
Terrella	Nevski	1932	T. curvata (Piper) Nevski (= *E. virginicus*)
Campeiostachys	Drobov	1941	C. schrinkiana (Fisch. & Mey.) Drobov
Semeiostachys	Drobov	1941	S. turczaninovii (Drobov) Drobov (= *E. gmelinii*)
Cockaynea	Zotov	1943	C. gracilis (Hook. f.) Zotov
Kengyilia*	Yen & Yang	1990	K. govicola Yen & Yang

*Kengyilia is not a synonym of *Elymus*, but the authors (Yen & Yang 1990a) claimed that it contained the SYP genomes which were found in some *Elymus* species, such as *E. alatavicus* and *E. batalinii* (Jensen 1990b, c).

Since the establishment of *Elymus*, the circumscription of the genus has varied widely, and *Elymus* sensu Löve (1984) has also been treated in vastly different terms by various authors. Table 1 lists the different taxa which are synonymous with *Elymus* published during the last two hundred years.

In Bentham's (1881) treatment, *Elymus* comprised about twenty species having multiple spikelets per rachis node and two or more florets per spikelet. He accepted the subdivision of the genus into three sections, i.e., sect. *Sitanion* Schult., sect. *Clinelyna* Griseb. (= *Elymus*), and sect. *Psammelyna* Griseb. (= *Leymus*). He also recognized the genus *Asperella* Humb. as an independent genus encompassing three species. Many other taxa having single spikelets per rachis node and multiple florets per spikelet were placed in Agropyron Gaertn., such as *A. caninum* (= *Elymus caninus*). He included *Roegneria* and *Anthosachne* into *Agropyron* because he considered that the former (e.g., *R. caucasicus* = *E. caucasicus*) was closely allied to *A. caninum* and the latter (e.g., *A. scabra* = *E. scabrus*) was closely allied to *A. semicostatum* (= *E. semicostatus*) and *A. longearistatum* (= *E. longearistatus*). Therefore, the circumscription of Bentham's *Elymus* was narrow, including only those species in sect. *Sitanion*, and sect. *Elymus* of *Elymus* sensu Löve (1984).

Nevski's (1934) *Elymus* had an even narrower delimitation containing only four species with multiple spikelets per rachis node, lanceolate glumes, and with large anthers, namely, *E. arenarius* L. [= *Leymus arenarius* (L.) Hochst.], *E. mollis* Trin. [= *L. mollis* (Trin.) Pilger]\, *E. villosissimus* Scribner [=*L. villosissimus* (Scribner) Tzvelev], and *E. giganteus* Vahl [= *L. giganteus* (Vahl) Pilger]. However, none of these species are included in the contemporary *Elymus sensu* Tzvelev (1976) or Löve (1984). Instead, all of them are placed in *Leymus* Hochst. (in sect. *Leymus*). On the other hand, Nevski (1934) recognized many small genera, such as *Anthosachne* (e.g., *A. longearistata*), *Roegneria (R. caucasica)*, *Asperella (A. komarovii* = *E. komarovii)*, and *Clinelymus (C. sibiricus* = *E. sibiricus*), all of which are now included in *Elymus* sensu Löve (1984). Besides, two species, namely, *A. alatavicum (= E. alatavicus)* and *A. batalinii (= E. batalinii)*, were included in *Agropyron* by Nevski (1934), but they were transferred to *Elymus* by Löve (1984). Nevski's taxonomic treatment exerted considerable influence in the neighboring countries.

The Chinese agrostologists basically followed Nevski's (1934) treatment of Triticeae. Keng (1959) recognized *Asperella, Roegneria, Elymus* and *Clinelymus* as independent genera. Keng's generic circumscription of *Elymus* followed Nevski's (1934) concept. A few Elymus species with multiple spikelets per rachis node were put in *Clinelymus* and the majority of species (more that 60 taxa) with single spikelets was included in *Roegneria* (Keng 1959; Keng & Chen 1963). The concept of *Elymus* in the latest Chinese flora (Kuo 1987) was equivalent to *Clinelymus sensu* Keng. Likewise, *Hystrix (Asperella)* and *Roegneria* were recognized as independent genera, and the latter encompassed the majority of *Elymus* species *sensu* Löve (1984).

Tzvelev (1987) treated *Elymus* in a wider context as consisting of caespitose and self-fertilizing species regardless of number of spikelets per rachis node. He

included *Roegneria* in *Elymus*, but recognized *Hystrix* as an independent genus. Two species, *E. batalinii* and *E. alatavicus sensu* Löve (1984), were place in *Elytrigia* by Tzvelev (1976). In his later treatment (Tzvelev 1989), and under the influence of the genomic system of classification, he agreed to include *Sitanion* into *Elymus* but still kept *Hystrix* as an independent genus.

Hitchcock (1951) followed essentially Bentham's classification, and his concept of *Elymus* was also based on multiple spikelets per rachis node and multiple florets per spikelet. Hitchcock (1951) treated *Elymus* as a genus including all *Leymus* and *Taeniatherum* species. He also recognized *Hystrix*, and separated *Sitanion* as a distinct genus from *Elymus*, even though all *Sitanion* species have multiple spikelets per rachis node. Many other species with single spikelets per rachis node, which are now placed in *Elymus* by contemporary taxonomists, were included in *Agropyron*. Working on North American plant material, Gould (1947) had a very wide circumscription of *Elymus*. Strongly influenced by cytological evidence, particularly that provided by Stebbins et al. (1946). Gould (1947) circumscribed the genus *Elymus* completely regardless of annuals or perennials, self-or cross-fertilization, and number of spikelets per rachis node, and other morphological characters. Gould (1947) not only included *Agropyron, Sitanion* and *Hystrix* in *Elymus*, but also lumped all the species which are now treated in *Pseudoroegneria, Elytrigia, Pascopyrum, Thinopyrum, Leymus*, and *Taeniatherum sensu* Löve (1984) into *Elymus*.

Significantly, Pilger (1954) emphasized the morphological characters in grass systematics, and he recognized *Elymus, Hystrix* and *Sitanion* as independent genera, although all species in the genera have multiple spikelets per rachis node. *Leymus* was treated as a genus distinct from *Elymus* as well. He also recognized *Agropyron* which supposedly contained species with single spikelets per rachis node. However, since no species names were given in his system, it is difficult to judge his generic delimitation. Runemark & Heneen (1968) in "*Elymus* and *Agropyron*, a problem of generic delimitation: presented the results from morphological, anatomical, and cytological studies on some perennial wheatgrasses. They suggested a very broad treatment, combining *Agropyron, Elytrigia, Roegneria, Clinelymus, Terrella, Hystrix*, and *Sitanion* into *Elymus*. This treatment has exerted a large influence on the treatment of the tribe Triticeae in the European floras. For example, in "Flora Europaea" by Melderis et al. (1980), the genus *Elymus* also included all the *Elytrigia* species *sensu* Tzvelev (1976), and those species with single spikelets formerly placed in *Agropyron*, such as *A. caninum* (= *Elymus caninus*). Clayton & Renvoize (1986) circumscribed *Elymus* in a very broad sense. They not only treated *Terrella, Roegneria, Clinelymus* and *Anthosachne* as synonyms of *Elymus*, but also amalgamated *Elytrigia, Pseudoroegneria, Thinopyrum, Lophopyrum, Pascopyrum*, and *Festucopsis* with *Elymus*.

In the genomic system of classification in the Triticeae, Löve (1984) has converted the available genomic data into a comprehensive worldwide taxonomic treatment of *Elymus*. In the "Conspectus of the Triticeae", Löve basically followed Tzvelev's (1976) circumscription of *Elymus*, but he included *Hystrix*, and *Sitanion*, and added three more sections into *Elymus*. Approximately 150 species were included in this treatment, where the genus *Elymus* was further subdivided into eleven sections, i.e., 1) sect. *Elymus* L.; 2) sect. *Turczaninovia* (Nevski) Tzvelev; 3) sect. *Macrolepsis* (Nevski) Jaaska; 4) sect. *Goulardia* (Husnot) Tzvelev; 5) sect. *Hystrix* (Moench) Löve; 6) sect. *Sitanion* (Rafin.) Löve; 7) sect. *Clinelymopsis* (Nevski) Tzvelev; 8) sect. *Anthosachne* (Steud.) Tzvelev; 9) sect. *Stenostachys* (Turcz.) Löve & Connor; 10) sect. *Dasystachyae* Löve; and 11) sect. *Hyalolepsis* (Nevski) Löve.

It is obvious that the main arguments relating to the circumscription of *Elymus* proposed by various authors revolved around whether to employ single vs. multiple spikelets per rachis node as a key character in the classification. Some taxonomists delimited *Elymus* by including all species with multiple spikelets per rachis node, and thus placed those species with single spikelets per node into other genera, such as *Agropyron* or *Roegneria*, e.g., the treatments by Bentham (1881), Nevski (1934), Hitchcock (19151), Keng (1959), and Baum (1983). In these treatments, *Elymus* had rather narrow circumscription, and encompassed few species. However, other taxonomists did not consider this morphological feature significant in generic circumscription, e.g., the treatments by Pilger (1954), Runemark & Heneen (1968), Tzvelev (1976), Melderis (1980), Löve (1984), Dewey (1984), and Clayton & Renvoize (1986). They included species in *Elymus* regardless of the number of spikelets per rachis node, and their circumscription was therefore much wider and included many species. Even today there is no complete agreement on the circumscription of the genus. The reason, apart from the above-mentioned different opinions of criteria, is that various taxonomists dealt with *Elymus* species from different geographical and historical perspectives. The various taxonomic classifications were based on the material from different geographic regions, and users tend to follow the classification systems established by their own taxonomists. For example, in North America Hitchcock's (1951) definition of *Elymus* still prevails today, in China agrostologists essentially follow Keng's (1959) treatment of the Triticeae, in Russia and its neighboring countries, Tzvelev's (1976) circumscription of *Elymus* is followed to a great extent, and in Europe the classification system by Melderis et al. (1980) plays a major role.

Generic circumscription

The generic circumscription of *Elymus* adopted in this paper follows that of Löve (1984), in which *Roegneria, Hystrix*, and *Sitanion* are included. Following this generic delimitation, *Elymus* contains approximately 150 taxa. The

reason to follow Löve's (1984) circumscription here is because Löve (1984) compiled *Elymus* species worldwide in his taxonomic treatment. It is hence easier to compare the alternative generic treatments by different authors worldwide.

Elymus comprises relatively short-lived perennial and exclusively polyploid grass species. Most of the species are densely to loosely caespitose, or rarely rhizomatous, small anthered, and self-pollinating. The culms are 20-200 cm tall with 3 to 7 nodes, smooth or rarely scabrous to pubescent below the spikes. The basal leaf-sheaths are usually hairy, the leaf-sheaths smooth to sparsely scabrous, covering 1/2 to 3/4 of the internodes. The leaf-blades are flat or involute, glabrous, scabrous, or pilose on one or both surfaces, and with short ligules. The spikes are erect or nodding with a tough rachis. The spikelets are solitary or in groups of two or more at each rachis node, with two to several perfect florets. The rachillas are usually fragile and disarticulate above the glumes and beneath the florets. The glumes are unequal, broadly to linearly lanceolate or ovate, rarely reduced or absent, with none to several main veins, and with or without divaricate or straight awns; subsessile and adnate to the rudimentary pedicle. The callus at the base of the floret is broad and rounded or narrow and pointed, more or less hirsute, rarely glabrous. the lemmas are lanceolate, elliptic, or narrowly ovate, shorter or as long as the lemmas, or slightly longer than the lemmas, and with ciliated keels. The anthers are yellow to dark purple. The caryopses are oblong or oblong-linear, flat or subconvex on the inner side.

Genomic relationships

Even though information such as the chromosome numbers of many species in the genus was unknown, Löve (1984) accommodated two basic genomes (haplomes), i.e., the S and H, into *Elymus* species. The basic S genome originated from *Pseudoroegneria* (Dewey 1967), whereas the H genome from Hordeum s.l. (Dewey 1971). Löve (1984) suggested four genomic combinations, i.e., HS, HHS, HSS or SSHH as the constitution of *Elymus*. Dewey (1984) accepted Löve's (1984) circumscription of the genus. However, he pointed out that apart from the basic S and H genomes, the basic Y genome (of unknown origin) was also represented in many Asiatic tetraploids and some hexaploids (Dewey 1984). Therefore, he recommended that the genomic constitutions of Elymus should be SH, SY, or SYH and the combinations of the segmental alloploids. Recently, two more basic genomes have been identified from hexaploid species. The fourth basic P genome, which comes from *Agropyron,* was found in some Central Asiatic hexaploids, e.g., *E. kengii, E. longiglumis, E. alatavicus* and *E. batalinii* (Jensen 1990b, c). The fifth basic W genome, derived from *Australopyrum* Löve, was identified in an Australasian hexaploid *E. scabrus* (Torabinejad & Mueller 1993).

As an exclusively allopolyploid genus, *Elymus* has its origin from other groups, and thus it has close relationships with other genera in the Triticeae. The S genome is so far as known represented in all *Elymus* species, and hence is the most important composition of the genus. The S genome donor, *Pseudoroegneria,* is a perennial genus containing about fifteen species of diploids (S) and tetraploids (SS or S1S2 or SP) (Dewey 1974, 1984; Löve 1984; Wang et al. 1986: Jensen et al. 1992). Many intergeneric hybrids between diploid *Pseudoroegneria* and tetraploid *Elymus* species with the SH and SY genomes have been made, and the hybrids were relatively easy to produce, also without the assistance of embryo rescue. Meiotic pairing data indicate that the S genomes in the two genera have very high homology (up to 7 bivalents per cell), although with slight modifications in some species (Dewey 1982; Löve & Connor 1982; Jensen 1989, 1990a; Lu et al. 1991, 1994).

The H genome is present in many tetraploid and hexaploid *Elymus,* and its donor genus, *Hordeum* s.l., includes approximately 40 annual or perennial taxa, and comprises four basic genomes, viz, the H, I, X, and Y (cY in *Elymus).* About 90% of the *Hordeum* species share the H genome (Bothmer it al. 1986, 1987). Many intergeneric hybrids between *Hordeum* and *Elymus* species have been produced, and usually the hybridization was easy to perform with the aid of embryo rescue. Meiotic pairing (up to 6 bivalents per cell) in some of the triploid hybrids (SHH) suggested relatively high homology between the H genomes in the two genera, but the tetraploid hybrids (SYHH) showed certain modification of the H genomes (Dewey 1971, 1980b; Lu & Bothmer 1990a). The genomic affinity between the S and H genomes was reported to be very low with an average of 0.2 chiasmata per cell (Torabinejad et al. 1987). Other triploid hybrids between *Hordeum* and SY genome *Elymus* species were reported to have extremely low meiotic pairing at MI, suggesting the absence of the H genome in these *Elymus* species and the extremely low homoeology among the H and S or Y genomes (Lu & Bothmer 1990a; Lu, B. R., in prep.). The discovery of low affinities between the S and Y genomes, or among the three unrelated genomes (SYH) were also supported by the cytogenetic studies of the dihaploids and a trihaploid of several *Elymus* species (cf. Sakamoto 1964; Lu & Bothmer 1989; Lu et al. 1990a; Lu 1992a, 1993a). Hybrids between either the tetraploid or hexaploid *Elymus* species and *H. vulgare* or H. *bulbosum*, both containing the I genome, usually exhibit extremely low chromosome associations (with less than one bivalent per cell), which suggests an extremely low homoeology between the I genome and any of the genomes in *Elymus* (Mujeeb-Kazi & Rodriguez 1982; Mujeeb-Kazi 1985; Lu & Bothmer 1990a; Lu 1991b).

The donor of the P genome in some Central Asiatic hexaploid *Elymus* species was expected to be *Agropyron,* a genus containing perennial species of diploid, tetraploids, and hexaploids with the P, PP, and PPP genomes (Dewey 1982). Although, hybrids between and SYP genome *Elymus* species and *Agropyron* were obtained, no observations on meiotic pairing have been reported from the hybrids (Jensen 1990b). Consequently, no direct assessment of the genomic relationships between the two genera has been made. The presence of the P genome in these hexaploids was evidenced by the high chromosome pairing in hybrids between these hexaploids and *Pseudoroegneria tauri* (Jensen 1990b), a tetraploid perennial containing the SP genome (Wang et al. 1986). The S genome has been reported to have relatively high homoeology with the P genome (Napier & Walton 1982; Wang et al. 1985). The W genome has only been found in the Australasian hexaploid *E. scabrus*, apart from its presence in the donor *Australopyrum.* The W genomes in the two genera were reported to have fairly high homology, but the W genome has very low homoeology with any of the other genomes, e.g., the S and Y, in *Elymus* (Torabinejad & Mueller 1993).

Intergeneric hybrids have been reported between *Elymus* and many other genera in the Triticeae, and genomic relationships of *Elymus* with these genera have been estimated thereby. Only a few genera, such as *Elytrigia* (SSH genomes), *Thinopyrum* (JE), and *Pascopyrum* (SHNX), have relatively high genomic affinities to *Elymus* with a certain amount of chromosome pairing in their hybrids (Dewey 1970, 1984; Löve & Connor 1982; Napier & Walton 1983). Considerable low genomic affinities are assessed between *Elymus* and other genera, such as *Psathyrostachys* (N. Dewey 1967; Lu 1991a; Park & Walton 1990), *Leymus* (NX, Stebbins & Walters 1949; Dewey 1970; Sakamoto 1985), *Triticum* (ABD, Mujeeb-Kazi & Bernard 1985; Sharma & Baenziger 1986; Lu & Bothmer 1989, 1991b), and *Secale* (R, Lu et al. 1990a; Lu & Bothmer 1991a).

The genus *Elymus* in Asia

Asia, particularly the Central Asiatic mountain region, is an important center for the diversity of *Elymus*, which is reflected by the following facts: 1) more than half of the world number of *Elymus* species occurs in this area; 2) many different morphological forms are found in this region; 3) all known polyploid levels, 2n=4x=28, 2n=6x=42, and 2n=8x=56, have been reported from Asia (Tzvelev 1976), although the existence of the octoploid taxa needs to be confirmed; and 4) most genomic combinations known in this genus are found in Asia. Furthermore, the Y genome, which is present in the majority of Asiatic *Elymus* species, was considered to have its origin in Central Asia or the Himalayan region (Dewey 1984).

During the past 20 years, extensive cytogenetic studies, particularly genomic analysis, have been carried out in *Elymus*. Sakamoto & Muramatsu (1966a, b) made several interspecific hybrids, including tetraploids (2n=4x=28), i.e., *E. ciliaris, E. gmelinii, E. yezoensis, E. semicostatus*, and hexaploids (2n=6x=42), i.e., *E. tsukushiensis*, and *E. humidus* (as *Agropyron humidorum*). They concluded through the study of chromosome pairing at metaphase I of meiosis in the hybrids that the two genomes were very similar in all the tetraploids, and that the two hexaploids shared three nearly identical genomes. However, no genomic designation resulted therefrom. Similar cytogenetic studies were reported subsequently by Sakamoto (1964, 1982).

The earliest genomic designation for Asiatic *Elymus* species was conducted by Dewey (1968). In that study two Asiatic tetraploids, i.e., *E. caninus* (as *Agropyron caninum*) and *E. semicostatus* (as *A. striatus*) were included. The genomic formula of *E. caninus* was given as SX, in which the proposed X genome was later found to be derived from *Hordeum*. The symbol X was then replaced by H (Dewey 1971). The genomes of *E. semicostatus* were designated as SY, where the origin of the Y genome was unknown. Subsequently, more *Elymus* tetraploid species from Asia were included in genomic analysis, and they were found to be composed of either the SH genomes, e.g., *E. sibiricus, E. mutabilis,* and *E. trachycaulus* (Dewey 1968, 1974, 1984), or the SY genomes, e.g., *E. fedtschenkoi, E. tibeticus, E. pendulinus, E. ciliaris, E. abolinii, E. burchan-buddae*, and *E. antiquus* (Dewey 1980a; Liu & Dewey 1983; Jensen & Hatch 1988, 1989; Jensen 1989, 1990a; Lu et al. 1988, 1990c, 1991; Lu & Bothmer 1990b, 1993a, b; Lu & Salomon 1993a; Salomon 1993b).

Dewey (1972) determined the origin of a hexaploid *E. transhyrcanus* (as *Agropyron leptorum*) containing the "SSH" genomes. Later Dewey (1980b) investigated a central Asiatic hexaploid, *E. drobovii*, and discovered that it contained the tree unrelated SYH genomes. Subsequently, the genomic constitution of more Asiatic hexaploid *Elymus* taxa were determined, e.g., *E. tsukushiensis, E. himalayanus, E. schrenkianus, E. dahuricus,* and *E. nutans*, and were also found to contain the SYH genomes (Dewey 1984; Lu & Bothmer 1990a, 1992; Lu 1993b). Another rare group of hexaploid *Elymus* with dense and hairy spikes and more or less awnless lemmas occurring mainly in Central Asia, such as *E. alatavicus, E. batalinii, E. grandiglumis,* and *E. kengii*, were cytologically investigated by Jensen (1990a, b), and they were shown to possess the SYP genomes. Further genomic combinations were subsequently discovered in the hexaploid *Elymus* species. For example, recently the SSY genomes were determined in *E. tschimganicus* and *E. glaucissimus* by Jensen et al. (1994) and Lu et al. (1993). To summarize, there are four basic genomes (S, H, Y, and P) known in different combinations, which comprise the Asiatic *Elymus* species. These are the SH, SY, SYH, SSY, SSH, and SYP genomes.

A compilation of the most important Asiatic floras (Bor 1960, 1968, 1970; Ohwi 1965; Tzvelev 1976; Cope 1982; Kuo 1987; Melderis 1987), together with recently described *Elymus* species by Salomon (1990), Lu (1992b),

and Lu & Salomon (1993a), indicates that more than 140 *Elymus* species sensu Löve (1984) are native to Asia. Many of the species were though included in other genera, such as in *Roegneria, Agropyron, Hystrix*, or *Elytrigia* by the different authors. However, since the different authors worked separately on the *Elymus* species in different historical periods and geographical regions, and also because some of them had very narrow species concepts, many species have been separately described by different authors several times under different names. For example, a species from western China and Nepal has been described as *Agropyron antiquus* Nevski (1932), *Roegneria parvigluma* Keng (1959), and *Agropyron microlepis* Melderis (Bor 1960), and another Tibitan species has been described as *Agropyron tibeticus* Melderis (Bor 1960), *Roegneria stricta* Keng (1959), *R. varia* Keng (1959), and *R. sinica* Keng (1959), respectively. Similar examples can be found in the treatments of many other species. Based on the extensive examination of the available specimens (especially the types) from several major herbaria, and the results of cytogenetic investigation and taxonomic revision of *Elymus* species (Salomon 1993a, b; Lu 1993c, 1995; Salomon & Lu 1993; Lu & Salomon 1993b), some taxa have been treated as one species. The number of species in Asiatic *Elymus* is thus reduced. Approximately 105 species and 6 interspecific hybrids in the genus *Elymus* originating from Asia are recognized by the author, although more taxa may be merged after the species relationships have been clarified and taxonomic revisions have been made with a consequent reduction in the number of species. The Asiatic *Elymus* species with their chromosome numbers and genomic constitutions are summarized in Table 2.

The general distribution of the Asiatic *Elymus* is presented in Fig. 1, with an approximate indication of numbers of species in the different regions. Generally, species containing different genomes have separate distribution areas, although with considerable overlaps. The SH and SSH genome species occur mainly in northern boreal Asia with some off shoots to the Central Asiatic mountain region (Fig. 2). The SH genomes have considerably high homology only with minor chromosome structural changes (translocations) between various tetraploids indicated by occasional presence of 1-2 multivalents in the interspecific hybrids (Lu, B. R. & Salomon, B., unpubl.). The SY, SSY, and the SYH genome species overlap to a considerable extent, and they are found mostly in Central and Eastern Asia (Figs. 3 and 4). Compared with the SH, the SY genomes have largely differentiated, including reduction of homology and chromosome structural changes, i.e., translocations and inversions between the tetraploids and hexaploids revealed by drastic decrease of meiotic pairing, frequent presence of multivalents, chromatid bridges and fragments in the interspecific hybrids. The differentiation of the SY genomes is in accordance with the geographic distribution of these *Elymus* species (Lu & Salomon 1992; Lu & Bothmer 1993a; Lu 1993b, c). the SYP genome species are found only in Central Asiatic mountain region (Fig. 5).

Perspective of the taxonomic treatment for *Elymus*

So far, there is no general agreement for the taxonomic treatment of the genus, although some solutions have been achieved owning to the application of new approaches, such as chromosome karyotyping, genomic analysis, isozyme electrophoresis, etc. For example, the most ambiguous genus *Agropyron* has finally been delimited to a small group of species containing the basic P genome, and other genera, like *Pseudoroegneria* (S genome), *Taeniatherum* (T genome), *Pascopyrum* (SHNX genomes), and *Leymus* (NX genomes) have also been distinctly separated from *Elymus*.

Most of the arguments about the circumscription of *Elymus* in Asia seem to focus on whether to recognize the

Table 2. ***Elymus*** species native to Asia with their general information on chromosome number, genomic constitution.

2n =	Known genomes	Example of species	Approx. no. of species	%*
28	SH	***E. sibiricus***	10	18.2/9.0
28	SY	***E. semicostatus***	31	56.4/27.9
42	SYH	***E. drobovii***	7	12.7/6.3
42	SSY	***E. tschimganicus***	2	3.6/1.8
42	SSH	***E. transhycanus***	1	1.8/0.9
42	SYP	***E. alatavicus***	4	7.3/3.6
			55	49.5
28	??	***E. lenensis***	6	5.4
42	???	***E. edelbergii***	10	9.0
?	?	***E. calcicolus***	40**	36.0
			56	50.5
Total			111	

* Percentage of species known genomes/Percentage of total species.
** Including natural interspecific hybrids.

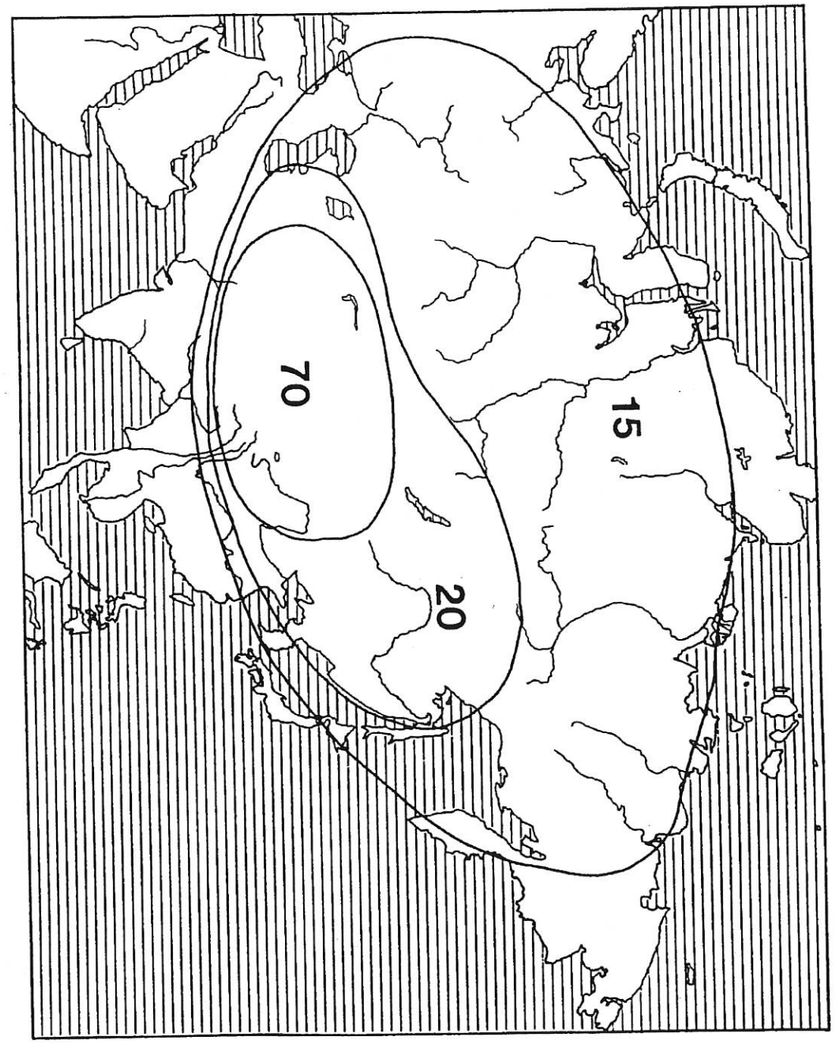

Fig. 1. A general distribution map of *Elymus* in Asia with isochores indicating the number of species in different geographic areas. The numbers represent the minimum number of species occurring in a particular area.

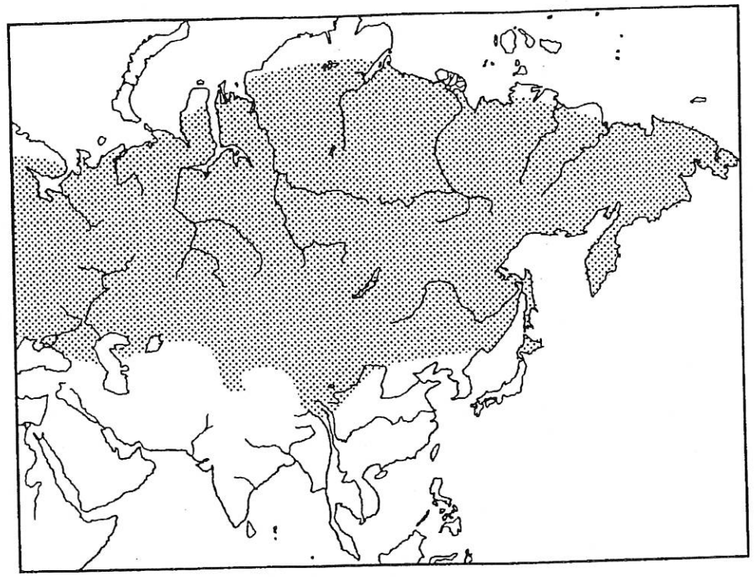

Fig. 2. A general distribution map of SH- and SSH-genome *Elymus* species in Asia.

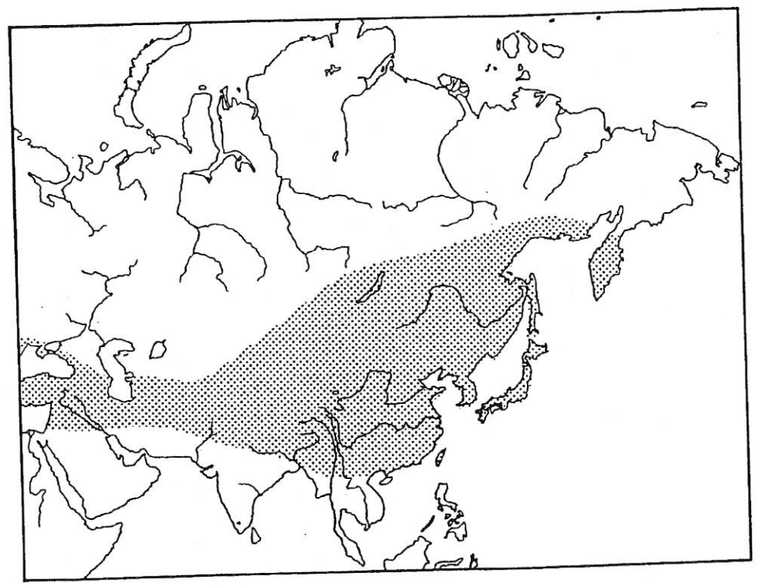

Fig. 3. A general distribution map of SY- and SSY-genome *Elymus* species in Asia.

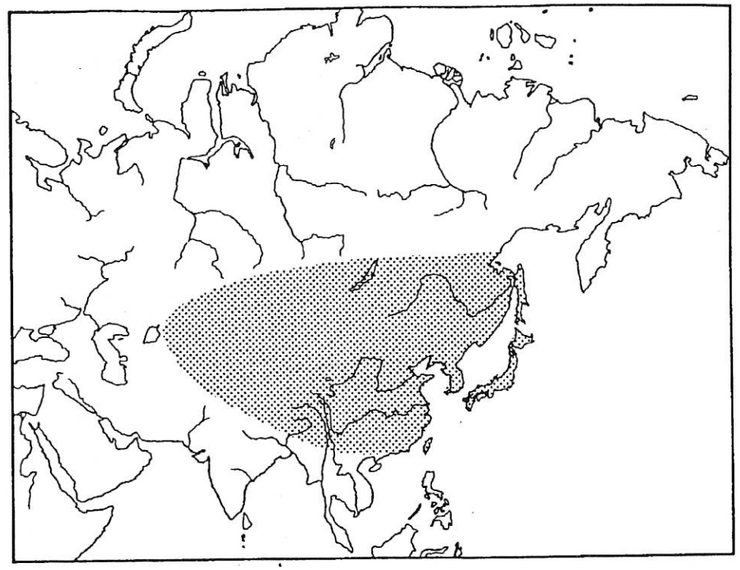

Fig. 4. A general distribution map of SYH-genome *Elymus* species in Asia.

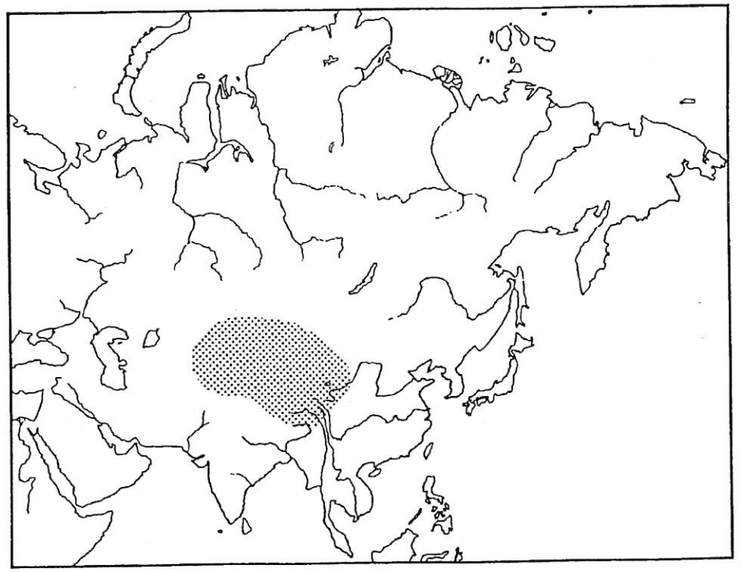

Fig. 5. A general distribution map of SYP-genome *Elymus* species in Asia.

genus *Roegneria* (cf. Löve 1984; Kuo 1987; Tzvelev 1989; Baum et al. 1991). As mentioned earlier, morphological characters, such as single vs. multiple spikelets at each rachis node, have been adopted by some taxonomists, like Nevski (1934), Keng (1959), Kuo (1959), Kuo (1987), and Baum (1983) as the most important key character to separate *Roegneria* (with single spikelets) from *Elymus* (with multiple spikelets). However, cytogenetic evidence, isozyme electrophoresis, and molecular data have demonstrated that the number of spikelets per rachis node has little or simply no biological relevance to the phylogenetic relationships of the species, although such a character is useful in the field and suitable for taxonomic keys. There are several examples showing the disadvantages of using such a character in delimitation of *Elymus* and *Roegneria*. Many tetraploid species, e.g., *E. caninus*, *E. confusus*, *E. mutabilis*, *E. ciliaris*, *E. pendulinus*, and *E. abolinii*, have all been placed in *Roegneria* by the above-mentioned taxonomists because of the single spikelets per rachis node, whereas *E. sibiricus*, *E. virginicus*, and *E. canadensis* have always been kept in *Elymus* because of their multiple spikelets per rachis node. The problem is that *E. caninus*, *E. confusus*, *E. sibiricus*, and *E. canadensis* all contain the SH genomes, and they have been shown to have a very close genomic relationship (Dewey 1968, 1974; Lu et al. 1994). It is therefore illogical to separate these species into different genera, providing that the classification should reflect phylogenetic relationship. On the other hand, *E. ciliaris*, *E. pendulinus*, and *E. abolinii*, which all contain the SY genomes, were included by the above-mentioned authors in the same genus (*Roegneria*) with *E. caninus*, *E. mutabilis*, and *E. confusus*, but the fact is that *E. ciliaris*, *E. pendulinus*, and *E. abolinii* have only one genome in common with *E. caninus*, *E. mutabilis*, and *E. confusus*. Therefore, they are not as closely related as indicated by the above taxonomic treatments. Likewise many hexaploid species, such as *E. dahuricus*, *E. nutans*, *E. himalayanus*, *E. schrenkianus*, and *E. tsukushiensis*, have always been treated separately either in *Elymus* (*E. dahuricus* and *E. nutans*) or in *Roegneria* (*E. himalayanus*, *E. schrenkianus*, and *E. tsukushiensis*), simply because of their single or multiple spikelets per rachis node. However, the fact that all these species contain the same genomes (SYH) and have very high genomic affinities (Lu & Bothmer 1992; Lu 1993b) does not support the separation of these species into different genera. Results from isozyme, RFLP, and immunology studies of Triticeae material also supported above cytogenetic data. Therefore generic circumscription based merely on such a character as number of spikelets per rachis node incorrectly reflects the relationships of the species.

To circumvent questionable characters such as single vs. multiple spikelets per node in their classification, Baum et al. (1991) used other characters such as relative length of paleas (compared to lemma) and shape of palea tip for their taxonomic key to *Elymus* and *Roegneria*. They also intended to link the genus *Roegneria* with the species containing SY genomes. However, the above-mentioned palea features were found to be applicable only to tetraploid Asiatic *Elymus* species (Lu & Salomon 1989; Salomon & Lu 1992). The authors suggested in this finding that the Asiatic tetraploids of *Elymus* having paleas shorter than or equal to the lemmas with obtuse or retuse tips more likely contained the SY genomes, whereas those having slightly longer or equal paleas with acute tips usually possessed the SH genomes (Salomon & Lu 1992). Unfortunately, these characters cannot be generalized to the whole genus *Elymus sensu* Löve (1984), because the palea features of *Elymus* species at higher ploidy levels are found to be far more complicated. Also, the American *Elymus* species do not follow this rule (B. R. Lu unpublished data). The genus *Roegneria* as defined by Baum et al. (1991) was actually a complex which not only contained *Roegneria* species with the SY genomes, such as *R. caucasicus*, *R. ciliaris*, and *R. turczaninovii* (=*E. gmelinii*), but also included many species having other different genomic combinations, these are: 1. the SH , [*E. confusus*, *E. mutabilis* (as *R. angustiglumis*), and *E. macrourus*]; 2. the SYH genomes (*E. tsukushiensis*, *E. himalayanus*, and *E. schrenkianus*); 3. the SSY genomes (*E. tschimganicus*); 4. the SSH genomes (*E. transhyrcanus* (as *R. leptoura*)]; 5. the SYP genomes [*E. kengii* (as *R. hirsuta*) and *E. grandiglumis*]; and 6. the SYW genomes (*E. scabrus*). Furthermore, *Roegneria* sensu Baum et al. (1991) also included species having the SS genomes, i.e., *R. elytrigioides* Yen et J. L. Yang (Lu 1994) and probably *R. alashanica* Keng. Both of them should then be included in *Pseudoroegneria*. Therefore, the generic circumscription of *Roegneria* by Baum et al. (1991) should not be recommended either from a morphological or genomical point of view.

Elymus also tends to be split into several genera by strictly following the concept of genomic genus proposed by Löve (1984). It was suggested that all species with the SH genomes should be remained in *Elymus*, all species having the SY genomes should be transferred to *Roegneria*, the hexaploids with the SYH genomes should be included in a new genus *Horderoegneia*, while those with the SYP of SP genomes should be placed either in the genus *Kengyilia* or *Parakengia*, respectively (Yen & Yang 1990a, b). However, generic delimitation according to the concept of one particular genomic combination circumscribing one genus is not without problems. Firstly, there are many *Elymus* species of which even the chromosome number is unknown. It is therefore difficult to see how such species can be classified without a knowledge of their genomic constitution. Secondly, since the fifth basic W genome was found in the Australasian hexaploid *E. scabrus* (SYW), five genomic combinations, not including the autoallopolyploid genomic combinations, are presented in *Elymus* sensu Löve (1984). A new genus name should then be used for the SYW genome species. If more genomic combinations are found in these species, than the number of genera will increase endlessly, so creating unnecessary taxonomic terminology. Thirdly, the differentiation of genomes is

evident, like the SY genomes in Asiatic *Elymus* species which have diverged to a great extent (Lu & Salomon 1992; Lu & Bothmer 1993a; Lu 1993b, 1995). How should taxonomists relate such genomic diversity to the genomic genera? In general, attempting to associate generic delimitation strictly with genomic constitution will produce more confusion and is likely to bring the classification of *Elymus* into chaos.

In contrast to the treatment of splitting *Elymus* into several genera, Assadi (1994) put all perennial species containing the S genome into *Elymus*, including *Elymus sensu* Löve (1984) and *Elytrigia* s. l. (incl. *Elytrigia sensu* Löve, *Thinopyrum* Löve, *Trichopyrum* Löve, *Pseudoroegneria* (Nevski) Löve, and *Pascopyrum* Löve), even though species in the *Elytrigia* complex are all large-anthered, cross-pollinating, and usually awnless. This generic delimitation of *Elymus* essentially agrees with those by Runemark & Heneen (1968) and Melderis (1980). To confirm the propriety of such combination for *Elymus*, Assadi (1984) emphasized that (1) the S genome had a dominant influence on the morphology of taxa in which it is present, and the pattern of morphological differentiation within the group having the S genome is more or less continuous; (2) *Elytrigia repens* (L.) Nevski, the type taxon of the genus *Elytrigia*, contained the SSH genomes which are present in many *Elymus* species, therefore, he considered the inclusion of *Elytrigia* s.l. in *Elymus* to be natural either from a morphological or genomical point of view.

However, based on our present knowledge of *Elymus*, the author believes that until we fully understand genomic relationships of these perennial species and discover the "true" relationship between morphological characters and genomic constitutions of the species, it is advisable and convenient to retain the circumscription of *Elymus* proposed by Löve (1984) at present. Actually, if we neglect the number of spikelets per rachis node, then species in the genus *Elymus sensu* Löve (1984) have considerable similarities in morphology, compared with other genera, such as *Agropyron*, *Psathyrostachys*, and *Leymus*. We should allow more than one genomic combination to be present in the genus *Elymus*, just as we permit four basic genomes, namely, the I, H, X (c X in *Elytrigia*) and Y (c Y in *Elymus*), to be present in the genus *Hordeum* (Bothmer et al. 1986, 1987), and three genomic constitutions (A, AB, and ABD) to exist in the genus *Triticum* (Kihara 1975). The acceptance of *Elymus* as a genus also avoids the need of nomenclature change of these species. On the other hand, it is advantageous that the genomic data revealed by the cytogenetical studies in *Elymus* provides us with an opportunity to understand the biosystematic relationships below generic level.

Traditionally, the taxonomic subdivision of *Elymus* was essentially based on morphological characteristics, such as the attitude of spikes (erect vs. nodding), number of spikelets per rachis node (single vs. multiple), the size of glumes, and awned or awnless lemma, although studies have shown very little value of using these characters to indicate biosystematic relationships of *Elymus* species (e.g., Salomon & Lu 1992). Even so, Löve's (1984) subdivision of *Elymus*, based on the traditional treatment, particularly on that of Tzvelev (1976), presents the current taxonomic subdivisions within *Elymus*. However, current investigations of the genomic relationships of *Elymus* species have demonstrated that the present subdivision of *Elymus* (Löve 1984) cannot reflect the evolutionary relationships between the *Elymus* species. This is illustrated by the fact that species containing the same genomes have been placed in different sections, whereas species possessing different genomic combinations have been

Table 3. Taxonomic treatment within *Elymus* sensu Löve (1984) and their known genomic constitutions.

Section	Genomic combination	Example of species
1. *Elymus*	SH	*E. sibiricus*, *E. confusus*
	SY	*E. antiquua*, *E. altissimus*
	SYH	*E. nutans*, *E. schrenkianus*
	SS?	*E. caesifolius*
2. *Turczaninovia*	SYH	*E. dahuricus*
3. *Macrolepsis*	SH	*E. canadensis*, *E. virginicus*
4. *Goulardia*	SH	*E. caninus*, *E. mutabilis*
	SY	*E. panormitanus*, *E. semicostatus*
	SYH	*E. tsukuchiensis*, *E. humidus*
	SYP	*E. kengii*, *E. grandiglumis*
5. *Hystrix*	SH	*H. hystrix*
6. *Sitanion*	SH	*E. elymoides*
7. *Clinelymiopsis*	SY	*E. caucasicus*
8. *Anthosachne*	SY	*E. brevipes*, *E. longearistatus*
	SSY	*E. tschimganicus*, *E. glaucissimus*
	SYH	*E. himalayanus*
	SYW	*E. scabrus*
9. *Stenostachys*	SH	*E. narduriodes*
10. *Dasystachyae*	SH	*E. lanceolatus*
	SHH	*E. patagonicus*
11. *Hyalolepsis*	SYP	*E. batalinii*, *E. alatavicus*

treated in the same section (Table 3). Obviously, the traditional subdivision of species in *Elymus* based on morphology does not agree with the grouping of the species based on genomic relationships indicated by meiotic pairing. Therefore, the traditional subdivision within the genus should also be revised. It is recommendable that the subdivision into sections within *Elymus* is made according to the genomic combinations of the species, i.e., one particular genomic combination delimits a section. For example, all species containing the SH, SSH and SHH genomes could be included in the same section, and all species containing SY and SSY could be treated in another, preferably if there exists an obvious morphological indication. These sections could mirror more appropriately the phylogenetic relationships of the different groups of species in *Elymus*.

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Somatic Dimorphism of Caryopsis Color in *Dasypyrum villosum* (L.) Candargy: Some Reproductive and Ecological Relationships

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ABSTRACT

Natural populations of *Dasypyrum villosum* produce two distinct kinds of caryopses: small, red and large, white. Counts on spikes from three natural populations revealed that the lowest floret within a spikelet produced small, red caryopses 42-66% of the time, whereas the upper floret produced large, white caryopses 57-80% of the time. The floret sterility ranged from 13 to 30% in the lower floret and from 14 to 34% in the upper floret. White caryopses were always heavier than red caryopses, but the difference was greatest for caryopses from the upper floret (8.5 and 5.6 mg, respectively) than those from the lower floret (7.0 and 6.1 mg, respectively). The ratio of white to red caryopses produced by individual plants varied between plants, but the correlation between the ratio in parent plants and their offspring was not significantly different from zero. Red caryopses had post-maturity dormancy, took longer to germinate than white caryopses, and had shorter colepotiles, but there was no significant difference between red- and white-derived plants in culm height and number, nor in their allele frequencies for the *Got-V2* and *Got-V3* loci. The caryopsis dimorphism of *D. villosum* provides greater variability in the germination requirements in the soil seed bank, a beneficial attribute for plants growing in Mediterranean climates. Since red caryopses may have longer storage life, they may be favored for long term maintenance in genebanks.

INTRODUCTION

Caryopsis polymorphism, in the narrow sense, refers to the production on a single plant of morphologically distinct caryopses. The morphs may differ in shape, color, embryo size, or dormancy. The resulting plants may differ in growth pattern and fitness (Silvertown 1984; Venable 1985). Caryopses of *Dasypyrum villosum* (L.) Candargy, an annual grass native to Mediterranean countries, eastern Europe, and the Caucasus (De Pace *et al.*, 1988), show strong somatic dimorphism for color and size. It is an annual pollinated species (De Pace, 1987) and the dispersal unit is the spikelet. Usually a spikelet of *D. villosum* has three (rarely four to five) florets and caryopses are generally set only on the two basal florets. The caryopsis color may be either white or red (Fig. 1B). The white caryopses are larger than red ones (Meletti and Onnis, 1961). Previous work, summarized in Table 1, has shown that the caryopses of the two morphs differ in germination ability, ascorbic acid metabolism, mitotic cycle, and histone/DNA ratio, and the seedlings derived from them differ for the amount of DNA and copy number of the subtelomeric 396-bp tandem repeats.

The association between the caryopses morph and the phenotype of mature plants has not been explored despite the potential consequences of such an association on the variation pattern of natural populations and the contents of genebanks. The importance of *D. villosum* as a genetic resource for forage production and for improvement of grain yields of wheat by transfer of disease and stress tolerance genes (Qualset *et al.*, 1993) make it important to develop a better understanding of the occurrence, inheritance, and consequences of the caryopsis dimorphism on mature plants and population structure. Using plants sampled in natural populations, this study was designed to answer four questions relating to this overall goal:

- Is there a developmental pattern to the distribution of the two caryopses morphs within spikes or spikelets?
- Is the degree of caryopsis dimorphism present in a plant inherited by its progeny?
- Do the two morphs differ in caryopsis size, germination ability, and coleoptile growth?

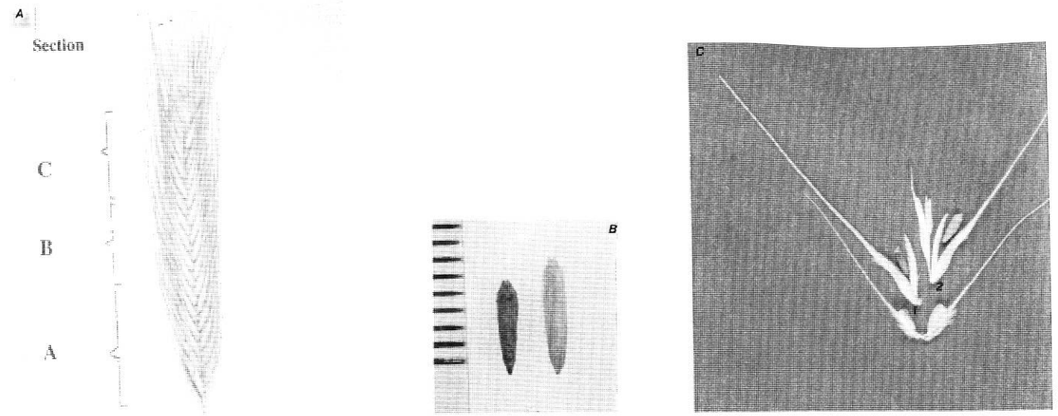

Figure 1. Spike, spikelet and caryopese of *Dasypyrum villosum*. A. spike sections. B. Red and White caryopses. C. Lower (1) and upper (2) floret positions within a spikelet.

Table 1. Genotypic and phenotypic traits for dimorphic caryopses and their derived seedlings of *Dasypyrum villosum*.

Trait	Caryopsis color: White	Red	Reference
Caryopsis size	Large	Small	Meletti and Onnis (1961)
Germination ability[a]	2	7	Stefani and Onnis (1984)
Dormancy period:			
(a) days from seed harvest to 50% germination ability	0-15	0-15	Stefani and Onnis (1984)
(b) days from seed harvest to 100% germination	0-15	0-30	Stefani and Onnis (1984)
Ascorbate content (AA) in 4-day-old seedlings (m moles/g fresh wt.)	2.74	2.68	Paciolla et al. (1991)
Enzyme activity in 4-day-old seedlings:			
Ascorbate free radical reductase (n moles AA formed/min/mg prot)	<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[a]	Paciolla et al. (1991)
Ascorbate peroxidase (n moles AA ox/min/mg prot) in seedlings from:			
1-year-old seeds	343[b]		
	270±15[c]	334[b]	Paciolla et al. (1991)
5-year-old seeds	100	155±18[c]	Paciolla et al. (1991)
		320	De Gara et al. (1991)
Caryopsis longevity:			
Germination after 3 years	90%	90%	De Gara et al. (1991)
Germination after 5 years	10%	80%	De Gara et al. (1991)
Germination after 7 years	0%	28%	De Gara et al. (1991)
Duration of mitotic cycle	12h	9.5h	Innocenti & Bitonti (1983)
Histone/DNA ratio in 2C nucleus of root meristematic cell during aging	Increase	Constant	Innocenti & Bitonti (1980)
Basic amount of nuclear DNA (in mean Feulgen absorption) in two-day-old seedlings	2185	1904	Frediani et al. (1994)
Copy number of subtelomeric 396-bp tandem repeats in:			
- resting embryos	$2.5x10^3$	$1.5x10^3$	Frediani et al. (1994)
- 10-day-old seedlings	$15.5x10^3$	$6.0x10^3$	Frediani et al. (1994)
- 50-day-old seedlings	$8.0x10^3$	$3.5x10^3$	Frediani et al. (1994)

[a] Number of days for 90-100% germination in nondormant 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).

- What is the relationship between the two morphs and the biochemical and quantitative characters of seedlings and mature plants?

MATERIALS AND METHODS

The plant materials used in this study were from collections made by the authors in Italy (Fig. 2). The distribution of the two caryopsis morphs within individual plants was examined using plants collected from three populations, I-86 (16 plants), I-27 (13 plants), and I-147 (12 plants). The collection sites were roadsides in Puglia (I-27 and I-86) and Toscana (I-147) regions. The relationship between the caryopsis dimorphism of parents and their offspring was explored using caryopses from plants I-27.3 and I-27.9a, which were sampled in population I-27 for their extreme ratios of white to red caryopses at each floret position (I-27.3) or for having short spikes (I-27.9a). Germination time and coleoptile length were examined using 11 plants from each of populations 81-2M, 81-3M, 81-5M, and 81-7M which were collected along different roadsides in Puglia. In each case, caryopses from three spikes per plant were used. Data for caryopsis size and 11-day-old seedling weight were obtained from the 12 populations used by Zhong and Qualset (1993) and 11 populations studied by De Pace (1987). To study the variability for morphological and biochemical traits, subpopulations of plants from the two caryopsis morphs of three populations collected in Puglia (pop. Alberobello and pop. Castellaneta) and Lazio (pop. Flaminia) regions, were used.

Relationships between floret position and caryopsis morphology

Three spikes were randomly chosen from each of the plants collected for use in this part of the study. Each spike was arbitrarily divided into three approximately equal portions: A - the lowest node to the eighth spikelet; B - ninth to the sixteenth spikelet; and C -seventeenth to the apical spikelet. Within each spikelet, only the first two florets were considered, the few caryopses found in more distal florets being ignored.

The florets from each spike section were separated into an upper floret sample and a lower floret sample. The color and weight of each caryopsis was then recorded, and the total number of caryopses per sample counted. The proportion of upper florets producing a given morph was obtained by dividing the number of caryopses of that morph in the sample concerned by the total number of upper florets in that sample; the figures for lower floret samples were calculated similarly. The average proportion and weight of the two morphs were then calculated for each sector of every individual of each population. Weighted averages were used to arrive at the population values. Averages over all populations were weighted for the number of plants analyzed for each population.

A nested analysis of variance was conducted to obtain information on the relationship between the various factors examined and caryopsis morph. The factors examined were (in order of increasing precision): population, spike, spike section, and floret position. The analysis was carried out for the proportion and for the weight of each morph in the two floret positions.

Relationship between the caryopsis dimorphism of parents and their offspring.

Caryopses from each of two mother plants (I-27.3 and I-27.9a) from population I-27 were sorted according to the 2 morphs, 2 floret positions, and 3 spike sections. Twelve and eight groups of caryopses were obtained from the mother plant I-27.3 and I-27.9a, respectively; the spikes of I-27.9a were shorter and had only spikelets in the A and B sections. Each group contained 6 to 9 caryopses and represented a different 'treatment' on the offspring derived from those mother plants for a total of 20 treatments. The caryopses of the same treatment were sown in two-row plots 1 meter long at the Experimental Farm of the University of Tuscia, Viterbo, Italy. The plants were grown to maturity under open pollinating conditions. At maturity, the height and number of culms produced by each plant in each plot were recorded. Caryopses from the three tallest culms of each of these plants (offspring) were then sorted by morph, floret position, and spike section for the calculation of the average proportion of each morph produced by each floret in each section.

Variation in morphological and biochemical traits of plants grown from the two morphs.

Caryopses from three populations (Alberobello, Castellaneta, and Flaminia) were separated according to their morph and then planted in the field at the Experimental Farm, University of Tuscia, Viterbo, Italy. The six entries were arranged in a randomized block with two replications. For each entry, 40 plants of each morph were randomly chosen and tagged. At the tillering stage, leaf samples were taken from each tagged plant and frozen at -80 °C until their sap could be extracted for electrophoresis. The occurrence of glutamate-oxaloacetate-transferase (GOT) isozymes in two electrophoretic zones was examined, and the allelic frequencies were estimated from the zymogram phenotypes in each zone, following the procedures of De Pace (1987). Culm length, number of culms, and number of spikes per plant were evaluated for each tagged plant at maturity. Student's t-test was used to assess the significance of both the difference between the means of the morphological traits and allelic frequency difference of the plants from the two caryopsis morphs.

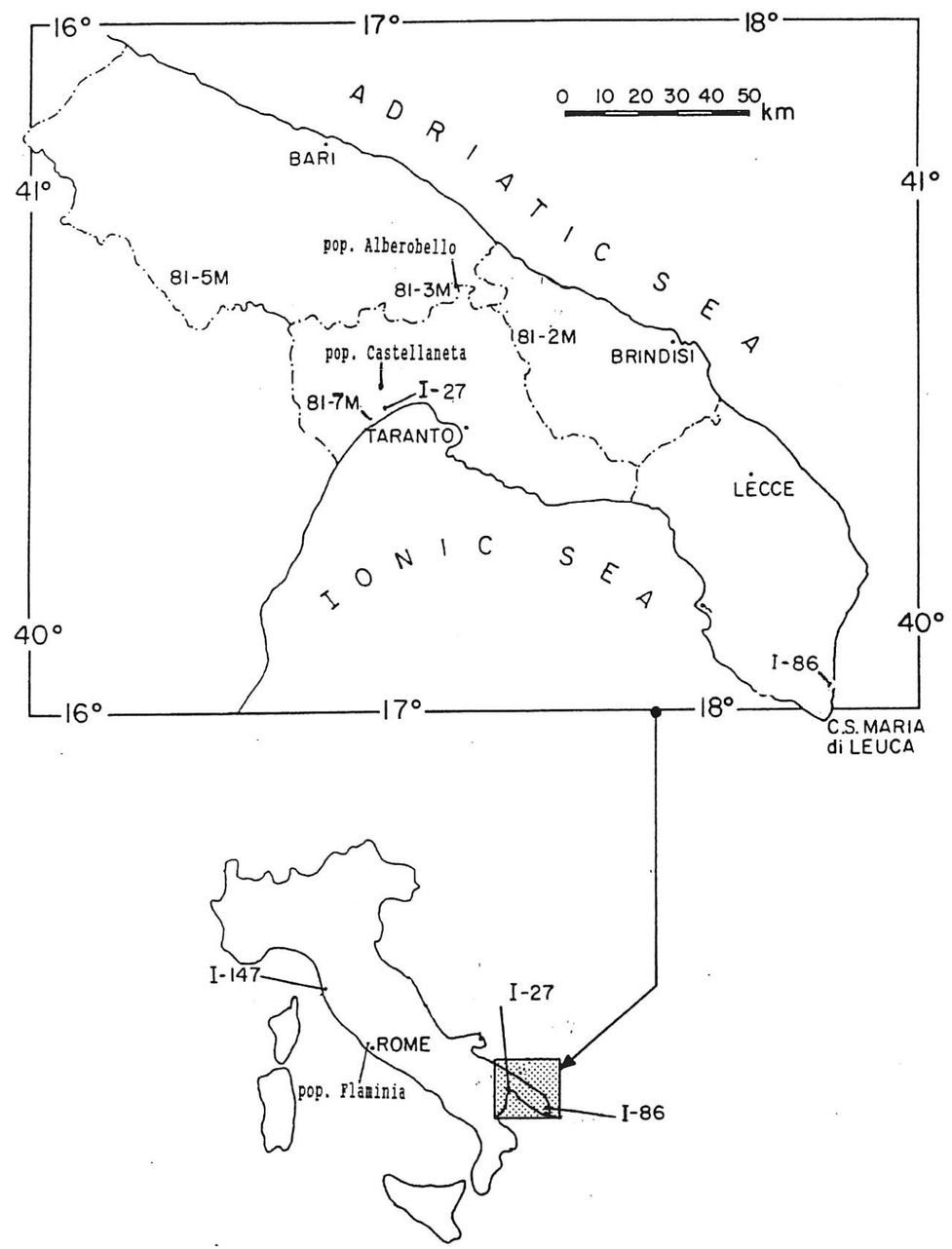

Fig. 2. Geographic locations of collections of *Dasypyrum villosum* made in Italy.

Germination rate and time and coleoptile length.

Caryopses from three spikes of 11 plants from four populations (81-2M, 81-3M, 81-5M, and 81-7M) were hand-threshed, pooled together for each population, and then sorted according to their caryopsis morph. Sixty caryopses of each morph were germinated using the slant-board technique (Jones and Cobb, 1963). The trays were kept in a growth chamber (16 h light, 25°C; 8 h dark, 20°C). A caryopsis was considered to have germinated when its coleoptile was 3 mm long. Germinability was estimated as the percentage of caryopses that produced a coleoptile at least 3 mm long. The length of the coleoptile was measured after the first leaf emerged. Analysis of variance according to mixed factorial-nested model was performed on the data collected for the three traits. Means were compared using the least significant difference.

Relationship between caryopsis weight and seedling growth.

Caryopsis weight and the weight of 11-day old seedlings were measured for 10 caryopses of each morph sampled in 23 populations. Twelve populations were those described by Zhong and Qualset (1993) and 11 populations were those studied by De Pace (1987). The caryopses were germinated using the slant-board technique; the caryopses of the same population were germinated on the same slant board. The significance of the average difference between morphs for caryopsis and seedling weight was evaluated using the least significance difference method based on t-values at the 0.05 probability level. The correlation coefficient between caryopsis and seedling weight was also calculated.

RESULTS

Floret position and caryopsis morphs.

Both of the florets examined in the spikelets were capable of producing either or both caryopsis morphs, but the upper florets produced mostly white caryopses, whereas the lower florets produced mostly red caryopses (Table 2). The mean number of white and red caryopses varied somewhat according to the section of the spike and the population concerned, as indicated by the significant floret x section and floret x population interactions (Table 4). In all three populations examined, the lowest section of the spike produced the highest proportion of sterile florets. This was true for both the upper and lower florets. The average number of white caryopses produced in the upper floret was significantly higher than in the lower floret, especially in the upper and middle spike sections (Table 5). On the other hand, the average number of red caryopses was significantly higher in the lower floret than in the higher floret. This situation made the white: red caryopsis ratio in the upper florets to range between 13:1 in pop. I-27 to 3.9:1 in pop. I-147 (Table 2). The white: red caryopsis ratio in the lower floret varied between 0.31:1 in pop. I-27 to 0.66:1 in pop. I-86. The proportion of upper florets producing white caryopses in the middle and upper spike sections was similar within each population, but ranged from 63% in population I-86 to 86% in I-27. The percentage of red caryopses produced by the lower floret in these sections also varied little within a population, but considerably between populations, from 45% in I-86 to 72% in I-27. The between population variation was, however, significant at p=0.01 only for the frequency of white caryopsis production (Table 4).

The white caryopses were consistently heavier than the red caryopses (Table 3), with white caryopses produced in upper florets being 1.5 mg heavier than those produced in the lower florets (Table 3). For red caryopses, the reverse was true, the heavier red caryopses being those produced in the lower floret. In most instance, however, the difference was not significant (Table 5).

Comparison of caryopsis parents with their progeny.

In neither case examined was the ratio of the two caryopses morphs produced by a parent plant maintained in its progeny (Table 6). Mother plant I-27.3 was notable for its extreme ratios for each floret position in each section. None of the 12 progeny examined had such extreme ratios. Their ratios were generally between those for the parental plant and the population as a whole, but in some instances, they were below the population average. The second mother plant had such short spikes that there was no C section, but the spikes of all its progeny were longer. The ratios for the offspring of I-27.9a were intermediate to the parent and population means, but occasionally exceeded those values. Unlike the progeny of I-27.3, the ratios for the progeny of I-27.9a varied beyond the parental plant value, even on the lower two sections. In no case was the regression of the progeny ratios on the parent ratio significantly different from zero.

Comparison of plants derived from white and red caryopses.

Plants grown from white and red caryopses did not differ significantly in overall height nor the number of culms produced (Table 7). This was true for the progeny of I-27.3 and I-27.9a and for plants grown from caryopses obtained from the Alberobello, Castellaneta, and Flaminia populations (Table 8). There was also no difference in the allele frequencies at the gene loci governing isozyme variability in the GOT-2 and GOT-3 electrophoretic zones in plants derived from the different caryopsis morphs.

Germination of the two morphs.

White caryopses were quicker to germinate and produced a longer coleoptile (2 mm) than red caryopses (Table 9), but there was no difference between the two caryopsis morphs in the percentage of caryopses that germinated. Only the difference in coleoptile length was consistently significant at p=0.05.

Relationship between caryopsis weight and seedling growth.

The white caryopses were 5 mg (p 0.01) heavier than the the red ones. The 11-day-old seedlings grown from the white caryopses were 25 mg (p 0.01) heavier than those from the red caryopses. There was no significant correlation between caryopsis and seedling weights within either morph (Table 10).

DISCUSSION

The white caryopses appear in the upper floret to be 4 to 13 times more frequently than red caryopses and the white caryopses in the lower floret were 2 to 3 times less frequent than red caryopses. The same range of variability may be found in different spike sectors of the same spike, or between spikes of different populations, or between spikes of the mother and daughter plants. These data suggest that caryopsis morph frequency at each floret position was relatively stable, but variation about these frequencies occurred and were environment-dependent. The average frequency and range of variability of sterile florets was the same in the upper and lower florets, indicating that the different proportions of white and red caryopses produced on the upper and lower florets do not depend on the fertility level within a spikelet.

The different times of caryopsis (spikelet) disarticulation does not seem to be related to the time of caryopsis germination. The lower spikelets, corresponding to those of spike section A, are retained by the mother plant for at least 1 month after caryopsis maturity and show the same distribution of dark and light caryopses as the upper spikelets which are released just after caryopsis maturity. Caryopsis germination rate is then related only to caryopsis color and size and not to the position of the spikelet carrying the caryopsis.

The ecological significance of the white and red caryopses becomes relevant when considered relative to several factors such as dormancy within the spikelet dispersal unit and the climatic conditions at the time of germination and establishment. As matter of fact, there are indications that red and white caryopses differ in dormancy. A preliminary germination test carried out on a white and a red caryopsis pool from population 81-7M a few days after collection showed an average of 5 days to germination for white caryopses and of 11 days for red caryopses. However, after 2 months storage at room temperature, the difference in days to germination of white and red caryopses did not exceed one day. Similar results were obtained by Onnis (1967) and Stefani and Onnis (1984). Therefore, a prolonged caryopsis dormancy for red caryopses in *D. villosum* can be the crucial link between generations which may have evolved in response to the probability of an adaptation to dry/wet habitat. Harper (1977) indicates that somatic polymorphism is the optimum strategy adopted by the fugitive annuals of disturbed habitats if the environments are very different. In the Mediterranean region rainfall is uncertain and plants arising from caryopses that are triggered into germination by an early rain after the dry season may die if the next rain is delayed. The presence of two caryopsis morphs in *D. villosum* may exert a buffering effect against this occurrence since the caryopses that will germinate after an early rainfall will more likely be white than red. If the following rain is too late to support seedling establishment, the red caryopses will germinate and compensate for the mortality of seedlings from white caryopses. This dormancy behavior has been called "wild type" regulation of germination in wild cereals by Zohary (1969). It endows strong fitness to the daughter generation, assuring a gene pool in which some progenies from each spike will survive.

The floret position in which the caryopses develop is the most important feature regulating the production of the two caryopsis morphs in *D. villosum* as well as other noncultivated Triticeae species evaluated by the authors (Table 11). This influence is expressed as caryopses size and/or color dimorphism. It is then expected that differences between the two morphs more likely will be seen in early establishment stages and be affected by environmental factors and length of time in the soil seed bank than for agronomic traits expressed in later stage of growth. In fact, it has been observed that the survived seedlings developed from white and red caryopses formed two pools of plants sharing similar average phenotypic values for plant height and number of culms, and similar allele frequencies at isozyme loci. Some other consequences of caryopsis dimorphism in *D. villosum* have been described in Table 1; similar information is lacking for the other Triticeae species that exibit this trait. The information obtained in this and previous studies may have relevance for conservation *D. villosum* germplasm. For example, De Gara *et al.* (1991) have shown that red caryopses have longer viability and therefore would be desirable for long-term storage.

Table 2. Proportion of white (W) and red (R) caryopses and sterile (S) florets in the upper and lower florets of spikelets from base (A), middle (B), and top (c) spike sections of spikes from three *D. villosum* population collected in Italy in 1984 (values in brackets are standard errors).

Pop.	No. plants	Spike section	Proportion					
			Upper floret			Lower floret		
			W	R	S	W	R	S
I-27	13	A	0.68 (0.038)	0.09 (0.023)	0.23 (0.012)	0.25 (0.033)	0.58 (0.052)	0.17 (0.011)
		B	0.86 (0.040)	0.07 (0.038)	0.07 (0.010)	0.19 (0.020)	0.72 (0.060)	0.09 (0.004)
		C	0.85 (0.040)	0.03 (0.013)	0.12 (0.012)	0.19 (0.033)	0.69 (0.088)	0.13 (0.018)
		Mean W/R	0.80 13.3	0.06	0.14	0.21 0.32	0.66	0.13
I-86	16	A	0.42 (0.048)	0.15 (0.034)	0.43 (0.019)	0.31 (0.034)	0.33 (0.060)	0.36 (0.021)
		B	0.65 (0.047)	0.08 (0.020)	0.27 (0.098)	0.27 (0.038)	0.49 (0.067)	0.24 (0.091)
		C	0.63 (0.052)	0.05 (0.025)	0.32 (0.021)	0.25 (0.048)	0.45 (0.071)	0.30 (0.020)
		Mean W/R	0.57 6.3	0.09	0.34	0.28 0.66	0.42	0.30
I-147	12	A	0.36 (0.055)	0.24 (0.080)	0.40 (0.027)	0.32 (0.042)	0.48 (0.081)	0.20 (0.035)
		B	0.75 (0.073)	0.14 (0.062)	0.11 (0.003)	0.25 (0.066)	0.62 (0.082)	0.13 (0.002)
		C	0.81 (0.063)	0.05 (0.032)	0.14 (0.002)	0.29 (0.032)	0.64 (0.097)	0.07 (0.001)
		Mean W/R	0.65 4.6	0.14	0.22	0.30 0.52	0.58	0.13
Weighted Mean		Mean W/R	0.67 7.4	0.09	0.24	0.26 0.48	0.54	0.20

Table 3. Average weight of white (W) and red (R) caryopses in the upper and lower floret of spikelets from base (A), middle (B), and top (c) spike selections of spikes from three *D. villosum* populations collected in Italy in 1984 (values in brackets are standard errors).

Pop.	No. plants	Spike section	Average weight of caryopses (mg)			
			Upper floret		Lower floret	
I-27	13	A	6.2 (0.38)	4.2 (0.58)	4.8 (0.54)	4.9 (0.30)
		B	8.2 (0.74)	5.3 (0.36)	7.2 (0.83)	5.8 (0.34)
		C	8.7 (0.24)	5.3 (0.22)	8.2 (0.49)	5.7 (0.29)
		Mean W/R	7.7 1.6	4.9	6.7 1.23	5.4
I-86	16	A	8.6 (0.68)	5.7 (0.50)	7.7 (0.82)	5.9 (0.41)
		B	9.9 (0.78)	6.5 (0.27)	7.2 (0.95)	7.0 (0.42)
		C	9.8 (0.79)	5.7 (0.62)	7.0 (0.79)	6.7 (0.34)
		Mean W/R	9.4 1.6	6.0	7.3 1.1	6.6
I-147	12	A	7.3 (0.91)	5.1 (0.06)	5.7 (0.80)	5.9 (0.45)
		B	9.4 (0.89)	8.2 (1.01)	7.3 (0.67)	7.0 (0.72)
		C	8.1 (0.41)	3.8 (0.51)	7.7 (0.44)	5.6 (0.28)
		Mean W/R	8.3 1.4	5.7	7.0 1.1	6.2
Weighted mean		Mean W/R	8.5 1.5	5.6	7.0 1.1	6.1

Table 4. Mean squares from the analyses of variance of caryopses number per spikelet and caryopses weight of white (W) and red (R) colored seeds from upper and lower floret positions on three spike sections of Italian *D. villosum* populations I-27, I-86, and I-147.

Source of variation	Degrees of freedom	Mean no. caryopses/spikelet		Mean caryopses/weight	
		W	R	W	R
Population (P)	2	0.119**	0.243	57.52	20.98
Error (a)	38	0.019	0.106	25.23	8.48
Section (S)	2	0.401**	0.053	59.55**	29.95**
S x P	4	0.087**	0.011	4.04	4.58
Error (b)	68	0.014	0.027	4.98	1.97
Floret (F)	1	9.583**	10.405**	60.01*	8.97*
F x P	2	0.382**	0.366**	0.85	1.42
F x S	2	0.597**	0.472**	0.50	3.50
F x P x S	4	0.080	0.030	2.09	1.17
Error (c)	92	0.048	0.031	2.58	1.18

*, ** Significant at the 0.05 and 0.01 probability levels, respectively.

Table 5. t-values from two-tailed t-tests for comparing pairs of means of upper (U) and lower (L) floret samples for average number and weight of white and red caryopses from A, B, and C spike sections of individuals from three Italian *D. villosum* populations.

Population	df	Spike section	Average no. of White caryopses U vs. L	Average no. of Red caryopses U vs. L	Average White caryopses weight (mg) U vs. L	Average Red caryopses weight (mg) U vs. L
I-86	30	A	1.89	3.17**	0.31	0.61
		B	6.55**	5.28**	1.11	0.20
		C	5.92**	5.79**	1.71	1.55
I-27	24	A	8.60**	8.42**	2.90**	0.26
		B	17.55**	7.18**	0.74	1.15
		C	13.46**	7.98**	2.09*	8.48**
I-147	22	A	2.00	1.05**	1.28	1.09
		B	5.05**	4.66**	2.49*	1.69
		C	4.44**	5.78**	0.69	4.31**

*, ** The differences between the U and L means is significant at the 0.05 and 0.01 probability levels, respectively.

Table 6. Frequency of white (W) and red (R) colored (C) caryopses in the upper and lower floret of spikelets from base (A), middle (B) and top (C) section sof spikes from the *D. villosum* mother plants 27.3 and 27.9a and

Caryopses in the mother plant					Spike section: A				B				C				Whole spike			
					Upper floret		Lower floret		Upper floret		Lower floret		Upper floret		Lower floret		Upper floret		Lower floret	
Mother plant	Spike section	Floret posit.	C	Freq.	W	R	W	R	W	R	W	R	W	R	W	R	W	R	W	R
I-27.3	A	Upper	W	0.66	0.34	0.12	0.13	0.34	0.60	0.08	0.17	0.49	0.61	0.03	0.15	0.50	0.52	0.08	0.15	0.44
		"	R	0.16	0.31	0.25	0.12	0.42	0.47	0.12	0.31	0.37	0.40	0.20	0.00	1.00	0.39	0.19	0.14	0.60
		Lower	W	0.08	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
		"	R	0.83	0.31	0.03	0.13	0.27	0.52	0.06	0.27	0.27	0.58	0.00	0.29	0.37	0.47	0.05	0.23	0.30
	B	Upper	W	1.00	0.28	0.06	0.19	0.25	0.58	0.08	0.18	0.45	0.80	0.03	0.16	0.64	0.55	0.06	0.18	0.45
		"	R	0.00	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
		Lower	W	0.04	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
		"	R	0.95	0.40	0.03	0.03	0.28	0.64	0.08	0.12	0.52	0.42	0.00	0.04	0.33	0.49	0.06	0.06	0.38
	C	Upper	W	0.96	0.42	0.07	0.16	0.27	0.71	0.06	0.11	0.54	0.71	0.00	0.35	0.32	0.61	0.06	0.21	0.38
		"	R	0.00	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
		Lower	W	0.00	0.45	0.16	0.12	0.54	0.84	0.00	0.00	0.79	0.83	0.00	0.00	0.83	0.71	0.05	0.04	0.72
		"	R	0.92	0.34	0.11	0.20	0.30	0.57	0.04	0.13	0.49	0.41	0.00	0.03	0.39	0.44	0.07	0.12	0.39
	Weighted mean				0.35	0.10	0.13	0.33	0.61	0.06	0.16	0.49	0.59	0.03	0.12	0.54	0.53	0.09	0.14	0.46
I-27.9a	A	Upper	W	0.79	0.76	0.17	0.32	0.66	0.86	0.11	0.29	0.85	0.73	0.00	0.03	0.87	0.78	0.09	0.21	0.83
		"	R	0.04	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
		Lower	W	0.20	0.37	0.08	0.12	0.43	0.82	0.00	0.00	0.60	0.47	0.00	0.00	0.63	0.55	0.03	0.04	0.55
		"	R	0.66	1.00	0.00	0.27	0.50	1.00	0.00	0.00	0.87	0.57	0.00	0.00	0.42	0.86	0.00	0.09	0.60
	B	Upper	W	0.86	0.64	0.01	0.03	0.63	0.62	0.00	0.04	0.69	0.70	0.00	0.14	0.63	0.65	0.00	0.07	0.65
		"	R	0.08	0.80	0.00	0.25	0.62	0.54	0.00	0.00	0.46	0.57	0.00	0.00	0.71	0.64	0.00	0.08	0.70
		Lower	W	0.21	0.65	0.00	0.19	0.65	0.68	0.00	0.24	0.67	1.00	0.00	0.00	0.67	0.78	0.00	0.14	0.67
		"	R	0.78	0.72	0.02	0.15	0.68	0.71	0.00	0.10	0.67	0.70	0.00	0.02	0.63	0.71	0.00	0.09	0.66
	Weighted mean				0.69	0.04	0.17	0.65	0.73	0.02	0.09	0.69	0.68	0.00	0.03	0.65	0.71	0.02	0.10	0.66

-: no data were available for this offspring due to the lack or very small number of plants raised.
*: for each floret position (upper or lower) the difference [1-(freq. w. car. + freq. R car.)] accounts for the frequency of sterile florets.

Table 7. Means and standard errors (values in brackets) for plant height and number of culms in the progeny from white (W) and red (R) caryopses harvested from the upper and lower florets of the mother plants I-27.3 and I-27.9a.

	Progeny from:			
	Upper floret		Lower floret	
Plant no.	W	R	W	R
I-27.3				
Plant height (cm)	84.8 (4.6)	84.0 (1.0)	86.2 (3.7)	87.1 (5.1)
Number of culms	10.5 (1.2)	8.0 (0.5)	8.9 (2.4)	7.4 (1.2)
I-27.9a				
Plant height (cm)	102.4 (3.7)	108.0 (4.8)	103.7 (6.0)	98.8 (6.9)
Number of culms	9.4 (1.4)	10.0 (4.9)	11.6 (0.8)	9.6 (1.1)

Table 8. Mean values and standard errors (values in brackets) for the morphological traits and allele frequency at loci controlling Glutamate-oxaloacetate transaminase (GOT-2 and GOT-3) isozyme phenotypes in the progenies from white (W) and red(R) caryopses of three *D. villosum* populations collected in Italy.

	Site					
	Flaminia		Alberobello		Castellaneta	
Character	W	R	W	R	W	R
Plant height (cm)	146.4 (9.7)	153.7 (4.5)	111.0 (9.5)	117.6 (5.2)	140.0 (6.5)	137.6 (7.6)
Number of culms	37.0 (4.4)	34.6 (3.1)	37.0 (3.7)	34.4 (4.9)	53.7 (5.8)	52.4 (5.9)
Number of spikes	35.0 (3.9)	32.6 (4.2)	32.0 (3.5)	31.4 (4.0)	48.8 (5.1)	49.0 (4.3)
Allele frequency *Got-V2S*	0.8	0.9	0.9	0.9	0.6	0.8
" " *Got-V2F*	0.2	0.1	0.1	0.1	0.3	0.2
" " *Got-V3S*	0.8	0.8	0.7	0.8	0.8	0.9
" " *Got-V3F*	0.2	0.2	0.3	0.2	0.2	0.1

Table 9. Mean values for germination percentage, duration of germination time, and coleoptile length of white (W) and red (R) colored caryopses from 50 spikes from four populations of *D. villosum* collected in 1981.

	Germination %		Days to germination		Coleoptile length, mm	
Population	W	R	W	R	W	R
81-3M	99	96	3.3	3.5	17.4*	14.7
81-5M	99	99	3.1	3.5	18.6*	15.7
81-7M	81	83	4.2	4.7	16.0*	14.6
81-2M	71	74	4.5	4.6	15.1*	14.0
Mean	88	88	3.8	4.1*	16.8*	14.8
L.S.D. (0.05)	17	11	0.42	0.11	1.33	0.74

* Significantly different from white or red-colored caryopses at P = 0.05.

Table 10. Average caryopsis weight and 11-day-old seedling weight from the white (W) and red (R) caryopses, t-values for the difference of W- and R-caryopses mean values and correlation coefficients between caryopses and seedling weight in 23 populations of *D. villosum*.

	Caryopsis morph		t
Trait	W	R	
Caryopsis weight (mg)	13.4 ± 0.59	8.4 ± 0.43	6.86**
Seedling weight (mg)	85.6 ± 5.1	60.0 ± 4.00	3.94**
r	0.23 ns	0.33 ns	

** P < 0.01.

Table 11. Caryopses color dimorphisms and weight in *Triticum* and *Aegilops* species.

		Caryopses in Upper floret		Caryopses in Lower floret		
Species	Genome(s)	Color[2]	Size	Color[2]	Size	Size difference
			(mg)		(mg)	(Uf - Lf)
Triticum boeoticum	A	ster.		W	34.0	
		W	14.5	R	8.0	6.5*
Triticum monococcum[2]	A	ster.	--	W	10.9	
Triticum urartu	A	W	19.0	W	10.5	8.5*
Aegilops speltoides	S	R	6.0	R	3.0	3.0*
Aegilops longissima	S^l	W	6.2	R	6.1	0.1 ns
Aegilopsis bicornis	S^b	R	6.0	R	5.0	1.0 ns
Aegilops searsii	S^s	W	10.0	W	6.0	4.0*
Aegilops sharonensis	S^h	R	10.0	R	6.3	3.7*
Aegilops tauschii	D	W	12.0	W	7.2	4.8*
Triticum araraticum	AG	W	20.1	R	18.0	1.9 ns
Triticum timopheevi	AG	W	38.0	W	38.0	0.0
Triticum dicoccum	AB	W	45.0	W	48.0	-3.0 ns

[1] *T. monococcum* can produce two caryopses under favorable conditions.
[2] R may indicate either red, purple or blue color according to the species evaluated.

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The Phylogeny of *Psathyrostachys* Nevski (Triticeae, Poaceae) - Are We Able to See the Wood for the Trees?

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ABSTRACT

Molecular sequences and phylogenetic hypotheses based upon individual sequences continue to accumulate at a steadily increasing rate. It is, however, a contentious issue how differences among these molecular phylogenies, and between molecular phylogenies and morphology, are resolved. These questions are explored using phylogenies based upon a number of different sequences obtained both from the chloroplast (cpDNA) and nuclear genome (nDNA), and from the morphology of *Psathyrostachys*.

INTRODUCTION

As long as different sets of characters, derived from the same organisms, share a common evolutionary history it is to be expected that when reliable phylogenetic methods are used, they will all give the correct evolutionary tree. Consequently, the evolutionary trees derived from each data set should in principle be congruent. However, the increased availability of molecular data sets has made differing phylogenetic hypotheses for the same taxa a rather common phenomenon, and they cannot all be "true" at the same time.

This raises the fundamental question whether such apparent conflicts are "real", e.g. whether they are unavoidable, or only "spurious", e.g., due to sampling error or to inappropriate assumptions or analytical methods (Hillis 1987).

One way of solving the problem of conflicting phylogenies is taxonomic congruence, e.g., to make the best fitting hypothesis for each data set, and derive a consensus for these topologies. Another is total evidence, e.g., using character congruence to find the best fitting hypothesis for all synapomorphies. In order to subject these different solutions to critical examination the genus *Psathyrostachys* Nevski was selected as a model system. The ultimate goal is, however, to offer guidelines for an analysis of the phylogeny (based on different genes and morphology) of the monogenomic Triticeae.

As *Psathyrostachys* has recently been revised (Baden 1991) it has been fairly easy to produce a phylogenetic hypothesis of the genus based on morphology.

A large series of different genes could have been chosen as targets for phylogenetic analysis. In plant molecular biology the gene of choice is without doubt the chloroplast (cpDNA) encoded gene for the large subunit of rubisco (rbcL, ribulose-1,5-bisphosphate carboxylase/oxygenase). *rbc*L (e.g., Doebley et al. 1990) and cpDNA genes in general are often considered to be too conservative to resolve relationships at lower taxonomic levels; however, this need not be the case, and to some extent hinges on the choice of phylogenetic methodology. Thus, we have selected three different cpDNA genes for this study: *rbc*L, *rpo*A, and *rpo*C2 (the genes for the and " subunits of RNA polymerase).

Nuclear encoded genes show much greater variation than plastid genes (Curtis and Clegg 1984, Wolfe et al. 1987). However, nuclear genes are only rarely used in studies of plant phylogeny, and those that are usually belong to multicopy families (e.g., ITS1 and ITS2, Internal Transcribed Spacers (Scoles et al. 1988, Baldwin 1992)).

A few investigations have used the single copy nuclear encoded gene for alcohol dehydrogenase 1 (*Adh*1) in phylogenetic studies (e.g., Gaut and Clegg 1993). *Adh*1 is a

NAD+-dependent dimeric oxidoreductase which catalyses the oxidation of a wide range of alcohols. It belongs to a group of alcohol dehydrogenases which also includes *Adh*2 and *Adh*3. In the Poaceae (incl. *Hordeum* L.) *Adh*1 is organized into 10 exons and nine introns. *Adh*1 is located on chromosome 4 in *Hordeum vulgare* L.

MATERIALS AND METHODS

Morphology

The data from morphology were extracted from Baden (1991) and may be found in Table 1. The data matrix shown in Table 2 was used as input to PAUP (ver. 3.1.1; Swofford 1993). All characters except one were binary; all were treated as unordered. Due to the small size of the data set it was possible to use the Branch and Bound option, ensuring that all equally parsimonious trees were found. *Hordeum* was used as outgroup (Frederiksen and Seberg 1992).

Sequencing

A list of the plants sequenced in the present investigation is found in Table 3. In two rare species DNA was extracted from herbarium material. For various reasons *P. juncea* (Fischer) Nevski and *P. kronenburgii* (Hack.) Nevski were unavailable. Total DNA was extracted from fresh leaves following the method of Doyle and Doyle (1987). Prior to sequencing the DNA was amplified via the polymerase chain-reaction (PCR) (Saki et al. 1988). Double-stranded amplifications were followed by single-strand amplification, securing enough copies of the DNA fragment for dideoxy termination sequencing (Sanger et al. 1977, Gyllensten and Erlich 1988). Amplifications were performed in 50 l reactions running 20-40 cycles, and

Table 1. Morphological data set (Baden 1991)

List of characters and character states	
1. Habit	10. Lateral spikelets
0: stoloniferous	0: pedicellate
1: caespitose	1: sessile
2. Root diameter	11. Spikelet type
0: thin (< 1 mm)	0: heterospiculate
1: thick (> 1 mm)	1: homospiculate
3. Culm indumentum	12. Glume length
0: glabrous	0: 2 x length of lemma
1: pubescent	1: approx. as long as lemma
4. Leaf sheath margins	13. Glume indumentum
0: free	0: glabrous-scabrous
1: joined	1: pubescent
5. Nodes	14. Triplet length
0: glabrous	0: < 20 mm
1: pubescent	1: > 20 mm
6. Aestivation	15. Awn length
0: involute	0: < 6 mm
1: flat, margins involute	1: > 6 mm
2: flat	16. Lemma indumentum
7. Leaf indumentum	0: glabrous-scabrous
0: glabrous	1: pubescent
1: pubescent	17. Anther colour
8. Leaf epidermis (long cells)	0: violet
0: thin straight walls	1: yellow
1: thick sinuous walls	18. Chromosome size
9. Auricles	0: small
0: absent	1: large
1: present	

Table 2. OTU-character matrix for *Psathyrostachys*, based on the characters found in Table 1.

	Character no. 1-5	6-10	10-15	16-
P. fragilis				
ssp. *fragilis*	11111	01111	11011	011
ssp. *secaliformis*	11110	00111	11011	011
ssp. *villosus*	11110	00111	11111	111
P. caduca	11111	21111	10111	00?
P. huashanica	01010	20101	10000	011
P. rupestris	11010	10101	10?01	?1?
P. lanuginosa	11010	20011	10100	111
P. kronenburgii	11010	20001	10100	10?
P. juncea	11010	20001	10?00	?11
P. stoloniformis	01010	10001	10000	011
Hordeum erectifolium	10000	01110	00001	010

Table 3. The taxa of *Psathyrostachys* and *Hordeum* sequenced

Accession no.	Species	Country	Locality	Chromosome no.	Collector
H 6700	*P. caduca*	Afghanistan	Westnuristan	--	A. Scheibe
H 6702	*P. caduca*	Afghanistan	Deh Kundi	--	K. H. Rechinger
H 917	*P. fragilis* ssp. *fragilis*	Iran	Mazandera	14	R. von Bothmer
H 4348	*P. fragilis* ssp. *secaliformis*	Turkey	Bitlis	28	GP & M
H 4372	*P. fragilis* ssp. *villosus*	Turkey	Kars	14	GP & M
H 4375	*P. fragilis* ssp. *villosus*	Turkey	Kars	14	GP & M
H 4378	*P. fragilis* ssp. *villosus*	Turkey	Kars	14	GP & M
H 3087	*P. huashanica*	China	Hua-Shan Pass	14	J. Yang
H 8803	*P. lanuginosa*	China	Xinjiang	14,28	J. Yang
H 6701	*P. rupestris*	USSR	Dagestan	--	?
H 6703	*P. rupestris*	USSR	-	--	?
H 9182	*P. stoloniformis*	China	Ganzu	14	CS
H 1150	*H. erectifolium*	Argentina	Bahía Blanca	14	N. Jacobsen

GP & M = G. Petersen & M. Ørgaard, CS = China-Scandinavian Collecting Mission

with a 1:50-100 dilution of one primer in the single-strand reactions. The primer sequences will be published elsewhere, and the sequences will be submitted to Genbank.

The individual sequences were subjected to a parsimony analysis using PAUP (ver. 3.1.1; Swofford 1993). Due to the size of the data sets only the heuristic search option was used. *Hordeum* was used as outgroup, and the characters were treated as unordered. To increase the likelihood that all equally parsimonious trees were found, the data matrices were run 25 times with random input order of the OTUs. Manipulation of the trees was done using MacClade (ver. 3.04; Maddison and Maddison 1992). Subsequently a semistrict (Bremer 1990) consensus tree was calculated from the suite of equally parsimoniuos trees that were usually found.

Finally, the different tree topologies were compared and all data sets were combined into a "total evidence analysis".

Alcohol dehydrogenase 1 (*Adh*1)

Primers were designed to amplify a segment of *Adh*1, spanning exon 5 to exon 8. The primers were constructed from the *Adh*1 sequences published by Trick et al. (1988). Care was taken to secure that there was a minimal match in the 3'-end with *Adh*2 and *Adh*3. The segment chosen covers approx. 550 bp. To increase the probability that only *Adh*1 was captured by the primers the double-strand amplification was done by running two successive double-stranded amplifications, one catching the whole segment and one using this product as template utilizing internal, overlapping primers in exon 7.

The sequences were aligned using MALIGN (ver. 1.85; Wheeler and Gladstein 1993), with a gap cost of 3 and a transition-transversion bias of 1:2. The transition-transversion bias was determined empirically from the sequences. The gaps were all in the introns. The settings were chosen following several different alignments using different parameters.

The *Adh*1 sequences were partitioned into four different subsets and subjected to phylogenetic analysis: The whole sequence, exons only, and introns only (with gaps coded as informative or gaps excluded, respectively).

The α subunit of RNA polymerase (*rpo*A)

Primers were designed to amplify the entire *rpo*A gene and the intergenic regions between *rpo*A and *rps*11 and *pet*D, respectively (approx. 1360bp). They were constructed from the published sequences from rice, wheat, and maize (Hiratsuka et al. 1989, Hird et al. 1989, Ruf and Kössel 1988). The sequences could easily be aligned by eye.

The grass-specific insert in the β subunit of RNA polymerase (*rpo*C2)

Primers used were designed by Cummings et al. (1994). Only a 250 bp fragment was obtained, which could easily be aligned by eye.

The large subunit of rubisco (*rbc*L)

Primers designed by Dr. G. Zurawski (DNAX Research Institute, Palo Alto, California), but slightly reduced in length from the 5'-end, were used for the ampli-fications. Additional primers were produced from the *Hordeum*-sequence and from *Zea mays* L. (Zurawski et al. 1984). Attempts were made to sequence the whole gene, but in some cases only smaller fragments of the gene were obtained.

As deletions are very rare in *rbc*L, alignment was done by eye. The weighting function of Albert et al. (1993) was used in the phylogenetic reconstructions.

RESULTS

Morphology

The morphological data set (Table 2) resulted in 32 equally parsimonious trees (length = 29, c.i. = 0.66, and r.i. = 0.69). The semistrict consensus tree is shown in Fig. 1A; in the strict consensus tree the clade including *P. lanuginosa* (Trin.) Nevski, *P. kronenburgii*, *P. juncea*, and *P.*

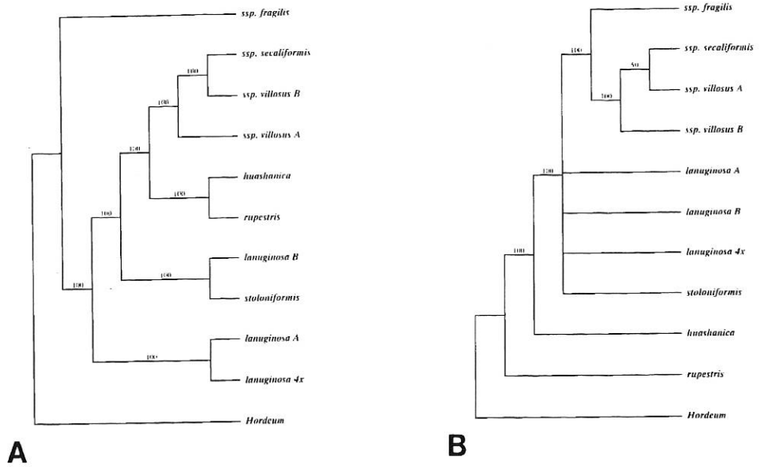

Fig. 2. Total evidence trees for *Psathyrostachys* based on A. All data and B. cpDNA data plus morphology. For abb. see fig 1.

stoloniformis C. Baden collapses into an unresolved bush. That the three subspecies of *P. fragilis* (Boiss.) Nevski constitute a monophyletic group is one of the possible resolutions of the polychotomy also involving *P. caduca* (Boiss.) Melderis.

Sequences

Alcohol dehydrogenase 1 (*Adh*1)

Even very extensive manipulation with the *Adh*1 sequences results in more or less the same tree topologies. If the whole sequence is subjected to analysis (transition-transversion bias = 1:2, gap penalty = 1) the outcome is 6 trees. If gaps are considered informative (see below) there are 80 informative sites, if not, only 52. The semistrict consensus tree is shown in Fig. 1B. The most remarkable features of the tree are that in no resolutions of the consensus tree do the three subspecies of *P. fragilis* nor the three accessions of *P. lanuginosa* constitute a clade, and that *P. fragilis* ssp. *fragilis* is the sister group of the remaining *Psathyrostachys* species.

Though *P. lanuginosa* does not form a clade, the two cytotypes of P. lanuginosa (2x, 4x) are included in the same clade. If the exons alone are run (transition- trans-version bias = 1:2, 234 equally parsimonious trees) parts of the resolution of the phylogeny disappear but among the possible resolutions are the one shown in Fig. 1B. This also applies if the introns are run alone with gaps considered a fifth state (9 equally parsimonious trees).

If the introns are run with the gaps considered uninformative exactly the same tree topology as the one shown in Fig. 1B is found (18 equally parsimonious trees).

The α subunit of RNA polymerase (*rpo*A)

The *rpo*A sequences only had four informative sites, and led to 933 equally parsimonious trees. Their semistrict consensus tree is shown in Fig. 1C. The only notable features of this consensus tree are that the three subspecies of *P. fragilis* may be comprised in a monophyletic group and that the two accessions of *P. caduca* constitute a monophyletic group. Both these clades are embedded in a larger clade that includes all other species of *Psathyrostachys*, except *P. rupestris* (Alex.) Nevski, which is the sister group of them all.

The grass-specific insert in the β subunit of RNA polymerase (*rpo*C2)

There are only two informative sites in grass-specific insert in *rpo*C2 and both indicate that *P. rupestris* is the sister group of the remaining species of *Psathyrostachys*.

The large subunit of *rbc*L

The *rbc*L sequences have 18 informative sites and resulted in 126 equally parsi-monious trees. The semistrict consensus tree is rather well resolved (fig. 1D). Among the more curious features of the consensus tree are that, even though the three subspecies of *P. fragilis* constitute a clade, one of the accessions of ssp. *villosus* Baden is the sister group of the rest of the ssp. *villosus* accessions plus ssp. *secaliformis* Tzvel., and that the position of an accession of *P. lanuginosa* makes the species paraphyletic. However, in both instances the peculiar positions would disappear on the strict consensus tree.

DISCUSSION

One obvious weakness associated with the morphological reconstruction of the phylogeny is that several of the characters are not qualitative, but quantitative. Though the quantitative characters used show a very distinct bimodal distribution with no or almost no overlap between the states, it is an evident possibility that added sampling will close the gaps. Even though the distinction between these two qualities of data is not as clearcut as is frequently thought (Thiele 1994), we have only used the quantiative characters as a last resort (Chappill 1989). If the five quantitative characters (see Table 1) are removed from the matrix, the number of equally parsimonious trees decreases from 32 to 8 (length = 22, c.i.= 0.64, and r.i. = 0.67), but the three subspecies of *P. fragilis* become scattered on the cladograms as their only synapomorphy is large glume length. However, there is no congruence with the gene tree based on the *Adh*1 sequences (see Fig. 1B), which also resulted in a non-monophyletic *P. fragilis*.

Great care has been taken to secure that only *Adh*1 has been amplified. In cases where double sequences were obtained it was checked whether the sequences could have been derived from *Adh*2 or *Adh*3, but this seemed never to be the case. The possibility of gene polymorphism, which has been amply documented in *Pennisetum* Rich. ex Pers. (Gaut and Clegg 1993), has been neglected in the present study. Though this weakens the conclusions it seems highly unlikely that this could explain the peculiar positions of the three accessions of *P. lanuginosa* and the three subspecies of *P. fragilis* (see below).

The *rpo*C2 sequences do not include any extra coding regions, as have been reported for several grass genera (Cummings et al. 1994), and the information obtained from these sequences amounts to a few substitutions. The level high level of information in the *rbc*L sequences is unexpected. Though *rbc*L could not resolve the phylogeny of *Psathyrostachys* completely the semistrict (and strict) consensus tree is fairly well resolved. Compared with *rpo*A, which is a gene of comparable length (approx. 1000 and 1400 bp, respectively) the information content of rbcL is much higher.

Taxonomic congruence

The congruence between the different tree topologies is very small, and a strict consensus tree would amount to nothing but an unresolved bush. The complete collapse of the consensus tree when the five data sets are combined is partly caused be the nature of the consensus methods (Swofford 1991).

However, in all trees (Fig. 1A-D), except the tree based on Adh1, the three sub-species of *P. fragilis* either make a clade of their own or this possibility is among the possible resolutions of the polychotomies they are involved in. Both *rpo*A and *rpo*C2 place *P. rupestris* as the sister group to the rest of the *Psathyrostachys* species, whereas the sister group of all the other species are *P. caduca* plus *P. huashanica* Keng on the *rbc*L tree and *P. fragilis* ssp. *fragilis* on the *Adh*1 tree. On all the gene trees (incl. the *Adh*1 tree) it is a possibility that the tetraploid cytotype of *P. lanuginosa* is an autotetraploid.

The *Adh*1 gene tree deviates considerable from all the other trees. It is most surprising that the three ssp. of *P. fragilis* are distributed all over the tree (Fig. 1B) and the three accessions of *P. lanuginosa* not are in the same clade.

It is difficult to give an explanation of the structure of *Adh*1 gene tree. It seems rather unlikely that the taxa have been misidentified, but the morphological groups may not correspond to natural entities. Neither can the possibility that the primers do not capture the correct gene, but a pseudogene be ruled out. No less than five *Adh*1 loci have been mapped in *Hordeum vulgare* L. (Good et al. 1988, Kleinhofs and Kilian 1994) using a cDNA probe. It is at least difficult to believe that the structure of the *Adh*1 trees could be caused by polymorphism, e.g., that different haplotypes of the same species should group with different other species.

Total Evidence

Unfortunately there is not exact correspondence between the taxa (and accessions) that have been sequenced. In some cases amplification of one or the other gene was not successful. Hence, in order to combine the data sets it has been necessary to reduce them to their least common multiple. When sequences from more than one accession of the same species or subspecies were included the morphological characters of the species or subspecies were simply multiplied in the morphological matrix. In cases (e.g., *P. rupestris*) where more than one partial sequence of the same gene was available from different accessions, and the taxa were each other's sister groups, a consensus sequence was made by combining the two sequences. In all such instances no conflicts in base-composition were seen between the over-lapping parts of the sequences that were combined.

If all the data sets are combined the dominating effect of the highly variable Adh1 sequences is clearly seen and the single total evidence tree obtained suffers from the same inexplicable deficiencies as the *Adh*1 gene tree (fig 2A).

If the *Adh*1 sequences are removed and only the cpDNA encoded sequences plus morphology are run a much more easily explicable result is obtained (only a single tree was found); the three subspecies of *P. fragilis* constitute a clade and one possible resolution of the polychotomy including the *P. lanuginosa* accessions is that they form a clade, too.

As emphasized by Eernisse and Kluge (1993) and by Kluge and Wolf (1993) there are at least four reasons for preferring a total evidence approach: 1. Consensus of different cladograms can be positively misleading (Barrett et al. 1991, Swofford 1991), 2. The different data sets are weighted equally, though being of different sizes, 3. There is no basis for achieving a consensus of suites of equally parsimonious, fundamental cladograms, and 4. The partitioning of evidence into classes is artificial.

It cannot, however, be neglected that lack of congruence between gene trees derived from different genes (e.g., *Adh*1 and the cpDNA genes) and morphology attracts attention to the need of further explanation.

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The Principle of Recombination Gene Pools (RPG) and Introgression Gene Pools (ITG) in the Biosystematic Treatment of *Elymus* Species

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ABSTRACT

Individual genotypes of *Elymus sibiricus*, *E. jacutensis*, *E. macrourus*, *E. caninus*, *E. ircutensis* and *E. dahuricus* complexes were identified by endosperm protein electrophoretic patterns. Genotypes within these complexes were then crossed to evaluate their recombination compatibility C_r (genotypic level) and reproduction ability of hybrids A_r (phenotypic level). Analysis of hybrid fertility in the F_1, F_2, and segregation of morphological characters provide an understanding of Recombination Gene Pools (RGP) and Introgressive Gene Pools (IGP) in the genus *Elymus*. The identification and marking of RGP and IGP make it possible to understand better both biological and taxonomic species definitions. A species should be considered as originating from one or more RGPs being combined on the morphological similarity (overlapping) of individuals belonging to the same adjoining RGPs with introgression of genetic material possible from one RGP to another.

INTRODUCTION

As additional genotypes of *Elymus* are included in biosystematic studies, it becomes more difficult to recognize genotypes and species using diagnostic keys especially since the inheritance of most morphological characters is unknown.

In an attempt to identify gene pools in breeding programs of wheat and other cereal cultivars with their wild relatives, the concept of primary (GP-1), secondary (GP-2) and tertiary (GP-3) gene pools was suggested by Harlan and de Wet [1971]. This concept suggests the possibility of easy utilization of the different systematic groups as genetic resources in cereal breeding. At the First International Triticeae Symposium (Helsinborg, Sweden, 1991) a system of gene pools in barley, cereals and forage grasses of the tribe Triticeae was presented [Bothmer et al. 1992]. A more detailed investigation of the real recombinative and reproductive relationships within natural taxa is necessary for an improved biosystematic treatment that recognizes the potential of wild grasses as a genetic resource in breeding programs. The ambiguity and complexity of the reproductive ability within *Elymus* species has been demonstrated in papers concerning *E. glaucus* [Snyder 1950, Stebbins 1957]. This paper attempts to develop an understanding of gene pools within the genus *Elymus*.

MATERIAL AND METHODS

The following plant materials (Fig. 1 collection sites in Republics of the ex-USSR) were used for crossing after individual genotypes were identification by SDS-PAG-electrophoresis of endosperm protein (Fig. 2, 3). *E. sibiricus* L. from Asiatic part of Russia: ALT-84-01 (Altai, Fig. 2-a, 3-a), KAZ-79-31 (Kazakhstan, Fig. 2-d), Bur-90-12 (Buryatia, Fig. 2-h), ZEJ-88-06 (Amurski region, Fig. 2-i), JAC-83-26 (Yacutia-Sakha, Fig. 2-j), KAM-92-19 (Kamchatka), SAK-91-13 (Sakhalin, Fig. 2-k), VLA-84-51 (Vladivostok, Fig. 2-l), SIC-90-62 (China, Sichuan, Fig. 2-m), short awn forms SH-252 and SH-236; accessions received form the Department of Crop Genetics and Breeding, Svalov, Sweden (the Swedish University of Agricultural Sciences): H10238 (Tajikistan, Fig. 2-c), H8800, H7570, and H7528 (China, Xinjiang, Fig. 2-e, 2-f, 2-g); *E. jacutensis* (Drob.) Tzvel. GAC-89-58 (North Altai mountain, Fig. 3-d), JAC-89-01 (Yacutia-Sakha, Fig. 3-f), AMU-90-01 (Amurski region, Fig 3-g); *E. macrourus* (Turcz.) Tzvel. GAL-89-59 (Central Altai mountain, Fig. 3-c), JAC-89-22 (Yacutia-Sakha, Fig. 3-e), JAC-83-13 and JAD-87-05 (both Yacutia-Sakha); *E. caninus* (L.) L.: ELC-83-06 and ACD-88-05 (Novosibirsk region), GAT-92-10 (Altai),

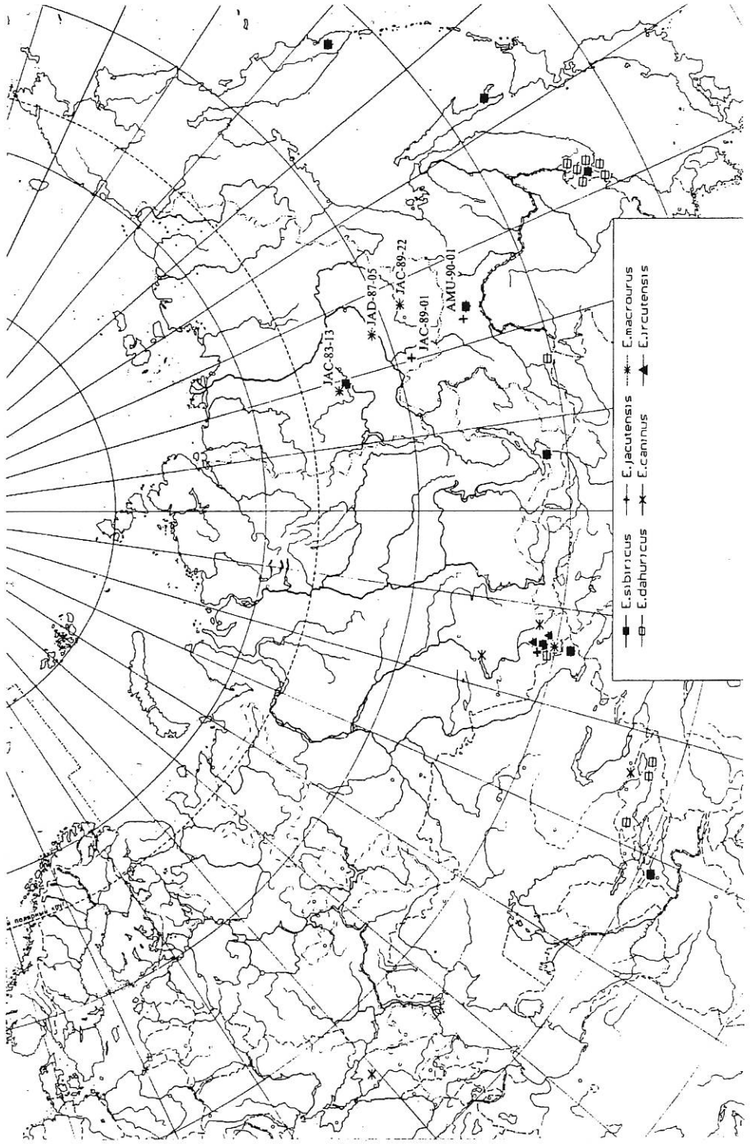


Fig.1. Collection sites of *Elymus* species in Republics of the ex-USSR. Numbers are given for genotypes of *E. jacutensis* and *E. macrourus*.

KAS-85-05 (North Tien-Shan, Kazakhstan); accessions received from the Forage and Range Research Lab. (USDA-ARS, Logan, Utah, USA): PI-172364 (Turkey), PI-251417 (Jugoslavia), PI-314205 (USSR, Krasnodar region). *E. ircutensis* Pesch. is morpholigicaly similar to *E. caninus*: GAC-89-21 and GAC-89-23 (Altai). Genotypes with the same letters have some morphological differences but are treated in the same species. *Elymus dahuricus* complex includes *E. exselsus* Turcz. ex Griseb., *E. woroschilowii* Probat., and *E. tangutorum* (Nevski) Hand.-Mazz. with the following distribution: (1) from Altai mountain (2 genotypes), Chita region (1 genotype), Primorski region (6 genotypes), West Tien-Shan (Kirghizstan, 1 genotype), Central Tien-Shan (2 genotypes); (2) accessions received from DCGB (the Swedish Univ. Agr. Sciences): H8113, H8363 (Tibet, China), H8068, H8107 (Sichuan, China). The latter is awnless phenotypicly.

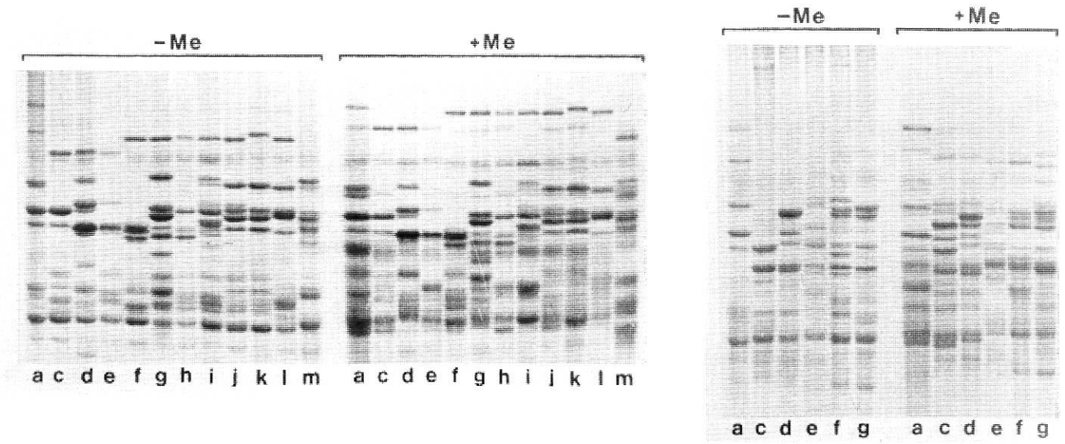
The crossing procedure followed that previously

described by Lu and Bothmer [1990]. Reproductive ability of hybrids between individuals belonging to same or different taxonomic species was studied in two or three generations. Plants of the F_1 and F_2 hybrids were grown in the greenhouse at the Swedish Univ. Agr. Sciences, Svalov and on field plots near Novosibirsk, Russia. Under these conditions back-crossing was excluded, but cross-pollination with other species was not excluded. Some hybrids were isolated from foreign pollen. Identification of hybrid was determined by morphological features and/or electrophoretic banding patterns. Three grains of F_1 plants were analysed by SDS-PAG electrophoresis [Agafonov and Agafonova 1992, Agafonova 1995, this volume] to observe genetic segregation and endosperm protein bands and to make sure that they were true hybrids.

RESULTS AND DISCUSSIONS

It was found that certain genotypes within species of *Elymus* varied in their ability to form fertile hybrids, thus leading to the terms "Recombination Gene Pool" (RGP) and "Introgression Gene Pool" (IGP). The recombination compatability of a pair of genotypes (Integrated value C_r) reflects general homology of parental genomes and is realized in phenotypes through the mediation of hybrid fertility as the reproduction ability A_r. The pollen formation process is sensitive to only minor abberrations in meiosis, whereas the female gametophyte can withstand moderate meiotic irregularities and still have viable gametes. Most hybrids are typically sterile with reference to pollen viability. This pecularity is the basis for the proposed approximate scale of recombinative (genotypic level) and reproductive (phenotypic level) relationships that illustrates the principle of RGP and IGP in *Elymus* (Fig. 4). Normal fertility estimates (value of seed set) should be determined for each species and genotype because of the variation being observed within species. A value A_r is less than would be expected theoretically under optimal environmental conditions, suggesting the need for a coefficient of realization k or = 1.

A-level is defined as the Recombination Gene Pool (RGP) and corresponds to the entire gene pool where



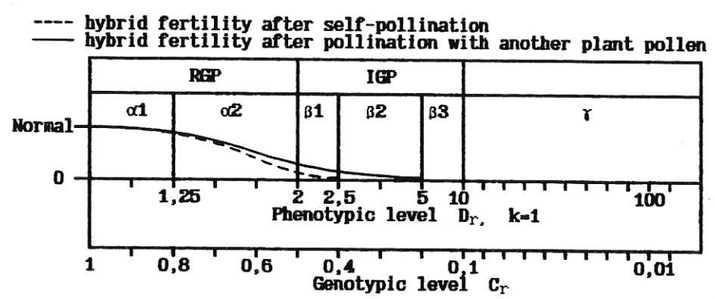


Fig.2. SDS-PAGE patterns of endosperm protein in *E. sibiricus* genotypes have been crossed. Electrophoretic variants -Me and +Me.
Fig.3. SDS-PAGE patterns of endosperm protein in *E. jacutensis* and *E. macrourus* genotypes have been crossed. Electrophoretic variant -Me and +Me).
Fig.4. Illustration of RGP and IGP principle in genus *Elymus* concerning other *Triticeae* genotypes.
C_r - Recombination compatibility of a pair of genotypes
$A_r = kC_r$ - Reproduction ability of a hybrid in the F_1

Table 1: Seed fertility in hybrids of *E. sibiricus* in the F_1 and F_2.

No	Cross combination Determination*		Highest value of seed set % F_1	Seed set % F_2 Pl. 1	Seed set % F_2 Pl. 2	Level of C_r
1.	ALT-01 X SAK-13	E	75.5			α1
2.	ALT-01 X H 7570	E	19.9			α2
3.	KAZ-31 X ALT-01	E	32.1			α2
4.	KAZ-31 X JAC-26	E	27.4			α2
5.	reciprocal	E	17.7	38.3	25.8	α2
6.	H 10238 X VLA-51	E	6.1	24.6	19.9	α2
7.	reciprocal	E	18.8			α2
8.	H 10238 X SH-236	M	22.2			α2
9.	reciprocal	M	28.6	17.6	32.1	α2
10.	H 7528 X H 8800	E	75.6			α1
11.	H 8800 X SAK-13	E	25.5	44.3	30.9	α2
12.	JAC-26 X SAK-13	E	73.5			α1
13.	JAC-26 X ALT-01	E	47.4	56.8	48.0	α1
14.	JAC-26 X H 7570	E	34.6			α2
15.	SH-252 X ALT-01	M	49.4			α1
16.	SH-252 X KAZ-31	M	33.0	36.5	44.2	α2
17.	SH-252 X ZEJ-06	M	78.3	68.7	73.4	α1
18.	SH-252 X JAC-26	M	70.4	76.1	78.4	α1
19.	SH-252 X SAK-13	M	75.5	64.8	71.2	α1
20.	SH-236 X ALT-01	M	27.8	54.7	49.8	α2
21.	SH-236 X JAN-19	M	52.5			α1
22.	SAK-13 X H 7570	E	53.0	55.5	47.0	α1
23.	SAK-13 X BUR-12	E	49.5			α1
24.	VLA-51 X 252-6	M	38.2	35.7	38.0	α2
25.	VLA-51 X ZEJ-6	E	67.7			α1
26.	VLA-51 X SAK-13	E	57.6			α1
27.	VLA-51 X H 7570	E	31.1	24.7	40.2	α2
28.	H 7362 X JAC-26	M	0.0			?
29.	H 7362 X SH-236	M	0.0			?
30.	H 7362 X H 10238	M	0.0			?

* Determination of crossing: E = electrophoretic, M = morphological

genetic recombination can take place. The border between 1 (free recombination) and A2 (limited recombination) in most species is not established. Within A1-level, meiosis is normal. Segregation of Mendelian characters in the F_1 is normal. The major difference in A2-level is the potential for F_2 segregation.

B-level refers to the Introgression Gene Pool (IGP). Within B1-level, fertility of self-pollinated hybrids is more than zero, with plants in the F_2 becoming completely sterile. Sexual reproduction is possible within B2-level; however, multiple back-crosses will be necessary for genetic introgression to take place. Within B3-level, hybrids are completely sterile despite the pollination type. Fertility can be restored by doubling the hybrids; however, this often creates a whole new RGP with additional problems.

C-level includes crosses that have resulted in hybrids that exist in the vegetative state only. Return to sexual reproduction is impossible at this level. In the genotypic hybrids within *E. sibiricus* their fertility and their approximate levels of C_r are shown in Table 1.

The data suggests that *E. sibiricus* complex represents an indivisible RGP throughout the Asiatic area. Genotype SIC-90-62 from Sichuan, China differs from other accessions by endosperm protein pattern (Fig. 2). It has a chromosome number 2n=42, and should belong to another RGP composed of *E. nutans* and SHY-genome taxa. However, the process of gamete introgression as it relates to speciation is not yet well defined making it

Table 3: **Highest values of seed fertility in some hybrids of *E. jacutensis* and *E. macrourus* in generations F_1-F_3.**

No	Cross combination	Plant No	Seed set % F_1	N*	Seed set % F_2	N*	Seed set % F_3
5.	JAC-01# X JAC-13*	3	6.1	8	53.6	-	-
6.	JAC-01# X JAC-22*	2	7.2	14	42.2	3	61.5
7.	reciprocal	1	3.8	3	46.9	-	-
12.	JAC-22* X GAC-58#	1	2.7	5	6.5	2	13.9
14.	JAC-22* X GAL-59*	3	0.22	2	15.4	2	21.7
15.	reciprocal	3	2.15	2	13.2	-	-

N* number of plants analyzed

Table 2: **Seed fertility in hybrids of *E. jacutensis* and E. *macrourus* in the F1**

No	Cross combination	Seed set % Greenhouse 1st veget. Pl. 1	Pl. 2	Field 2nd veget. Pl. 1	Pl. 2	Pl. 3	Highest value of seed set	Level of C_r
1.	AMU-01# X JAC-01#	21.1	18.7	24.4	42.7	29.5	42.7	α1
2.	AMU-01# X JAC-22*	44.2	42.1	25.9	28.3	44.6	44.6	α1
3.	AMU-01# X GAC-58#	0.2	0.0	0.4	0.0	0.0	0.4	β1?
4.	AMU-01# X GAL-59*	0.0	-	0.0	0.3	0.0	0.3	β1?
5.	JAC-01# X JAC-13*	2.6	-	4.0	-	6.1	6.1	α2
6.	JAC-01# X JAC-22*	1.9	3.6	5.9	7.2	4.7	7.2	α2
7.	reciprocal	1.8	0.6	3.8	3.3	0.0	3.8	α2
8.	JAC-01# X GAC-58#	0.0	0.0	0.0	0.0	0.0	0.0	β1?
9.	reciprocal	-	-	-	-	0.3	0.3	β1?
10.	JAC-01# X GAL-59*	0.0	0.0	0.0	0.0	0.0	0.0	β1?
11.	JAD-05* X JAC-22*	-	-	-	-	10.1	10.1	α2
12.	JAC-22* X GAC-58#	0.7	1.0	2.7	0.87	-	2.7	α2
13.	reciprocal	-	-	-	-	0.6	0.6	α2
14.	JAC-22* X GAL-59*	-	-	-	-	0.22	0.22	α2
15.	reciprocal	0.45	0.5	1.2	0.7	2.15	2.15	α2
16.	GAC-58# X GAL-59*	37.3	29.5	33.4	27.6	17.8	37.3	α1?
17.	reciprocal	-	-	-	-	23.9	23.9	α1?

*# = E. jacutensis; * = E. macrourus*

difficult to assign taxonomic rank based on IGP. A segregation of morphological characters in the F_2 after self-pollination can be used as indicators of genetic recombination in hybrids as well as segregation patterns of endosperm protein bands on electrophoretic gels. For instance, a character "long awns" in *E. sibiricus* is controled by two loci with independent inheritance. The phenotypic ratio of awned to awnless in the F_2 population is 15:1. In an attempt to transfer the awnless character from genotypes SH-252 and SH-236 to other genotypes was accompanied by a segregation of a number of Mendelian and polygenic morphological characters.

Studied genotypes of *E. jacutensis* (#) and *E. macrourus* (*) demonstrated reproductive relationships which do not correspond to their taxonomic rank (Table 2). Morphological difference between these species (length of awns) is controlled by a single locus, as demonstrated by the 1:2:1 phenotypic ratio (34 awned: 73 short awned: 36 awnless, hybrid AMU-01# x Jac-22* in the F_2). The characters "glaucous stem" and "hairy stem" also appear to be controlled by a single locus regardless of their cytoplasm. All above-mentioned characters exhibit independent inheritance patterns from each other. Genotype JAC-89-22* is more or less compatible with all studied genotypes of *E.jacutensis-E. macrourus* complex. There is some variation in hybrid seed set within the same hybrid combinations. Seed set can be improved in most hybrids when advanced from the first generation to the second. Because of this, reproduction ability of a combination of hybrids should be evaluated according to the highest seed set, which is closer to a theoretically possible coefficient of realization k = 1.

Based on F_1-F_2 hybrid fertility all genotypes of *E. jacutensis* and *E. macrourus* studied should be included in the same RGP (Table 3). Recombinative and reproductive relationships of genotypes correspond to the A-level. This RGP consists of two geographical subunits or "recombination nuclei" of a sort. One of them is distributed in West-Southern Siberia and another in the North and East regions of Siberia. Electrophoretic patterns of endosperm protein support the existence of two geographical subunits (Fig. 3).

Little is known about genotypes from the Russian Far East; however, they are known to occur there (Susud. Rast. Sov. Dal. Vostoka, 1985). It is very likely that *E. zejensis* Probat. also belongs to RGP of *E. jucutensis-E. macrourus* complex.

Data regarding seed fertility of infraspecific hybrids in *E. caninus* (17 combinations) and *E. dahuricus* complex (28 combinations) support the existence of seperate RGP and IGP.

E. caninus represents a widespread RGP which includes genotypes from Siberia, Kazakhstan and the South-European part of Russia as a major recombination nucleus (Table 4). The genotypes from Turkey and Yugoslavia form another nucleus. Recombination between the two groups is at the A2-level. The genotype of *E. ircutensis*, GAC-89-21, has probably diverged from the main recombination nucleus of *E. caninus* recently. This accession differs from *E. caninus* by having hairy lemmas and longer glumes with awns up to 5mm in length. An additional accession, GAC-89-23, is even more isolated and appears to be in its own RGP based on genetic introgression with other accessions of *E. caninus*.

All studied genotypes of *E. dahuricus* represent a single RGP, suggesting that regardless of the geographical region, collections will reflect the basic genotypes wordwide. Within the RGP of the *E. dahuricus* complex, the most distant genotypes were *E. excellsus* from the Far East Russia and *E. dahuricus* from Sichuan China, based on hybrid fertility in the F_1 and F_2 hybrids. A slight decrease in hybrid seed fertility was observed in hybrids along a geographical transect through Siberia, Tien Shan mountains of the USSR, Tibet, and Sichuan Provences of China.

Genotype ARS-87-06, *E. dahuricus* from Far East Russia, had a low recombination compatibility with any other genotypes within this complex. Its hybrids had very

Table 4: **Highest values of seed fertility in hybrids of *E. caninus* and *E. ircutensis* in the F_1 and F_2**

No	Cross combination	N*	Seed set % F_1	N*	Seed set % F_2
1.	ACD-05 X ELC-06	2	39.2	5	74.2
2.	GAT-10 X ELC-06	3	57.4	3	86.4
3.	KAS-05 X ELC-06	3	44.1	3	94.1
4.	KAS-05 X GAT-10	3	43.0	3	93.6
5.	PI-205(K) X ELC-06	3	36.1	3	79.7
6.	PI-205(K) X GAT-10	3	67.6	3	80.5
7.	PI-205(K) X KAS-05	3	59.0	3	93.5
8.	PI-205(K) X PI-364(T)	3	7.2	3	5.7
9.	ELC-06 X PI-364(T)	2	8.0	37	22.0
10.	ELC-06 X PI-417(J)	3	5.9	3	14.9
11.	GAT-10 X PI-364(T)	2	8.6	12	18.8
12.	KAS-05 X PI-364(T)	3	3.4	4	2.8
13.	PI-364(T) X PI-417(J)	2	77.4	1	85.0
14.	GAC-21 X ELC-06	2	8.5	16	13.3
15.	ELC-06 X GAC-23	2	0.6	4	0.0
16.	GAC-21 X GAC-23	2	4.9	2	0.0
17.	reciprocal	1	4.2	1	0.1

N* number of plants analyzed

little seed set in the F_1 and F_2 generations in most combinations. It is not improbable that the genotype has some substantial chromosomal modification being fixed in the homozygous state. However, until further data is available, this genotype should be assigned to the larger IGP with other genotypes of the *E. dahuricus* complex.

The character "long awns" in *E. dahuricus* is controled by two loci as well as in *E. sibiricus*. The diognostic character of *E. woroschiloii* which has glaucous stem and leaves, is controled by a single dominant gene.

The use of RGP and IGP provides an additional tool to better understand phytogenetic relationships in the genus *Elymus*. A taxonomical species is an artificial category combining one or more RGPs on the basis of morphological resemblance of the different individuals. Accordingly, introgression is a sexual transfer of genetic material from one RGP to another. From this, it follows that the term "biological species" in *Elymus* should be substituted for the non-taxonomic category "recombination gene pool". It is proposed then, that it would be incorrect to divide one RGP into two or more species because of their high degree of genome homology indicating genotypes to be closely related phylogeneticaly. Furthermore, the potential of genotypes within RGP for crossing and recombination of genetic material may result in intermediate phenotypes and a segregation of characters in subsequent generations.

Electrophoretic identification (or any precision one) of parental genotypes and hybrids is an essential part of the RGP-IGP principle. Combined characteristics of studied plant accessions would make up the basis for a prediction of recombination compatibility of genotypes that have not been crossed.

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