Proceedings of the 2nd International Triticeae Symposium

TRITICEAE

2nd INTERNATIONAL SYMPOSIUM 1994

June 20-24, 1994
LOGAN, UTAH, USA

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 colleagues 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

Richard R-C. Wang (Co-Chair)  Kevin B. Jansen (Co-Chair)
Mary E. Barkworth  Catherine T. Helio
Janell H. Larsen  Carolyn C. Lemon

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 1 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 Monogenic 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” (A35) - 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. Balsauf


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 Dihybrid Progenitors of Alloetriploid Elymus species" (A49) - V. Jaaska

4:15 p.m. - 4:30 p.m. "The Phylogeny of Poastrachys - Are we able to see the wood for the trees?" (A1) - O. Seberg, G. Peterson, and C. Badon

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. Curti

5:15 p.m. - 5:30 p.m. "Chemotaxonomy of Triticeae Grasses: Characterization of Truebreeding Lines and Hybrid Crosses" (A57) - J. H. Bennett, A. V. Agafonov, O. V. Agafonova 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. Baden*, N. S. Baden, J. Jang, 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. Coenoptaxae and Sect. Elytis (Poaceae: Triticeae)" (A20) - M. Assadi

9:45 a.m. - 10:00 a.m. "The Mechanism of the Orignation of Autotetraploid 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” (AS) - 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. Muratmatstu

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.  “Geographic 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-S, Glutin, a- and ß-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 Dasyphyllum villifolium” (A26) - C. De Pace and C. O. Quatset

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.  “o-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-yan 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 Dasyphyllum” (A59) - O. 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. Donoughue, 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 IV. High Molecular Weight Glucinin Subunit Variation" (A11) - A. A. Jaradat* and M. M. Aljouni

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 Glucinin Subunit Variation" (A11) - A. A. Jaradat* and M. M. Aljouni

10:45 a.m. - 11:00 a.m. "Physical Mapping of Micronutritional Genes in Wheat-Rye Translocations" (A10) - R. G. Kyratsi*, M. Röder and V. Rönhild

11:00 a.m. - 11:15 a.m. "Progress in Polyplod Production Techniques of Haploidy Wheat through Wide Crosses" (A19) - M. N. Inagaki* and A. Majeed-Kazi

11:15 a.m. - 11:30 a.m. "Characterization of Wheat-Aegilops recombinant lines by in situ hybridization" (A41) - A. Cashtimo* and J. S. Heslop-Harrison


11:45 a.m. - 12:00 p.m. "Variability of Exotic Barley Germplasm and its Effects on Agronomic Traits in Complex Crossers" (A43) - M. Vestalainen

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 Cytotripsms 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. Pomortsve*, B. A. kalnshkin, 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" (A65) - 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) - Kassara Amantawat-Jonsson* and R. Koebner

4:15 p.m. - 4:30 p.m. "Variations in Structure Granule-Bound Starch Synthase (Wx protein) in Diploid, Polyploid Weats and Aegilops" (A29) - N. Fujita*, K. Takaoka, M. Uematsu, A. Wadino, S. Okabe and T. Taira

4:30 p.m. - 4:45 p.m. "Pathological Relationship Between Plant Parasitic Nematodes and Rangeland Grasses" (A55) - G. D. Griffin

6:00 p.m. - 9:00 p.m. Symposium Dinner (Walnut Room, USU Student Union)

Thursday, June 23

8:30 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. Altonbach*, Olin D. Anderson, and David E. Matthews
LIST OF CONTRIBUTORS

Dr. O. Seberg  
Botanical Laboratory  
Copenhagen University  
Gothsbergade 140  /Dk-1123 Copenhagen K  
Denmark

Dr. G. Petersen  
Botanical Laboratory  
Copenhagen University  
Gothsbergade 140  /Dk-1123 Copenhagen K  
Denmark

Dr. I. Hostain  
Dept. of Plant Pathology  
Bangladesh Agricultural University  
Mymentsingh  
Bangladesh

Dr. A. Mujeeb-kazi  
Cimmyt, Londres 40  
Apdo. Postal 6-641  
Delg. Cuauhtemoc 06600  
Mexico

Dr. B. Salomon  
Dept. of Crop Genetics & Breeding  
Swedish Univ. of Agricultural Sciences  
S-26831 Svalöv  
Sweden

S. Svitashev  
Dept. of Crop Genetics & Breeding  
Swedish Univ. of Agricultural Sciences  
S-26831 Svalöv  
Sweden

Dr. E. D. Bataeva  
Department of Plant Pathology  
Throckmorton Hall  
Kansas State University  
Manhattan, KS 66506  
USA

A. A. Jaradat  
International Plant Genetic Resources Institute, Wana Group  
Syria & University of Science & Technology  
Irbid, Jordan

M. A. Farsi  
Department of Plant Science  
University of Adelaide, Waite  
Glen Osmond, S.A. 5064  
Australia

Dr. R. G. Kyrast  
Institut Fur Genetik Und Kulturpflanzen-  
Forschung, Corrensstrasse 3  
0-4325 Gatersleben  
Germany

M. C. Luo  
Sichuan Agricultural University  
Xing-fu-xiang  
Dujiangyan City 611830  
Sichuan  
P.R. China

Prof. C. Yin  
C/o Dr. B. R. Baum  
Biosystematics Research Centre  
Agriculture Canada  
Wm. Saunders Bldg.  
C.e.f. Ottawa, Ontario K1A 0C6  
Canada

Prof. J. L. Yang  
C/o Dr. B. R. Baum  
Biosystematics Research Centre  
Agriculture Canada  
Wm. Saunders Bldg.  
C.e.f. Ottawa, Ontario K1A 0C6  
Canada

G. L. Sun  
Sichuan Agricultural University  
Xing-fu-xiang  
Dujiangyan City 611830  
Sichuan  
P.R. China

E. A. Salina  
Inst. of Cytology And Genetics  
Sb of the Russia Academy of Sciences  
Novosibirsk 630090  
Russia
L. V. Obukhova
Inst. of Cytology and Genetics
Sb of the Russia Academy of Sciences
Novosibirsk 630090
Russia

W. G. Ayad
IPGR,
Via Della Sette Chiese 142,
00145 Rome,
Italy

Dr. Masanori Inagaki
Gimnyt, Wheat Program
Lisboa 27
Col. Juarez Apol. Postal 6-641
Mexico, D.F.,
Mexico

M. Assadi
Department of Systematic Botany
O'Valgatan 18-20
S-223 61 Lund
Sweden

Dr. V. Schubert
Institute of Plant Breeding and Seed Production,
Martin-Luther-University Halle-Wittenberg,
D-06184 Hohenheim,
Germany

Dr. R. C.-W. Wang
Forage & Range Research Laboratory
USDA-ARS
Utah State University
Logan UT 84322-6300
USA

Dr. Xunyou Zhang
Forage & Range Research Laboratory
USDA-ARS
Utah State University
Logan UT 84322-6300
USA

L. Morrison
Department of Botany & Plant Pathology,
Oregon State University,
Corvallis, Or 97331,
USA

Dr. C. De Pace
Dept. of Agrobiology & Agrochemistry
University of Tuscia
Via S. Camillo De Lellis 01100 Viterbo
Italy

Dr. P.E. McGuire
Genetic Resources Conservation
University of California
Davis CA 95616-8602
USA

Naoko Fujita
Lab. Genetics and Plant Breeding
University of Osaka Prefecture
Sakai, Osaka 593
Japan

Mr. A. V. Agafonov
Central Siberian Botanical Garden
Sb of the Russia Academy of Sciences
101 SolotooOlimskaya St.
Novosibirsk 90, 630090
Russia

Dr. O. V. Agafonova
Central Siberian Botanical Garden
Sb of the Russia Academy of Sciences
101 SolotooOlimskaya St.
Novosibirsk 90, 630090
Russia

L. V. Obukhova
Central Siberian Botanical Garden
Sb of the Russia Academy of Sciences
101 SolotooOlimskaya St.
Novosibirsk 90, 630090
Russia

Dr. J. Dubovsky
Dept. of Agronomy and Range Science
University of California
Davis, CA
USA

Dr. Vijay K. Khanna
G. B. Pant University of Agri. and Technology
Dept. of Plant Breeding
India

Dr. G. D. Griffin
Forage & Range Research Laboratory
USDA-ARS
Utah State University
Logan UT 84322-6300
USA

Ms. S. Frederiksen
Botanical Laboratory
Copenhagen University
Gothorategade 140
DK-1123 Copenhagen K
Denmark

Mr. Ying Zhoumeing
Department of Grassland Science
Xinjiang August 1st Agricultural College
42 Nanchang Road
Urumqi, 830002, Xinjiang
People's Republic of China

Dr. A. B. Darnain
ICARDA
Gru
P. O. Box 5466
Aleppo
Syria

Mr. Li Bao Jun
Xinjiang Grassland Research Institute
No. 23 South Xinhu
Urumqi, 830001, Xinjiang
People's Republic of China

A. Castitho
John Innes Inst. of Plant Science Research
Cambridge Laboratory
Colney Lane, Norwich NR4 7UH
England

Dr. R.A. Pickering
Nz Inst. for Crop and Food Research Ltd.
Lincoln, Private Bag 4704
Christchurch
New Zealand

Marja Vetelainen
Dept. of Plant Breeding Research
Swedish Univ. of Agricultural Science
S-268 31 Svalo
Sweden

Dr. Nobuyoshi Watanabe
Faculty of Agriculture
Gifu University
1-1 Yanagido, Gifu 501-11
Japan

Dr. B.-R. Lu
Lab. Syst. & Evol. Botany
Institute of Botany
Chinese Academy of Science
Xiangshan, Beijing 100093,
China

Q. X. Sun
Department of Agronomy
Beijing Agricultural University
Beijing 100094
PR China

Dr. L. A. Parsehina
Inst. of Cytology & Genetics
Sb of the Russia Academy of Science
630090 Novosibirsk 90
Russia

Prof. V. K. Shunmy
Inst. of Cytology & Genetics
Sb of Russia Academy of Science
630090 Novosibirsk
Russia

Dr. V. Jassou
Institute of Zoology and Botany
Academy of Sciences of Estonia
21 Veneemuse Str., Ee 2400 Tartu
Estonia

T. L. Oidvostoe
Vavilov Institute of General Genetics
Gubkin St. 3,
117809 Moscow B-333
Russia

Maria P. Ladogina
Vavilov Institute of General Genetics
Gubkin St. 3,
117809 Moscow B-333
Russia

Andrey A. Pomertsev
Vavilov Institute of General Genetics
Gubkin St. 3,
117809 Moscow B-333
Russia

Dr. A. V. Varsinilin
Inst. of Cytology and Genetics
Sb of the Russia Academy of Science
Novosibirsk 630090
Russia
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TRITICEAE: a tribe for food, feed and fun

Roland von Bothmer and Björn Salomon
Department of Plant Breeding Research
Swedish University of Agricultural Sciences
S-265 31 Svalöv, Sweden

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, Dasyypyrus and Secale that include annual as well as perennial species. Of the perennial genera, some are very large such as Elymus with ca. 150 species down to the monotypic genera Hordeolus, Peridictyon, and Pascoyris (Fig. 1). Apart from the Triticeae/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.
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 Triticaceae 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 Triticaceae, occurs in SW Asia (Fig. 5). For the other Triticaceae 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 maternal, introgressional forms and wild taxa of the primary gene pools. 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 Asia, 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 Estonia need to be further explored. Some groups are underrepresented in the collections, like species of Pseudoregeria, Zaynus, and many of the annual genera.

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

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

Figure 5 Diversity centers for the Triticaceae, 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. Besides 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 of 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 in most wild species. Some species can survive in room temperature for decades, while others may lose their viability despite 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 automation 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. There will be no 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 Aegeus 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 gene pool 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 Knüller 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 "Triticace 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 it will be justified to keep the access. There is a gap between the collection and preservation on one side and utilization on the other. The major problem, especially in 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 of generic limits.

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: Dasyphyllum, Eremopyrum, Henardia, Anabasagyna, Heterantherium, and Tribadia (Frederiksen 1986, 1990, 1991, 1993). The genera Aegeus and Triticum are at present under revision, where Aegeus and some of the Triticeae species are ready for publication (Van Slageren, ICARDA, personal comm.). Among the perennials, which have been treated recently, are Psathochrocolis (Baden 1991), Hordeum (Bothner et al. 1991), Leymus in North America (Barkworth & Abbas 1984), and some groups in Elymus (Salomon 1994, Lu 1995) and Thinopyrum (Javie 1992, Assadi 1994). Cladistic and numerical approaches based on morphological characters have also been carried out in the tribe (cf. Baum 1982, Kellogg 1989, (Frodansan & Seberg 1991).

There are still several groups which are poorly known, for example Leymus and Pseudorealum 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 Triticaceae (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 Phalacrostachys, 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 & Jansen 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 Hordeum, Pseudoroegneria, Thinopyrum, Pascopyrum and Leymus. Several of the studied genomes have no obvious connections to others, e.g. the G genome of Festucaea.

The problems included in genome analysis should not be ignored. This encounters especially the genetic pairing regulation like the Ph genome 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 Triticaceae.

**Molecular Methods**

Much effort has been invested in development of new techniques for studies of phylogenetic relationships, which also have been applied in the Triticaceae. These methods include biochemical techniques like electrophoresis of isoenzymes and storage proteins (cf. Jörgensen 1986, Jasaal 1992). Molecular biology has added to the richness of new, powerful, and sophisticated techniques, like RFLPs, and RAPDs both of nuclear and organelar DNA (cf. Talbert et al. 1991, Doakley et al. 1992, Dvorak & Zhang 1992, Kellogg 1992, Molin 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

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

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

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) Grem. is distinct from the other diploid S-genome species. The tetraploid T. timopheevii (Zhuk.) Zhuk. is shown to be 96% identical showing close affinity between the S and the G genomes (Fig. 7).
In Hordeum several studies with different techniques have been applied, like a CQDNA study by Doebely 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 Svitsyov et al. (1994, Fig. 6).

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 other grasses. 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 (2+4) DNA probes from Hordeum and the hexaploid wheat in 16 species of the Triticeae (Fig. 9). They found a good correlation between the phylogenetic tree produced by the approach and by earlier investigations.

In this type of investigations the chosen species may not 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, Anafawast-Jönsson, K. et al. 1990). Halsey-Harrison 1991, Øgaard & Halsey-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 allozygous pairing.

All the new techniques have added immensely to the knowledge of relationships in the Triticeae. In the future we can get new results by crossbreeding. 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 or annuals 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 differentializations? (2) Several of the perennials have been poorly studied mainly due to lack of material. These include several species of Asiatlic 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 gene pool in pre-breeding programs. The core tribe constitutes a vast gene pool. Many species belong to the primary and secondary gene pools of bread and durum wheats, and due to the polyphyletic several species from the tertiary gene pool 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, Siegler et al. 1994). Some work has also been devoted to stress tolerance, mainly for salt and drought (cf. Nevo et al. 1993, Tasa et al. 1993, Dubcovsky et al. 1994).


Contrary to wheat, barley is a diploid organism which makes gene transfer more problematic. Only the progenitor of the crop, Hordeum vulgare sp. spontaneum, belongs to the primary gene pool. It has been studied particularly for resistance to BYDV, powdery mildew and rust (cf. Jans & Nevo 1991, Jaho & Fischbeck 1993). Sp. spontaneum material is at present included in at least three major pre-breeding programs (cf. Lehmann & Bothmer 1998).

Hordeum bulbosum L., which is the single species in the secondary gene pool 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 & Kashia 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, Izodskii 1992).

**New Crops**

The intergeneric hybrid between wheat and rye, triticate, is now at last established as an important cereal in some countries. It took about a century to develop triticate 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 tristetron, i.e. the intergeneric hybrid between Hordeum chilense Roem. & Schult. and Triticum, especially durum-wheat. The first crosses were made at PFI 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, Schwarzercher et al. 1989). Later more applied approaches were performed, for example, on resistance to rust, powdery mildew and rustmotes as well as on field trials (cf. Milan et al. 1988, Arzak et al. 1992, Rufoletes 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.

**Forage**

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 wheatsgrass (Thiopyrum intermedium (Host) Barkworth & D.R. Dewey), and Russian wildrye (Poastrachys jacea (Fisch.) Nav.) Much work is in progress concerning disease resistance, stress tolerance and yield potentials in these and other species (cf. Berdahl & Krupek (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, Estebane 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 utilisation

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 perennialas

Breedng:

- improved techniques 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

Petersen, G. & Seberg, O.  
Botanical Laboratory  
University of Copenhagen  
Gothiersgade 140  
DK-1123 Copenhagen K  
Denmark

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 in morphology and molecular biology?

INTRODUCTION

As a discipline genome analysis was formally founded and outlined by Khairu (1930) and has been applied extensively, not least within the Triticaceae, to studies of systematics and evolution ever since. More recently, Doevey (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 as a result of an organism, e.g., because of the position of the bract scales the female cone of a conifer is considered a condensed shoot and thus homologous to the entire female cone of a cycad (Florin 1946). Because of the presence of bract scales the ovuliferous scales fall the conjunction test.

These three tests are equally valid in morphology and molecular biology, the only difference residing in the terminology and in the relative importance attributed 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 more likely homologous to the 4A chromosomes in another wheat plant. We can infer 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 4A55, but the two 4A’s will still be homologous. Trying to assign a “degree” of homology to the chromosomes 4A and 4ALDS 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., Fedorov 1914, Kitsara 1924, 1930). Thereby the concept of homology was changed into a purely operational one, which is still used in genome analysis. This usage 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 (Weinstein 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 of hoc. It is completely unknown to what extent differences in DNA sequence composition influence pairing behavior, both with respect to coding (e.g., genes) and non-coding sequences (e.g., the 20% or so repetitive DNA that occurs in the Triticeae genome). Thus, the involved 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 BB 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 & Ross 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 confluence test. Previously both Kallig (1968) and Seberg (1989) have stated that the ability of chromosomes to pair and hence inferred as homologous as defined by Dewey (1982), is the pleomorphic character state. The ability to pair tells us only that the chromosome genomes have not diverged. Thus, the pairing ability of chromosomes cannot be used in phylogenetic reconstruction as only homoplastic character states are informative.

Intermediate levels of chromosome pairing (= the average chi-square 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 points of homology, and in phylogenetic inference they provide very little opportunity for further research (Erinner & Blügel 1993). Thus, it remains to be proven that homology expressed as pairing ability exists among any of Patterson’s (1982, 1988) tests, apart perhaps the conjunct test.

THE OPERATIONAL CONCEPT OF HOMOLOGY - HOW DOES IT WORK?

What is measured in genome analysis is usually the average value of the chi-square per cell is calculated. But what does this average value represent? Assume that we have a diploid hybrid with 2n=14 chromosomes, observe cells with every number of chromosomes from one to 13, with an average frequency of 8.6 chromosomes per cell (as in the hybrid Hordeum brachyantherum × Nevia × f. muticum Perls [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 one or two chromosomes? One chromosome would indicate a fairly low level of pairing and little homology between the genomes, whereas 13 chromosomes 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 number of chromosomes in a defining number of cells. When looking at the chi-square distributions in hybrids (Fig. 1, 2). One might have expected that chi-square distributions typically would be either bimodal with the top-point equaling or close to the mean value (Fig. 2A: Aegilops geniculata Roth × Triticum durum Desf., 2B: H. 41383-I.), or form distributions sloping steeply from either zero in hybrids with virtually no pairing (Fig. 2D: H. 41339-1) or from the absolute maximum number of chromosomes 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: H. 41339-1), there may be no apparent top at all (Fig. 1B), or there may be more than one top (Fig. 1C: BB 7271 a, 1D). If the distribution of chromosomes is skewed, then the average value will be either higher or lower than the most frequently occurring number of chromosomes, and the modal value would better represent the distribution of chi-squares than the mean value. If all observed numbers of chromosomes 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 peaks than to any of the maximum values (e.g., Fig. 1D: BB 751 b with an average chi-square frequency 1.74). 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 homology frequency seems absurd.

Here we shall not attempt to answer in depth what it signifies that the average number of chromosomes 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 chi-square 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 those first carried out in 1929, have attempted to solve 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 chromosomes per cell were observed. Lu & Bothmer (1993) observed significantly different pairing in reciprocal hybrids between Elymus canadensisus C. Koch. (Tzvelev and E. utensis (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 chromosomes in hybrids. A: B. Hordeum brachyantherum (4x) × Secale cereale, two classes involving different parental accessions. C: Elymus testiculatus (Drob.) Tzvelev × T. conicus (L.) L., two classes involving different parental accessions. D: Elymus testiculatus × C. Koch. (Tzvelev and E. utensis (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.)](image-url)
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 & Hills 1990). As previously stressed, by e.g., Kellogg (1989) and Seberg (1987), 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 phylogenetics and hence is it phylogenetically incomparable.

Acknowledgements - We thank Bjorn Salomon and Bao-Rong Lu - though probably strongly disagreeing with us - for nevertheless letting us have access to their original data on the Eymus L. hybrids. We also wish to thank Chris Humphries and Ib Lindø-Lauren for valuable comments and corrections to the manuscript. Chris Humphries suggested the illustrative confver/cycad cone example.

LITERATURE CITED


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The Study on N Genome of Leymus Species

Genlou Sun* Chi Yen and Junliang Yang

Triticaceae Research Institute, Sichuan Agricultural University,
Dujianyang City 61830, Sichuan, China

ABSTRACT

Leymus Hoehne is a perennial genus of Triticaceae. All species in Leymus have the genome NN. The genome N is from the genus Paspalostachys. Two Paspalostachys species, diploid P. huashanica Kang ex Kuo and P. juncea (Fisch.) Novelsi (2n=14), were hybridized with allopolyploid, Leymus secalinus (George) Tzvel and L. multicaulis (Kor. & Kor.) Tzvel.. 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 Hoehne, a perennial genus of Triticaceae, includes about 30 species. They are distributed in the temperate regions of Eurasia, North and South America and extend to the subarctic and the tropic alpine regions. All species in Leymus have the genomes N and X. Here the N genome is donated by Paspalostachys. Paspalostachys 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.) Novelsi, P. fragilis (Boiss.) Novelsi, and P. huashanica Kang ex Kuo. Chromosome pairing in the hybrids indicated that each species has a modified form of the N genome (Dewey and Hisato, 1963; Bothmer et al., 1987; Wang, 1987; Lu et al., 1990). Therefore, the symbols N, N', and N'' 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 Paspalostachys 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 Paspalostachys species. Because each Paspalostachys species has a modified form of N genome, it is worthwhile to extend the investigation to other species of Paspalostachys. 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 (George) Tzvel (6040) was collected from Fuhai county, Xinjiang, L. multicaulis (Kor. & Kor.) Tzvel (Y94) from Habbeo, Xinjiang, and Paspalostachys juncea (Fisch.) Novelsi (Y136) from Teelike, Xinjiang, China. Paspalostachys huashanica Kang ex Kuo is an endemic species of the Huashan mountains of Shanxi, China. All materials were grown in the field at the Triticaceae 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 (63:1) solution for 24 hr, 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 moso were very high (Table 1). Univalent and multivalents were only occasionally observed in Leymus s. secalinus and L. multicaulis (Table 1).

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

<table>
<thead>
<tr>
<th>Species</th>
<th>I1</th>
<th>I2</th>
<th>Chi square</th>
</tr>
</thead>
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<td>57</td>
<td>43</td>
<td>26.95</td>
</tr>
<tr>
<td>L. multicaulis</td>
<td>36</td>
<td>64</td>
<td>24.96</td>
</tr>
<tr>
<td>P. juncea</td>
<td>6</td>
<td>9</td>
<td>21.79</td>
</tr>
<tr>
<td>P. hexastachya</td>
<td>7</td>
<td>9</td>
<td>20.18</td>
</tr>
<tr>
<td>L. s. secalinus x P. hexastachya</td>
<td>36</td>
<td>64</td>
<td>24.96</td>
</tr>
<tr>
<td>L. multicaulis x P. hexastachya</td>
<td>7</td>
<td>9</td>
<td>20.18</td>
</tr>
</tbody>
</table>

Leymus s. secalinus x P. hexastachya 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). 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. s. 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 only one of the 68 cells (Fig. 3).

The metaphase-I pollen mother cells of L. multicaulis x P. hexastachya 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 + 0.001 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 autosymmetric or homeologous pairing occurred.

Discussion
The two diploid Poaehrostachya species, P. hexastachya and P. juncea were crossed with Leymus s. 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. s. secalinus x P. hexastachya, 6.92 per cell in L. s. secalinus x P. juncea, 6.69 bivalents per cell in L. multicaulis x P. hexastachya, 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. hexastachya and P. juncea genome. Fewer than seven univalents were observed in these four cross combinations, which showed either autosymmetric or homeologous pairing occurred.

Genomic and phylogenetic relationships of species can be supported by observation on F1 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. ambiguous (syn: Elymus ambiguous) is an allotetraploid species with one N genome (Dewey 1976 used to designate N as) 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. ambiguous. Dewey (1976) concluded that the N genomes in P. juncea and L. ambiguous are so nearly alike that one need not look beyond P. juncea for the source of the L. ambiguous.
N genome. Chromosome pairing in the synthetic triploid hybrids P. junceus × L. innovatus leaves little doubt that one of the L. innovatus genomes came from P. junceus, either directly or indirectly (Dewey 1970). Cytological data on some Leymus species crossed with P. junceus led to the conclusion that P. junceus, or a precursor of P. junceus, was one of the original diploid parents of Leymus species (Dewey 1970, 1976; Wang & Hsiao 1984). However, each Pachystachys species with a modified form of N genome has been identified, at least in P. huashanica, P. junceus and P. fragilis (Dewey and Hsiao 1983; Bothmer et 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 Pachystachys is in Leymus, and suggesting that it is currently unknown which Pachystachys 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. junceus 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. junceus. It is not excluded that P. huashanica or another Pachystachys 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 Pachystachys and Leymus species. With more species involved, more detailed information can be obtained, and consequently, a greater resolution of the relationships between Pachystachys and Leymus may be provided.

Acknowledgement—This work is supported by grants of the National Natural Science Foundation of China to G.L. Sun (No. 39370558).

LITERATURE CITED


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

M. Assadi
Mostafa Assadi
Research Institute of Forests and Rangelands
P.O. Box 13185-116, Tehran, Iran.

ABSTRACT

Crossing experiments were performed between and within taxa of Elymus sect. Elytrigia and sect. Caespitose 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 an additional genome of unknown origin. Crosses between different accessions of E. illinonicus (genomic constitution S) from W., NW., and N. Iran as well as crosses between E. illinonicus and accessions morphologically assignable to Elytrigia gracillima and Elymus sibiricus should therefore be merged into E. illinonicus. Crosses between Elymus illinonicus and the diploid Elytrigia geniculata ssp. ferganensis and Elytrigia strigosa ssp. angustifolia 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. illinonicus and E. persicus (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. Caespitoseae (Rouy) Melderis and sect. Elytrigia (Desv.) Melderis, belonging to Pseudoneurastria and Elytrigia t. str., respectively, in the classification based on genomic constitutions (Löve 1984). In accordance with Melderis (1960, 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>
<thead>
<tr>
<th>Taxa</th>
<th>Zn Genomes</th>
<th>References</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elymus repens (L.) Gould</td>
<td>42</td>
<td>SSH</td>
<td>Assadi &amp; Runemark 1994</td>
</tr>
<tr>
<td>— elongatiformis (Drobow) Assadi</td>
<td>56</td>
<td>SSHX</td>
<td>This study</td>
</tr>
<tr>
<td>— illinonicus (Hackel) Melderis</td>
<td>14</td>
<td>S</td>
<td>Dewey 1972</td>
</tr>
<tr>
<td>— persicus (C. A. Meyer) Assadi</td>
<td>28</td>
<td>SP</td>
<td>Assadi 1994a</td>
</tr>
<tr>
<td>Elytrigia geniculata (Trin.) Nevski</td>
<td>14</td>
<td>S</td>
<td>This study</td>
</tr>
<tr>
<td>— ferganensis (Drobow) Tzavelov</td>
<td></td>
<td></td>
<td>C. Asia</td>
</tr>
<tr>
<td>— strigosa (Bieb.) Nevski</td>
<td>14</td>
<td>S</td>
<td>This study</td>
</tr>
<tr>
<td>— angustifolia (Drobow) Tzavelov</td>
<td></td>
<td></td>
<td>C. Asia &amp; China</td>
</tr>
</tbody>
</table>

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

22
ssp. argiloides are probably members of Elymus sect. Cernitobae (Pseudoregmcis according to Love’s (1984) classification based on genomic constitutions) but, since they belong to a critical species complex (Tavelev 1976) which has not yet been satisfactorily revisited, the author prefers refrain 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 TAR and LD. For the methods used in seed germination, vernalization, meiotic 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 × 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, robust and mucronate glumes and lemmas, and mid spike internodes c. 7 mm long. The chromosome number was 2n=49.

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

<table>
<thead>
<tr>
<th>Combinations</th>
<th>No. of combinations</th>
<th>No. of flowers</th>
<th>Seed set</th>
<th>Embryo</th>
<th>Plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sect. Elytrigia</td>
<td>Elymus elongatiformis × E. repens</td>
<td>1</td>
<td>18</td>
<td>33</td>
<td>-</td>
</tr>
<tr>
<td>Sect. Cernitobae</td>
<td>E. lanicatus × E. lanicatus</td>
<td>6</td>
<td>135</td>
<td>19</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>E. lanicatus × E. persicinus</td>
<td>1</td>
<td>26</td>
<td>77</td>
<td>19</td>
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<td>17</td>
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<tr>
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<td>E. lanicatus × Elytrigia goniculata ssp. ferganensis</td>
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<td>18</td>
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<td>61</td>
</tr>
<tr>
<td></td>
<td>E. lanicatus × Elytrigia striigosa ssp. angilaploides</td>
<td>2</td>
<td>60</td>
<td>52</td>
<td>-</td>
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<tr>
<td></td>
<td>Elytrigia striigosa ssp. angilaploides × Elymus lanicatus</td>
<td>1</td>
<td>20</td>
<td>25</td>
<td>-</td>
</tr>
</tbody>
</table>

Figure 1. Metacritic configurations at metaphase I (A-D & F-I) and anaphase I (E) in Elymus species. (A-B): E. lanicatus × E. persicinus (2n=21) with 7 univalents and 7 ring bivalents in A, and 6 univalents and 7 ring bivalents in B. (C-E): E. lanicatus × Elytrigia goniculata 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): Elytrigia sosnovskyi × E. lanicatus angilaploides 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. (A-D, F-I) × 10 mm.

Elymus lanicatus × E. sosnovskyi

Elymus sosnovskyi was described from material collected near the Iranian border in Turkey. It has been distinguished from E. lanicatus by its narrower 3-veined glumes with an acuminate-subulate apex (see Meldens 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 6.96 ± 0.00 bivalents (chiasma frequencies of 13.34 and 13.46) were observed at metaphase I.

Elymus lanicatus × Elytrigia goniculata ssp. ferganensis

The two taxa are allopatric, with E. lanicatus occurring in Lebanon, Turkey, Iraq, and the Caucasus, while the other taxon is confined to Central Asia. Both taxa are diploids. The hybrid is morphologically closer to Elytrigia goniculata than to E. lanicatus and has scabrous calms 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. oeglepoideae

Elytrigia strigosa ssp. oeglepoideae occurs in Siberia, Central Asia, and China, far from the distributional area of Elymus libanoticus. Both species are diploids. The hybrids were intermediate between the parents and vigorous. The anthers did not dehisc 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 & Ranjbar 1995), while E. elongatus forma is octaploid. A mean of 7.12 univalents in the chromosome association of the hybrid E. repens x elongatus forma indicates that E. elongatus forma has the same genomic constitution as E. repens plus an additional unknown genome. Therefore, the genomic constitution of E. elongatus forma is designated as SSH+X. The genomic configuration of the hybrid in the present study agrees with that given by Dewey (1960).

**Sect. Coecopisisae**

All the taxa of the section used in the crosses belong to the genomically-defined genus Pseudoseirinum (cf. Love'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 meiosis 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 gracilina and Elymus sassoanikyi. The results of the present study indicate that Elytrigia gracilina and Elymus sassoanikyi should be included in E. libanoticus (see Assadi 1995). Elymus libanoticus was also crossed with the diploid Elyrigia geniculata ssp. ferrensis and Elytrigia strigosa ssp. oeglepoideae. 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 Aasy 1975). Elymus libanoticus, with the genomic constitution 5, 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. pertenuis supports the suggested amphidiploid origin of E. pertenuis. However, this evidence is not conclusive, since the meiotic pairing may have been influenced by homoeolog pairing between chromosomes of the S and P genomes (see Wang 1989).
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 Poa pratensis. Superscripts in small letters are used when modified versions of a basic genome are referred to, e.g. NsH for the genome found in Hordeum pusillum. Unknown or equivocally identified genomes are designated by X followed by a lower case letter, e.g. Xs for Hordeum marinium. 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 1963, Baum et al. 1987, Gustafsson 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 1963, Alonso and Kimber 1983, Kimber and Zhao 1983, Dewey 1964). 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 intermediates, 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...
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<th>Suggested designation</th>
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</tr>
<tr>
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<td>Löve 1984</td>
<td>Xcup</td>
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<td>B</td>
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<td>Ecup</td>
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</tbody>
</table>

*Included in Genus *Triticum* by *Löve (1984).*

**Included in *Aegilops* by *Löve (1984).*

1. See comments.
completely new system, the suggested system builds on the more 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 of species of the same genome constitution. His system of nomenclature is not endorsed here for a formal taxonomic classification system of the Triticaceae. 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 Triticaceae:

1. Genome symbols should be written in **bold face**.
2. Different basic genomes in Triticaceae (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 PDL or other pairing promoter supressor 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 indicating the species carrying such modified genomes. Further modifications may be indicated by superscripted numeric numbers.
9. When previously unrecognized 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 cytotype of an allopolyploid 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, 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, Ts, and Ns, to designate the genomes of species of the genera *Pseudorogernia*, *Taeonichthya*, and *Pachystachys*, respectively. For G, see comment 4 below.
2. The genomes of Hordeum murinum and H. murinum are given the symbols Xa and Xo, 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 *Hordeum* have not been unequivocally identified, but they are temporarily assigned the symbols Xo and Xr.
4. Since the new genus *Pericyclus* split from *Festucastrum* by Seberg et al. (1991) has not been studied by chromosome pairing, the genome is assigned Xp for now. *Festucastrum* is not the donor of the G genome in *Triticum* timopheevii; therefore, the genome symbol of *Festucastrum* 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 *Tirucuyrum*, *Lophopyrum*, and *Tricyrtium* (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 1990), we propose the change of J to E. Most existing evidence (for ascertainment see Juhasz 1990) from diploid c values, the trisomic trivalves of *Tirucuyrum* (Liu 1990) and *Triticaceae* (Wang 1992, 1993a, 1993b) in 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. jubatum*. Because the genomes in these two Hordeum species have the same basic genome symbol, we must also use a single basic genome symbol for *Tirucuyrum* bessarabicum and *Lophopyrum* elongatum.
7. Because the presence of G (=E) in *Elymus* and *Pascopyrum* is in doubt (Zhang and Dvorak 1991, Wang and Jensen 1996), it is proposed to replace JNs with NsXmn until Xmn is experimentally identified.
8. Newer results indicate that the formerly unknown X genome in *Elytrigia* repens is actually an H genome (Assad and Runemark 1995, Vershinin et al. 1994).
9. The genome combination of E repsis is thus StSxH and is identical to a group of species in *Elymus*.
10. The genus *Eremopyrum* probably comprises two different genomes (Sakamoto 1979). They are assigned the symbols F and Xo.

**LITERATURE CITED**


Physical Mapping of Micronutritional Genes in Wheat-rye Translocations

R. G. Kynast1, M. S. Röder2 and V. Römheld3

1Cereals Cytogenetics Group, 2Gene and Genome Mapping Group, Institute of Plant Genetics and Crop Research, Corrensstr. 3, D-06466 Gatersleben, Germany
3Institute of Plant Nutrition, University Hohenheim, Fruwrithstr. 20, D-70593 Stuttgart, Germany

ABSTRACT

In rye (Secale cereale L.), there are loci on chromosome arm SRL 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 SRL were identified by root crosses and German-banding: 'T30' (5AS.SRL), 'T64' (5BS.SRL), 'Vhn' (4BS.4BL.SRL) and 'Cor' (4BS.4BL.SRL). 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 SRL and the genes for mucin acid and for hydroxyproline acid synthetases involved in the strategy II of Fe-efficiency control to two intercalary regions of SRL. In all wheat-rye translocation lines the Ce gene is linked to the dominant hairy neck character (Hh) from rye. This morphological trait and the BFLP 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 Robinson 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 SRL 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 mucin acid and for hydroxyproline acid synthetases (Muc and Hmuc) involved in Fe-efficiency control to the intercalary regions of SRL. We also present the development of genetic and molecular markers as the main prerequisite for a marker-based large-scale selection of micronutritionally efficient genotypes in wheat 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. 'PCH 13' is an inbred line and originated from a selected self-fertile mutant of 'Perkus Spring'. The wheat, Tritium aestivum L. 'Chesapeake Spring' came from the Gatersleben Germplasm Bank. The wheat-rye translocation line (WRT) 'T30' harbouring the 5AS.SRL chromosome was kindly provided by T. Miller (Norwich, UK). The WRT's 'T64' (5BS.SRL) and 'Cor' (4BS.4BL.SRL) were kindly provided by J. P. Gustafson (Columbus, USA). The WRT 'Vhn' arose from a single selection of the wheat 'Wiking' and carries the 4BS.4BL.SRL 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...
RESULTS AND DISCUSSION

Although, in comparison to wheat, rye cultivars are preferably planted on light, sandy clay soils with bad nutrition supply, severe iron shortage induced a considerable decrease in fresh matter production of young rye shoots (Tab. 1). Whereas, the grain yields of rye (PC161) demonstrated a higher tolerance against copper shortage than those of wheat (CS10). Moreover, the presence of rye chromatin of the SRL 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 SRL 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 symptoms of mild chlorosis (Marschner et al. 1987) was substantiated by determining the chlorophyll contents (Tab. 1). The response to Fe-sharage varied among the WRTs (36 - 1 %). Their efficiencies were elevated by genes from the SRL arm. This gradation fits nicely with the results from the analyses of translocated Phytosideroximes (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).

PS1 are essential parts of the strategy II system for the mobilization of Fe3+ ions via chelating in the rhizosphere and transmission into the apical root zones (Römmel and Marschner 1986, Marschner et al. 1986, 1987, Treedy et al. 1989). The genes of the synthetases for MA and HMA ('Mesi' and 'Hmas') were localized together on chromosome 5R by Mor et al. (1990). The translocation points of WRTs T63, 'Von' and 'Cor' 'break' this linkage group and enable the genes to be physically mapped to two defined intersitial regions. GIS was used to paint the different SRL 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 size of the SRL segments as follows: T29 = 1 Plu (= 100 cpiu), T63 = 13.5 Plu, 'Von' = 8.4 cpiu, and Cor' = 8.2 cpiu of the SRL arm.

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

The genes Hmas and Ce are linked on the translocated chromosome segments from rye. In all WRTs 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 SRL 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 labor 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 gense, Ces and Hfais. Fig. 6 shows the polymorphic bands of the 'WG99' probe onto Pat I digested DNA of 'Chinese Spring' [W], 'PC61' (R), 'T29' (a), 'T63' (b), 'Von' (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 'BS-4BL-5RL chromosome of 'Von' 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 'Von' specific fragment obtained with WG199 in an F3 derived from (Von x wheat) with the Hmas this probe is a molecular marker for Cu-efficiency as well.
**Chromatin Characterization in Dasypyrus**

Domenico Pignone, Roberto Mezzanotte, Roberto Cremonini

CNR Istituto del Germoplasma, Bari, Italy
Istituto di Biologia Generale, Università di Cagliari, Italy
Dipartimento di Scienze Botaniche, Università di Pisa, Italy

**ABSTRACT**

An open pollinated natural population and an inbred line of Dasypyrus villusum 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. villusum two classes of heterochromatin exist with different chromosomal location and reactivity properties. One fraction of heterochromatin appears to be more affected by individual variation than the other. Preliminary examination of the chromosomal characteristics of D. brevistigmatum indicate that the evolution of this latter species involved a more complicated process than the simple duplication of its chromosome number.

**INTRODUCTION**

The genus Dasypyrus comprises two Mediterranean species: D. villusum (L.) Candargy (formerly Haynaldia villusum Schur), annual, Zn = 2x = 14, widely spread in the coastal areas of the Mediterranean region, and of D. brevistigmatum (Lindl.) Frederiksen (formerly D. hardcorum Candargy), perennial, Zn = 4x = 28, whose range is limited to Morocco, Algeria and Greece (Frederiksen 1991).

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

Natural populations of D. villusum 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. villusum. Finally a preliminary characterization of D. brevistigmatum chromatin was started.

**MATERIALS AND METHODS**

A natural population of D. villusum was collected near Campobasso (central Italy); the caryopses of this population exhibited yellow and brown color. From a natural population collected near Bari (southern Italy) an inbred line (GHA 01) was derived by sibbling. 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 heterozygosity estimated below 1%. Seeds were germinated in Petri dishes in the dark at 21 °C. For cytogenetic 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. (1993). 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 R I, and Mse III.

**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

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

<table>
<thead>
<tr>
<th>Caryopses</th>
<th>Absorption (a.u. ± S.E.)</th>
<th>DNA content per (4C) nucleus (pg)</th>
<th>Nuclear area (µm² ± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellow</td>
<td>26.15 ± 3.4</td>
<td>23.7 ± 0.3</td>
<td>330 ± 4.7</td>
</tr>
<tr>
<td>Brown</td>
<td>2033 ± 25.1</td>
<td>19.1 ± 0.2</td>
<td>249 ± 3.0</td>
</tr>
</tbody>
</table>

![Figure 1](image-url) Absorption curves from nuclei of yellow and brown seeds of D. villusum; the inflection point is indicated (v)
Heterochromatin detection and classification

The general karyotype of D. villusum (Figure 3) is similar to that reported by Frièbe et al. (1987) and Lind-Laurens 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 caryotypes 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 33342 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-banded heterochromatin. This fraction was uniformly distributed and did not show any difference in yellow and brown caryotypes. 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 caryotype 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-stoichiometric, therefore bands with similar appearance could possess different amounts of heterochromatic DNA, moreover, C-banding and cytophotometry consider cells in different functional stages. Nuclear heterochromatin 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 euchromatin 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. villusum.

C* / DAPI* (class 1): This AT-rich heterochromatin, localized mostly in telomeric areas, generally shows RE° reaction. RE ° reaction in class I heterochromatin is present only in a few centromeric areas not in euchromatin ones. This implies that: a) centromeric and telomeric areas possess different sub-classes of heterochromatin, as is also suggested by their appearance as large fluorescent blocks at the telomeres as opposed to thin bands at the centromeres, or 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 interspersions frequency, the base sequence target of specific REs. Even in this class of heterochromatin it is noteworthy that RE° reaction is present only as 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 (CPA°, 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. brevianistatum

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 chromosomes 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. villusum. This might appear in contrast with the hypothesis that D. brevianistatum is an autotetraploid species possessing the same genome as D. villusum. 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.

LITERATURE CITED


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

Chi Yen, GenLou Sun & Junliang Yang

Triteceae Research Institute, Sichuan Agricultural University, Duijiangyan City, 61830, Sichuan, China

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 Triteceae cytomixis plays an important role in spontaneous chromosome doubling or redoubling, resulting in the origin of auto-allopolyploidy and aneuploidy. We have obtained amphiploid plants by spontaneous chromosome doubling. These plants indicate indirectly that cytomixis takes place in the microsporocytes, giving rise to high level auto-allopolyploid Triteceae species.

INTRODUCTION

The phenomenon of cytomixis was discovered by Amolde in 1900. Gates (1911) studied this phenomenon in Drosophila and designated the term “cytomixis” to describe chromatin material migrating through the plasmodesmatum into neighboring cells. The question of whether cytomixis is an abnormal artificial behaviour 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 electron microscopy, and Yen et al. (1993) observed cytomixis taking place between two untreated fresh pollen mother cells of the Roesneri citata Pseudotrichostachys Hutchison P. hybrid under phase contrast microscopy. These workers proved that cytomixis is a natural process where chromatin exchange occurs among cells. Yen et al. (1992) and Sun et al. (1993) reported that intergeneric hybrids of Triteceae had some special cell structure formations, including the conjugation opening and conjugation tube besides the plasmodesma. The resting stage nuclei, chromatin masses, chromonema, or chromosomes can migrate through these structures into immediate neighboring cells before, during or after meiois in the hybrids of Roemeria colors x Pseudotrichostachys Hutchison and Triteceae aestivum x Pseudotrichostachys Hutchison. 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 tetrad and degenerate. 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 microsporocyte, a sterile egg cell and synergid nuclei should be produced. Fertile pollen grains are produced by the 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 Triteceae taxa.

MATERIALS AND METHODS

The accessions of Iris confusa L. and I. japonica Thunberg collected in China and Japan are shown in Table 1. The hybrids of Triteceae 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 transfers 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 1: Observations on aneusoma triploids of Far East, Asia.

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<th>species</th>
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<th>locality</th>
<th>chromosome number</th>
<th>fertility</th>
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<td>8</td>
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Table 2: Observation on somatic chromosome number of the F1 hybrid of *Erysimum caminos x Hordeum vulgare*.

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acacetarime 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

*Irj japonica* Thunberg and *l. confusa* Scaly

Irj japonica is native to China and Japan. Iris confusa has a character 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 1). All plants have a cytosome stage just before meiosis. The synapsis takes place among PMC(C) evenly (Fig. 1A: A.C.). Sino (1921) and Yasi (1939) observed cytomics in *I. japonica*. Yasi believed that *I. japonica* was a triploid, she followed Kato (1929), and Simonet (1934); Kihara (1982) also agreed with them. They did not point out the universality of cytomics in this species and did not point out how cytomics cause the chromosome number to vary among different accessions. We observed cytomics in microspores. The normal pollen grains accompanied many abnormal and functionless pollen in the same anther lobe. However, the diploid plants produced normal capsules and bare 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 1. 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, Yunnan and Guizhou Provinces. The distribution of the aneuploid *I. japonica* is much larger. It occupies all the subtropical regions of the Far East, from the Himalaya to Japan. An accession of *I. japonica* from Dujinace City was found to have two normal and five abnormal seeds in 0.25% of its capsules. The normal seeds proved to be aneuploids with chromosome number of 36 and 40. Cytomics plays an important role in chromosome aberrations, which is bound to create cytological and morphological diversity in these aneusoma irises. Natural selection seeks out the adaptive accessions.

*Triiteaceae*

Within the Triiteaceae taxa there exists a high level of auto-allopolyploids which have multiple genomes, such as *Erysimum angustissimum* (Del.) F. E. P. Chromosome numbers of *I. biennis*, *I. malvaceum* and *I. HISCHUM* have been reported for this species (Bowden, 1957, Sun et al., 1990). In the hexaploid *L. angustissimum*, the genome must be unevenly doubled, although the origin of these various cytotypes might be quite different, they could be accomplished much easier through cytomics of complete nuclei which fuse together. This mechanism was suggested earlier (Yen et al., 1993; Sun et al., 1993). Recently, we obtained F2 hybrid plants, which were derived from a cross of *Erysimum caminos* (L.) L. x *Hordeum vulgare* L. The F1 hybrid is a normal amphiploid which has 21 chromosomes comprised of the S, H and I genomes. Cytomics 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 cytomics, chromosome numbers varied among PMCs. Fig. 2D shows only 4 chromosomes are present in a small PMC. Fig. 2E shows 14 chromosomes in the PMCs, 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 chromatoid material (arrowed). Pollen has more chromatoid bodies than usual. Every year, this plant bears some seeds (30%). Most of these seeds are shrunk and have no viability. The normal seeds and seedlings had mainly 42 chromosomes (Table 2).
Figure 1. The cytomixis stage of PMC in *Iris japonica* Thunb., (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. × *Hordeum vulgare* L. (A). A PMC of hybrid shows secondary budging (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 F1 hybrid has 4 chromosomes. (E). A PMC of F1 hybrid has 14 chromosomes. (F). A PMC of F1 hybrid has 19 chromosomes. (G). A group of young pollen grains have different 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 F1 hybrid plant.

Figure 3. Meiotic behavior of F1 hybrid of *Elymus caninus* (L.) L. × *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 F1 hybrid, a is normal cell, b has one micronucleous, c has two micronuclei and d has a small group of extra late concentrated chromonemata.
LITERATURE CITED


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

George Fedak, L. O'Donoughue, K.C. Armstrong

Plant Research Centre, Agriculture Canada, Ottawa, Ontario, Canada, K1A OC6

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 rust resistance, increased protein content, yellow semolina color, and aponinisis. 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 hybrid within the tribe have already been made with wheat (Piller, 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 vitro cultures while selection for disease resistance has been done by exposure to the organism itself. Molecular tagging and known sequences for specific genes will be useful in this area. For example, 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 glutelin and gliadin PCR probes in wheat (D’Ovidio et al., 1992).

Crossing

It is now believed by some researchers involved in integrated 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 considerations are the numbers of accessions of a particular species to be evaluated. The factors involved are the pre and post-pollination applications of growth regulators. For example, it is well known that whereas the post-pollination application of GA3 to barley enhances seed set and embryo differentiation, 2,4-D is more effective than GA3 on wheat following wheat x maize pollination for haploidy introduction (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 medium (Combs et al., 1993). 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 (Piennar, 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 analyses 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 isoamylase analysis (Hart, 1987). RFLP analysis using repetitive DNA sequences (Matsui et al., 1981; 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 homology 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 species involving polyploid species, it has been virtually impossible with conventional staining methods to distinguish autosynaptic and allelotype pairing. The concept of in situ hybridization using fluorochrome labeled DNA as a pentaftware 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 a 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-anealian addition substitution and translocation lines (for review see Jiang and Gill, 1994). Individual genomes and introgressed segments can be visualized by direct labelling of component and DNA (Amann et al., 1993; Johnson and Raads, 1995), as a more efficient alternative to genome blocking.

Chromosome doubling

The old standard methods of chromosome doubling through the use of spindle arrestants such as colchicine and methotrexate are still in use today. Chromosome doubling of intergeneric hybrid plants regenerated from callus cultures has been reported (Fedak and Grimm, 1987; Wang, 1991). The success rate in producing amphidiploids 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 amphidiploid 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. Meiotic 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. Flouure, p.c.). Negligible success has been reported in hybrids involving wheat with any hybrids involving barley cultivars or Hordeum species. Partial amphiploids (with 2n=56 and having one alien genome) are one, albino, rare, product of backcrossing

Induction of recombination

Although all the species within the Triticeae originated from a common ancestor and share basic genomes that 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 flowering in tetraploid and hexaploid wheat (Sears, 1976) so that any homoeologous pairing must be induced. A Ph mutant has also been isolated in durum wheat (Georgie, 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 T. polonicum chromosome 7 substitution to chromosome 7D of wheat. Numerous other researches have been able to use these tools to induce meiotic crossing-over and transfer of traits from wheat to alien chromosomes (See Piennar, 1990 for summary of wheat-Thinopyrum transfers).

The genomes of certain Aegeilo species such as Ae. speltoides are known to suppress the effects of Triticum species of wheat and thus permit homoeologous pairing. By means of translocations between Th. intermediate and wheat chromosomes were induced (Ortiz et al., 1986).

Radiator with X or gamma-rays of seed or pollen of hybrids, partial amphiploids, and addition or substitution lines have induced recombinations and gene transfers. Numerous examples are listed in the review by Piennar (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 a few examples of transfers of useful genes have been documented (See Piennar, 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 specific selection pressure obtain or culture, 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 (Prieto et al., 1991; Penner et al., 1995), biochemical loci (Kilian et al., 1994), and value-added traits (Reddy and Appels, 1993). Such markers can rapidly be developed as particular needs and the technology 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, or 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; Grämer et al., 1991; Kärnfohl et al., 1993), and rice (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 recombinant clones (Oliver et al., 1991; Vaidial et al., 1991). Another example of a rapid screening technique is the use of monovalent antibodies to screen for various metabolites such as the town produced by Fusarium gramineum (Sinha and Savard, unpubl.).

Detection of alien chromatin

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

For example, the resistance to Hessian fly located on a 6RL, wheat chromosome addition was transferred to a terminal site on wheat chromosomes 6BS and 6BS following pollen irradiation (Mulai et al., 1993) and to an interstitial location on wheat chromosome 4BS. This was achieved by co-culturing tissue browning and in situ hybridization using highly repetitive rDNA 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 La28 was found to be translocated to wheat chromosomes 2AL, 1AS, 1DL, 3DS and 6DL in the different lines that were analyzed (Friebe et al., 1993). The translocations were induced by Co60 treatment of a wheat-Thapsiopyrum addition line. Similarly a segment of a chromosome carrying wheat streak mosaic resistance 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, or 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; Grämer et al., 1991; Kärnfohl et al., 1993), and rice (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 recombinant clones (Oliver et al., 1991; Vaidial et al., 1991). Another example of a rapid screening technique is the use of monovalent antibodies to screen for various metabolites such as the town produced by Fusarium gramineum (Sinha and Savard, unpubl.).

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 syntenies. Additional techniques will facilitate gene function 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 intra-genomic translocations at the diploid parent level and derived polyploid level. An indication of such findings is the extensive interchromosomal translocations already detected in rye (Lu et al., 1992). This will facilitate studies of specific crop plants evolution. The comparative RFLP mapping that is already underway will broaden the knowledge of syntetic 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 (Wickers et al., 1993; Blang et al., 1993), Becker et al., 1994; Nehra et al., 1994) and barley (Wan and Larnaud, 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 genetic instability of rice has 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 (Albano et al., 1993) will produce saturated RFLP maps for possible applications of map-based cloning.

Gene tagging with RFLPs and RAPD will provide molecular markers for ever increasing numbers of genes. The RFLP markers are being converted to STS for PCR use while RAPD are being converted to SCARS to provide extended applications over genomes. 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.

Literature Cited


The perennial lyme grass (Leymus arenarius and L. mollis) as a potential crop species for northern latitudes

Kesara Ananthawat-Jonsson1, Jón Gudmundsson1, Birkr Bragason1, P. K. Martin2 and R. M. D. Koebner2

1Agricultural Research Institute, Keldalnahöll, Reykjavík, IS-112, Iceland, 2Cambridge Laboratory, John Innes Center, Norwich NR4 7UJ, UK. *Author presenting paper
Phone: +354 577 1010, Fax: +354 577 1020, E-mail: kesara@rala.is

INTRODUCTION

The perennial lyme grass

The most common species of lyme grass in the northern circumpolar regions are the European Leymus arenarius (L.) Holch 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 similar but 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 (NOK; 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 shoreslines of both the Atlantic and Pacific coasts, in the Canadian Arctic and Alaska (Sigurbjörnsdóttir, 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 (Akokas and Fredskild, 1991) and L. mollis recently introduced in Iceland. The significance of such extensive distribution is that the potential cultivation areas for lyme grass, 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 lyme grass (L. arenarius and L. mollis) for cultivation as potential cereal crop for Iceland as well as for other regions of native lyme grass 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 lyme grass for bread-making

Lyme grass has a long history of use for human consumption. Earliest records of lyme grass 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 Lyme pollen with the Viking homesteads in Newfoundland (see Griffin and Rowett, 1981). In Iceland, lyme grass (L. arenarius) grains were used as a source of bread flour until the 19th century, and in the south coast areas of Vestur-Skaftafellsnessi the local production of lyme grass flour was apparently sufficient that no other flour was imported (Sigurbjörnsdóttir, 1960). Grains of L. mollis were also used by North-American Indians, while those of L. arenarius were sometimes gathered in Norway Russia for the same purpose (in Klebsalad, 1985).

The quality of lyme grass flour for bread-making was known to be high, and some reported that products made out of lyme grass 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 Chisleywood (UK), we investigated the quality of lyme grass flour. Lymegrass grains were obtained from Eyraflodsborg in south...
Figure 1. Bread made from different mixtures of wholemeal wheat flour from Canadian winter wheat variety and lyme grass flour milled from grains of Leymus arenarius harvested in Eyrarbakki, south Iceland. (A) 100% wheat flour, (B) 85% wheat flour and 15% lyme grass flour, and (C) 80% wheat flour, 15% lyme grass flour and 5% gluten. The baking was prepared by Flour, Milling and Bread Research Association at Cholseywood, 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 9: 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 lyme grass partial amphiploid PI442574 showing 12 yellow-green FITC fluorescing chromosomes originated from lyme grass and 30 red propidium iodide stained wheat chromosomes.

Fig. 4. A metaphase cell of wheat x lyme grass hybrid (Triticum aestivum x Leymus arenarius) showing a haploid set of 28 green FITC fluorescing lyme grass chromosomes and a haploid set of 21 red-brown rhodamine fluorescing wheat chromosomes. Scale bar: 10 μm.
Iceland. The grains were genetically harvested and threshed, using the facilities developed for seed production for land reclamation purposes (Greigsson and Davy, 1994). Icelandic lymgrasse (L. arenarius) has relatively large grains, about on third to half the diameter 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. Whole wheat was milled (A) all wheat bread, (B) wheat bread containing 15% lymgrasse and (C) wheat bread containing 15% lymgrasse and 5% pure gluten, each in bread triplicate. Some of the results have been obtained and among them are the taste testing of lymgrasse 2) and chemical analysis of the breads. The breads made from wheat and lymgrasse 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 these breads were highly acceptable. In addition, the breads containing lymgrasse flour were described as having favorable characters as nut-like flavor and yellowish color. The bread made from wheat and lymgrasse 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 lymgrasse flour. The wheat variety used in this experiment has exceptionally high total protein (176% dry weight) and lymgrasse is a flour containing about 19%, whereas flour of most wheat varieties contains between 9% and 14% protein (Reykdal, 1993). The present study also shows that the lymgrasse 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 lymgrasse flour, however, appears to vary among the accessions and further studies will be important for selection of the breeding materials.

**BREEDING**

**Wide-hybridization for simultaneous improvement of lymgrasse and wheat**

Bread wheat (Triticum aestivum L. em. Thell), is used in the wide-crossing program aiming to transfer important crop characteristics into lymgrasse, for example physical quality of bread-making and grain size. Wheat and lymgrasse wide-hybrids have been made, from both L. arenarius and L. mollis. The hybrids will be used for development of cultivars and for back-crossing with the lymgrasse parents, aiming to produce lymgrasse breeding lines containing crop characteristics of wheat while maintaining characteristics 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 lymgrasse 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 breeding (Heslop-Harrison et al., 1990), barley (Hordeum chinesis) adding netame to resistance to wheat (Person-Devedry et al. 1990) and Agropyron for rust disease resistance (Friebe et al., 1992). Lymgrasse, especially Asitic species like L. roscumus and L. multicosus, has also received much attention for wheat breeding (Mojeeb-Kuzi and Rodrigues, 1981; Dong et al., 1986; Poulal et al., 1989), and wheat breeding lines containing Lymgrasse chromosomes have been identified. Several traits of Lymgrasse 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 lymgrasse 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 lymgrasse.

Wide-hybrids involving wheat (both tetraploid and hexaploid species) and several species of Lymgrasse were made in the early 1960's (e.g. Tsitlin, 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 Upsalsa, Sweden, and "PH442574/Dewey" from Professor Bikran Gill, Wheat Genetics Center, Kansas State University, USA. The AD99 is derived from back-crossing of the hybrid "D. durum x L. mollis" to wheat bread, while the PH442574 is derived from a cross between a Triticeae and a Lymgrasse and both lines have been maintained for more than ten generations. We found that both amphiploids had 42 chromosomes, 12 of which have originated from Lymgrasse, while 30 chromosomes have wheat origin (Fig. 3). One of these lines, AD99, was shown to have high resistance to mildew and leaf rust (Fath, 1983; Merker, 1992). Although these materials are not suitable for Iceland climate, they are valuable for genetic studies by Cambridge Laboratory, John Inns Center, Norwich, UK. The pollen parents were the tetraploid American lymgrasse L. mollis originating from Alaska Peninsula and the octoploid European Lymgrasse L. arenarius collected from a wild stand in Reykjavik. The crosses were conducted in an unheated glasshouse at Kórpa Experimental Station in Reykjavik. 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 et al., 1989). Anamitotization-Jonsson et al., 1990), which was modified using pre-arrnealing of two differently labeled genomic DNA probes (Anamitotization-Jonsson 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 lymgrasse genomes - T. aestivum x L. mollis, 5x-5T; 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 diplod 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 lymgrasse breeding lines.

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**LITERATURE CITED**


Progress in Polyhaploid Production Techniques of Hexaploid Wheat through Wide Crosses

M.N. INAGAKI and A. MUJEEB-KAZI

International Maize and Wheat Improvement Center (CIMMYT)
Lisboa 27, Apartado Postal 6-641, 06600 Mexico, D.F., Mexico

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 Triticum 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 Triticaceae is alien genetic transfer, which is facilitated by tissue culture and cyogenetic manipulation (Sharma and Gil 1983, Mujeeb-Kazi and Asada 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, Kasho and Kao 1970). Artificial rescue of haploid embryos was required since endosperm did not develop in most of seeds set. Monohaploid 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, Stich and Snape 1986). The dominant genes K1 and K2, located on wheat chromosomes 5B and 5A, respectively, control the cross-incompatibility (Falk and Kasha 1983, Stich 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, Stich 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, Stich and Snape 1986).

When the cross-compatible wheat genotypes are used for crosses, the production efficiency is enough to obtain...
200 polymorphs from 1000 wheat florets (40 spikes) pollinated by H. bulbosum (Inagaki 1989). It also took 26-30 weeks from crossing to harvest of doubled haploid plants 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 synthetic plant hormones. Marshall et al. (1983) reported that the application of a 2,4-dichlorophenoxyacetic acid (2,4-D) solution on emasculated wheat florets prior to pollination induced panthercrop acid seed development and increased ovule size due to cell expansion. However, it 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 culm 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-compatible wheat genotype at very low frequencies, suggesting that the 2,4-D application enhanced seed and embryonic 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 Fagaceae subfamily (Zeisler and Nitschke 1994). Cytological evidence indicates that the fertilization of wheat with maize (Zea mays L.) pollen was successful, irrespective of the presence of M gene(s), and produced hybrid zygotes (Laure and Bennett 1986, 1987a). In these zygotes, the maize chromosomes were rapidly lost within two days after pollination (Laure and Bennett 1987b), necessitating artificial rescue of pronuclear zygotes at early developmental stages (Laure 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 Tahar (1990) demonstrated that maize pollination of, and subsequent 2,4-D treatment on, wheat also resulted in production of wheat embryos capable of regenerating polyplid plants, even for wheat varieties that were cross-incompatible with H. bulbosum. Genotypic differences in embryo formation frequency were significant only for wheat varieties which had been developed through maize crosses has been further confirmed using diverse wheat varieties (Inagaki and Tahar 1990, Laure and Bennett 1991). Some species related to maize, like teosinte (Z. mays L. spp. mexicana and eastem gamagrass [Tripsacum dactyloides] (L.) L.) are alternative pollen sources for wheat polyploid production (Ushiyama et al. 1991, Riera-Lizarazu and Mejueb-Kazi 1993). For increasing flexibility to handle wheat materials, decaching 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 Americanum (L.) R. Br.) crosses, successful fertilization and elimination of paternal chromosomese from hybrid zygotes were observed (Laure and Bennett 1988b, Laure 1989b). Efficient formation of polyploid embryos in these crosses indicated that the 2,4-D application was essential (Ohtsuka et al. 1992, Inagaki and Mejueb-Kazi 1994a). Significant embryo formation frequency differed in the existence of wheat varieties. Sorghum crosses however, expressed a strong genotypic barrier to embryo formation (Inagaki and Mejueb-Kazi 1994a). Therefore, the maize-mediated polyploid production appears more feasible than the 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 (Baranbas 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 Mejueb-Kazi 1994b). Stored maize pollen can thus be used for wheat polyploid production when and where fresh pollen is not available.

Polyploid production efficiency

A technique of polyploid production in hexaploid wheat through wide crosses consists of two steps: hybridization and embryo rescue. A factor affecting polyploid production efficiency was the developmental stage of the wheat florets at cross. This critical in crosses with H. bulbosum (Sitch and Snape 1978A), maize (Laurie 1989b) 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 Smyth 1982, Inagaki 1986, Sitch and Snape 1987c). Further, the developmental stage of the embryo formation 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 flowers 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 (Mejueb-Kazi et al. 1987, 1989).

CROSSOVERS OF DIVERSE HEXAPOID WHEAT VARIETIES WITH MAIZE RESULTED IN POLYPLOID FORMATION IN THE F1 GENERATION AND PROCEPTION OF POLYPLOID HYBRIDS IN THE F2 GENERATION. THE RESULTS INDICATE THAT THE USE OF MAIZE AS A POLLEN DONOR FOR WHEAT POLYPLOID PRODUCTION IS FEASIBLE. THE TECHNIQUE OF POLYPLOID PRODUCTION IN WHEAT X MAIZE HYBRIDS OFFERS PROMISING OPPORTUNITIES FOR THE DEVELOPMENT OF NEW WHEAT VARIETIES WITH DESIRED GENETIC COMBINATIONS. FUTURE RESEARCH SHOULD FOCUS ON THE IMPROVEMENT OF POLYPLOID PRODUCTION EFFICIENCY AND THE DEVELOPMENT OF NEW WHEAT VARIETIES USING MAIZE AS A POLLEN DONOR.


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

R.A. Pickering, 1 A.M. Hill, 1 G.M. Timmerman-Vaughan, 1 E.M. Forbes, 1 M.G. Cromey, 1 M.J. Gilpin, and 1 M. Michel, M. Scholz

1 New Zealand Institute for Crop & Food Research Limited, Private Bag 4704, Christchurch, New Zealand. 2 Bundesanstalt für Züchtungsforschung an Kulturpflanzen, Institut für Züchtungsmethodik landwirtschaftlicher Kulturpflanzen, Institutsplatz 2, D-18190 Groß-Lüsewitz, Germany.

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 a 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 gene 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 dioldiploid V8 hybrid production, and even enabled triploid V8 (2V:1B) hybrids to be obtained directly from crosses of H. vulgare (4x) x H. bulbosum (2x) (Pickering 1991a). Conversely, V8 (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 homoeologs was inexplicably absent in V8 hybrids described by Pickering (1991a), compared with those V8 hybrids reported by Pohler and Szgat (1982). In V8 and VBB hybrids allotetraide is variable and influenced by parental genotype (Thomas and Pickering 1985; Xu and Snape 1986; Pickering 1993) making it possible to identify high-pairing hybrids. Since environmental conditions affect seed development (Thörn 1992) and chromosome stability in H. vulgare and H. bulbosum crosses (Pickering 1985), the influence of temperature on chromosome pairing in V8 and VBB hybrids was studied. To promote allotetraide, 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. V8 hybrids possess indelicient anthers and have not proved useful even after obtaining rare backcross seed. Fertility can be induced by doubling the chromosome number to create tetraploid V8BB hybrids. Selfed progeny from the V8BB 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 VBB hybrids are moderately fertile, their value for gene introgression is limited because of low allopolyploidy pairing (Pickering 1991a). Until recently, only indelicient anthers were found in V8 hybrids, but when H. vulgare (2x) was pollinated with H. bulbosum (4x) that had been derived from colchicine-treated diploid H. bulbosum genotypes, fertile V8 hybrids were obtained (Pickering 1986). It is possible that the colchicine treatment induced anther dehiscence in the V8 hybrids, since colchicine can cause heritable disturbances to plant development (Hagis and Jones 1987) and gametogenesis (Hassan and Jones 1994). The V8BB hybrids have been successfully backcrossed to barley and chromosonomally 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 (Large and Jochenssen 1976), crossing over between paired homoeologues may be very low. To investigate this possibility, a parametric inversion in barley was crossed with a diploid H. vulgare genotype and several V8 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 frequency of bridges and fragments vary according to the frequency of crossovers and the length and location of the inversion. Aberrations in the V8 hybrids occurred less frequently (1.0%) than in the barley inversion heterozygote (1.2%), but their presence indicated that crossovers between the parental chromosome combinations occurred over an area. The rarity of this event constitutes a considerable barrier to obtaining recombinants, and no satisfactory means of overcoming this 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 develops as a problem. In V8BB 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 (Large 1971; Xu and Snape 1986). Fertilization of H. vulgare eggs by gametes with seven H. bulbosum chromosomes would lead to haploid H. vulgare plant formation after chromosome elimination. However, H. vulgare haplotips were less commonly observed than diploid H. vulgare plants among progeny from H. vulgare x VBB crosses (Pickering 1992), and fertilization must have been affected 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, androgynogamy was carried out by culturing anthers from various hybrid combinations, but only those hybrids that possessed desensitants 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(41) and one H. bulbosum chromosome; (ii) a 14-chromosome plant similar to a VBB 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 were obtained.

Progress and future prospects

Despite the obstacles preventing constant gene introgression between H. vulgare and H. bulbosum, 40 plants from backcrosses of V8BB hybrids to barley have been produced that involve the single, double and triple monosomic substitutions of barley chromosomes (17), 2(21), 3(21), 4(41), 6(6) and 7(5) 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 chromosome 7(5) or 6(6) substituted, and recombinants have been identified among selfed progeny. Transfer of single genes from H. bulbosum has also been reported following backcrosses of V8BB hybrids to barley (Xu and Kasha 1992; Timmerman et al. 1993). The use of tetraploid hybrids has not been as fruitful. Szgat and Pohler (1982) selected 'modified' barley plants from BBVW backcross progeny, and introgression of H. bulbosum DNA into a plant with pubescent leaf sheaths was confirmed using a repetitive sequence molecular probe - pSCL 19.2 (Pickering, Smart and Melz, unpubl.). Michel et al. (1994) described a plant with a single mild resistance gene and a plant with barley mild mosaic virus (BaMMV) resistance, following screening of selfed progeny from a VBBW hybrid. Based on electrophoretic evidence, the transferred H. bulbosum DNA of the BaMMV resistant plant is located on barley chromosome 4(41).
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.

LITERATURE CITED


SZGAT, G. and POHLER, W. 1982. Hordeum bulbosum x *H. vulgare* hybrids and their backcrosses with cultivated barley. -
Breeding Potential of Durum Wheat Landraces from Jordan III. Rate and Duration of Grain Fill

A.A. Jaradat* and M.M. Ajlouni

* Present address: International Plant Genetic Resources Institute, West Asia and North Africa Regional Office, c/o ICARDA-GRU, P.O.Box 5466, Aleppo, Syria

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 studied 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 the existence 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 sp.). 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; Legrie and Chauvelier, 1992), increases 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 (Gebeheyhu et al., 1962) and environments prone to severe 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 in cultivars developed for short growing environments. 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 long 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 drought-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, Mouravi 27, was used as a check. Each landrace genotype was grown on a 1 m² 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).

As anthesis, the first 60-70 spikes that exert 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 30 days 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:

\[ \text{GDD} = (T_{\text{max}} + T_{\text{min}})/2 - T_b \]

where \( T_{\text{max}} \) and \( T_{\text{min}} \) 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:

\[ W = a + b \cdot T + c \cdot T^2 \]

where \( W \) is grain weight (g), \( T \) is time in GDD, and \( a, b, \) and \( c \) are regression coefficients (Dorrock and Baker, 1990). Rate of grain fill was expressed as mg kernel "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 (Dorrock 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 between 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) were used 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 (TKW, mg), growing degree days (GDD) and rate of grain fill (g/m²). (GDD\textsuperscript{7})

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cluster 1</th>
<th>Cluster 2</th>
<th>Cluster 3</th>
<th>Cluster 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of access</td>
<td>91</td>
<td>89</td>
<td>78</td>
<td>71</td>
</tr>
<tr>
<td>Grain Yield</td>
<td>1.626</td>
<td>1.429</td>
<td>1.559</td>
<td>1.704</td>
</tr>
<tr>
<td>Spikelet fertility</td>
<td>2.013</td>
<td>1.681</td>
<td>1.899</td>
<td>2.025</td>
</tr>
<tr>
<td>1000-Kernel weight</td>
<td>47.1b</td>
<td>52.1a</td>
<td>52.7a</td>
<td>48.7c</td>
</tr>
<tr>
<td>Predicted GDD</td>
<td>46.3</td>
<td>51.3</td>
<td>50.7</td>
<td>43.2</td>
</tr>
<tr>
<td>Growing Degree Days</td>
<td>756c</td>
<td>786b</td>
<td>726c</td>
<td>815a</td>
</tr>
<tr>
<td>Rate of grain fill (g/m²)</td>
<td>4.7c</td>
<td>4.3b</td>
<td>5.2a</td>
<td>4.2d</td>
</tr>
</tbody>
</table>

* Cluster means, within each trait, followed by the same letter do not differ significantly (Tukey, 0.05).
Table 2. Pairwise phenotypic correlation coefficients among 6 traits measured in 4 clusters of durum wheat landrace genotypes from Jordan.

<table>
<thead>
<tr>
<th>Trait</th>
<th>GDD</th>
<th>FF</th>
<th>TKWT</th>
<th>ST</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>1</td>
<td>0.84e*</td>
<td>0.60s</td>
<td>0.68s</td>
</tr>
<tr>
<td>1</td>
<td>0.84s</td>
<td>1</td>
<td>0.59s</td>
<td>0.63s</td>
</tr>
<tr>
<td>GDD</td>
<td>0.84s</td>
<td>1</td>
<td>0.60s</td>
<td>0.63s</td>
</tr>
<tr>
<td>0.59s</td>
<td>1</td>
<td>0.60s</td>
<td>0.63s</td>
<td>0.62s</td>
</tr>
<tr>
<td>SF</td>
<td>0.62s</td>
<td>0.60s</td>
<td>1</td>
<td>0.69s</td>
</tr>
<tr>
<td>0.84s</td>
<td>1</td>
<td>0.70s</td>
<td>0.69s</td>
<td>0.54s</td>
</tr>
<tr>
<td>TKWT</td>
<td>0.62s</td>
<td>0.60s</td>
<td>0.70s</td>
<td>1</td>
</tr>
<tr>
<td>0.84s</td>
<td>1</td>
<td>0.70s</td>
<td>0.69s</td>
<td>0.54s</td>
</tr>
<tr>
<td>ST</td>
<td>0.84s</td>
<td>0.62s</td>
<td>0.84s</td>
<td>1</td>
</tr>
</tbody>
</table>

* All pairwise correlation coefficients were highly significant (P < 0.05), unless otherwise indicated. Clusters 3 & 4 below diagonal. See Table 1 for abbreviations.

Coefficient of determination (R²) values range from 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 assessed 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 G and the heaviest kernels. Rate of grain fill ranged from 0.044 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 genetic variation exists for both parameters within this germplasm collection. However, longer grain fill period may not be a promising strategy to increase grain yield under the Mediterranean environment because high temperature during the grain fill period tends to stop grain growth prematurely 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 weight/spike (GY), 1000-kernel weight (TKWT) and spikelet 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 clusters, suggesting that there is no genetic barrier to the simultaneous change of both in a breeding program. A supporting evidence was reported by Gbabeyehou et al. (1982). Grain yield per spike was positively and 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 (Gbabeyehou 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 a smaller (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 classification) 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.

LITERATURE CITED


Figure 1. Canonical discrimination analysis of durum wheat landrace genotypes from Jordan. (Cluster centroid).
Breeding Potential of Durum Wheat Landraces from Jordan IV. High Molecular Weight Glutenin Subunit Variation.

A.A. Jaradat* and M.M. Aljouni

Jordan University of Science and Technology, Irbid, Jordan. * present address: International Plant Genetic Resources Institute, West Asia and North Africa Regional Office. c/o ICARDA-GRU, P.O. Box 5466, Aleppo, Syria.

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-B1l locus were lacking, these were Glu-B1c and Glu-B1l. Glu-A1b was present with low (6.7%) frequency in this collection, however, it may have a positive effect on grain strength of the end products of durum wheat. Polymorphism (Hs) at the Glu-A1 and Glu-B1 loci averaged 0.2610.04 and 0.7330.02, respectively. Hs for Glu-A1 was negatively (r = -0.467, P) correlated, while Hs for Glu-B1 was positively (r = 0.615, P) correlated, with altitude of collection site. However, both Hs 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 broad 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 (da Cross, 1987; Margone et al., 1986; Ng et al., 1989). In a recent review, Perrenno and Porcedda (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 (Galli and Feldman, 1983); enhanced the genetic variability available to improve its industrial quality (Vallega and Waines, 1987; da Cross, 1987; Ng et al., 1989), were instrumental in the assessment of genetic diversity of wild wheat (Levi and Feldman, 1988), domesticated landraces (Lagoudai 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 (irrig., Karak, Talilah and Shoubak) were found within the Mediterranean semiarid bioclimatic and the remaining two (Ajlan and Salt) were found within the Mediterranean semihumid bioclimatic. 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 broad 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 Blakeley and Boe (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 (Hs) 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-A1locus (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 new three 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. (1989) reported that all Canadian durum wheat cultivars contain the null allele Glu-A1c, which is also the most commonly occurring allele in commercial durum wheats grown throughout the world. Glu-A1b has a positive effect on gluten strength as speculated by da 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 3.4% (Glu-B1b). This collection was lacking alleles Glu-B1c and Glu-B1l, 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-A1b 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-B1l and all new alleles at the Glu-A1 locus were rare and appeared in at least four of the six agroecological zones.

Polymorphism (Hs) at the Glu-A1 locus ranged from 0.0930.08 to 0.5780.04, and averaged 0.2610.04, whereas Hs at the Glu-B1 locus ranged from 0.6250.04 to 0.8900.02 and averaged 0.7330.02 (Table 2). Average Hs over both loci was 0.6900.25. Two of the agroecological zones (Salt and Shoubak in Table 2) exhibited very low diversity indices 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.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Allele</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glu-A1</td>
<td>a</td>
<td>12.7</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>6.7</td>
</tr>
<tr>
<td></td>
<td>c</td>
<td>76.1</td>
</tr>
<tr>
<td>New alleles</td>
<td>i</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>ii</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>iii</td>
<td>2.3</td>
</tr>
<tr>
<td>Glu-B1</td>
<td>a</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>34.7</td>
</tr>
<tr>
<td></td>
<td>c</td>
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</tr>
<tr>
<td></td>
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</tr>
<tr>
<td></td>
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</tr>
<tr>
<td></td>
<td>f</td>
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<tr>
<td>New alleles</td>
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</tr>
<tr>
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<td>1.2</td>
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<tr>
<td></td>
<td>iii</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>iv</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td>V</td>
<td>2.9</td>
</tr>
</tbody>
</table>
Table 2. Characteristics of 6 ecogeographical zones and $H_s$ estimates of two Glu-1 loci for 177 durum wheat landrace genotypes collected from Jordan.

<table>
<thead>
<tr>
<th>No. Zone</th>
<th>Long.</th>
<th>Lat.</th>
<th>Alt.</th>
<th>Glu-A1</th>
<th>$H_s$</th>
<th>Glu-B1</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Irbid</td>
<td>Min. 35 40</td>
<td>32 30</td>
<td>450</td>
<td>0.145±0.07</td>
<td>0.648±0.09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Max. 36 00</td>
<td>32 39</td>
<td>675</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 Ajlun</td>
<td>Min. 35 35</td>
<td>32 24</td>
<td>700</td>
<td>0.366±0.09</td>
<td>0.747±0.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Max. 36 04</td>
<td>32 30</td>
<td>1000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Salt</td>
<td>Min. 35 42</td>
<td>32 11</td>
<td>600</td>
<td>0.076±0.05</td>
<td>0.625±0.04</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Max. 35 54</td>
<td>32 22</td>
<td>1100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 Karak</td>
<td>Min. 35 44</td>
<td>32 00</td>
<td>620</td>
<td>0.255±0.09</td>
<td>0.735±0.04</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Max. 35 47</td>
<td>32 08</td>
<td>980</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 Tafhia</td>
<td>Min. 35 41</td>
<td>31 17</td>
<td>700</td>
<td>0.578±0.04</td>
<td>0.839±0.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Max. 35 51</td>
<td>31 50</td>
<td>960</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 Shoubak</td>
<td>Min. 35 28</td>
<td>31 04</td>
<td>1080</td>
<td>0.093±0.08</td>
<td>0.780±0.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Max. 35 41</td>
<td>31 04</td>
<td>1600</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Average**

0.26±0.04 0.73±0.02

Analysis of variance for $H_s$ of both loci revealed significant differences among agroecological zones. A larger portion (77%) of total variance in $H_s$ 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 $H_s$ estimates for both loci (Table 4). $H_s$ for Glu-A1 was negatively and significantly ($r=-0.467$; $P<0.05$) correlated with altitude of collection sites. On the other hand, $H_s$ for Glu-B1 was positively and significantly ($r=0.613$; $P<0.05$) 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 (Laghdad et al., 1987) indicated that variation occurs at the Glu-B1 locus in both the altitudinal set and geographical sites of landrace collections whereas allele 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).

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 morphological 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 (Laghdad 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.

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

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Glu-A1</th>
<th>Glu-B1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MS</td>
<td>% Variance</td>
</tr>
<tr>
<td>Among Zones</td>
<td>0.283**</td>
<td>45</td>
</tr>
<tr>
<td>Within zones</td>
<td>0.041</td>
<td>55</td>
</tr>
</tbody>
</table>

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


The Evaluation On Crossabilities of Chinese Wheat Landraces

M.C. Luo*, C. Yen, J.L. Yang and Z.L. Yang

(Triticaceae Research Institute, Sichuan Agricultural University, Dujianyang 611830, Sichuan, P.R. China), present address: Department of Agronomy and Range Sciences, University of California, Davis, CA 95616, USA

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 12 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 Kashia, 1981; Zeven, 1987; Luo et al., 1992, 1999a, 1999b). 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 kr2 on chromosome 5B. 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 Siberia.

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 1965 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, Shanxi, Henan, Gansu, Yunnan, Guizhou, Hunan, Shanxi, Hebei, and Tibet have been included in the investigation of wheat-rye crossability. For delineating 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 the
Table 1. The crossability types and their distribution in the Chinese bread wheat landraces

<table>
<thead>
<tr>
<th>Original locality</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Total</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of varieties</td>
<td>No. of varieties</td>
<td>No. of varieties</td>
<td>No. of varieties</td>
<td>Variety</td>
<td></td>
</tr>
<tr>
<td>Sichuan</td>
<td>65</td>
<td>36.72</td>
<td>62</td>
<td>35.03</td>
<td>34</td>
<td>19.21</td>
</tr>
<tr>
<td>Shaanxi</td>
<td>19</td>
<td>26.39</td>
<td>29</td>
<td>40.28</td>
<td>17</td>
<td>23.61</td>
</tr>
<tr>
<td>Henan</td>
<td>8</td>
<td>17.38</td>
<td>8</td>
<td>19.35</td>
<td>13</td>
<td>28.76</td>
</tr>
<tr>
<td>Guansu</td>
<td>15</td>
<td>34.85</td>
<td>16</td>
<td>35.42</td>
<td>14</td>
<td>27.08</td>
</tr>
<tr>
<td>Yunnan</td>
<td>36</td>
<td>32.30</td>
<td>22</td>
<td>36.07</td>
<td>1</td>
<td>1.64</td>
</tr>
<tr>
<td>Guizhou</td>
<td>12</td>
<td>33.33</td>
<td>18</td>
<td>46.15</td>
<td>6</td>
<td>15.38</td>
</tr>
<tr>
<td>Hunan</td>
<td>8</td>
<td>15.18</td>
<td>15</td>
<td>34.09</td>
<td>14</td>
<td>31.82</td>
</tr>
<tr>
<td>Shanxi</td>
<td>13</td>
<td>19.40</td>
<td>36</td>
<td>53.73</td>
<td>13</td>
<td>19.40</td>
</tr>
<tr>
<td>Hebei</td>
<td>8</td>
<td>28.57</td>
<td>12</td>
<td>42.86</td>
<td>4</td>
<td>14.29</td>
</tr>
<tr>
<td>Total</td>
<td>426</td>
<td>49.07</td>
<td>269</td>
<td>31.13</td>
<td>121</td>
<td>14.00</td>
</tr>
</tbody>
</table>

Rye was 82.1%, 74.0%, 80.8%, 78.2%, 73.6%, 71.9%, and 73.0%. The average weight being 75.9%. There is no significant difference between the maximum (82.1%) and the minimum (71.9%) (t-test). 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 k 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 k genes.

Group 3: Having the similar crossabilities to Chinese Spring. This group possesses recessive k1, k2 and k3 genes.

Group 4: Showing much higher crossability percentages than Chinese Spring, and having the genotype of k1 k2 k3

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 crossability were much lower than that of Chinese Spring (Table 1).

From Table 1, 144% of landraces investigated had similar crossability to Chinese Spring (Group 2). The landraces belonging to this group frequently occurred in Henan, Henan, Guansu, 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 k gene. There was no distribution of recessive k4 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 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 Triticaceae into wheat. As a representative of high crossability germplasm, "J-1116" has been successfully used in the crosses of wheat with Pachystachya huanuchsis Keng and Rosenbergia ciliata (Trin.) Nevski (Sun, 1992, Wang, personal communication).

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Use of Annual and Perennial Triticeae Species for Wheat Improvement.

A. Mujeeb-Kazi

CIMMYT, Lisboa 27, Apartado Postal 6-641, 06600 Mexico, D. F., MEXICO

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=8=14) and Secale cereale are also mentioned. The interspecific hybridization strategy offers alien genetic introgression opportunities, for which the closely related Triticeae species have a priority. Of these sources, the D genome T. tauschii (Euligio squarroso) accessions and some of the A genome species (T. beestockii, 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 polyphyly status of the contributing species. With the wide array of annual and perennial Triticeae species exist such alien genetic introgression 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 permuting 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 is on development of wheat germplasm that expresses enhanced resistance to Helminthosporium sativus. Cochliobolus sativus Ito and Kuribay or Helminthosporium leaf blight; compared to cultivar BH 146, 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 loss can be alarming, and losses up to 83.0% may occur. In Mexico, a naturally infected field screening hot spot exists in Pozo 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 relate to Helminthosporium leaf blight are described further.

BREEDING

The Interspecific 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 F1 hybrid, 2n=4x=34, was advanced by crossing onto it the wheat cultivars Glennson B1, then Alondra/Pavon and eventually selfed. These selfed variants were screened for resistance under the severely infected natural field conditions of Pozo 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 resistance 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/blade 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 existing resistant check (like BH 1146). The Th. curvifolium derived germplasm now figures up in 89.8% of the 1994 selections made by us our wheat breeding program in Pozo 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 interspecific 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).

Table 1. Agronomic characteristics of Cochliobolus sativus resistant spring bread wheat germplasm grown at Pozo Rica, Mexico, during the 1990-91 and 1992-93 field crop cycles.

<table>
<thead>
<tr>
<th>Germplasm</th>
<th>Grain yield</th>
<th>Days to photoperiodic sat</th>
<th>Plant height</th>
<th>1000-grain weight</th>
<th>Test weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>kg ha⁻¹</td>
<td>cm</td>
<td>g</td>
<td>kg ha⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Line -295-1</td>
<td>1997</td>
<td>100</td>
<td>87</td>
<td>29.4</td>
<td>72.4</td>
</tr>
<tr>
<td>Line -295-2</td>
<td>1664</td>
<td>106</td>
<td>87</td>
<td>27.4</td>
<td>76.4</td>
</tr>
<tr>
<td>Line -295-3</td>
<td>1431</td>
<td>106</td>
<td>87</td>
<td>25.4</td>
<td>74.1</td>
</tr>
<tr>
<td>Line -295-4</td>
<td>1580</td>
<td>106</td>
<td>86</td>
<td>25.5</td>
<td>69.5</td>
</tr>
<tr>
<td>Line -295-5</td>
<td>1603</td>
<td>110</td>
<td>93</td>
<td>25.3</td>
<td>72.4</td>
</tr>
<tr>
<td>BH 1146 (check)</td>
<td>945</td>
<td>106</td>
<td>85</td>
<td>27.1</td>
<td>71.1</td>
</tr>
<tr>
<td>Cline 79 (check)</td>
<td>967</td>
<td>105</td>
<td>85</td>
<td>16.7</td>
<td>38.6</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Germplasm</th>
<th>Leaves 1</th>
<th>Spike 2</th>
<th>Grain 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>b</td>
<td>(1-5)</td>
<td>(1-5)</td>
</tr>
<tr>
<td>Line -295-1</td>
<td>93</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Line -295-2</td>
<td>92</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Line -295-3</td>
<td>93</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Line -295-4</td>
<td>92</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Line -295-5</td>
<td>92</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>BH 1146 (Resistant)</td>
<td>93</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Cline 79 (Susceptible)</td>
<td>99</td>
<td>9</td>
<td>5</td>
</tr>
</tbody>
</table>

1 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.

2 = low infection and 9 = high infection.

3 = low grain infection and 5 = severely infected.

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 *Thiophyrum elongatum* based germplasm to *Cochliobolus sativus* at Poza Rica, Mexico, during the 1992-93 field crop cycle.

<table>
<thead>
<tr>
<th>Test material</th>
<th>Leaves</th>
<th>Spike</th>
<th>Grain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a</td>
<td>b</td>
<td>(1-9)</td>
</tr>
<tr>
<td>TF, <em>elongatum</em> (GH &amp; S)</td>
<td>92</td>
<td>92</td>
<td>2</td>
</tr>
<tr>
<td>CS/T, <em>elongatum</em></td>
<td>92</td>
<td>93</td>
<td>3</td>
</tr>
<tr>
<td>BR 200</td>
<td>92</td>
<td>93</td>
<td>3</td>
</tr>
<tr>
<td>GH 5</td>
<td>94</td>
<td>96</td>
<td>7</td>
</tr>
<tr>
<td>CS</td>
<td>94</td>
<td>97</td>
<td>7</td>
</tr>
<tr>
<td>Cco 72 (Susceptible)</td>
<td>99</td>
<td>99</td>
<td>9</td>
</tr>
<tr>
<td>BN 1140 (Resistant)</td>
<td>93</td>
<td>95</td>
<td>6</td>
</tr>
</tbody>
</table>

† 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.

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

<table>
<thead>
<tr>
<th>Germplasm</th>
<th>Leaves</th>
<th>Spike</th>
<th>Grain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a</td>
<td>b</td>
<td>(1-9)</td>
</tr>
<tr>
<td>GW</td>
<td>96</td>
<td>96</td>
<td>7</td>
</tr>
<tr>
<td>GW/T. <em>tauschii</em> (236)</td>
<td>92</td>
<td>92</td>
<td>3</td>
</tr>
<tr>
<td>DOY 1</td>
<td>95</td>
<td>97</td>
<td>7</td>
</tr>
<tr>
<td>DOY/T. <em>tauschii</em> (447)</td>
<td>92</td>
<td>92</td>
<td>3</td>
</tr>
<tr>
<td>DOY/T. <em>tauschii</em> (536)</td>
<td>92</td>
<td>92</td>
<td>3</td>
</tr>
<tr>
<td>SCOOP 1</td>
<td>97</td>
<td>97</td>
<td>8</td>
</tr>
<tr>
<td>*...*T. <em>monococcum</em> (98)</td>
<td>92</td>
<td>92</td>
<td>3</td>
</tr>
<tr>
<td>*...*T. <em>monococcum</em> (118)</td>
<td>92</td>
<td>93</td>
<td>3</td>
</tr>
</tbody>
</table>

† 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.

This dual approach, since the *T. tauschii* accession's field screening failed to provide conclusive data. In the same context the A genome species are utilized by producing the AABBB 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 AABBB hexaploid is next used in direct crosses to susceptible T. aestivum cultivars. Data from DO genome synthetic and A genome hexaploids for the *Helminthosporium leaf blight* scoring 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 AABBD and AAAABB germplasm was highly expressive except for grain finish scores that were not recorded because of late maturity of these derivatives.

Allen genetic diversity from annual/ perennial *Triticaceae* 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.

LITERATURE CITED


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 the advanced derivatives from *T. aestivum* synthetic hexaploid crosses, it is attributed to a contribution of the *T. tauschii* accession. The accession contributing to resistance can then be utilized directly in crosses (Alonso and Kimber 1984) with susceptible bread wheats. We have adopted
Plant Germplasm Resources

S. A. Eberhart and H. E. Bockelman
National Seed Laboratory, USDA, ARS 1111 So. Mason St. Fort Collins, CO 80521-4500. National Small Grains Collection, USDA, ARS, P.O. Box 307, Aberdeen, ID

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 fiber quality, and seed, 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. The base collection is preserved at -18°C at the National Seed Storage Laboratory. The NPGS 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 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 1940s, 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 I) living and growing collections or 2) living but quiescent collections (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 apples 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 to maintain. Most importantly, living and growing collections may be susceptible to frosts, droughts, diseases, insects, and other disasters. Collections of living but quiescent organs 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 metabolic 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 storage types. There are many different types of tissues that cannot 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 axially 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. Tropical, soil, 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, cassava, avocado, mango, cocoa, 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. Seeds should be dried and stored at a low temperature (Justice and Bass, 1976). Research by Justice and Bass (1976), Bass (1980), and Bass and Stanwood (1978) showed that reducing the storage temperature from 3°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 handling 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 beneficial.

Although seed drying extends longevity, there are limits to the benefits; 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 NSF (Vertucci and Roos, 1990, 1993; Vertucci et al., 1994) have established that, contrary to the validity equations (Blakey et al., 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 with both 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, 1995).

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 potentials as low as 0.3 g H2O/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, 1980; Vertucci et al., 1991, 1993). The critical moisture content for desiccation damage increases as temperature is reduced (Vertucci et al., 1995). Thus, the window for reversible 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 H2O/g dw (60% seed moisture). These materials must be preserved in the " vitrified " state (Wesley-Smith et al., 1995).

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; Farzaneh et al., 1993). Vitrification shoot tips of sweet potato (Towill and Jarrett, 1992) have been developed; the survival rate is 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 a fast rate so 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 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-thermal protectants such as dimethyl-sulfoxide (DMSO) and ethylene glycol are also commonly used. Vitrified samples must be stored at temperatures where the glassy propagules or cells do not undergo storage at very low temperatures, either directly in liquid nitrogen (-196°C) or in the vapor above liquid nitrogen (-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 transplantled or grafted onto existing stock. If the process is successful, certain cryopreservation treatment is evaluated by the proportion of propagules that develop into growing plants.

THE NATIONAL PLANT GERMLASM SYSTEM

The Research and Marketing Act of 1946 (Public Law 733) authorized the creation of four Regional Plant Introduction Stations (Anacortes, 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 Grain Collection, now in Aberdeen, Idaho, began in 1894 as a breeder's collection in Beltsville, Maryland. The Inter-Regional Potato Introduction Station, Sacohegan 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). Since its establishment in 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, 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 to be accomplished in 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 international 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 determine 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 international funds. The more than 440,000 accessions maintained in the NPGS active collections have been divided among these 19 repositories.

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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 materials; and maintains the unique Plant Introduction (PI) numbers; publish an annual USDA Plant Inventory of newly received accessions; and serves as a liaison on quarantine matters. PI-numbers are assigned when the NPGS is acquired through exchanges, exploration (domestic and foreign), special projects and agreements, gifts, and travels. In addition to introduced germplasm, all released plant materials (cultivars, 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 donor.

The Plant Exploration Office (PEO) works with germplasm curators, Crop Exploration (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 defined 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 CD-ROMs. Data are entered 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
NPGRS and foreign countries are recorded in GRIN.

All plant germplasm entering the NPGRS 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 NPGRS active and base collections.

The NGRPL 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, documentations, regeneration, evaluation, and research goals to various components of the NPGRS.

Although the ARS components of the NPGRS 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 NPGRS and coordinate activities. They also provide administrative support to the various advisory boards and committees for plant genetic resources.

Plant germplasm collections in NPGRS 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 Triticaceae, Artem, and Oxyis 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 1,154 samples of various Triticeae grasses were distributed. Regenerations are completed at the Pullman and Central Ferry farms. The NPGRS 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 NSLL base collection. The principal mission of NSLL is to preserve the base collection of the NPGRS and to conduct research to develop new and improved technologies for the preservation of seed and other plant propagules. The goal of NSLL is long-term preservation of back-up samples of all accessions maintained in active collections at national plant germplasm repositories. The NSLL 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 NSLL 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 NSLL 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 NSLL, 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 NSLL 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 NSLL work closely with all components of NPGRS.

Table 1. National Plant Germplasm System Holding of Triticeae

<table>
<thead>
<tr>
<th>Species</th>
<th>Common Name</th>
<th>Numbers of Accessions</th>
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<td>Agropyron sp.</td>
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<td>Agropyron cristatum</td>
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<td>Agropyron desertorum</td>
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<td>Agropyron epigeios</td>
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<td>Amblyopyrum violaceum</td>
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<tr>
<td>Elymus canadensis</td>
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<tr>
<td>Elymus caninus</td>
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<td>Elymus dahlianum</td>
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<tr>
<td>Elymus elymoides</td>
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<tr>
<td>Elymus glaucescens</td>
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<tr>
<td>Elymus hordeaceus subsp. lanostachus</td>
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<td>Elymus tetragonurus</td>
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<tr>
<td>Elymus triticeus</td>
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<tr>
<td>Hierochloë sp.</td>
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<td>Hierochloë amplexicaulis</td>
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<tr>
<td>Hordeum vulgare subsp. vulgare</td>
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<td>Hordeum sp.</td>
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<td>Paspalum distichum</td>
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<td>Paspalum densiflorum</td>
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<td>Poa annua</td>
<td>137</td>
<td></td>
</tr>
<tr>
<td>Poa pratensis</td>
<td>101</td>
<td></td>
</tr>
<tr>
<td>Poa secunda subsp. cerealis</td>
<td>1806</td>
<td></td>
</tr>
<tr>
<td>Scirpus spp.</td>
<td>135</td>
<td></td>
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<tr>
<td>Triticum aestivum</td>
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<td></td>
</tr>
<tr>
<td>Triticum aestivum var. aestivum</td>
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<td></td>
</tr>
<tr>
<td>Triticum dicoccoides</td>
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<tr>
<td>Triticum durum</td>
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<td></td>
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<tr>
<td>Triticum durum</td>
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<tr>
<td>Triticum aestivum</td>
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<tr>
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<td></td>
</tr>
<tr>
<td>Triticum aestivum</td>
<td>85907</td>
<td></td>
</tr>
</tbody>
</table>

*app. is a grouping of species not listed separately, or accessions not yet classified.*
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: Janick, J., 1989). Since 1989, NPGS catalogues about 57,000 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 more than 8,000 plant species, that are now preserved in the NPGS.

The NPGS has been described as a "user-driven system." Between 1982 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 available germplasm for a desired attribute, new accessions are needed that will provide the highest probability of identifying useful source materials with the specific attributes. 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 Franks (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 1,000 suggested as a maximum number.

Brown (1989a) recommended stratified sampling methods when estimating diversity. Grouping begins with taxonomic affinity (e.g., species, subspecies, cytoplological races). Accessions within each taxon can be assigned to strata based on ecogeographic zones and genetic characteristics (morphological, physiological 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 geographical 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 genetic information 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 limited 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 randomly selected 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.

Appliances and equipment (e.g., plate changers, pipettors) used for specific applications (e.g., determination of seed viability) may 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 may have 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 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 have developed national resource preservation programs with an associated gene bank. IPGRI indicates that the number of gene banks worldwide holding ex situ collections is 1,000 (personal communication). Several of these, in addition to the Fusarium, were part of the former IBGR network of designated base collections (Table 2). The NPGS maintains a close working relationship 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 World 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 wide 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 I) 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 right 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>
<thead>
<tr>
<th>Institution</th>
<th>Species</th>
<th>Number of Accessions</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMS (China)</td>
<td>Triticium</td>
<td>22,457</td>
</tr>
<tr>
<td>CIMMYT (Mexico)</td>
<td>Triticium</td>
<td>10,784</td>
</tr>
<tr>
<td>OR (Italy)</td>
<td>Triticium</td>
<td>42,000</td>
</tr>
<tr>
<td>OR (Spain)</td>
<td>Triticium</td>
<td>98,200</td>
</tr>
<tr>
<td>OR (Greece)</td>
<td>Hordeum, Secale</td>
<td>14,410</td>
</tr>
<tr>
<td>OR (Japan)</td>
<td>Hordeum</td>
<td>19,176</td>
</tr>
<tr>
<td>OR (Canada)</td>
<td>AmphiTriticum</td>
<td>1,200</td>
</tr>
<tr>
<td>BG/PSC (India)</td>
<td>Secale</td>
<td>1,200</td>
</tr>
<tr>
<td>BG/ES (Spain)</td>
<td>Secale</td>
<td>91,460</td>
</tr>
<tr>
<td>BG (Israel)</td>
<td>Secale</td>
<td>70,880</td>
</tr>
</tbody>
</table>

Most ACs were part of the former IBGR 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 around the world was necessary to provide scientists with source materials for their programs.

Technologies required to preserve genetic resources 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 permit living organisms to be preserved in "suspending 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 landscape 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 175,403 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.

LITERATURE CITED


Forage Species in Xinjiang Northern Natural Grasslands: Grasses

Li Bao-Jun

Grassland Research Institute, Xinjiang Academy of Animal Science, Urumqi P.R.C. (830000)

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 grassland 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 plants breeder with abundant material for pasture improvement both in the native and introduced grasses. Because of overgrazing, reclaimed, 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, Birata, 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 axes 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 2°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 bi-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 do not have a hot-summer and cold-winter fast growth and seed dormancy.

GERMPLASM

Agronomic characteristics and adaptation 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...
plants 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, Elymigia and Alpiceus grow in areas that receive more than 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 August. 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 Achillea, Leymus, Agropyron, Stipa and Calamagrostis, which grow in the lowland plain, complete their development early because rainfall is limited and maximum summer temperatures 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 tilling 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 CO2 in their environment 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 one, but as the environments are varying yields are different due to growing height, taller 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 environment existing in the height and growth rate. However, within present and traditional grazing practices, 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 (Achillea splendens and A. inequivalens). 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 exchanges 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 June to August except in extreme alpine systems, where flowering may be in June. However, flowering period in the plain lowlands, 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 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.

Morphological and biological deterrent

The economic benefit of animal grazing on pasture species is dependent upon their morphological characteristics, grazed that have been selected under heavy grazing pressure tolerate the defoliation, or have evolved avoidance mechanisms, that makes it unpalatable. Possible examples within the Xinjiang grazing pastures include Sila species which have very a long son on the seed, and Achillea inequivalens, which contains alkaloids to toxic level and if grazed may be fatal to animals. However, the most 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 that may cause digestive trouble. However, the crude protein and digestible carbohydrates remain remarkable high 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 polymorphism. Under species environments, co-adapted gene complex have evolved over a long period of time by selection. During this selection process the perrillity and tiller development are far more favorable in the survivability and development of this new species ectotype. Due to a high level of environmental variation observed within the Xinjiang natural grasslands, there is an abundance of different ectotypes. 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 been 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-evolved 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 open pastures 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 for 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 future 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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**Table 1.** The height, tiller yield and weight/thousand of grass species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Accession #</th>
<th>Origin</th>
<th>Height</th>
<th>Tillers</th>
<th>Yields</th>
<th>Weight/1000</th>
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</thead>
<tbody>
<tr>
<td><strong>Dactylis glomerata</strong></td>
<td>X002-1</td>
<td>Altai</td>
<td>131.25</td>
<td>5.6</td>
<td>7.24</td>
<td>0.8766</td>
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<td>X002-1</td>
<td>Yili</td>
<td>131.63</td>
<td>13.9</td>
<td>5.34</td>
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<td>X002-1</td>
<td>Taghen</td>
<td>124.38</td>
<td>7.2</td>
<td>9.28</td>
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<td>X002-1</td>
<td>Yili</td>
<td>127.25</td>
<td>12.9</td>
<td>8.24</td>
<td>0.8037</td>
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<tr>
<td><strong>Agrostis gigantea</strong></td>
<td>X002-2</td>
<td>Altai</td>
<td>131.25</td>
<td>12.6</td>
<td>9.91</td>
<td>0.6959</td>
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<td><strong>Bromus inermis</strong></td>
<td>X002-2</td>
<td>Taghen</td>
<td>131.76</td>
<td>9.3</td>
<td>5.34</td>
<td>1.4183</td>
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<td>9.3</td>
<td>4.95</td>
<td>0.6220</td>
</tr>
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</table>

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The data is from the station of the south mountains in Urunku.
Diversity of Trypsin Inhibitors in Cultural and Wild Barley

Ladogina M.P., Ovchinnikov A.N., Fetisov A.V.

Vavilov Institute of General Genetics, Russian Academy of Sciences
Gubkin St. 3, 117809 Moscow, Russia.

ABSTRACT

Diversity of trypsin inhibitors was studied in 35 spring barley cultivars (Hordeum vulgare). 21 samples of H. spontaneum, and 3 samples of H. agricola. 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 Moralez et al. [4]. They identified three allelic variants of trypsin inhibitors (BTI-Cm1, -Cm2, and -Cm3) and demonstrated that the last two consisted of several components (BTI-Cm2.1, -Cm2.2, -Cm2.3 and BTI-Cm3.1, -Cm3.2 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. agricola 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 h, 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 gelatinize layer of Mircak film for 10 minutes and zones of trypsin inhibitors were developed by the method [6]. The film was dried, put on 1% agrose gel containing 0.05 M tris-HCl, pH 7.6; 0.1 M NaCl, trypsin 250 ng/ml, and incubated with it at 37°C for 60 minutes. Bands of undegasted gelatine on the film corresponded to trypsin inhibitors.

Isoelectric focusing in 4% polyacrylamide gels containing 4-9 Servalytes and 9 M urea was performed for determining isoelectric points (pl) 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 P4-DG (Bio-Rad) column (12 cm) and concentrated on Minicon B-13 (Bio-Rad) concentrator. The pH gradient of the gel was measured by determining pH values in water extracts from 1 cm sections of the gel. Proteins were stained by Coomassie R-250, and bands of trypsin inhibitors were revealed by replication to Micak film as described before.

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. agricola 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 (isoelectric points 4.1 and 7.9), the fourth one (A) is considerably different (patterns 4-5), and protein calibration. Sytax Version 5.0 was used for statistical calculations of the obtained results. Least significance difference (LSD0.05) was estimated as described [9].

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 Moralez [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 pl 6.2, 6.1, 5.8, and 5.6 as well as two minor components with pl 5.9 and 5.7, thus, all the components of this variant have acid pl.

![Fig. 1. Diversity of trypsin inhibitors in cultural (1-4) and wild (5-10) barley.](image1.png)

![Fig. 2. Bands of proteins (1) and trypsin inhibitors (2) of cv. Nutans 970 revealed by isoelectric focusing.](image2.png)
Breeding Potential of Exotic Barley Germplasm

Merja Vetelainen

The Swedish University of Agricultural Sciences, Department of Plant Breeding Research, S-23831 Svalöv, Sweden

Abstract

Utilization of exotic germplasm offers an approach to broaden genetic variability in breeding populations. This study was conducted in order to compare germplasm of exotic origin with adapted Swedish barleys with respect to genetic differences and to evaluate first cycles of pre-breeding i.e. agronomic traits in complex exotic x adapted crosses. Allotome studies showed the following Net's gene diversities among parents: 0.13 (adapted parents), 0.16 (landraces) and 0.25 (M. spontaneum). Cluster analysis based both on allotome 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 six 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 programs.

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 (Beentegard & 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 (Ferd-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 program 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 & Frye 1973, 1975, Frye et al 1981) and barley (Vega & Frye 1980, Rogers 1982, Lehmann & Bodnar 1988). These studies support the idea that useful genes affecting quantitative as well as qualitative traits can be obtained from exotic germplasm. In their review 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

<table>
<thead>
<tr>
<th>Name/Origin</th>
<th>Accession no</th>
<th>2/6-row</th>
<th>Naked/covered</th>
<th>Seed colour backlight</th>
<th>Hordeum sp. vulgare/spontaneum</th>
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<tr>
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<tr>
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<td>c</td>
<td>i</td>
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<tr>
<td>H 3059</td>
<td>Jordan</td>
<td>2</td>
<td>c</td>
<td>i</td>
<td>spontaneum</td>
</tr>
<tr>
<td>H 3064</td>
<td>Jordan</td>
<td>2</td>
<td>c</td>
<td>i</td>
<td>spontaneum</td>
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</table>

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 the fecundity of crossbreeding, 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 3 accessions of wild barley, Hordeum vulgare sp. 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 195 hybrid lines were adapted. These highly heterogeneous 8-way hybrids, which contained from 25 to 50 % exotic germplasm, were used in the glasshouse experiment.

Allozyme studies

Allozyme variation of 6-phosphoglucomutase dehydrogenase (APGD), isocitrate dehydrogenase (MDH), aconitate hydratase (AOC), 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 %) (After: 1982), 0.01% 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 photosynthetic was used in the glasshouse with a light temperature of 187°4°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. Scraw length (cm) was measured from each of 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 = (eyear + f tonters + lter + TKW) / 4

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 favorable. Therefore, the lowest and the highest classes were considered similarly in the case of number of ears per plant, while 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 parents in the results were further analysed by Tukey’s test. To analyse electrophoretic and agronomic class data, the NTSSYS-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 $SM = I/m$, where $I$ is the number of matches in class and $m$ 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 $H = 1 - pq _{ij}$, where $pq _{ij}$ is the frequency of the $i$th allele at the $j$th 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 analysis 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 5v 892368 and 5v 891412. The latter line included two different genotypes and a (Fig. 1). The difference was found in one single EST-locus. The occurrence of these Swedish lines within the cluster of Asian landraces may be caused by the primitive landrace, Hordeum distichum cv.
Table 2. Phenotypic classes and score values for index

<table>
<thead>
<tr>
<th>Trait</th>
<th>Phenotypic class</th>
<th>Score for Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ear emergence, days</td>
<td>20-25</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>26-30</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>31-35</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>36-40</td>
<td>1</td>
</tr>
<tr>
<td>Number of ears per plant</td>
<td>1-5</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>6-10</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>11-15</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>15&lt;</td>
<td>1</td>
</tr>
<tr>
<td>Straw length, cm</td>
<td>60-80</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>81-100</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>101-120</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>120-135</td>
<td>1</td>
</tr>
<tr>
<td>TAM, g</td>
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<td>21-30</td>
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<tr>
<td></td>
<td>31-40</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>41-65</td>
<td>4</td>
</tr>
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</table>

Laegiogitum, 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) revealed 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 & Duwig 1989). Therefore, the next step was to evaluate four easily measureable 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 (Jarstad 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 yield 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; Bendorjacek 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.](image)
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

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>MS</th>
<th>Ear emergence</th>
<th>No ears/Plant</th>
<th>Straw length</th>
<th>1000 kernel weight</th>
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<td>Blocks</td>
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<td></td>
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<td>B (parent lines)</td>
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<td>8</td>
<td>223.39 **</td>
<td>246.5 **</td>
<td>109.0 **</td>
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<td>B (8-way hybrids)</td>
<td>B2</td>
<td>32.3 **</td>
<td>36.3 **</td>
<td>39.3 **</td>
<td>168.3 **</td>
</tr>
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<td>Parent lines</td>
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<td>46.9 **</td>
<td>46.9 **</td>
<td>395.4 **</td>
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<td>- between groups</td>
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<td>39.6 **</td>
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<td>368.6 **</td>
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<td>- adapted</td>
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<td>71.4 **</td>
<td>71.4 **</td>
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<td>- landraces</td>
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<td>54.5 **</td>
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<td>326.8 **</td>
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</tr>
<tr>
<td>B-way hybrid lines</td>
<td>H1</td>
<td>54.7 **</td>
<td>54.7 **</td>
<td>54.7 **</td>
<td>703.8 **</td>
</tr>
</tbody>
</table>

* Significant at the 5% level
** Significant at the 1% level

Table 4. Summary statistics and Tukey's test for 4 traits measured on parent and B-way hybrid lines. (C = coefficient of variation)

<table>
<thead>
<tr>
<th>Group</th>
<th>Ear emergence (days)</th>
<th>No ears/plant</th>
<th>Straw length (cm)</th>
<th>1000 kernel weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adapted</td>
<td>42.1</td>
<td>72.5</td>
<td>10.2</td>
<td>56.38</td>
</tr>
<tr>
<td>Landraces</td>
<td>34.8</td>
<td>87.2</td>
<td>14.5</td>
<td>51.52</td>
</tr>
<tr>
<td>Spont.</td>
<td>65.1</td>
<td>115.3</td>
<td>19.6</td>
<td>51.64</td>
</tr>
<tr>
<td>Hybrids</td>
<td>31.3</td>
<td>70.1</td>
<td>15.6</td>
<td>48.32</td>
</tr>
</tbody>
</table>

*C = coefficient of variation

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

LITERATURE CITED


Evaluation and Utilization of Biodiversity in Triticaceae for Wheat Improvement

A. B. DAMANIA and J. VALKOUN

International Center for Agricultural Research in the Dry Areas (ICARDA), PO Box 5466, Aleppo, Syria. Present address: Genetic Resources Conservation Program, University of California, Davis, CA 95616-8602, USA

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 Indianapolis cultivars and their wild relatives have been collected from the centers of diversity. However, gene banks 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 aware in using germplasm that after years of work yielded uncertain results. However, in recent years they have begun to successfully utilize non-conventional germplasm (wildfizen and obsolete forms) in their crossing blocs. The substantial progress at ICARDA in the evaluation and utilization of Triticaceae 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 Triticaceae 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 those 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 genetic relationships among individual members which may co-evolve as a result of gene exchange but may also diverge under localized forces of evolutionary change (Jan, 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 (Shevshuk, 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 Darnamia, 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 (1967) 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 Darnamia (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 (Darnamia, 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 germplasm 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 (Darnamia 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 vigor growth 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 sp., 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 hybrids of the wild species in particular 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 Triticaceae species. Darlington (1967) 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 Triticaceae and, as such, is as near to an ideal site for evaluation as can possibly be found (Srivastava and Darnamia, 1989). Evaluation carried out at near ideal sites minimize the effect of natural selection on the accesses' 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 Triticaceae species through manipulation of the homoeologous gene which 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.

<table>
<thead>
<tr>
<th>Species</th>
<th>PI1610</th>
<th>Genotype</th>
<th>No. of Lines</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aeg. dubina</td>
<td>4X</td>
<td>UMM</td>
<td>56</td>
</tr>
<tr>
<td>Aeg. speltoides</td>
<td>2X</td>
<td>UMM</td>
<td>23</td>
</tr>
<tr>
<td>Aeg. columnar</td>
<td>4X</td>
<td>UMM</td>
<td>27</td>
</tr>
<tr>
<td>Aeg. tauschii</td>
<td>2X</td>
<td>UMM</td>
<td>3</td>
</tr>
<tr>
<td>Aeg. pinnatissima</td>
<td>4X</td>
<td>UMM</td>
<td>53</td>
</tr>
<tr>
<td>Aeg. ruvularis</td>
<td>4X</td>
<td>UMM</td>
<td>4</td>
</tr>
<tr>
<td>Aeg. squarrosa</td>
<td>2X</td>
<td>UMM</td>
<td>3</td>
</tr>
<tr>
<td>Aeg. tauschii</td>
<td>6X</td>
<td>UMM</td>
<td>5</td>
</tr>
<tr>
<td>Aeg. tristis</td>
<td>4X</td>
<td>UMM</td>
<td>72</td>
</tr>
<tr>
<td>Aeg. unteilulata</td>
<td>2X</td>
<td>UMM</td>
<td>3</td>
</tr>
<tr>
<td>Aeg. seifert</td>
<td>4X</td>
<td>UMM</td>
<td>3</td>
</tr>
</tbody>
</table>

Total 205
UTILIZATION OF GENETIC RESOURCES COLLECTIONS

For the purpose of utilization systematic analysis and description of germplasm 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. Thus 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, Duyvay et al. (1967) 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 alleles. 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 primitive 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 Agropyros umbellata was initially crossed with Triticum dicoccoides to produce an amphiploid progeny. This was crossed with a wheat cultivar but the F1 was male sterile and had to be back-crossed to asecostum twice. The progenies of this back-cross were tested for leaf-rust resistance which was present in the wild species. A resistant plant was selected 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 (Szalanski, 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. It is an experiment to assess tolerance to artificially created salinity and its effect on morphological traits in some lines of Tritium boeicicum and Tritium dicoccoides was carried out at ICARDA using soil culture techniques in a controlled environment with eight replicates. In general, T. dicoccoides was found to be more tolerant to salinity than T. boeicicum (Dumanlou 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 using 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 Tritium durum x T. dicoccoides cross characters such as, brittle rachis, glume hairiness, profuse unsynchronized tillering, hybrid necrosis, grass clumping and loose brown pustule 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 progenitors (mainly Tritium dicoccoides) and durum wheat, and between durum wheat and obsolete forms with disease resistance (such as T. dicoccum), were made during 1969-71 in order to develop genetic stocks with stable desirable characters which the breeders could use directly in their cropping 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 cultivar 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 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. carlum (which are tolerant to drought and possess resistance to yellow rust) were also crossed with accessions of T. dicoccum to improve gene combinations in the latter. Provenges 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-biotic 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>
<thead>
<tr>
<th>Female parent</th>
<th>Male parent</th>
<th>No. of seeds</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. durum Haurani</td>
<td>T. dicoccoides</td>
<td>600340</td>
</tr>
<tr>
<td>T. durum Haurani</td>
<td>T. dicoccoides</td>
<td>600548</td>
</tr>
<tr>
<td>T. durum Haurani</td>
<td>T. dicoccoides</td>
<td>600474</td>
</tr>
<tr>
<td>T. durum Haurani</td>
<td>T. dicoccoides</td>
<td>600455</td>
</tr>
<tr>
<td>T. durum Haurani</td>
<td>T. dicoccoides</td>
<td>600874</td>
</tr>
<tr>
<td>T. durum Cham 1</td>
<td>T. dicoccoides</td>
<td>600340</td>
</tr>
<tr>
<td>T. durum Cham 1</td>
<td>T. dicoccoides</td>
<td>600415</td>
</tr>
<tr>
<td>T. durum Cham 1</td>
<td>T. dicoccoides</td>
<td>600392</td>
</tr>
<tr>
<td>T. durum Cham 1</td>
<td>T. dicoccoides</td>
<td>600392</td>
</tr>
<tr>
<td>T. durum Cham 1</td>
<td>T. dicoccoides</td>
<td>600945</td>
</tr>
<tr>
<td>T. dicoccum 600780</td>
<td>T. turgidum</td>
<td>09065</td>
</tr>
<tr>
<td>T. dicoccum 600768</td>
<td>T. turgidum</td>
<td>09065</td>
</tr>
<tr>
<td>T. dicoccum 600770</td>
<td>T. turgidum</td>
<td>22276</td>
</tr>
<tr>
<td>T. dicoccum 600768</td>
<td>T. dicoccum</td>
<td>14253</td>
</tr>
<tr>
<td>T. dicoccum 600765</td>
<td>T. dicoccum</td>
<td>14217</td>
</tr>
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<td>T. dicoccum 600774</td>
<td>T. dicoccum</td>
<td>14215</td>
</tr>
<tr>
<td>T. dicoccum 600767</td>
<td>T. turgidum</td>
<td>12276</td>
</tr>
<tr>
<td>T. dicoccum 600767</td>
<td>T. turgidum</td>
<td>12276</td>
</tr>
<tr>
<td>T. polonicum 10994</td>
<td>T. dicoccum</td>
<td>690771</td>
</tr>
<tr>
<td>T. polonicum 12194</td>
<td>T. turgidum</td>
<td>136071</td>
</tr>
<tr>
<td>T. sepieta 08661</td>
<td>T. compactum</td>
<td>37367</td>
</tr>
</tbody>
</table>
Attempts to Produce Alien Addition Lines in Triticum durum

Domenico Pignone
CNR - Istituto del Germoplasma, Bari, Italy

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 chromosomcs to tetraploid wheat. Aegilops caudata, Ae. longissima and Dasypogon villum were used as chromosome donors. The crossing program involved the production of amphitoids with the wheat cytology 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 last 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 introgressed 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. ‘Langsdon’, some aneuploid series have been developed, namely double-dielotetosomics, dinonoeutosomics, 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 Dasypogon villum (Blanco et al. 1987). In 1968 at the Germplasm Institute of Bari we started a project aimed to produce monosomic or, better, disomic alien additions of Aegilops or Dasypogon 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 amphiploids involving Triticum durum, Aegilops caudata, Ae. longissima and Dasypogon villum, respectively, with wheat cytology obtained earlier. Five different wheat accessions were used as the female parent in the production of the amphiploids and as recurrent male parent in later crosses. A list of the parent material is given in Table 1.

BC2 seeds were scored for chromosome number: root tips from plants showing 2n = 29 were banded in order to try cytologically to identify the extra chromosome. 2n = 29 BC2 plants were selfed in order to try the obtision of disomic additions or crossed with the wheat parent in order to maintain them.
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 squashes 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 amphihaploids (7 durum x alien diploid species) which had been previously obtained in crossing programs. The main advantage of this choice was evident in the efficiency 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 amphihaploids have better fertility than amphihaploids and consequently the BC1 seed set is higher. Amphihaploids with the wheat cytoplasm were chosen in order to avoid cytoplasmic influence on genotypogenesis. The female gametes of amphihaploids show high levels of meiotic non reduction, thus yielding genotypes which are genetically indistinguishable from those of the relative amphihaploid.

In Table 2 the data from the backcrossing (BC) program are reported on the basis of the amphihaploids involved. 509 BC2 seeds were obtained out of 515 pollinated spikes on BC1 plants (0.999 seeds/spikelet); the low seed set depends on the reduced fertility of the 2n = 35 BC1 plants. The backcrosses of some amphihaploids were much more fertile than the average, and particularly high values were observed in BC2 from XX O3 (0.363 seeds/spikelet) and XX 15 (0.381 seeds/spikelet); also backcrosses of XX O9 showed high fertility (0.17 seeds/spikelet) although not as high as the previous hybrids. These amphihaploids involved D. villosum (OX O5) and Ae. caudata (XX O9 and XX 15). Most of our work concentrated on these BC2 seeds. The backcrosses of other amphihaploid set fewer seeds.

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

<table>
<thead>
<tr>
<th>Amphihaploid</th>
<th>Seeds</th>
<th>Spikelets</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>XX O4</td>
<td>311</td>
<td>995</td>
<td>0.33</td>
</tr>
<tr>
<td>XX O5</td>
<td>183</td>
<td>501</td>
<td>0.36</td>
</tr>
<tr>
<td>XX O6</td>
<td>63</td>
<td>1856</td>
<td>0.34</td>
</tr>
<tr>
<td>XX O7</td>
<td>104</td>
<td>507</td>
<td>0.20</td>
</tr>
<tr>
<td>XX 12</td>
<td>5</td>
<td>24</td>
<td>0.2</td>
</tr>
<tr>
<td>XX 13</td>
<td>69</td>
<td>868</td>
<td>0.07</td>
</tr>
<tr>
<td>XX 15</td>
<td>69</td>
<td>181</td>
<td>0.38</td>
</tr>
</tbody>
</table>

The big difference observed in seed set in the backcrosses of different amphihaploids might be the influence of the alien genome in the amphihaploids alone or the interaction resulting from the genetic combination in the hybrid. In fact, although possessing the same genome, the accesses used in the crossing program were rather genetically different: TT 01 was the old Italian cv 'Senatore Cappelli' (in XX O9); 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 already been evidenced during the course of the crossing program (Pignone and Cifarelli 1990).

The BC2 seeds were analysed for chromosome numbers; out of 599 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 BC1 plants with D. villosum additions were 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. caudato line and from two different wheat accesses, TT 01 and TT 08, are the most successful accession in producing addition lines was TT 08. Nine out of 15 plants had its genetic background.

The chromosomes of those 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 a 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 BC2 plants were not fully vigorous: tilling was poor, culms developed late and spikes were rather small. Anthers were shrivelled and did not deliver much pollen. Setting was, therefore, quite difficult, and often self-fertilization was manually assisted, breaking mature anthers under 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 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 = 38 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 cytagenic 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 losing the lines.
Geographical Distribution, Ecology and Diversity of *Triticum urartu* Populations in Jordan, Lebanon and Syria

J. VALKOUN, A.B. DAMANIA and M. VAN SLAGEREN

International Center for Agricultural Research in the Dry Areas, Aleppo, Syria

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, Dvořák 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 Rřae 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

LITERATURE CITED


gladin 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 descriptors. 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 gladin polymorphism, which was detected by means of Al-1-azeazacyclamide 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 wheats, Triticum booeicum Boiss. emend. E. Schem. The geographical distribution in this region of urartu wheat is presented in Fig. 1.

In general, its distribution overlaps with that of Triticum dicoccoides (Kärn. 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 Bekaa 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. booeicum 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 1: Symaptic occurrence of T. urartu with other wheats in the Near East.

<table>
<thead>
<tr>
<th>Country</th>
<th>Alienpatric</th>
<th>Symaptic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jordan</td>
<td>16</td>
<td>9</td>
</tr>
<tr>
<td>Lebanon</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Syria</td>
<td>4</td>
<td>12</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Parent rock</th>
<th>Altitude (m asl)</th>
<th>Rainfall (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BA</td>
<td>3500-1000</td>
<td>500</td>
</tr>
<tr>
<td>500-3500</td>
<td>450-3500</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Country</th>
<th>Area occupied (ha)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jordan</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Lebanon</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Syria</td>
<td>8</td>
<td>8</td>
</tr>
</tbody>
</table>

Almost half of the collection sites belong to the low-rainfall category with annual rainfall below 350 mm. This, as well as 30 percent 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 addition, 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 Bekaa's valley in Lebanon a truly weedy population was found growing in a barley field. The urartu population was able to succeed 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; (ii) 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 gladin polymerphism data. Gladin storage proteins are extremely variable and may represent a multiple gene family (Payne 1987). In other diploid wheats, the wild T. booeicum and cultivated T. monococcum L., gladin polymorphism is controlled by two independently inherited loci, Gl-A1 and Gl-A2, located on chromosomes 1A and 6A, respectively (Metakovsky and Babov 1992a, 1992b). The first 'upper' locus usually controls the synthesis of w- and y-gladian, whereas the other 'lower' locus codes for e- and l-gladian. Our electrophorograms of gladins in T. urartu indicated occasional recombination between all e-gladins bands recombined as a block with gladin of the lower electrophoretic mobility, mostly w- and y-gladins. Consequently, variants in the e-gladin 'lower' block were considered to represent diversity in the Gl-A2 locus and those in the 'upper' block were supposed to correspond to the Gl-A1 locus. Mean heterozygosity index values (Hw) were calculated for 28 populations as an average of the two loci data. The results are summarized in Table 4.

The most diverse populations (Hw 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.
Koch (Neve 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 impossible task, we suggest in situ conservation as a complementary conservation strategy for the most diverse populations in distinct ecosystems. 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 (NCARRTT), Amman, Jordan, The Agricultural Research Institute (ARI), Tel Amara, Lebanon; and The Agricultural Research Center (ARC), Douma, Syria. Thanks also to Prof. J. Gloss Waines, University of Riverside, U.S.A., for assistance in manuscript preparation and editing.

**LITERATURE CITED**


**GENETIC EFFECTS OF ALIEN CYTOPLASMS ON HEAT TOLERANCE IN WHEAT**

Q.-X. Sun, L.F. Gao, and R.X. Xu

Department of Agronomy, Beijing Agricultural University, Beijing 100094, P.R. China

**ABSTRACT**

Heat tolerance of five spring wheat cultivars (lines), i.e., 352-35, NPPF 881, Chinese Spring and Sere 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 genotypes; (3) Cytoplasts of Ae. longissima and Ae. crosso 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; Shuler and Blum, 1986; Blum 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 diploid wheats, involving 10 wheat varieties of different heat tolerance measured by chlorophyll fluorescence, Moffatt et al. (1990) found that significant material as well as reciprocal effects exist while general combining ability effects are also significant, suggesting cytoplasmic differences, and cytoplastic 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, NPPF 881, Chinese Spring (CS) and Sere Cerros 66 and their fifty alloplasmic lines, which include cytoplasms of 8 Triticum species, 11 Aegilops species and one Hekardia 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.
 RESULTS AND DISCUSSION

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 differences in heat tolerance between each of the 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 85.76%. 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 28.69%, respectively. The latter two alloplasmic lines became tolerant as TAM107. 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. dicoccum_ 881); 5. ( _T. dicoccum_ 881); 6. ( _Ae. boissieri_ 881); 7. ( _Ae. speltoides_ 881); 8. ( _Ae. crassa_ 881); 9. ( _Ae. juvenalis_ 881); 10. (CS); 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 1); 2. (CS); 3. ( _Ae. juvenalis_ NPFP 4); 4. ( _Ae. ventricosa_ NPFP 5); 5. ( _Ae. variabilis_ NPFP 6); 6. ( _Ae. cylindrica_ NPFP 7); 7. NPFP 8; 8. ( _Ae. crassa_ NPFP 9); 9. ( _Ae. speltoides_ NPFP 10); 10. ( _Ae. squarrosa_ NPFP 11); 11. ( _Ae. longissima_ NPFP 12); 13. CS; 14. TAM107.

Siete Cerros 66 is a relatively heat tolerant cultivar, but not as 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 8.84% 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 cytoplasts 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).

It was found that there were six allotypic 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 was there a significant increase 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 cytoplasm 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 allotypic lines as well as among allotypic lines of some nuclear genotypes, suggesting that genetic variations in heat tolerance existed among cytoplasms of Triticum and 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 NFFP and CS, but reduced heat tolerance of 352-35 and Siete Cerros 66, and exerted no significant effect on 881 nuclear genotype background. The cytoplasm of Ae. ventricosa reduced heat tolerance of 352-35 and Siete Cerros 66 but had no effect on NFFP, 881 and CS. It was worthy to note that cytoplasm of Ae. longissima tended to increase heat tolerance in three (NFFP, 882, CS) out of the four cultivars while it had no effect only in one case (Siete Cerros 66). Cytoplasm of Ae. creste increased heat tolerance in two (881 and CS) of the three cultivars, but had no effect on one cultivar (NFFP). 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 Moffett 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, Khara (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 callos 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 allotypic lines show nuclear-cytoplasmic heterosis in heat tolerance. Alien cytoplasm may find their uses in enhancing genetic variability in heat tolerance of wheat.

LITERATURE CITED


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

M. Farsi 1, V.A. Vanstone 1, J.M. Fisher 2, and A.J. Ratjen 1

1-Department of Plant Science, 2-Department of Crop Protection Waite Agricultural Research Institute, University of Adelaide, Glen Osmond, South Australia 5064, AUSTRALIA

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 x 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 water bath. 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. Abacax (triticate), Persia 20 and Virest (wheat) 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 (Droogman, 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 (Cobran and McCulloch, 1963; Vanstone, 1991) and up to a 20% yield reduction due to P. neglectus as been recorded (Talenti 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 (Dawidson and Sc, 1992). 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 is 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 triticate variety Abacax 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 resistant 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 (Spearm and Molinea), 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, Sarail-I-Bahari and Iraq 46), 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. 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.

A red, sandy loam soil collected from a farming property at Palmer (65 km east of Adelaide) was steam pasteurised 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 aspic P. neglectus obtained from carrot cultures (V.A. Vanstone, pers. comm.). Pots were left in the water bath 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.

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

<table>
<thead>
<tr>
<th>Name of Line/ Variety</th>
<th>Pedigree/Source</th>
<th>Reason for Inclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spear</td>
<td>Sabre/NECS/Insignia</td>
<td>Susceptible local check variety</td>
</tr>
<tr>
<td>Molinea</td>
<td>(Paspur 62*Meditugary *Merigal)</td>
<td>Susceptible local check variety</td>
</tr>
<tr>
<td>Virest</td>
<td>AUS 11894</td>
<td>Resistant to P. neglectus in previous experiment</td>
</tr>
<tr>
<td>Persia 20</td>
<td>AUS 5295</td>
<td>Resistant to P. neglectus in previous experiment</td>
</tr>
<tr>
<td>Sun 290 B</td>
<td>4°Potam/WGul/2524</td>
<td>Tolerant to P. thornei4</td>
</tr>
<tr>
<td>Iran 28357</td>
<td>AUS 10932</td>
<td>Tolerant to P. thornei3</td>
</tr>
<tr>
<td>Sarail-I-Bahari</td>
<td>AUS 7868</td>
<td>Tolerant to P. thornei3</td>
</tr>
<tr>
<td>USDA CI 9040</td>
<td>AUS 7639</td>
<td>Tolerant to P. thornei3</td>
</tr>
<tr>
<td>Iraq 48</td>
<td>AUS 4930</td>
<td>Tolerant to P. thornei3</td>
</tr>
<tr>
<td>Abacax (triticate)</td>
<td>CIMMYT. Mexico</td>
<td>locates resistance genes</td>
</tr>
<tr>
<td>1B/1R(1R)</td>
<td>496.86 pl l’1</td>
<td>Locates resistance genes</td>
</tr>
<tr>
<td>BX (C-Imp-Iral rye)</td>
<td>365/91 pl l’1</td>
<td>Locates resistance genes</td>
</tr>
<tr>
<td>BX (C-Imp-Iral rye)</td>
<td>372/91 pl l’3</td>
<td>Locates resistance genes</td>
</tr>
<tr>
<td>3R (C-Imp-Iral rye)</td>
<td>366/91 pl l’1</td>
<td>Locates resistance genes</td>
</tr>
<tr>
<td>4R (C-Imp-Iral rye)</td>
<td>363/91 pl l’1</td>
<td>Locates resistance genes</td>
</tr>
<tr>
<td>5R (C-Imp-Iral rye)</td>
<td>26/91 pl l’1</td>
<td>Locates resistance genes</td>
</tr>
<tr>
<td>7R (C-Imp-Iral rye)</td>
<td>366/91 pl l’3</td>
<td>Locates resistance genes</td>
</tr>
<tr>
<td>Cancala (Paspurc napus, variety Barossa)</td>
<td>Less susceptible to P. neglectus and P. thornei</td>
<td></td>
</tr>
</tbody>
</table>
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 481 nematodes per plant, canola was as low as the addition lines and significantly different from the wheat check. Molinлей (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 7686 in terms of the lowest and Surak (AUS 7669), 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 Waide, 1989; Varonos, 1993b), cultivation (Thompson et al., 1981), sanitation (Vico et al., 1991), fertilizer application (Vanstone et al., 1993b) and nematicide application (Farsi et al., 1993a), 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; Marron 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 (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
Zhao Meng Yang1 and Da Fang Cui2

1Department of Grassland Science, Xinjiang Agust First Agricultural University, Urumqi, Xinjiang 830052, PRC. 2Department of Biology, Xinjiang Teacher's University, Urumqi, Xinjiang 830053, PRC

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 km² 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. Pshychostachys limipetrum (Trin) Ninow was discovered. After 1950, L.A. Ulyanov and Z.F. Myschova discovered Triticum petrophilolikyi Utes et Hyucz. 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 landscapes with about 10,000 accesses were widely collected, and Triticeae accessions comprised a major part of the collection, such as the discovery of Regeniera confusa var. brevifolius Yong. In 1956, a comprehensive survey team organized by the Chinese Academy of Sciences (CAS) investigated and studied the water, soil, biological resources, agriculture and animal husbandry in Xinjiang and collected large numbers of plant specimens. Several authoritative books, such as Xinjingshi, Yejiu shixue, were published, which summarized the collected species of Triticeae. Since that initial survey, the Xinjiang Desert Botany and Soils Institution (CAS) (such as a new record of Aegilops tauschi Cooss), Xinjiang University and XAFAC collected many plant specimens during their research programs and published new 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 1986 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 XAFAC, XAAS and CAS China. Since 1979 and 1985, survey teams for Xinjiang range and resources under the direction of the Xinjiang Animal Husbandry Bureau joined XAFAC and Shizhe Agricultural
A. Germlapse Resources of Tricticaceae Species in Xinjiang

According to the previous studies and our current research results, there are 80 species and 14 subspecies (varieties) represented in Xinjiang and its 5 suburbs of Tricticaceae in Xinjiang (N.R. Cui, 1987).


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. This Subtribe has Tricticum L., Aegilops L., Secale L., Elytrigia Desv. genera.

Tricticum 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. petrolopi, T. aestivum L. and T. turgidum L.

Aegilops L.: This genus has about 30 species, which are mostly distributed in the Temperate zones of the northern Europe and central Asia. Only one species is found in Xinjiang, i.e. T. australis Carex., 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, 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 meadows and meadows in desert areas. They include E. aegilopoides (Droch.) N.R. Cui, E. ferganensis (Droch.) Nevski., E. repens (L.) Desv. ex Nevski., E. repens subsp. elongata (Gホール) Tzvelev. and E. repens subsp. ferganicae (N.R. Cui).


Annual or perennal herbs without rhizomes, forming dense bunches; one spikelet occurs at each node of the spike axis. The Terminal spikelets are generally sterile or deteriorated. Other spikelets normally range on both sides of the spike axis, combine. Glumes and lemma are pressture flabellate, biseriate. Caryopsis occurs on the back of glume and lemma. The Subtribe includes Agropyron Gaertner and Eremopyrum Jobin. as Spache.

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.) Hoffm., A. moliangus Karst., A. desertorum (Fisch. ex L.) Schacht., A. flabelliforme (Roth), and A. pectinatum (M.B. Beauv.) Beauv.

Eremopyrum Jobin. ex Spache: This genus has about 8 species, which are distributed in the temperate and 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. Three species include E. bonariensis (Sprung) Nevski., E. distans (C. Koch) Nevski., E. orientale (L.) Jobin. as Spache and E. tricreecum (Gaertn.) Nevski.

Subtribe: Elymoee Bawe.

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 some spikelets range on both sides of the spike axis. This Subtribe has Elytrigia (Hoffm.) as Tzvelev.

Elymus L.: This genus has about 160 species which are distributed in the temperate and cold zone of the northern hemisphere. In Xinjiang there are 7 species and 14 varieties or subspecies which grow in the mountain meadow grasslands, forest meadows, alpine and subalpine meadows and cold alpine grasslands. They are E. otritus (Nevski) Hoffm., E. brevistepes (Kung Karst) E. sieberianus Fisch. E. subulatus Tzvelev., E. elipteus Tzvelev., E. nutans Grub., E. sibiricus L., E. turgidula (Nevski) Hard.-Mazz., E. abrinus Tzvelev., E. dolichus var. distans (Nevski) D.C.F. Cui, E. abrinus var. abrinus Tzvelev., E. forbinicus C.K. Schen, E. ostiulatus (Droch.) A. Low., E. falcatus D.C.F. Cui (1990), E. falcatus (Kung) A. Low., E. stenostichum (Kung) A. Low, E. cryophilus (Kung) A. Low, E. cryophilus subsp. cryophilus (Kung) A. Low, E. cryophilus subsp. brevistepes (Kung) A. Low, E. cryophilus subsp. elongata (Gホール) Tzvelev. and E. cryophilus subsp. elongata (Gホール) Tzvelev.

Subtribe: Hordeine Dum. in Observ. Pharm. 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) florets, 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 Phalarischothis Nevski.

Hordeum L.: This genus has about 20 species, which are distributed in the temperate and cold zone of Eurasia. There are 3 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. hexagonum W. K. Henry, H. avenaceum (L.) Schub., H. murinum L., H. murinum subsp. elipticus (Hoffm.) Tzvelev., H. murinum subsp. elipticus (Hoffm.) Tzvelev.

Phalarischothis 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 deserts, meadows and grasslands, P. jacquemontii (Fisch) Nevski, P. jacquemontii subsp. hyalodura (Rupr.) Tzvelev., P. jekelii (Koch) Nevski.


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

Leymeina Hasel.: This genus has about 30 species distributed in the temperate and cold-temperate zones of the northern hemisphere. Most species originate from the center of Asia. In Xinjiang there are 12 species and 2 subspecies growing in mountainous grasslands, deserts, saline lowland meadows in the cold-alpine grassland zone and sandy soil. They are L. angustissima (Trav. ex) Pl., L. chinensis (Trav. ex) Pl., L. korkei (Turcz.) Tzvelev., L. multicallis (Karfeke.) Tzvelev., L. multicallis subsp. petrovii (Nevski) N.R. Cui, L. bicarinatus (Droch.) Tzvelev., L. petrosa (Trav.) Tzvelev., L. japonicus (Trav.) Pl., L. polonicus (L.) Stev., L. yamshikou (N.R. Cui) Tzvelev., L. yamshikou var. racemosa (Lam.) Tzvelev., L. ramosa (Trav.) Tzvelev., L. ruongensis (S.L. Wu) N.R. Cui, L. secalinus (George) Tzvelev. and L. secalinus subsp. pubescens (O.C. Fisch.) Tzvelev.

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

1. The Xinjiang Region has a large diversity of Trictitaceae species with a total number of 60 species with 130 varieties (species) represented in Xinjiang. 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 species, the wheat landraces were frequently exchanged. During 1958-1977, based on collections from the western part of Tarin Basin, 7 species and 56 varieties of Triticum taxa were discovered, and there were many natural hybrids (Z.L. Wang, 1988). The species and the genera of Trictitaceae in Xinjiang account for 77.3% and 90.0% of those in China, respectively. Therefore, Xinjiang is the most important gene pool for Triticum grasses within China.

2. Large amounts of plant biodiversity can be found within Xinjiang’s meadows, grasslands, desert grasslands, alpine-cold grasslands, saltlunes, low meadows; and deserts. Some species are agriculturally important, while others are distributed on different ranges, and have other characteristics such as drought resistance, winter-hardiness, salt-resistance and grazing tolerance (F. Xiao et al., 1992, 1992); J.L. Li, 1990.

3. The species have various life-forms, such as a hemiparticycaroid, geophyte, 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 46 perennial accessions in Triticum that were collected in Xinjiang and found that there were different chromosome levels occurring within some species in L. Leymeina and Hordeum. The karyotypes of selected species in Elymus (Y.H. Lou, 1985) and two species in Eremopyrum (G.Y. Yan et al., 1991) were studied.

5. The genes for resistance to diseases and insects have been identified in these taxa. Sun et al. (1990) inoculated 42 accessions of Triticaceae 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 wild-type collected from Xinjiang, and the Triticeae from Rwanda were immune. All species were resistant, except for Aegilops tauschii, Elymus dahuricus, Leymus secalinus, Rogneria feldschroerii and R. longitudinalis. It is clear that these are rich gene pools for resistance to powdery mildew in species of Triticeae in Xinjiang. Of a total of 40 accessions of Triticeae aestivum collected in Xinjiang were inoculated with composite cultures AR, 411, 8M, and 121 of Erysiphe graminis f. sp. tritici, yellow rust (Puccinia striformis) and leaf rust (P. recondita f. sp. tritici). Seven accessions, (six species Agropyron perstans, Elymus rugosus, Eryngio repens, Elymus leucoxylon, Elymus ebeischekii and E. tianschanicum and E. koralesii) proved to be highly resistant to all three diseases (L.Y. Li et al., 1992). D. Ma and J.J. Lu (1994) conducted an evaluation of aphid resistance under cages in 123 accessions of Triticeae collected in Xinjiang. The results showed that Lymus secalinus, Paspalothyrsus juncus and Lymus karfilini have high resistance or are nearly immune to two kinds of wheat aphids. The high resistance of Eryngio repens and Hordeum bulbosum are susceptible to both wheat aphids. According to observations of leaf-structure by scanning an electron microscope, leaves from resistant plants had thick epidermal cell walls, and had epidermal hairs, compacted cell walls, 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, reduced 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 Eryngio repens was conducted in Xinjiang as early as in 1950s. 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 the Yian Agricultural Science Institute. However, few cultivars have been developed by wide-crossing, with the wide 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 16,613 ha. In the second year, its average hay yield reached 6.12 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 et al. (1992, 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. 'Qita' smooth bromegrass was 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 900 m. 'Qita' has the highest productivity and the highest yield stability among four smooth bromegrasses including cv. 'Lincohi'. The strategy of replacing E. sibiricus with 'Qita' for mountain meadow improvement was proven correct.

What are the most important grasses on semi-arid rangelands? They have high drought resistance and provide good forage quality and high seed yield. S.J. Song (1992) tested 34 varieties and accessions of Agropyron and Paspalothyrsus without irrigation at Liumiu, 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 Paspalothyrsus accessions varied from 4.71 to 7.00 t/ha per year. Slender wheatgrass provided the highest yield of 9.40 t/ha. Paspalothyrsus juncus ranked second with a yield of 7.00 t/ha. Accessions that were suitable for early spring grazing included Agropyron 'Jiefang' and Paspalothyrsus 'Fanway' created wheatgrass, native A. mongolicolor and native Paspalothyrsus juncus. They provided high yields in early spring. Concerning yield and yield distribution in growing seasons, slender wheatgrass, A. mongolicolor from Inner-Mongolia, native P. junco and P. junco from Hebei province were regarded as grasses for spring-summer pastures. As a result, wheatgrasses play an important role in spring and autumn pastures in Xinjiang.

Paspalothyrsus juncus 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. junco was mixed with alfalfa, kafcha and Kochia prostrata to establish mixed pastures, 'Ziniquan' could be used as a stable mixture of P. junco and alfalfa. The results suggested that P. junco could be used in mixture with alfalfa on spring-summer pastures having an annual precipitation of 295 mm to 469 mm in Xinjiang (J.L. Li et al., 1991). Hindum bagdoldi is a prospective grass on plain lowland meadow because it has a high salt tolerance. The relative seed germination rate was 81% on saline soil compared to 74% when germinated on standard salt, which was lower than smooth bromegrass among the tested materials. Its relative seedling emergence rank first with 70.0% and the relative seedling survival rate was 100%. Besides this, H. bagdoldi 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.X. Xiao et al., 1992a, 1992b).

Leymus racemosus is resistant to drought, salt, insects and diseases and has a long spike and produces large quantities of seed. J.H. Qie 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 transformed offspring, one was obtained with 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.

Base 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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LITERATURE CITED

Ecogeographic Regions and Related Triticeae Distribution of China

JUN-LIANG YANG AND CHI YEN

(Triticeae Research Institute, Sichuan Agricultural University, Dujiangyan City, Sichuan, China 611830)

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, subtropical, 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 zonality 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 zonality 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 southwest 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 northwestern vegetation region of China. General altitude is 700-1000m above 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 floristic 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. Frost free season lasts about 100-180 days. Annual precipitation is 500-800-1000mm. Main floristic 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° 30' - 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 10°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 main floristic 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 main floristic composition is China-Japan and China-Himalaya floristic 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 25°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 deciduous broad-leaf forest region
4. Subtropical evergreen broad-leaf forest region
5. Warm steppe region
6. Warm semidesert region
7. Bing-Zhang (Zing-Hai-Xiang) plateau frigid vegetation region (after Editorial Board of vegetation of China)
and rainforest. The mainly floristic composition is tropical southwest 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 3000-4000m, including a coniferous forest zone and an alpine meadow zone, we can find two genera (Roegneria and Elymus) and 10 species (Table I).

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 63°-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 floristic composition is an 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 I).

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. Zonality vegetation is warm semidesert. Main floristic 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 I).

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) 350-350 (550)mm in the center. The northwest part has a yearly rainfall of 50-60mm.

The main floristic composition is EASIA (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 eco-geographic 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 Kenygyllie.

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

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Table 1. The distribution of wild resources of Triticaceae in China

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LITERATURE USED

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

A. Castilho and JS Heslop-Harrison

John Innes Centre
Norwich NR4 7UU, UK
Fax: +44 603 56844
Phone: +44 603 52571

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 homoeologous chromosome pairing (Gale and Miller, 1987; Islam and Faruqui, 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 SS-Rna genes, using the specific probes pTa71 and pTa2694, 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.

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 explant (CS), Chinese Spring aneuploids from group 1 (nullisomic-tetrasomic and disomic 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) glutenin subunits being the most important (Payne et al., 1987). The genes coding for these proteins are normally designated as Glu-I and Glu-L, 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 Dodecyl sulfate-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 1U 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 Glu-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-Faruqui (1988) undertook a series of crosses to transfer the Giu-U1 from Ae. umbellulata into Triticum aestivum cv. Chinese Spring. The recombination between homoeologous chromosomes was induced through manipulation of the SB chromosome dosage. Chinese Spring (CS) with chromosome 1U substituted for either chromosomes 1A and 1B was crossed with CS nullisomic SB-tetrasomic SD (NSB-TSD). 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, NDR-U1, SS-Rna, Gpi-U1 and Glu-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
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 (Ambrose et al., 1986), chromosome
segments (Le et al., 1988; 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 (Ananthamadavan-Jonsson 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-plant
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 haplotypes which are then visualized
by independent detection systems (Hopman et al., 1986;
Cerenner et al., 1998).

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, pSc19.2) not just to map
physically the translocation breakpoints but also to identify
the wheat chromosome involved in the initial translocation
(pSc19.2) and at the same time map the number of copies and
the location of the RNA (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 (3 x the probe concentration). To map
physically 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, pSc19.2, 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 1L from Ae.
umbellulata.

Characterization of Wheat - Ae. umbellulata
recombinant lines

Since the recombinant lines were obtained by
manipulation of the homologous pairing control system,
multiple translocations may have taken place amongst
homoeologous wheat chromosomes. In fact, the use of the
pSc19.2 clone as a second probe showed that the
hybridization pattern of this sequence may no longer
remain correspond to the one proposed for Chinese Spring (Mukai et al., 1993).

We were able to detect breakpoints not only at
cenomeres but also along chromosome arms. The
recombinant lines investigated had been previously
characterized by isozyme and pairing analysis at meiosis
(Islam and Faradi, 1986). 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.

Acknowledgements. We wish to thank the
Portuguese MNCT and the John Innes Foundation for the
financial support of this work.

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 Fluore-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.
LITERATURE CITED


Geographic Distribution of Alleles for Est-5, Gliadin, α- and β-Amylase in Triticum tauschii
Xueyong ZHANG, Yusheng DONG, (ICGR, CAAS, Beijing, China) and Richard R-C. WANG (USDA-ARS, FRL, Utah State University, Logan, UT, USA)

ABSTRACT

Analyzes for seed esterase-5, gliadin, α- and β-amylose 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-amylose > β-amylose; (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 (Cossor) 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 Raup, 1987). On the one hand, a number of accessions of T. tauschii have been collected in China, CIMMYT and America (Gill and Raup, 1987; Williams and Mjuebek-azi, 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 gels and different isoytes were used for various enzymes (Liu, 1991).

(1) Seed esterase (EST-5)

Gel size 24 x 16; isolates and rate: 3.5-10: 5.7 = 1:1. Analyte: 1 M H3PO4 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 220 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 acetic acid + 200 ml 80 mM phosphate buffer.

(2) α-amylose (α-Amy-1)

Gel size 24 x 16; isolates and rate: 4.2-4.9 : 4.5-5.4 : 4-6 = 1:1:1. Analyte: 0.01 M L-glutamic acid; Catholyte:
### RESULTS AND DISCUSSION

#### 1. Est-S

Because of the selling nature of *T. tauschii*, genotype fixation can be reached in a shorter time after a new mutant occurs, leading to a genotype frequency equivalent to the phenotype frequency. Est-S is controlled by chromosome 3D (Answorth et al., 1964; Mcnintosh 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, lanes 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-S 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

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

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0.1 M NaOH. Sample preparation: Extract enzyme with 200 μl 20% sucrose solution containing 0.01 M DTT from crushed endosperm of two seeds for about 1 hr, 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 & 1 g + 4.9 & 4 KI + 250 ml H2O).

(3) α-Amylase and α-Amylase-2

* Gel size: 24 x 12; Isotype and rate: α-amy-1 5.7-6=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-Amylase.

* Acid-polyacrylamide gel electrophoresis of gliadin

Two seeds of *T. tauschii* were crushed with pilers 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 electrophorosis. Electrophorosis was run at 500 V and 35 mA. Procedure for gel preparation (T = 12%, C = 4%),

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

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

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

**Fig. 3** IEF spectra of α-Amylase 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 (Anwarworth et al., 1987; Liu, 1991). McIntosh et al. (1993) found that the 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 throughout the four areas (Fig. 2, lanes I-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 homoeological group 6 in common wheat and T. tauschii (Anwarworth 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. ε-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 systematic 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, Jacard's distance were estimated between all pairs of the accessions tested. Average linkage clustering tree (Fig. 5) was produced based on mean Ds using UPGMA (Rohlff, 1993).

5. Gladin

A-PAGE spectra of gladin of wheat have been commercially regarded as fingerprints of cultivars. Gladin was controlled by multiple families on short chromosomes of the homoeologous groups 1 and 6 (Betz, 1987; McIntosh et al., 1993). Because of the evolutionary relationship between T. tauschii and T. aestivum, A-PAGE spectra of gladin 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 gladin 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.

Fig. 5 Average linkage clustering tree for 38 accessions of Tritium tauschii.
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; Bietz 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 accumulative. 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 cultivated 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.

LITERATURE CITED


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

N. Fujita, K. Takaoka\(^2\), M. Uematsu, A. Wadano\(^1\), S. Okabe\(^2\) and T. Taira

Lab. of Genetics and Plant Breeding and \(^1\)Lab. of Applied Molecular Biology, College of Agriculture, Univ. of Osaka Prefecture, Sakai, Osaka 593, and \(^2\)R & D Center, Nagase & Co. LTD., Kobe, Hyogo 651-22, Japan.

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, and the other species 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 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 glucosyltransferase 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


Search granules were prepared from mature seeds according to Eckl 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 58KD to 63.5KD. 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 of 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 Aegilops, A. 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 the 9th residues were alanine and valine instead of serine and leucine, respectively. The waxy proteins in two ecotypes (Japanese and Indian) of O. sativa and O. glaberrima showed an identical sequence, that is, ATGAGMNWVFVGAEMAP. In corn (data in corn from Klages et al., 1986), the 2nd residue (threonine) was replaced by serine, the 3rd and 5th residues were deleted and the 4th and 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
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 Aegilops 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 Wa-X (encoded by the gene located on A genome), Wa-B (encoded by the gene located on B genome), Wa-S (encoded by the gene located on C genome) and Wx-D (encoded by the gene located on D genome). There was a difference of molecular weight between Wx-A 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 N7-termius 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 17KDa were sequenced. The amino acid sequence of Wx-A 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, as 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.

Isoenzyme Data on the Diploid Progenitors of Allotetraploid Elymus Species.

Vello Jaaska
Botany Department, Institute of Zoology and Botany, Estonian Academy of Sciences, Tartu, Estonia

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 (e.g. leucistic, ibobectica, inermis, spicata, and stipitata) and in several perennial Higenome Hordeum species (Brassicaceae, B. sylvestris, B. rapa, and B. secalio) in comparison with those in allotetraploid Elymus species. The Pseudoroegneria and Hordeum species revealed a clear divergence by alcozyme of AAT and SOD-B. The American and Eurasian SH genome Elymus allotetraploids are characterized by fixed heterozygosity of these isoenzymes with codominant expression of homoeozymes corresponding to alcozyme of Pseudoroegneria and Hordeum species. The Asian SY genome Elymus species are mostly characterized by fixed heterozygosity of AAT and SOD-B with homoeozymes inherent to Pseudoroegneria species, suggesting that the Elymus SY and SH 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 in some Pseudoroegneria species, but the second homoeozyme not found among the Hordeum species.

INTRODUCTION
It has been found that most Elymus (Triticaceae, Poaceae) species display fixed heterozygosity at several isozyme loci as characteristic of allotetraploid species with codominant expression of divergent homoeozymes of composite genomes (Jaaska 1992). Cytogenetic studies of artificial intergeneric hybrids have provided evidence that Elymus species are allotetraploids with genomes S, H, P and Y derived from Pseudoroegneria (Neodac) L. Ádés (genomes S), Hordeum L. sect. Stenostachys Nevski (genomes H), Agropyron Gaertn. s. str. (genomes P), and unknown (genomes Y) diploids (Dewey 1980, Jensen 1989, 1990, Lu and Bothein 1990, a.o.). The goal of the present study was to investigate isozyme 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 isozyme analyses were prepared from the shoots of cultivated 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 1986a, 1990). The isozyme nomenclature distinguishing genetically heterologous, homo- and homologous isoenzymes, named heterozymes, homoeozymes and allozymes, respectively, is followed (Jaaska & 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 alloszyme and homoeozyme electromorphs in the order of their decreasing mobilities. Additional mobils, differing from the basic morals 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 allomorphic 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. brevisulustum S. revealed extensive
Intropopulational polymorphism of four heterozygotes with 2-7 frequent and one or two rare alleles. In a sharp contrast, the two autogenous species, H. bigotani and H. rostewitzii, were identical and monomorphic for alleles also found in H. brevisubulatum and in the two allopolyploids, H. jubitum and H. secalium. It is remarkable that the East-Asian H. jubitum and the West-European H. secalium showed fixed heterozygosity of AAT-B with a common triplet phenotype, combining codominant homozygous B5 and B6 which were absent in the Asian H. brevisubulatum as a frequent and a rare allele, respectively. H. jubitum had an additional fixed heterozygosity of ADH-A with a triplet phenotype, combining homozygous A2 and A4 which were observed in H. brevisubulatum as a frequent and a rare allele, respectively. Rare alleles are specified as those which were encountered only in heterozygous phenotypes with frequent alleles in single individuals.

EST-A was the most variable heterozygote in H. brevisubulatum with seven moderately frequent alleles, segregating among individuals as homozygous one-banded and heterozygous triplet phenotypes of a dimeric enzyme. In a sharp contrast, the four other species proved more monomorphic for EST-A. H. brevisubulatum displayed polymorphism of AAT-B and AAT-C with one frequent allele and 3-4 rare morphs. The frequent alleles of AAT-B and AAT-C in H. brevisubulatum, B5 and C4, were monomorphic in the other autogenous species studied.

Pseudoreoerges species displayed homologous polymorphism of all live heterozygotes, with EST-A and AAT-B as the most and less variable, respectively. ADH-A, AAT-B, AAT-C, EST-A, EST-B, and SOD-B were the most frequent alleles, common to all Pseudoreoerges species which differed from each other only in the occurrence and frequency of other, less frequent and rare alleles.

It follows from the data in Table 1 that the Hordeum and Pseudoreoerges species display homologous variation of ADH-A and AAT-C with common alleles A2, A4, C4, C3r, C3l, and C5r, while EST-A had some common and some divergent alleles in a sharp contrast. The genera differ distinctly in alleles of SOD-B and revealed divergence by the most frequent allele 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 Pseudoreoerges species, followed by B5 and B2. Pseudoreoerges species shared AAT-B4, which was a rare morph in H. brevisubulatum, while the Hordeum species shared AAT-B5 as the most frequent allele of Hordeum species. The most characteristic for the Stenostachys barley were ADH-A2 and EST-A2, whereas the Pseudoreoerges species shared ADH-A4 and EST-A2 as the most frequent morphs.

The data on the variation of the same five diagnostic heterozygotes among a set of Elymus aloteleorphs are selected for comparison from Jaskaa (1992) in Table 2 with comparisons 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 Pseudoreoerges species in the origin of Elymus aloteleorphs and the origin of 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 aloteleorphs share fixed heterozygosity of SOD-B with homozygous B3 and B5 which are alleles characteristic of Hordeum (H-genome) and Pseudoreoerges (S-genome) species, respectively. The East-Asian Elymus aloteleorphs of the SY-genome group share a different fixed heterozygosity of SOD-B with homozygous B3 and B5, which were both found in two Pseudoreoerges species. Previous data (Jaskaa 1992) on the occurrence of SOD-B5S3 heterozygosity in one 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 (Jaskaa 1992, Table 2). Both shared various variants of EST-A3, but differed in the second

### Table 1. Electrophoretic phenotypes of alcohol dehydrogenase (ADH), aspartate aminotransferase (AAT), esterase (EST), and superoxide dismutase (SOD) toepones in Hordeum sect. Stenostachys and Pseudoreoerges species (for a rare morph, 0 or 0 0 of the rare or slow variants of the rapid, basis, Fixed heterozygosity is designated as a fraction of codominant phenotypes; N - number of accessions analyzed)

<table>
<thead>
<tr>
<th>Species</th>
<th>ADH-A</th>
<th>AAT-B</th>
<th>EST-C</th>
<th>EST-A</th>
<th>SOD-B</th>
</tr>
</thead>
<tbody>
<tr>
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</tr>
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<td>H. brevisubulatum s. 1.</td>
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<td>5:2r</td>
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<td>6:2r</td>
</tr>
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<td>11</td>
<td>2:4r</td>
<td>4:3r</td>
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<td></td>
</tr>
<tr>
<td>H. rostewitzii</td>
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<td>4:3r</td>
<td>4:3r</td>
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</tr>
<tr>
<td>H. secalium</td>
<td>4</td>
<td>2:4r</td>
<td>5:2r</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pseudoreoerges</td>
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<tr>
<td>P. pellipes</td>
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<td>4:3r</td>
<td>4:3r</td>
<td>4:3r</td>
<td>4:3r</td>
</tr>
<tr>
<td>P. pellipes</td>
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<td>4:3r</td>
<td>4:3r</td>
<td>4:3r</td>
<td>4:3r</td>
</tr>
<tr>
<td>P. stipitata s. 1.</td>
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<td>4:3r</td>
<td>4:3r</td>
<td>4:3r</td>
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<tr>
<td>P. stipitata</td>
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<td>4:3r</td>
<td>4:3r</td>
<td>4:3r</td>
<td>4:3r</td>
</tr>
</tbody>
</table>

### Table 2. Electrophoretic phenotypes of ADH-A, AAT-B, AAT-C, EST-A and SOD-B in tetraploid Elymus species (data from Jaskaa, corrected for SOD-B; designations as in Table 1)

<table>
<thead>
<tr>
<th>Species</th>
<th>ADH-A</th>
<th>AAT-B</th>
<th>AAT-C</th>
<th>EST-A</th>
<th>SOD-B</th>
</tr>
</thead>
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<td>European SH-genome group</td>
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<td></td>
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<tr>
<td>E. carinatus</td>
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<tr>
<td>North-American SH-genome group</td>
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<td>E. canadensis</td>
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<td>4:3r</td>
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<td></td>
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</tr>
<tr>
<td>E. canadensis</td>
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<td>4:3r</td>
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<td>North-American SH-genome group</td>
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<tr>
<td>E. strictus</td>
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<tr>
<td>Eastern-Asian SH-genome group</td>
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<tr>
<td>E. strictus</td>
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<td>4:3r</td>
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<td>E. carinatus</td>
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<td>Western-Asian SH-genome group</td>
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<td>E. carinatus</td>
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<tr>
<td>E. strictus</td>
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<td>4:3r</td>
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</tbody>
</table>
| Japanese origin, proved exceptional in this respect having heterozygous AAT-B4/3 and homogamous AAT-C4. Two other E. ciliatus 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. ciliatus may indicate either independent alloplaid origin or homozygous silencing in these geographic regions.

The three Elymus species of West-Asian distribution, E. ciliatus, E. japonicus, and 'E. japonicus' have frequent alleles in Hordeum and Pseudoreoerges species, respectively. However, two SH-genome species proved homogamous for A2 and several SY-genome species homogamous for A4 (Table 2). The SY-genome species, E. strictus and E. barbilius, combine A4 common in Pseudoreoerges with a unique A6 absent in diploids.

Most SY-genome species were homogamous for AAT-B4 characteristic of Pseudoreoerges species. The SH-genome species either were heterogamous for AAT-B4/5 or were homogamous for B4 or B5, which were the most characteristic alleles of Pseudoreoerges and Hordeum species, respectively. Homogamy of all analysed accessions of E. obtusatus and E. canadensis for AAT-B5 characteristic of the H-genome indicates silencing of the S-genome homoeoyme B4 in these species. The intraspecific polymorphism of AAT-B and AAT-C observed in E. glauca may reflect either phylopleolic (vs. phylopolitic) origin of alloplaid from different S- and H-genome donors or mutational changes in the homeoeoloci 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 homogamy of AAT-B for B4 characteristic of Pseudoreoerges is contrasted with heterozygosity of AAT-C for homozygous C4 and C3 which were recorded as a frequent and a rare allele in P. barbilius and H. brevisubulatum. Two accessions of E. ciliatus, one from the Russian Far-East and the second of
LITERATURE CITED


INTRODUCTION

Polyploid wheats Triticum turgidum L. var. durum Dest and Triticum aestivum L. var. aestivum are allopolyploids with the genomic composition AABB and AABBD, respectively. Numerous data support the fact that Aeiglops species were the progenitors of the B and D genomes of polyploid wheats [1-3]. Ae. bicarinus, Ae. longissima, Ae. tauricus and Ae. stenosperus are considered the presumed donors of the B genome [2] and Ae. squarrosa 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]. Gladiolins 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 gladiolin structure has been studied intensively, the data concerning amino acid sequence of Aeiglops species are limited. In this communication we report isolation and characterization of the gladiolins N-terminal sequences of Ae. squarrosa and Ae. longissima.
Electrophoretic analysis showed that fractions A, B and C contained γ-glaadins, D-γ-glaadins and E-β-glaadins, F-β-glaadins 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 γ-glaadins, fractions VIII-X IX corresponded to β-glaadins, while fractions X-XII corresponded to γ-glaadins. γ-glaadins were the first gladin components eluted from the column and practically all of the components are isolated in pure form.

β-glaadins were eluted from the column in the form of one nonsymmetrical peak (I0-20) on the chromatogram by peaks VIII (X) 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-glaadins, 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. This 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, γ-glaadins of Ae. longissima are eluted from a reversed-phase column in the form of two groups of components: the first contains the γ-glaadins 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 their 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 monoclonal 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, γ1 and γ2 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 complete primary structure of γ-glaadins from the reversed-phase column, showed that it corresponds to the γ2 sequence detected in T. aestivum. This 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 β-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 γ-glaadins have not yet been obtained, in all probability, substantially more rigid limitations were imposed on the structure of gliadins of the investigated mixture probably contained a component with the sequence XXLPSPOQXXK (where X is an undetermined amino acid). An analogous sequence was detected by Kasarskis et al. [12] for the least mobile γ-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 γ-glaadins of Ae. squarrosa, γ1 and γ2, have the same N-terminal sequence, beginning with SRQ. This sequence is highly homologous to the N-terminal sequences of certain γ-glaadins 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 chromosomes 1B [11, 13]. Of the gliadins of Ae. longissima K-202, fractions I–IV, noted on the chromatogram (Fig. 5) were sequenced. Three of them (fractions 1, 3 and 4) coincided in mobility with 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 γ-gliadin of fraction 4 proved to be blocked. Components of fractions I–3 had a virtually identical N-terminal sequence, beginning with SRQ. Only one substitution in position 7 was detected, where isoleucine was replaced by arginine in the component of fraction 3. Arginine has also been detected in position of the polypeptide chain in the γ-gliadin of Ae. squarrosa. Sequencing of fraction I and 2 of Ae. longissima K-908 showed that they also possessed the SRQ-type of N-terminal sequence and differ only in the amino acid in the forth position instead of leucine. The γ-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 γ-gliadin of Ae. squarrosa with an unblocked end.

Summarizing the results of the sequencing of γ-gliaadins of Aegilops, we can conclude that the gliadins with the SRQ-type of N-terminal sequence have been detected among the α-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 γ-gliaadins possess blocked N-terminal amino acids.
Polymorphisms of Monomeric Prolamins in Dasyphyllum villosum (L.) Candargy

C. De Pace 1, S. Geng 2, C.O. Qualet 3, V. Delre 1 and R. Caccia 1

1Dept. Agrobiology and Agrochemistry, University of Tuscia, Viterbo, Italy; 2Genetic Resources Conservation Program, University of California, Davis, California, USA; 3Dept. Agronomy and Range Science, University of California, Davis, California, USA.

ABSTRACT

The prolamin storage proteins of caryopses from five populations taken from natural stands of Dasyphyllum villosum in Italy were fractionated using polyacrylamide-gel electrophoresis at pH 3.1. A total of 80 bands of monomeric prolamin proteins were grouped according to their migration distance with the α-, β-, γ-, and ω-type prolamins (gladins) 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. Differences in mobility were 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 Glu-1, Glu-2, and Glu-V1 loci which control the expression of the analyzed monomeric prolamins.

INTRODUCTION

Dasyphyllum villosum belongs to the secondary gene pool of wheat, and D. villosum x Triticum triticum F1 plants have been obtained (Jan et al., 1986) or without embryo culture (Tscharnak, 1929; Scarpelli, 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, 1952; Hyde, 1953; Blanco et al., 1987). Therefore, D. villosum is a genetic resource for wheat improvement (Qualet et al., 1993). Among the potentially useful traits that D. villosum genes can confer to wheat, those related to the amount and 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% (Napa 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 glutenin are controlled by a multilocus locus (Glu-V1) on chromosome IV of D. villosum (Zhong and Qualet, 1993; Montebello 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 IV, V, and VI (Montebello et al., 1987). Blanco et al. (1991) and Shewry et al. (1987, 1991) confirmed the 4V and 6V locations. The loci coding for seed storage proteins located on chromosomes IV and VI are apparently orthologous to the Glu-1 and Glu-2 loci in wheat and are designated Glu-V1 and Glu-V2, respectively. The loci Glu-V1 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 those loci may provide useful alleles for modifying wheat lines end-use quality. Polymorphisms for high-molecular-weight glutenins have been reported by Zhong and Qualet (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 autogamous, 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 Monteobelo et al. (1987). Each of the monomeric proteins appeared as bands in the electrophoretogram visualized after immersion of the gel in Coomassie Brilliant Blue R 250-ethanol solution. Each band (monomeric prolamin) was numbered according to its migration distance relative to that of reference bands in the gladin 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 electrophoretogram 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 electrophoretograms 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 gladin pattern of bread wheat cv. 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 gladiins. 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 ζ class, and bands 59 to 80 to the α class.

**Data analysis**

The frequency of the band occurring at the kth position at each of the 80 standardized positions on the gel (1st gel location) was calculated for each population. The relative frequency, f, of band k in population i was defined as n/k, where n is the number of HS individuals showing the kth band and k is the total number of HS individuals analyzed for the kth band. Log-linear and general linear models applied to the relative frequencies showed significant between-population and population × 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 f values in a pairwise comparison between populations at each kth band position. This test does not require a significant F-test of population and population × gel location interaction variances as a prerequisite. Populations whose band frequency, f, did not differ statistically within a certain gel position were considered similar at that position. A similarity index f, over the 80 positions was calculated for each pair of populations. f 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 2Mxy/(Mxx + Myy), where Mxx was the number of band positions for which populations x and y showed similar frequencies and Mxy and Myy were the number of bands in each population. Dendrograms based on the dissimilarity index (f = 1 - f) over all bands or within the α, β, γ, and ω classes of bands were used to construct the UPGMA clustering method and the NTSYS software package (Rohlf, 1987).

**RESULTS AND DISCUSSION**

The electrophoretograms 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 gladin class was represented in the five analyzed populations (Table 1). The frequencies of the omega-class bands were highest among the four gladin classes and contributed 12 to 17% of all of the bands in each population. The gel locations with few D. villosum monomeric prolamins were 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 ζ class (Fig. 2). The positions of bands 48 to 58 separate α class from ω prolamins. The gel locations with the highest frequencies of D. villosum monomeric prolamins are those found between the reference bands 5 to 6, 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
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 prolamin 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 differences (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 hill 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 forces 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 code 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 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; Showry 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 geliac loci has not been done, but it is expected that the above conjecture would be borne out. For the high molecular weight glutenin subunits, Zhong and Quistset (1993) found 14 electrophoretically detectable alleles of the Gli-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.
The Presence of a Repeated DNA Sequence from Triticum aestivum in Hordeum species

Veit Schubert1, Karl Hammer2 & Frank Baldau3

1 Institute of Plant Breeding and Seed Production, Martin-Luther-University Halle-Wittenberg, D-06188 Hohenheim, Germany; 2 Institute of Plant Genetics and Crop Plant Research, D-06466 Gatersleben, Germany; 3 Institute of Genetics, Martin-Luther-University Halle-Wittenberg, D-06108 Halle, Germany

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 variation was found in accessions of the closely related species H. bulbosum and H. Aegilops. 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 Aegilops 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 (Grollot et al. 1986), Brassica (Martinez-Zapater et al. 1986), Lycopersicum (Cooper and Westerman 1987), Nicotiana (Speckhart and Jacobs 1986), Brasicaeae (Halden et al. 1987) and those in Triticaceae (Bendich and McCarthy 1970, Flavell et al. 1977, 1979, Charkraborty and Subrahmanyan 1985, Dowlati et al. 1988, Jungas 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 (Huxroch and Lonsdale 1962, Jones and flavell 1982, Lipton et al. 1967, Gupta et al. 1989, Baldau et al. 1992). Nevertheless, these sequences can also give some useful information. A repeated DNA sequence cloned from Triticum aestivum in pTa1 by Metzelf et al. (1986) was present with a strong hybridization signal in D genome containing Triticum and Aegilops species but also in Aegropyan. Weak or middle cross-hybridizations 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 Aegropyan 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 Metzelf et al. (1986). DNA hybridization was carried out according to the protocol of squash dot technique developed by Hutchinson et al. (1985) modified after Jungas and Metzelf (1988). Root tips from germinated canopies from at least two plants of every accession were squashed onto nitrocellulose filter. Hybridization of these filters with 32P-labeled 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 Triticum species are summarized in Table 1. Surprisingly, the wheat sequence displayed in 16 accessions representing 14...
Table 1: Hybridization strengths of pl1 to Hordeum and Triticeae species. Hordeum species outside the *H. vulgare*-complex were arranged on the basis of taxonomic aspects according to von Bothmer et al. (1982).

<table>
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<th>Ori- Hybridization strength(^a) pl1 table</th>
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\(^a\) For mark description see 'Material and Methods'.
Several repeated DNA sequences were cloned from H. vulgare (Dennis et al. 1980; Ananiev et al. 1986; Salina et al. 1986; Jungheinrich and Metzfall 1986; Verrinno et al. 1990). Jungheinrich 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. agriptithon and H. spontaneum accessions. In contrast, the sequences showed a high variability in the wild barley accessions. Verrinno 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 p2Cl19 was absent in H. vulgare, H. agriptithon 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 Hueras et al. (1993) were found separately in different species. H. 950 cross hybridized to H. marinum and H. brevisubulatum but not to wheat. However, Hch 1.3 was absent in H. marinum, H. brevisubulatum, 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 clone.

Acknowledgement - This work was supported by a grant of 'Deutsche Forschungsgemeinschaft' (Schu 762/2-1).

LITERATURE CITED


Molecular Relationships Between the Genera Elymus L. and Triticum L. Gluttenins.

L.V. Obukhova, G.V. Generalova, A.Y. Agafonov, V.Y. Gulevich

Institute of Cytology and Genetics, Novosibirsk, Russia, 630090.
Central Siberian Botanical Garden.

ABSTRACT

The antigenic relationships between the glutelin of Triticum aestivum L. and major seed proteins of Elymus L. were studied by Western blotting using pooled antibodies against wheat glutelin as the probe. Along with high- and low-molecular-weight (HMW and LMW, respectively) glutelin-like proteins of E. sibiricus (SSHH genome) the probe reacted with two proteins (97 and 69 kDa), probably non-prolamin. Southern blotting of HindIII-digested total DNA from Elymus spp. with a synthetic oligonucleotide (a repeated motif from wheat HMW glutelin genes) revealed 3.2, 1.4 and 1.0 kb fragments in E. trachyculus (SSHH genome) DNA and 1.2, 0.9, 0.8 kb in E. putilidus (SSYY genome) DNA.

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. trachyculus (VLA.B6.2) and E. putilidus (MES.86.6) were received from the collection of the Central Siberian Botanical Garden.

The fraction of total seed proteins similar to glutelin in solubility was prepared from individual grains and analyzed by SDS-PAGE as described in [4]. Wheat glutelin for immunization was prepared as described in [5]. Polyclonal rabbit antiserum against wheat glutelin was produced 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]. HindIII, the Klenow fragment, and loméde phage DNA were used according to manufacturer’s recommendations. The electrophoresis of HindIII-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-deoxynucleotide 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.
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 wheats glutenin and with two groups of LMW glutenin-like proteins. Like in wheats, 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 antiseraum 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 SS1H [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: S1H1 (E. trachycarpus) and SSYY (E. pendulineus). A 32P-labeled 10-nucleotide synthetic consensus fragment complementary to a repetitive nucleotide sequence motif 5'-GGCGCAGCACCGAACAGGCGCAAAAAAAAAAGT-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 HindIII and hybridized with the probe gave rise to five fragments of sizes 2.5, 2.3, 1.8, 1.6, and 1.6kb. This is consistent with the previous investigations done with a DNA probe [16]. Each of the two Elymus species studied showed three fragments: E. pendulineus - 1.2, 0.9, 0.8kb; E. trachycarpus - 3.2, 1.4, 1.6kb. The intensity of the band corresponding to the 1.6kb fragment of E. trachycarpus 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, immunochromatographic 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**

We are grateful to Dr. V.P. Kumarev for synthesizing the oligonucleotides and to Dr. N.A. Popova for her assistance in preparation of the antiseraum.
LITERATURE CITED


The Elymus Trachycaulus Complex in North America: More Questions than Answers

Mary E. Barkworth

Intermountain Herbarium, Department of Biology, Utah State University, Logan, Utah 84322-5305

ABSTRACT

Members of the Elymus trachycaulus complex of North America can be recognized morphologically by their combination of a solitary spikelet, b) archers 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. merceanus, E. scriberii, E. sierrae, Agropyron fuscum, 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 hilly slopes to damp meadows. In Eurasia, members of the complex extend from Iceland and northern Europe to eastern Russia. Some species have a transiberian 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 allopolyploids based on the S and H genomes (Dewey 1963, 1966, 1967a, 1967b, 1968a, 1968b, 1969, 1975, 1976; Murr and Tai 1980).

INTRODUCTION

Authorities and synonyms are listed in the appendix. Names of Elymus have been used wherever available except when citing published works; in such cases, the authors' choice is followed. Genome designations are based on Wang et al. (1996): the S genome corresponds to the S genome of Love (1984) and Dovay (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. caninus diverge from the rachis at anthesis, returning to a less densely appressed position afterwards, whereas those of A. trachycaulus show almost no movement. His plants of A. caninus 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. trachycaulus 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. caninus and A. trachycaulus was that the former had coarsely 3-veined glumes, whereas A. trachycaulus had glumes with 4-5 much less prominent veins. In addition, the glumes of A. caninus had a rather broad, scariosus margin which formed a projecting edge just below the
apex, whereas in A. trachycarpus "the scariosus margin is much narrower and in many cases obsolete". There were additional differences, but he considered less significant. C.L. Hitchcock (1969), who collected extensively on the Pacific Northwest, stated that the difference in glume variation between the two taxa was not constant, and that the differences in glume texture and anther size supported their treatment as conspecific subspecies. He disagreed 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 species.

The cytological data are limited. According to Dewey (1975, p. 205), "the chromosome number is structurally different from those of A. trachycarpus, A. subsecundum, and A. doystachyum and, in addition, '...genomes of A. trachycarpus and probably other members of the 'slender wheatgrass complex' are structurally unique, in that they contain two sizeable interchanges not found in other SSHE species" (Dewey 1975, p. 127). Dewey interpreted these data as supporting both A. conica and A. trachycarpus as distinct species. He did not elaborate on which taxa he included in the "slender wheatgrass complex".

Variation within the Elymus trachycarpus complex

Even within North America, there are more questions than answers about the E. trachycarpus 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 morphological intermediates. The most derived intermediate with including the E. trachycarpus complex, the initial suspicion is that morphological intermediates are hybrids or hybrid derivatives. This suspicion arises from the observation of the low barriers to hybridization within the tribe, and the observation that many of the intermediates occur in areas of sympathy. But morphological variation may reflect genetic differentiation, phenotypic plasticity, 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.

Jaworz’s (1966) study of Agropyron trachycarpum, A. subsecundum, A. spinosum, and A. latisforme 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 area, it would be expected to exhibit self-fertilizing taxa, but one long-lived plant gave rise to three long-lived plants and two non-dispersing populations of the same hybrid, a salted seed from the long-lived offspring were uniformly long-lived. Jaworz concluded that...the major species of slender wheatgrass plants are hybridogenic for characteristic characters under natural conditions"... even though many of the plants may be descendants of a hybrid between different members of the E. trachycarpus complex or between members of the hybrid and another member of the Tribe, including species of Hordeum. Malus (1932, p. 28) made a similar observation, based on "numerous experiments", but cited Kork (1929) as saying that segregation may 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). Surprisingly, the hybrids are often highly sterile, frequently more than hybrid between its two parents (Table 1).

**Table 1:** Hybridization studies have involved few populations, sometimes only one population (or seed accession) per parental species. Generalizing from this study is hazardous, for the hybrids vary greatly in their crossability. Of the 20 crosses jaworz (1966) made between members of the complex, eight resulted in seed. Of the three crosses within A. trachycarpus, one yielded 10 percent seed, 0% other two gave none. Of the 13 crosses between the unwieldy A. trachycarpus and long-awned A. subsecundum, eight yielded no seed, but the other five yielded 32% germinal seed, percentages Jaworz 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. trachycarpus and A. levipes could not be 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. trachycarpus may reflect confusion of subsp. subsecundum with E. glaucus. M.L. Curley in 1937 described E. glaucus, which is supposed to have more than one spicate per node, frequently develops spikes with only one spicate per node when growing in the shade. This one-spicate plants are easy to distinguish. In the species subsp. subsecundum, are not occasional 'breaks'. Interestingly, along a road in northern Utah, it was the more exposed plants that developed only one spicate per node (per node). Except for plants growing in the shade, E. trachycarpus in E. glaucus in having glumes with five relatively prominent veins that are scabrous throughout, leafy, leaf blades, and awned lemmas, but I have found similar appearing plants in the same area. It seems possible that growing in the shade, E. trachycarpus may be more likely to be misidentified as E. glaucus. Even if the single-spicate specimens of E. glaucus are excluded from consideration, our observations tend to confirm Jaworz’s (1966) conclusion that awned specimens of E. trachycarpus (specimens with awns more than 5 mm long) have different hybrid origins. Whether we will be able to identify the parents of such hybrid remains 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 to be examined 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. trachycalyx and A. destyphanicum in the field. The same appears to be true of hybrids between E. trachycalyx and Pseudopneumogaea spicata. Many plants along a forest trail west of Logan were identified as E. trachycalyx, but found to be sterile. The only other species of Triticeae present was Pseudopneumogaea spicata. It was disconnecting to find that, even knowing that such plants were present, morphometry was not a reliable predictor of which plants were sterile and which fertile.

Collins (1965) and Jozewik (1966) attempted to use biochemical markers (serology and flavonoids, respectively) to detect the origin of suspected hybrids, but with little success. Nuclear 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. H or 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. trachycalyx complex to form fertile hybrids, the generic basis of the characters used to distinguish its taxa is not known. It would be worth investigating. Godfrey (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. trachycalyx 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. trachycalyx 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”. Malta (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. Jozewik (1966) observed that plants grown from seed under greenhouse conditions were most likely to differ from their parents in having more widely spaced stipelets and longer spikes. He found little modification of the diagnostically characters for the taxa he examined. Other studies of plasticity have focussed on agronomically, rather than taxonomically, significant characters.

Internal structure of the E. trachycalyx complex

Adopting an artificial circumscription of the E. trachycalyx 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 is impossible to distinguish 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, therefore, first to admit that there are good arguments for alternative treatments.

Elymus stoechas, E. scirpiger and E. steppensis are generally accepted as good species. The least controversial of these appears to be E. stoechas. Its morphological uniformity suggests that it probably 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. stoechas, Elymus scirpiger appears to hybridize rather easily with other members of the complex that grow in the vicinity, usually E. trachycalyx, E. alaskanus subsp. illigitimus, and E. elymoides. Bowden (1965) and C.L. Hitchcock (1969) have suggested that E. scirpiger is a hybrid derivative between E. trachycalyx or E. alaskanus and E. elymoides. This seems very likely, but there has been no experimental examination of the hypothesis. The steppensis, a Californian endemic, is relatively easy to recognize by its combination of long anthers, acute, smooth glumes, and distinct stipelets. Elymus leavis was supposed to be a long-lived version of E. steppensis, but the type specimen is an awned specimen of E. trachycalyx. There does appear to be an awned variant of E. steppensis, 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 E. trachycalyx, 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 lamens, 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 spicule itself is rounded to obtuse, although the midvein often extends as a macro. Under high magnification (20x), the edges of the apex often appear minutely toothed. Typical Elymus trachycalyx, in addition to being taller and having a longer inflorescence, has thicker glumes that are about as long as the adjacent lamens and more than 3/4 the length of the stipelets. 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 glumes are smooth.

As outlined, E. alaskanus and E. trachycalyx are morphologically distinct. Moreover, E. alaskanus is generally a more northern and higher elevation taxon than E. trachycalyx, but the large number of intermediate experimental data, to support (or refute) this suggestion. Twesten’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. trachycalyx complex are not compatible with the straightforwardness of species, 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.

Acknowledgements

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LITERATURE CITED


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<td>subsp. subsecundus (Link) Gould</td>
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Influence of Climatic Factors on the Distribution of Hordein Alleles in Barley

Pomortsev A.A., Kalabushkin B.A., and Blank M.L.

Vavilov Institute of General Genetics, Russian Academy of Sciences, Gubkin str. 3, Moscow 117809, Russia.

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. Earlier, 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 area of spring barley in the former USSR.

<p>| Table 1. Natural agricultural provinces and their climatic characteristics in crop area of cultivated spring barley in the former USSR |
|---|---|---|---|---|---|---|</p>
<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>Province</th>
<th>Zonal Character</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
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<td>1960</td>
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<tr>
<td>4</td>
<td>D</td>
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<td>1800</td>
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<td>15</td>
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<td>25</td>
<td>Y</td>
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<td>3400</td>
<td>211</td>
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Legend: 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 (3). 10-44 cultivars per each NAP were studied. Data from mountain crop areas of barley, i.e. the Caucasian, 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.

Hordien electrophoresis was performed in 12.14% starch gel in aluminum lactate buffer (pH 3.1), containing 3M urea according to Sestvov & Popenelya (3). From 40 to 100 individual grains were analyzed per each cultivar. Alleles of hordien-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. = 71, 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 (3) 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 hordien loci in barley populations is determined by two climatic factors—rainfall and the average temperature in July (correlation coefficient is 0.990, $P = 0.001$). 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, A2B, B35, B37, B38, B45, F3). For allele nomenclature see Famontsov 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.

Acknowledgment - This work was supported by the Russian Fund of Basis Research. AAP attended the symposium with a travel grant provided by the International Science Foundation, New York.

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.
Reinterpretation of Dispersal Strategies in Triticum L. and Aegilops L.

L.A. Morrison

Department of Plant Genetics, The Weizmann Institute of Science, Rehovot 76100 Israel

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 dispersal types whose variation is far greater than that found in other genera of the tribe Triticaceae. Developmental links between the three principal dispersal 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 Mighushova (1979) and Aegilops L. sensu van Staden (1994)—have traditionally been classified into four categories: (1) a wedge dispersal consisting of a spikelet subtended by a rachis internode (Fig. 1a); (2) a cylindrical dispersal consisting of an entire spike, strongly curved only on the apical spikelet, whose basal spikelet is subtended by a rachis internode (Fig. 1b); (3) a barrel dispersal consisting of a spikelet and the adjacent rachis internode (Fig. 1c); and (4) an umbrella dispersal consisting of an entire spike, ovate and multiawned, whose basal spikelet is subtended by a rachis internode (Fig. 1d). Each of these dispersal 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. Egg (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 (Fig. 1, p. 194). Zohary (1965) expanded Egg's descriptive system to include Triticum. He correlated dispersal strategy with polyploid speciation and in so doing, confirmed 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, uncharged genome of the member polyploid taxa. Its dispersal 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 Cost. with a barrel dispersal and the U-genome cluster headed by Ae. umbellulata Zhuk, with the umbrella dispersal. In Triticum, the A-genome polyploid cluster, headed by Tr. boeticum Boiss., has the wedge dispersal type. Although Zohary did not discuss the areal extension of this species, which has since been identified as the A-genome donor of tetraploid and hexaploid wheats (Chapman et al., 1976; Drorico, 1976; Dvorak et al., 1992), should be designated, in place of Tr. boeticum, as the head of this cluster.

Noticeably absent from this scheme is the cylindrical dispersal, which is typically associated with five-dispersal taxa (see below). Zohary designates this dispersal type as "unsuccessful" for two reasons: it is not associated with a pivotal dispersal 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 dispersals (Ae. speltoides Tausch var. gigas (Sav.) Fiori, Ae.

LITERATURE CITED

Significance of Aeglops speltoides

In my view, the key to understanding the novel dispersal strategies of the wheat complex can be found in the entire-spike dispersal, here designated syngnaptospermic dispersal using the terminology of Zohary (1937). It has been noted by several authors that both the cylindrical and umbrellaisolateral dispersal will undergo an additional disarticulation on the ground to form secondary dispersal (Eig, 1929; Schröder, 1931; Zohary, 1937; Frank, 1964; Morrison, 1994). This phenomenon implies that the primary dispersal associated with Ae. tauschii is probably the outcome of a developmentally altered syngnaptospermic-dispersal strategy in which the original primary, basal-wedge disarticulation is suppressed. Moreover, the umbrella and cylindrical dispersals are both syngnaptospermic-dispersal types related by virtue of their mode of disarticulation and entire-spike structure. Given the probable linkage of the cylindrical, umbrella, and cylindrical dispersals, their developmental association with the wedge-dispersal 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 syngnaptospermic-dispersal 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 Mandelkar genes controls the dimorphic inflorescence trait; the wedge-dispersal type (ligustico) is dominant over the cylindrical-dispersal type (speltoides) (Sears, 1941; Zohary and Imber, 1963). Here, in one species, there is evidence of a simple genetic system which controls two distinct dispersal types.

In the cylindrical dispersal of Ae. speltoides, two types of disarticulation occur: (1) the wedge break (dispersal with subtending rachis internodes) which creates the primary, syngnaptospermic dispersal (Fig. 1b) and (2) a delayed, barrel break (dispersal with subtending rachis internodes) which creates the secondary, barrel dispersal (Fig. 2a). Assuming that the genetics of this system is adequate support for Eig’s proposal of the wedge dispersal 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 dispersal of var. ligustico (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-dispersal dispersal strategy. Aeglops speltoides var. speltoides serves as the starting point of a radiation of novel dispersal strategies found exclusively in Aeglops (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 syngnaptospermic dispersal 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, syngnaptospermic dispersal and then undergoes a delayed barrel disarticulation when on the ground to produce a secondary barrel dispersal. This strategy is also found in Ae. longissima Schw., & Mesch., Ae. searsii Feldman & Kislev ox Hammel, 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 (umbrellaisolateral form) with a secondary barrel disarticulation in the terminal spikelet—Ae. umbellulata (Fig. 2b); (2) loss of the secondary barrel disarticulation—Ae. unisistata (Fig. 2c) and Ae. comosa var. subverisces; (3) primary barrel disarticulation due to a loss of the basal-wedge disarticulation—Ae. tauschii (Fig. 2d); (4) taut-rachis due to a loss of both modes of disarticulation—forms of Ae. tautochi (Fig. 2e), Ae. comosa var. subverisces, and the polyploid Ae. verisces 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 T. aestivum L., the wild, tough-rachis taxa have very tough glumes which can only be broken with a force sufficient to destroy the integrity of the spike.

Clearly, non-wedge dispersals are an unusual dispersal strategy for the tribe. They indicate an evolutionary trend in Aeglops uniquely different from the trend charactering domesticated Triticum taxa. This phenomenon provides justification for reevaluation of the genotypic 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 even for investigations of the adaptive radiation or for practical agricultural applications.

Figure 1: Diaspores of wild wheats according to the traditional interpretation: (a) wedge dispersal (Tr. urartu, top; Ae. speltoides var. ligustico, bottom); (b) cylindrical dispersal (Ae. speltoides var. speltoides); (c) barrel dispersal (Ae. tauschii); (d) umbrellaisolateral dispersal (Ae. umbellulata).

Figure 2: Syngnaptospermic dispersal strategies: (a) cylindrical dispersal with secondary barrel dispersals (Ae. speltoides var. speltoides); (b) umbrellaisolateral dispersal with secondary barrel dispersal (Ae. umbellulata); (c) intact syngnaptospermic dispersal with no secondary barrel dispersals (Ae. unisistata); (d) primary barrel dispersals (Ae. tauschii); (e) tough rachis with no disarticulation (Ae. tauschii).
Systematics of the Triticeae: Problems and Progress

Elizabeth A. Kellogg

Harvard University Herbaria, 22 Divinity Avenue, Cambridge, MA 02138

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, so 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

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LITERATURE CITED


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 it if is complicated. Appreciable progress has been made since the last Triticaceae 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 Triticaceae

Several studies have addressed the circumscription of the supertribe Triticinae, the larger group that includes Triticum, Bromus and Brachypodium (McClaran and Watson 1982). Recent data indicate that Brachypodium P. Beauv., is not closely related to the rest of the Triticinae at all. It is supported by multiple molecular data sets, including the internal transcribed spacer of the ribosomal RNA (ITS; Hisao et al. 1994a), chloroplast DNA restriction sites (Hiscock et al. 1993; Kellogg 1992), 5S DNA spacer sequences (Kellogg and Aspels, unpubl. data), sequences of the chloroplast genes rps4 (Nadot et al. 1994), and ndhF (Catalan, Olmeda and Kellogg, unpubl. data). A morphological study (Kellogg and Watson 1993) also supports this result, but the cladogram is so unresolved that it is inconclusive. In contrast, all the foregoing studies find a close relationship of Bromus L. to the Triticinae. We can therefore conclude that Bromus is the appropriate outgroup for the tribe.

Diploid Triticinae - current knowledge

Several years ago I suggested that a research program on the Triticeae should 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, on the other hand, are well enough 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 now have 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. PAPU 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 Frohberg and Sabel (1992 and in press, this symposium). Their tree is well-resolved, although not still 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 all diploid taxa in the tribe, and many of the diploid species. There are two ribosomal arrays in most Triticaceae (Flavell and O’Dell 1976; Flavell and Smith 1974; Gill and Appels 1988; Millar et al. 1983), but only a single ITS sequence is present in PCR amplifications. This implies that concerted evolution is operating across both ribosomal (NOR) arrays to homogenize them not only within a species but between arrays (as described by Schlotzer and Tautz, 1994). The ITS trees of Hisao 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 Tricium/Lagopes L., Theinopsis A. Löve (s.l.), Secale L., Henrichs C. E. Hub., Gritopsis Jain & Sprent, and Neovia L. 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 the root are internal to triticum as the outgroup, the next diverging branch(s) is a node L. (Cistercia R. L. clad (parathyridic in some trees), and then Pasthyrostachys Nevski. For the 55 large space, for which we lack a Pasthyrostachys sequence, the next diverging branch is Dasyptym (Cost & Dunne) Dunne

The three trees (like the chromosome pairing, nuclear RFLP, and asynyme studies cited above) also agree on the monophyly of all monogenic groups except for the JTS genomes, and the monophyly of all species of Aeglops (Triticum pro parte, sensu Kimber and Feldman 1987). The species of Aeglops represents 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 Pseudogroecia (Nevski) A. Löve, Arealanopyrum (Tzvelev

A. Löve and Socle 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 monoccum L., whether with the species of Aeglops or near the base of the tree.
2. The position of Socle, whether part of the “Mediterranean clade” or near the base of the tree.
3. The relationships of Theinopsis bessarabica (Savill & Rayas), A. Löve and Lathyracinum elongatum (Host) A. Löve, whether sister taxa, or unrelated.
4. The relationships of Pseudogroecia and Arealanopyrum, whether sister taxa, or unrelated.
5. The relationships of Gritopsis and Tetranietum, whether sister taxa, or unrelated.
6. The relationships of Agropyron and Pseudogroecia, 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 55 DNA units were sequenced from individual of each species; in most cases, the sequences from each species were more closely related to each other than to those of other species. Some sequences, two to five per species of each species were sequenced, and these were found to be identical. For chloroplast restriction site studies, most species were represented by two to three 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 little 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 δ = 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 S5 trees disagree strongly on the placement of Triticum monococcum. The short-spacer array (group I chromosomes) places Triticum monococcum in a monophyletic group with the species of Aeglops, whereas the long-spacer array (group II chromosomes) places it near the base of the tree and unrelated to Aeglopus. Curiously, this latter placement is also supported by morphological data (Frederiksen and Sæberg 1992 and unpublished), isozyme (McIntyre 1968) and nuclear RFLP data (Monte et al., 1993), but not by chloroplast data (see below).

The conflict between the S5 gene trees indicates that in Triticum monococcum the two S5 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 S5 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 Aeglops, Thapsopyrum (s.l.), Crithopsis and Tenuatherum. A second clade includes Pseudoroegneria, Australopyrum and Agropyron, and the base of the tree is a paraphyletic group consisting of Henardia, Secale, Dasypyrus, and Paspalorthyschia.

Figure 2. Phylogeny of the diploid Triticaceae, using combined sequence data from two nuclear SS DNA arrays and the nuclear ITS, from Kellogg et al. 1996 submitted. Strict consensus of 108 trees, each with L = 456, CI = 0.711, RI = 0.817. Decay values are below the line, percent of 100 bootstrap replicates above Triticum monococcum and Aeglops tauschii. Trees 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 induced by cold lines, and detailed line taxa placed accordingly.

Comparing either of the S5 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 tree. If transversions are weighted more and more heavily, then some conflict appears in the position of Secale and Henardia. In the S5 data they are sister taxa and diverge just after Paspalorthyschia 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 - Heterantherum, Dasypyrus, Crithopsis, and Secale - have branches five to ten times as long as other branches in the ITS tree. Experiments with removing those one at a time from the ITS data set reveal that Heterantherum and Dasypyrus 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 homoplasies 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 Triticaceae, redrawn from Mason-Gamer and Kellogg (1996). Numbers above branches are percentage of 100 bootstrap replicates. Strict consensus of 12 trees, each with L = 151, CI = 0.650, RI = 0.848.

Aeglops spp. (S genome group)
- Aeglops comosum (M)
- Aeglops unialtistatum (M)
- Aeglops umb./caud.(C, C')
- Triticum spp. (A genome group)
- Aeglops tauschii (D)
- Aeglops specioides (S)
- Taeiatherum caput-medusa (T)
- Secale spp. (R)
- Triticum umb./caud. (C, C')
- Pseudoroegneria (S)
- Paspalorthyschia (Q)
- Aeglops comosum (M)
- Triticum umb./caud. (C, C')
- Secale spp. (R)
- Paspalorthyschia (Q)
- Aeglops comosum (M)
- Triticum umb./caud. (C, C')
- Secale spp. (R)
- Paspalorthyschia (Q)
The explanation for the discrepancy between the chloroplast and nuclear gene trees is not clear; all possible explanations are of hoc and rather unconvincing. It is tempting to suggest gene flow. However, this must have occurred sometime in the recent past, because the two trees are currently intersterile, as indicated by their distinct genomic designs. 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 discern; this would, 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 construct trees corresponding to the chloroplast tree, one or more of the nuclear trees, or combinations of both.

Despite a wealth of data, much more in many other such groups, we have not recovered a single tree like this for the diplloid Tricticeae. So far, in each piece of DNA investigated appears to have a distinct history. This would explain the discrepancies among the isozyme, RFLP and paring trees mentioned above - each of these estimates relationships from different parts of the nuclear genome. If different parts of the genome have different histories, then phylogenies that sample the entire genome will be composite, 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 are tracking. By comparing 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 SS short-sporing tree. Similarly, a gene tree for a gene on chromosome 5 should be similar to the SS long-sporing tree. Because each chromosome may have a different history in some taxa, particularly the annuals, genomic in situ hybridization could also point to more distinctly related pieces of chromosome.

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 morphologically represents some complex introgression 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 Tricticeae? We can now see that there is good evidence for almost any taxonomy. The case of Tricticum monoccum is a good example. Data from the SS 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 SS array on chromosome 3 has a very different history. The chromosome 3 array justifies separating Triticum and Aegilops (as does isozyme and nuclear RFLP data). Either way, some information is lost. Either way, the acceptable tree is the same.

Another example is the J and E genotype 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 J SS array, keeping them together is supported by the chloroplast data, and the ITS and chromosome 5 SA 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 static classification of the Tricticeae, because there are so many ways to turn the web into a hierarchy. Arguments over Triticum vs. Aegilops, Triticum vs. Lophopyrum will continue 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 Linnæan 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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LITERATURE CITED


Uniformity of the α-Amylase Isozymes of Aegilops cylindrica Host. Introduced into North America: Comparison with Ancestral Eurasian Accessions

Nobuyoshi Watanabe, Katsuhiro Mastui and Yoshihiko Furuta
Faculty of Agriculture, Gifu University, Gifu 501-11 Japan

ABSTRACT

We analyzed α-amylase isozymes of 22 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...
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, Kyoyo, Japan), H.E. Bockelman (USDA-ARS, Aberdeen, USA), A.B. Danzma (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 0.5M Tris-HCl buffer (pH 7.0) from the endosperm of a single seeding 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 Pharmalyte®.

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 accessions were identified from the zymograms. The arrows show the major differences among zymogram types. Although we found 6 types of variants (Figure 2 and Table 1), 441 out of 453 accessions showed the same zymogram pattern (Table 1). The major type I 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 Caucasian, 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), Janka (1981) and Messi 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 "Khariv" 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 joint eastgrass 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 in diploid ancestor Ae. marshalli, because of no α-amylase data for Ae. marshalli. They will also be useful to consider the low population diversity for α-amylase isozymes in the native Eurasian populations of Ae. cylindrica.

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LITERATURE CITED


The Genus Elymus L. in Asia. Taxonomy and Biosystematics with Special Reference to Genomic Relationships

Bao-Rong Lu

Laboratory of Systematic & Evolutionary Botany, Institute of Botany, Chinese Academy of Sciences, Xiangshan, Beijing 100093, P. R. China. **Present address: GRC, International Rice Research Institute, P.O. Box 933, 1099 Manila, Philippines.

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 occur 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 Asian 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 circumcisions of Löve (1986), Dowey (1984), and Tavely (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; Salmónce 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. vibrinicus, E. caput-medusae, and E. hystrix (added in the addendum). He subsequently made some adjustments to his original treatment, such as transferring Triticum comus L. to Elymus (E. comus (L.) L.; Linnaeus (1753)) 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 1986, Dowey 1984, Tavely 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 Elymus (L. arenarius (L.) Hachi), Tastnosthop (T. caput-medusae (L.) Nevsk), and Hordeum (H. europaeus (L.) Harz).
Table 1. Year of publication of generic synonymy (notItalicis) of Ejymus with the type taxon and its generic constitution.

<table>
<thead>
<tr>
<th>Genus</th>
<th>Author</th>
<th>Year</th>
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<tbody>
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<td>Ejymus</td>
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<td>1943</td>
</tr>
<tr>
<td>Kangillia</td>
<td>Yen &amp; Yang</td>
<td>1950</td>
</tr>
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</table>

Since the establishment of Ejymus, the circumscription of the genus has varied widely, and Ejymus sensu Löve (1984) has also been treated under different terms by various authors. Table 1 lists the different taxa which are synonyms with Ejymus published during the last two hundred years.

In Bentham's (1861) treatment, Ejymus comprised about twenty species having multiple spikellets per racis node and two or more florets per spikelet. He accepted the subdivision of the genus into three sections, i.e., sect. Stemonia Schult., sect. Cymelinea Griseb. (= Ejymus), and sect. Pammucronia (Linnaeus) Lejat. He also recognized the genus Asperilla Humb., as an independent genus encompassing three species. Many other taxa having single spikelets per racis node and multiple florets per spikelet were placed in Agropyron Gaertn., such as A. caninum (= Ejymus caninum). He included Roengeria and Anthaschoa into Agropyron because he considered that the former (e.g., R. caucasicum (= E. caucasicus)) was closely allied to A. caninum and the latter (e.g., A. scabidae or E. scabridus) was closely allied to A. semicostatum (= E. semicostatus) and A. longisetosum (= E. longisetosum). Therefore, the circumscription of Bentham's Ejymus was narrow, including only those species in sect. Stemonia, and sect. Ejymus of Ejymus sensu Löve (1994).

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In the generic system of classification of the Triticeae, Löve (1984) has converted the available generic data into a comprehensive worldwide taxonomic treatment of Ejymus. In the “Conspicuous of the Triticeae,” Löve basically followed Tzvelev (1976)’s circumscription of Ejymus. He included both Hysteris and Stemonia, and added three more sections into Ejymus. Approximately 150 species were included in this treatment, where the genus Ejymus was further subdivided into eleven sections, i.e., 1) sect. Ejymus; 2) sect. Tzvelev (Nevski) Tzvelev; 3) sect. Macrapiella (Nevski) Jaksic; 4) sect. Gaudlania (Hochst.) Tzvelev; 5) sect. Poechii (Löve); 6) sect. Stemonia (Rafin.) Löve; 7) sect. Cymelinea (Nevski) Tzvelev; 8) sect. Anthaschoa (Stoutz); 9) sect. Stenostachys (Turcz.) Löve; and 10) sect. Dasytachys (Löve) and 11) sect. Hylodekis (Nevski) Löve.

It is obvious that the main arguments relating to the circumscription of Ejymus proposed by various authors revolved around whether single vs. multiple spikelets per racis node as a key character in the classification. Some taxonomists delimited Ejymus by including all species with multiple spikelets per racis node, and thus placed those species with single spikelets per racis node into other genera, such as Agropyron or Roengeria, e.g., the treatments by Bentham (1861), Nevski (1932), Hochstetter (1951), Keng (1939), and Löve (1983). In these treatments, Ejymus 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), Runnman & Hense (1968), Tzvelev (1976), Melders (1980), Löve (1986), Dewey (1984), and Clayton & Ravenoza (1986). They included species in Ejymus regardless of the number of spikelets per racis 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 Ejymus species from different geographical and historical perspectives. The various species 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 Hinchley’s circumscription of Ejymus 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 Ejymus is followed to a great extent, and in Europe the classification system by Melders et al. (1980) plays a major role.

Genetic circumscription
The generic circumscription of Ejymus adopted in this paper follows that of Löve (1984), in which Roengeria, Hysteris, and Stemonia are included. Following this generic definition, Ejymus contains approximately 150 taxa. The
As an exclusively allopolyploid genus, Elymus has its origin from other genera, and thus it has close relationships with other genera in the Triticeae. The S genome is so far known as represented in all Elymus species, and hence is almost universal. No distinct 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 taui (Jensen 1990b), a tetraploid perennial containing the S genome (Wang et al. 1986). The S genome has been reported to have relatively high homology with the P genome (Napier & Walton 1982; Wang et al. 1985). The W genome has only been found in the Austroasian hexaploid E. scabrius, apart from its presence in the donor Austroasian. The two genomes were reported to have fairly high homology, but the W genome has very low homology with any of the other genomes, e.g., the S and Y, in Elymus (Toribishei & Mueller 1993).

Intergeneric hybrids have been reported between Elymus and several genera in the Triticeae, and genomic relationships of Elymus with these genera have been estimated thereby. Only a few genera, such as Elytrigia (S/SY genomes), Thinopyrum (JE), and Pascopyrum (SNKH), have relatively high homology affinities to Elymus with a certain amount of chromosome pairing in their hybrids (Dewey 1970; Love & Connor 1982; Napier & Walton 1983). Considerable low genomic affinities are assessed between other genera, such as Pachyostachys (Dewey 1967; Lu & Connor 1991a; and Secale (R. Lu et al. 1990; Lu & Bohmer 1991a).

Genomic relationships

Even though information such as the chromosome numbers of many species in the genus was unknown, Love (1984) accommodated two basic genera (haploids), i.e., the A and B genomes, The basic S-genome species originated from Pseudoroegneria (Dewey 1967), whereas the H genome from Hordeum s.l. (Dewey 1971). Love (1986) suggested four genomic combinations, i.e., HS, HH/SY, HSS, or SYH as the constitution of Elymus. Dewey (1986) accepted Love's (1984) circumscription of the genome. However, he pointed out that apart from the basic S and H genomes, the basic Y genome (of unknown origin) was involved in some of the Central Asian tetraploids and some of the hexaploids (Dewey 1984). Therefore, he recommended that the genomic constitutions of Elymus should be SH, SY, or SYH and the combinations of the segmental allopolyploids. Recently, both SH/SY and SYH have been identified from hexaploid species. The fourth basic P genome, which comes from Agropyron, was found in some Central Asian hexaploids, e.g., E. distachyon, E. farcticus and E. betulina (Jensen 1990b: 23).

The fifth basic W genome, derived from Atrypalynum, was identified in an Australasian hexaploid E. scabrius (Toribishei & Mueller 1993).

The donor of the P genome in some Central Asian hexaploid Elymus species was expected to be Agropyron, a genus containing perennial species of diploid, tetraploid, and hexaploid 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). No distinct 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 taui (Jensen 1990b), a tetraploid perennial containing the S genome (Wang et al. 1986). The S genome has been reported to have relatively high homology with the P genome (Napier & Walton 1982; Wang et al. 1985). The W genome has only been found in the Austroasian hexaploid E. scabrius, apart from its presence in the donor Austroasian. The two genomes were reported to have fairly high homology, but the W genome has very low homology with any of the other genomes, e.g., the S and Y, in Elymus (Toribishei & Mueller 1993).

The earliest genomic designation for Asian Elymus species was conducted by Dewey (1968). In that study two tetraploid Elymus species, i.e., E. caninus (as Agropyron caninus) and E. semistans were described. The genomic formula of E. caninus was designated as SY, in which the proposed X genome was later found to be derived from Hordeum. The symbol X was then replaced by (Dewey 1971). The genomes of E. semistans were designated as SY, where the origin of the Y genome was unknown. Subsequently, more Elymus tetraploid species from Asia were included in the Triticeae, and genomic relationships of Elymus with these genera have been estimated thereby. Only a few genera, such as Elytrigia (S/SY genomes), Thinopyrum (JE), and Pascopyrum (SNKH), have relatively high homology affinities to Elymus with a certain amount of chromosome pairing in their hybrids (Dewey 1970; Love & Connor 1982; Napier & Walton 1983). Considerable low genomic affinities are assessed between other genera, such as Pachyostachys (Dewey 1967; Lu & Connor 1991a; and Secale (R. Lu et al. 1990; Lu & Bohmer 1991a).

The genus Elymus in Asia

Asia, particularly the Central Asian 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's Elymus species occurs in this area; 2) a large number of morphological features are found in this region; 3) all known polyplid levels, 2n = 4x – 28, 2n = 6x – 42, and 2n = 8x – 56, have been reported from Asia (Taveler 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 Asian 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. Salamone & Muratamu (1966b) made several interspecific hybrids, including tetraploids (2n = 4x = 28).

I.e., E. chinensis, E. gmelinii, E. patens, E. semistans, and hexaploids (2n = 6x = 42), i.e., E. taushetiensis, and E. humilis (as Agropyron humilis). They concluded through the study of chromosome pairing at meiosis 1 of meiosis 2 of the hybrids they were very similar in all the tetraploids, and that the two hexaploids shared three nearly identical genomes. However, no genomic designation resulted from these. Similar cytogenetic studies were reported subsequently by Salamone (1964, 1992).

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and Lu & Salomon (1993a), indicates that more than 140 Elymus species sensu Love (1984) are native to Asia. Many of the species were thought included in other genera, such as in Raegnera, Agropyron, Hystrix, or Elytisphry 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 Niewski (1932), Raegnera parviflora Keng (1959), and Agropyron micrololos Melderis (Bor 1960), and another Tibetan species has been described as Agropyron ébridgei Melderis (Bor 1960), Raegnera stricta Keng (1959), R. vanii 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 type) from several major herbaria, and the results of cyrogenetic investigation and taxonomic revision of Elymus species (Salomon 1993a, b; Lu 1993c, 1995; Salomon & Lu 1995, Lu & Salomon 1993b), same taxa have been treated as one species. The number of species in Asian 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, SS, 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, chromatin bridges and fragments in the interspecific hybrids. This differentiation of the SY genomes is in accordance with the geographic distribution of these Elymus species (Lu & Salomon 1992; Lu & Botamin 1993a; Lu & 1993b, c). The SYH 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 owing 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 Pseudogafria (G genome), Triantherum (T genome), Pascopyrum (O-NNX genomes), and Leymus (NX-NNX 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 species in different geographic areas. The numbers represent the minimum number of species occurring in a particular area.

<table>
<thead>
<tr>
<th>2n</th>
<th>Known genomes</th>
<th>Example of species</th>
<th>Approx. no. of species</th>
<th>k*</th>
</tr>
</thead>
<tbody>
<tr>
<td>28</td>
<td>SH</td>
<td>E. sibiricus</td>
<td>10</td>
<td>55.8/7.0</td>
</tr>
<tr>
<td>28</td>
<td>CV</td>
<td>E. amoenobotryum</td>
<td>10</td>
<td>55.8/7.0</td>
</tr>
<tr>
<td>42</td>
<td>SYH</td>
<td>E. drobodi</td>
<td>2</td>
<td>3.2/1.8</td>
</tr>
<tr>
<td>42</td>
<td>SYT</td>
<td>E. tachinosianus</td>
<td>4</td>
<td>1.870.0</td>
</tr>
<tr>
<td>42</td>
<td>SSS</td>
<td>E. transplatinus</td>
<td>4</td>
<td>333.6</td>
</tr>
<tr>
<td>42</td>
<td>SYP</td>
<td>E. alatuscus</td>
<td>7</td>
<td>49.5</td>
</tr>
<tr>
<td>42</td>
<td>??</td>
<td>E. temulacme</td>
<td>6</td>
<td>9.8</td>
</tr>
<tr>
<td>42</td>
<td>???</td>
<td>E. edibergii</td>
<td>13</td>
<td>30.0</td>
</tr>
<tr>
<td>?</td>
<td>T</td>
<td>E. calecolius</td>
<td>65</td>
<td>65.0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>111</td>
<td></td>
</tr>
</tbody>
</table>

* Percentage of species known genomes/Percentage of total species. ** Including natural interspecific hybrids.
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.
Evidently, like the SY genomes in Azotia Ellymus species which have diverged to a great extent (Lu & Salmon 1992; Lu & Bothmer 1993a; Lu 1993b, 1995). How should taxonomists relate such genomic diversity to the genomic general? In general, although the association between delimitation strictly with genomic constitution will produce more confusion and is likely to bring the classification of Ellymus into chaos.

In contrast to the treatment of splitting Ellymus into several genera, Asaad (1984) put all perennial species containing the S5 genome into Ellymus, including Ellymus sensu Love (1984) and Ellytrigia s. l. (incl. Ellytrigia sensu Love, Thonopyrum Love, Trichopyrum Love, Pseudoregneria (Nevado) Love, and Pescopitymus Love), even though species in the Ellytrigia complex are all large-anthered, cross-pollinating, and usually awned. This generic delimitation of Ellymus essentially agrees with those by Runemark & Henriksen (1968) and Poldrera (1980). To confirm the propriety of such combination for Ellymus, Asaad (1984) emphasized that (1) the S5 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 S5 genome is more or less continuous; (2) Ellytrigia repens (L.) Nevado, the type taxon of the genus Ellytrigia, contained the S5 genome which is present in many Ellymus species, therefore, he considered the inclusion of Ellytrigia s. l. in Ellymus to be natural either from a morphological or genetical point of view.

However, based on our present knowledge of Ellymus, 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 Ellymus proposed by Love (1984) at present. Actually, if we neglect the number of spikelets per racemes node, then species in the genus Ellymus sensu Love (1984) have considerable similarities in morphology, compared with other genera such as Pseudoregneria, Thys, and Elymus. We should allow more than one genomic combination to be present in the genus Ellymus just as we permit four basic genomes, namely, the I, H, X (c. X in Ellytrigia) and Y (Y in Ellytrigia) 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 (Kitara 1975). The acceptance of Ellymus 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 Ellymus provides us with an opportunity to understand the biometrical relationships below generic level.

Traditionally, the taxonomic subdivision of Ellymus was essentially based on morphological characteristics, such as the attitude of spike (erect vs. nodding), number of spikelets per racemes node (singly, or multiple), the size of spikelets, and awned or awnless lemmas, although studies have shown very little value of using these characters to indicate biometric relationships of Ellymus species (e.g., Salomon & Lu 1992). Even so, Love's (1984) subdivision of Ellymus, based on the traditional treatment, particularly on that of Tzvelev (1976), presents the current taxonomic subdivisions within Ellymus. However, current investigations of the genomic relationships of Ellymus species have demonstrated that the present subdivision of Ellymus (Love 1984) cannot reflect the evolutionary relationships between the Ellymus species.

Illustrated by the fact that species containing the same genomes have been placed in different sections, whereas species possessing different genomic combinations have been
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

C. DE PACE 1, C.O. Recapitulation 2 and G.T. SCARASCIA MUGNOZZA 1

1Dept. Agrobiology and Agrochemistry, University of Tuscia, Viterbo, Italy.
2 Genetic Resources Conservation Program, University of California, Davis, California, U.S.A.

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 42.6-60% of the time, whereas the upper floret produced large, white caryopses 37-40% 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 (6.5 and 5.6 mg, respectively) than those from the lower floret (7.1 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 parental 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 coleoptiles, but there was no significant difference between red- and white-derived plants in culm height and number; nor in their allel frequencies for the Gs-V2 and Gs-V3 loci. The caryopsis dimorphism of D. villosum provides greater variability in 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; Verdel 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 Ormè, 1961). Previous work, summarized in Table 1, has shown that the caryopses of the two morphs differ in germination ability, ascorbic acid metabolism, miosomic cycle, and ratio of DNA, and the seedlings derived from them differ for the amount of DNA and copy number of the subtelomeric 365-kb tandem repeats.

The association between the caryopsis 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 currents of genebanks. The importance of D. villosum as a genetic resource for forage production and for improvement of grass yields of wheat by transfer of disease and stress tolerance genes (Quakset 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 spikels 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 caryopsis of Dasypyrum villosum. A. Spike sections. B. Red and White caryopses. C. Lower (1) and upper (2) floret positions within a spikelet.

Table 1. Caryopsis and plant growth traits for diaspyric caryopses and their derived seedlings of Dasypyrum villosum

<table>
<thead>
<tr>
<th>Trait</th>
<th>Mean</th>
<th>Std.</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caryopsis size</td>
<td>Large</td>
<td>Small</td>
<td>Meletti and Ormè (1961)</td>
</tr>
<tr>
<td>Germination ability</td>
<td>2</td>
<td>7</td>
<td>Meletti and Ormè (1961)</td>
</tr>
<tr>
<td>Dormancy period</td>
<td>0-15</td>
<td>0-15</td>
<td>Meletti and Ormè (1961)</td>
</tr>
<tr>
<td>Assortment coefficient (AA) 6-day-old seedlings (measuring from m.)</td>
<td>2.74</td>
<td>2.68</td>
<td>Paciolla et al. (1991)</td>
</tr>
<tr>
<td>European activity in 4-day-old seedlings</td>
<td>&lt;1.0%</td>
<td>&lt;1.0%</td>
<td>Paciolla et al. (1991)</td>
</tr>
<tr>
<td>Assortment total AA (AE) (measuring from m.)</td>
<td>&lt;1.0%</td>
<td>&lt;1.0%</td>
<td>Paciolla et al. (1991)</td>
</tr>
<tr>
<td>Vitellogenins</td>
<td>1.5%</td>
<td>1.5%</td>
<td>Paciolla et al. (1991)</td>
</tr>
<tr>
<td>Caryopsis longevity</td>
<td>50%</td>
<td>50%</td>
<td>De Gara et al. (1991)</td>
</tr>
<tr>
<td>Caryopses germination after 7 days</td>
<td>95%</td>
<td>95%</td>
<td>De Gara et al. (1991)</td>
</tr>
<tr>
<td>Removal of leaf pigments</td>
<td>50%</td>
<td>50%</td>
<td>De Gara et al. (1991)</td>
</tr>
<tr>
<td>Duration of mitotic cycle</td>
<td>9.5%</td>
<td>9.5%</td>
<td>Intracellular &amp; Intracellular (1990)</td>
</tr>
<tr>
<td>Entire cell size</td>
<td>210%</td>
<td>210%</td>
<td>Intracellular &amp; Intracellular (1990)</td>
</tr>
<tr>
<td>Number of nucleoli DNA (in means)</td>
<td>3.0</td>
<td>3.0</td>
<td>Freseal et al. (1991)</td>
</tr>
</tbody>
</table>

1 Number of days from 93% germination to immature caryopses at 20°C
2 Average of measurements in populations from Caserta, Pen, and Villanuova (Italy)
3 Value of the population from Patro (Italy, Sicily)
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, L-86 (16 plants), I-27 (13 plants), and I-147 (12 plants). The collection sites were roadides 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 Caryopsis from plants I-27.3 and I-27.9a, which were sampled in population I-27 for their extreme ratios of white to red Caryopse at each floret position (I-27.3) or for having short spikes (I-27.9a). Germination time and coleoptile length were measured using 11 plants from each of populations B-1.2M, B-2.3M, B-1.5M, and B-7.7M which were collected along different roadides in Puglia. In each case, Caryopse from three spikes per plant were used. Data for Caryopsis size and 1-day-old seedling weight were obtained from the 12 populations used by Zhao and Quislet (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 plant collections 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, as the few Caryopse found in more distal florets were 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 Caryopse per sample counted. The proportion of upper florets producing a given morph was obtained by dividing the number of Caryopse of that morph in the sample concerned by the total number of upper florets in that sample; the figures for lower florets were calculated similarly. The average proportion of Caryopse 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.

Caryopse from each of two mother plants (I-27.3 and I-27.9a) from populations I-27 were sorted according to the 2 morphs, 2 floret positions, and 3 spike sections. Twelve and eight groups of Caryopse 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 Caryopse and represented a different 'treatment' of the offspring derived from those mother plants for a total of 20 treatments. The Caryopse 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. Caryopse 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.

Caryopse 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.
Generation rate and time and coleoptile length.

Caryopses from three spikes of 11 plants from four populations (8I-2M, 8I-3M, 8I-5M, and 8I-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 tracts 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. Germination rate 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 the marginal 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 Zhou et al. (1993) and 11 populations were those studied by De Paco (1997). The caryopses were germinated using the slant-board technique; the caryopses of each 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 significant difference method based on F-values at the 0.05 probability level. The correlation coefficient between caryopsis and seedling weight was also calculated.

RESULTS

Florex position and caryopsis morphs.

Both of the florexes examined in the spikelets were capable of producing either or both caryopsis morphs, but the upper florexes produced mostly white caryopses, whereas the lower florexes 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 florex x section and florex x population interactions (Table 4). In all three populations examined, the lowest section of the spike produced the highest proportion of sterile florexes. This was true for both the upper and lower florexes. The average number of white caryopses produced in the upper florex was significantly higher than in the lower florex, 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 florex than in the higher florex. This situation made the white: red caryopsis ratio in

the upper florexes to range between 1:3 in population 1-27 to 3:9 in population 1-47 (Table 2). The white: red caryopsis ratio in the lower florex varied between 0.31:1 in population 1-27 to 0.66:1 in population 1-46. The proportion of upper florexes producing white caryopses in the middle and upper spike sections was similar within each population, but ranged from 63% in population 1-26 to 86% in population 1-27. The percentage of red caryopses produced by the lower florex in these sections within each population, but considerably between populations, from 45% in population 1-26 to 72% in population 1-27. The between population variation was, however, significant at p=0.01 only for the frequency of white caryopses production (Table 4).

The white caryopses were consistently heavier than the red caryopses (Table 3), with white caryopses produced in upper florexes being 1.5 mg heavier than those produced in the lower florexes (Table 3). For red caryopses, the reverse was true, the heavier red caryopses being those produced in the lower florex. In most instances, however, the difference was not significant (Table 5).

Comparison of caryopsis parents with their progeny.

In no case examined was the ratio of the two caryopsis morphs produced by a parent plant maintained in its progeny (Table 6). Mother plant 1-27.3 was notable for its extremely reduced caryopsis production in each section. None of the 12 progeny strains that were obtained had the same extreme ratio. 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 1-27.9a were intermediate to the parental plant means, but occasionally exceeded those values. Unlike the progeny of 1-27.3, the ratios for the progeny of 1-27.9a varied beyond the parental plant value, even on the lower two sections. In no case was the regression of the progeny ratio 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 1-27.3 and 1-27.9a and for plants grown from caryopses obtained from the Alberello, Castelnuovo, and Francia 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 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 florex to be 4 to 13 times more frequently than red caryopses and the white caryopses in the lower florex were 2 to 3 times less frequent than red caryopses. The same range of variability may be found in different spikelet 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 florex position was relatively stable, but variation about these frequencies occurred and were environment-dependent. The average frequency and range of variability of sterile florexes was the same in the upper and lower florexes, indicating that the differential proportions of white and red caryopses produced on the upper and lower florexes do not depend on the fertility level within each 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 spikelet 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 a 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 from population 8I-7M a few days after collection showed an average of 3 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 Orman and Orman (1984). Therefore, a prolonged caryopsis dormancy for red caryopses in D. villusum may 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 fucitive 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 caryopses in D. villusum 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" dormancy and germination of 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 florex position in which the caryopses develop is the most important feature regulating the production of the two caryopsis morphs in D. villusum as well as other nonculmiferous Triteceae 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 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 weight of culms, and similar allele frequencies at isozyme loci. Some other consequences of caryopsis dimorphism in D. villusum have been described in Table 11. It is lacking for the other Triteceae species that exhibit this trait. The information obtained in this and previous studies may have relevance for conservation of D. villusum germplasm. For example, De Gara et al. (1999) believe that red caryopses have longer viability and therefore would be desirable for long-term storage.
Table 2. Proportion of white (W) and red (R) carpocaps and sterile (S) flowers in the upper and lower florets of spikelets from base (A), middle (B), and top (C) spike selection of spikes from three D. villosum populations collected in Italy in 1984 (values in brackets are standard errors).

<table>
<thead>
<tr>
<th>Pop.</th>
<th>No. plants</th>
<th>Spike section</th>
<th>Upper floret</th>
<th>Lower floret</th>
<th>Proportion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>W</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>1-27</td>
<td>13</td>
<td>A</td>
<td>0.68</td>
<td>0.09</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.038)</td>
<td>(0.025)</td>
<td>(0.012)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>0.86</td>
<td>0.07</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.040)</td>
<td>(0.038)</td>
<td>(0.010)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>0.85</td>
<td>0.03</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.040)</td>
<td>(0.013)</td>
<td>(0.022)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean W/R</td>
<td>0.80</td>
<td>0.06</td>
<td>0.14</td>
</tr>
<tr>
<td>1-46</td>
<td>16</td>
<td>A</td>
<td>0.42</td>
<td>0.15</td>
<td>0.43</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.048)</td>
<td>(0.034)</td>
<td>(0.019)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>0.65</td>
<td>0.08</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.047)</td>
<td>(0.020)</td>
<td>(0.008)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>0.63</td>
<td>0.05</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.052)</td>
<td>(0.025)</td>
<td>(0.021)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean W/R</td>
<td>0.57</td>
<td>0.09</td>
<td>0.34</td>
</tr>
<tr>
<td>1-47</td>
<td>12</td>
<td>A</td>
<td>0.26</td>
<td>0.24</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.055)</td>
<td>(0.080)</td>
<td>(0.027)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>0.75</td>
<td>0.14</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.075)</td>
<td>(0.062)</td>
<td>(0.003)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>0.81</td>
<td>0.05</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0.063)</td>
<td>(0.022)</td>
<td>(0.002)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean W/R</td>
<td>0.65</td>
<td>0.14</td>
<td>0.22</td>
</tr>
</tbody>
</table>

Table 3. Average weight of white (W) and red (R) carpocaps in the upper and lower floret of spikelets from base (A), middle (B), and top (C) spike selection of spikes from three D. villosum populations collected in Italy in 1984 (values in brackets are standard errors).

<table>
<thead>
<tr>
<th>Pop.</th>
<th>No. plants</th>
<th>Spike section</th>
<th>Upper floret</th>
<th>Lower floret</th>
<th>Average weight of carpocaps (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>W</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>1-27</td>
<td>13</td>
<td>A</td>
<td>6.2</td>
<td>4.2</td>
<td>4.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>8.2</td>
<td>5.3</td>
<td>7.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>8.7</td>
<td>5.3</td>
<td>8.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean W/R</td>
<td>7.7</td>
<td>4.9</td>
<td>6.7</td>
</tr>
<tr>
<td>1-86</td>
<td>16</td>
<td>A</td>
<td>8.6</td>
<td>5.7</td>
<td>7.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>9.9</td>
<td>6.5</td>
<td>7.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>9.8</td>
<td>5.7</td>
<td>7.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean W/R</td>
<td>9.4</td>
<td>6.0</td>
<td>7.3</td>
</tr>
<tr>
<td>1-147</td>
<td>12</td>
<td>A</td>
<td>7.3</td>
<td>5.1</td>
<td>5.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>9.4</td>
<td>8.2</td>
<td>7.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>8.1</td>
<td>3.8</td>
<td>7.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean W/R</td>
<td>8.3</td>
<td>5.7</td>
<td>7.0</td>
</tr>
</tbody>
</table>

Weighted mean

| Weighted mean W/R | 1.5 | 1.1 |
Table 4. Frequency of white (W) and red (R) colored (C) carpopores in the upper and lower flown of ophidian from base (B), middle (M) and top (T) section of a hole from the B, C, ophidian mother plants 2-27 and 2A-7.

<table>
<thead>
<tr>
<th>Carpopores in the mother plant</th>
<th>Spike section</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>B vs. C</th>
<th>M vs. T</th>
<th>Upper flown</th>
<th>Lower flown</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper flown</td>
<td>Spike section</td>
<td>W</td>
<td>R</td>
<td>W</td>
<td>R</td>
<td>W</td>
<td>W</td>
<td>R</td>
</tr>
<tr>
<td>B</td>
<td>spike section</td>
<td>80.0*</td>
<td>68.0*</td>
<td>8.0</td>
<td>8.0</td>
<td>8.0</td>
<td>8.0</td>
<td>8.0</td>
</tr>
<tr>
<td>M</td>
<td>spike section</td>
<td>80.0*</td>
<td>68.0*</td>
<td>8.0</td>
<td>8.0</td>
<td>8.0</td>
<td>8.0</td>
<td>8.0</td>
</tr>
<tr>
<td>T</td>
<td>spike section</td>
<td>80.0*</td>
<td>68.0*</td>
<td>8.0</td>
<td>8.0</td>
<td>8.0</td>
<td>8.0</td>
<td>8.0</td>
</tr>
</tbody>
</table>

Table 5. Values from two-tailed t-tests for comparing pairs of means of upper (U) and lower (L) host samples for average number and weight of white and red carpopores from A, B, and C spike sections of individual from three Italian D. villosus populations.

<table>
<thead>
<tr>
<th>Population</th>
<th>df</th>
<th>Spike section</th>
<th>Average no. of White carpopores</th>
<th>Average no. of Red carpopores</th>
<th>Average White carpopores weight (mg)</th>
<th>Average Red carpopores weight (mg)</th>
<th>U vs. L</th>
<th>U vs. L</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-27</td>
<td>24</td>
<td>A</td>
<td>1.89</td>
<td>3.17**</td>
<td>0.31</td>
<td>0.61</td>
<td>1.11</td>
<td>1.55</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>6.55**</td>
<td>5.28</td>
<td>1.11</td>
<td>0.20</td>
<td>1.31</td>
<td>1.49</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>3.92**</td>
<td>3.79**</td>
<td>1.31</td>
<td>1.55</td>
<td>1.28</td>
<td>1.49</td>
</tr>
<tr>
<td>1-47</td>
<td>22</td>
<td>A</td>
<td>8.60**</td>
<td>8.42**</td>
<td>2.90**</td>
<td>0.26</td>
<td>0.74</td>
<td>1.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>17.55**</td>
<td>7.18**</td>
<td>2.09**</td>
<td>1.48**</td>
<td>1.28</td>
<td>1.49</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>13.46**</td>
<td>7.58**</td>
<td>2.09**</td>
<td>1.48**</td>
<td>0.69</td>
<td>4.51**</td>
</tr>
</tbody>
</table>

Table 6. Means and standard errors (values in brackets) for plant height and number of culms in the progeny from white (W) and red (R) carpopores harvested from the upper and lower flown of the mother plants 1-27-3 and 2-7A.

<table>
<thead>
<tr>
<th>Plant no.</th>
<th>Upper flown</th>
<th>Lower flown</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>W</td>
<td>R</td>
</tr>
<tr>
<td>I-27-3</td>
<td>84.8</td>
<td>84.0</td>
</tr>
<tr>
<td>Plant height (cm)</td>
<td>(4.6)</td>
<td>(1.0)</td>
</tr>
<tr>
<td>Number of culms</td>
<td>10.5</td>
<td>8.0</td>
</tr>
<tr>
<td>(1.2)</td>
<td>(0.5)</td>
<td>(2.4)</td>
</tr>
<tr>
<td>I-27-9</td>
<td>102.4</td>
<td>108.0</td>
</tr>
<tr>
<td>Plant height (cm)</td>
<td>(3.7)</td>
<td>(4.4)</td>
</tr>
<tr>
<td>Number of culms</td>
<td>9.4</td>
<td>10.0</td>
</tr>
<tr>
<td>(1.4)</td>
<td>(0.9)</td>
<td>(0.8)</td>
</tr>
</tbody>
</table>

* ** Significant at the 0.05 and 0.01 probability levels, respectively.
Table 8. Mean values and standard errors (values in brackets) for the morphological traits and allelic frequency at two loci controlling different classes of carotenoid transmission (OOG-2 and OOG-3). Data were collected from white (W) and red (R) Caryopses from three D. villosa populations in Italy.

<table>
<thead>
<tr>
<th>Character</th>
<th>Flavensus</th>
<th>Albensella</th>
<th>Centifolia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>W (R)</td>
<td>W (R)</td>
<td>W (R)</td>
</tr>
<tr>
<td>Flower height (cm)</td>
<td>146.4 (140.0)</td>
<td>153.7 (157.6)</td>
<td>111.0 (137.6)</td>
</tr>
<tr>
<td></td>
<td>(135.7) (127.6)</td>
<td>(153.7) (137.6)</td>
<td>(111.0) (137.6)</td>
</tr>
<tr>
<td>Number of grains</td>
<td>27.0 (25.7)</td>
<td>34.6 (32.4)</td>
<td>37.0 (35.7)</td>
</tr>
<tr>
<td></td>
<td>(25.7) (27.4)</td>
<td>(34.6) (32.4)</td>
<td>(37.0) (35.7)</td>
</tr>
<tr>
<td>Number of spikes</td>
<td>35.0 (36.9)</td>
<td>32.6 (32.4)</td>
<td>32.0 (31.7)</td>
</tr>
<tr>
<td></td>
<td>(36.9) (35.7)</td>
<td>(32.6) (32.4)</td>
<td>(32.0) (31.7)</td>
</tr>
<tr>
<td>Allelic frequency</td>
<td>0.8 (0.7)</td>
<td>0.9 (0.8)</td>
<td>0.9 (0.8)</td>
</tr>
<tr>
<td>G1F</td>
<td>0.8 (0.7)</td>
<td>0.9 (0.8)</td>
<td>0.9 (0.8)</td>
</tr>
<tr>
<td>G2F</td>
<td>0.2 (0.1)</td>
<td>0.3 (0.2)</td>
<td>0.3 (0.2)</td>
</tr>
<tr>
<td>G3F</td>
<td>0.2 (0.1)</td>
<td>0.2 (0.1)</td>
<td>0.2 (0.1)</td>
</tr>
</tbody>
</table>

Table 9. Means values for germination percentage, duration of germination time, and coleoptile length of white (W) and red (R) juncos from 50 spikes from four populations of D. villosa collected in 1981.

<table>
<thead>
<tr>
<th>Population</th>
<th>Germination %</th>
<th>Days to germination</th>
<th>Coleoptile length, em</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>W (R)</td>
<td>W (R)</td>
<td>W (R)</td>
</tr>
<tr>
<td>81-3M</td>
<td>99 (96)</td>
<td>3.3 (3.5)</td>
<td>17.0 (14.7)</td>
</tr>
<tr>
<td>81-3M</td>
<td>99 (96)</td>
<td>3.3 (3.5)</td>
<td>17.0 (14.7)</td>
</tr>
<tr>
<td>81-7M</td>
<td>81 (83)</td>
<td>4.2 (4.7)</td>
<td>16.0 (14.6)</td>
</tr>
<tr>
<td>81-2M</td>
<td>71 (74)</td>
<td>4.5 (4.6)</td>
<td>15.1 (14.6)</td>
</tr>
<tr>
<td>Mean</td>
<td>88 (88)</td>
<td>3.3 (4.1)</td>
<td>16.8 (14.8)</td>
</tr>
</tbody>
</table>

L.S.D. (0.05) 17.11 0.42 0.11 1.35 0.74

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 Caryopsis and seedling weight in 53 populations of D. villosa.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Caryopsis weight (mg)</th>
<th>Seedling weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>W</td>
<td>15.4 ± 0.59</td>
<td>8.4 ± 0.43</td>
</tr>
<tr>
<td>R</td>
<td>8.4 ± 0.43</td>
<td>6.8 ± 0.35</td>
</tr>
<tr>
<td>t</td>
<td>0.23 ns</td>
<td>0.33 ns</td>
</tr>
</tbody>
</table>

** P < 0.01.
The Phylogeny of Psathyrostachys Nevski (Triticeae, Poaceae) - Are We Able to See the Wood for the Trees?

Seberg, O.¹, Petersen, G.¹ and Baden, C.²

¹Botanical Laboratory, Botanical Institute, University of Copenhagen
²Dept. of Botany, Dendrology and Forest Genetics, The Royal Veterinary and Agricultural University, Rolighedsvej 23, DK-1958 Frederiksberg C, Denmark

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 (cDNA), 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 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 monotypic 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, r-ribulose-1,5-bisphosphate carboxylase/oxygenase). rbcl, 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: rbcl, rpoA, and rpoC2 (the genes for the 2 and 4 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 (Scóles et al. 1988, Baldwin 1992)).

A few investigations have used the single copy nuclear encoded gene for alcohol dehydrogenase 1 (Adh1) in phylogenetic studies (e.g., Gutt and Clegg 1993). Adh1 is a
NAD+ dependent dimeric osidoreductase which catalyzes the oxidation of a wide range of alcohols. It belongs to a group of alcohol dehydrogenases which also includes Adh1 and Adh2. In the Poaceae (incl. Hordeum L.) Adh1 is found. Hordeum was used as outgroup (Friesdorfer and Seberg 1992).

**Sequencing**

A list of the plants sequenced in the present investigation is found in Table 3. In Table 2 two species DNA was extracted from herbarium material. For various reasons P. juncea (Fischer) Neevski and P. kronenburschi (Hack.) Neevski 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) (Saiki et al. 1988). Double-stranded amplifications were followed by one-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 reactions running 20-40 cycles, and with a 1:50-100 dilution of one primer in the single-strand primer. 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 semistree (Bremer 1990) consensus tree was calculated from the suite of equally parsimonious 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 I (Adh1)**

Primers were designed to amplify a segment of Adh1, spanning exons 5 to 8. The primers were constructed from the Adh1 sequences published by Trick et al. (1998). Care was taken to secure that there was a minimal match in the 3’-end with Adh2 and Adh3. The segment chosen covers approx. 550 bp. To increase the probability that only Adh1 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 Glidstein 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 Adh1 sequences were partitioned into four different subsets and subjected to phylogenetic analysis:

<table>
<thead>
<tr>
<th>Accession</th>
<th>Country</th>
<th>Locality</th>
<th>Chromo-</th>
<th>Collector</th>
</tr>
</thead>
</table>

The whole sequence, exons only, and introns only (with gaps coded as informative or gaps excluded, respectively).

**The n subunit of RNA polymerase (rpoN)**

Primers were designed to amplify the entire rpoN gene and the intergenic regions between rpoQ and rpoI and rpoD, respectively (approx. 1360bp). They were constructed from the published sequences from rice, wheat, and maize (Hiratsuka et al. 1989; Hirai et al. 1989, Ruf and Kössel 1988). The sequences could easily be aligned by eye.

The large subunit of ribulose (rbcL)

Primers designed by Dr. G. Zuravski (DNA Research Institute, Pala, California), but slightly reduced in length in the 3’-end, were used for the amplifications. Additional primers were produced from the Hordeum-sequence and from Zea mays L. (Zuravski 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 rbcL, 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 semistrect consensus tree is shown in Fig. 1A; in the strict consensus tree the clade including P. langenose (Trin.) Neevski, P. kronenburschi, P. junceae, and P.
Fig. 2. Total evidence trees for *Paathyrastachys* based on A. All data and B. cpDNA data plus morphology. For abb. see fig. 1.

A

<table>
<thead>
<tr>
<th>Species</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. grandis</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>P. exigua</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>P. chrysanthemifolia</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>P. glauca</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

B

The consensus tree is based on a cladistic analysis of the data presented in Table 1. The tree is a cladogram showing the relationships among the species of *Paathyrastachys*. The tree is divided into major clades, with the species forming discrete groups. The tree is supported by a high degree of bootstrap value, indicating strong statistical support for the relationships among the species.

**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 evidential possibility that added sampling will close the gaps. Even though the distinction between these two qualities of data is not as clear as it is frequently thought (Thiele 1994), we have only used the quantitative characters as a last resort (Chappell 1988). 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 = 32, 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 I sequences (see Fig. 1B), which also resulted in a non-monophyletic *P. fragilis*.

Great care has been taken to ensure that only Adh I has been amplified. In cases where double sequences were obtained it was checked whether the sequences could have been derived from Adh I or Adh II, but this seemed never to be the case. The possibility of gene polymorphism, which has been amply documented in *P. fragilis* (Klein and Klawans 1993), was at least difficult to believe that the structure of the Adh I 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. exigua*) where there was 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 the data sets are combined the dominating effect of the highly variable Adh I sequences is clearly seen and the single total evidence tree obtained suffers from the same inexplicable deficiencies as the Adh I gene tree (fig. 2A).
If the Adh1 sequences are removed and only the cDNA 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. jagiels constitute a clade and one possible resolution of the polyphyly including the P. elongatus accession 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; Swoford 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 artifical.

Acknowledgements - This study was supported by grants no. 11-0274 and 11-7722 from the Danish National Science Research Council and by grant BIO2 CT-920486 from the EU. The DNA-work was made at the Department of Population Biology, Institute of Zoology, University of Copenhagen. Charlotte Harwitz and Lisbeth Knudsen are thanked for their skilful technical assistance. Dr. G. Zurawski is gratefully acknowledged for putting the rbd-primer at our disposal. Dr. M. Cummings for letting us have access to the, at that time unpubliculated, morphologies of the rpoC2 primers.  

LITERATURE CITED


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Res. 17:6394.
The Principle of Recombination Gene Pools (RPG) and Introggression Gene Pools (ITG) in the Biosystematic Treatment of Elymus Species

AGAVONOV Alexander V.
Central Siberian Botanical Garden, Siberian Branch of Russian Academy of Sciences, Novosibirsk, 630090, Russia

ABSTRACT

Individual genotypes of Elymus sibiricus, E. jacutensis, E. macraus, E. camus, E. jacutis and E. deflexus complexes were identified by endosperm protein electrophoretic patterns. Genotypes within these complexes vary with respect to their recombination compatibility Cr (genotypic level) and reproduction ability of hybrids Av (phenotypic level). Analysis of hybrid fertility in the F1; F2, and segregation of morphological characters provide an understanding of Recombination Gene Pools (RPG) and Introgresive Gene Pools (IGP) in the genus Elymus. The identification and marking of RPG 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 RPGs being combined on the morphological similarity (overlapping) of individuals belonging to the same adjoining RPG with introgression of genetic material possible from one RPG 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 Hultin and de West [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 (Heidelberg, Sweden, 1991) a system of gene pools in barley, cereal and forage grasses of the tribe Triticeae was presented [Bothmer et al. 1993]. 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 identified by SDS-PAGE-electrophoresis of endosperm protein (Fig. 2, 3). E. sibiricus (from Asiat Ric 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), ZE-88-06 (Amurski region, Fig. 2-e), JAC-83-26 (Yacutila-Sakhia, Fig. 2-g), KAM-92-19 (Kamchatka), SAK-91-13 (Sakhalin, Fig. 2-k), MLA-84-51 (Vladivostok, Fig. 2-l), SIC-90-62 (China, Sichuan, Fig. 2-m), shortawn forms SH-152 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-e), H8800, H7570, and H7538 (China, Xinjiang, Fig. 2-e, 2-f, 2-g), E. jacutensis (Orlov.) Tzvel. GAC-89-58 (North Altai mountain, Fig. 3-d), JAC-89-01 (Yacutila-Sakhia, Figs. 3-a), AMU-90-01 (Amurski region, Fig. 3-g), E. macraus (Turkey) Tzvel. GAL-89-19 (Central Altai mountain, Fig. 3-c), JAC-89-22 (Yacutila-Sakhia, Fig. 3-e), JAC-83-13 and JAD-87-05 (both Yacutila-Sakhia); E. camus (L.) L. ELC-83-06 and ACD-88-05 (Novosibirsk region), GAT-92-10 (Altai), KAS-85-05 (North Tien Shan, Kazakhstan), accessions received from the Forage and Range Research Lab. (USDA-ARS, Logan, Utah, USA), PI 72364 (Turkey), PI-251417 (Jugoslavia), PI-314205 (USSR, Krasnodar region). E. jacutensisPers. is morphologically similar to E. camus: 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 deflexus complex includes E. exsexus Tzvel. ex. Griseb., E. warochskii Probst., 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 (Kyrgyzstan, 1 genotype), Central Tien Shan (2 genotypes); (2) accessions received from DCGS (the Swedish Univ. Agr. Sciences); HBR-13, HBR-363 (Tibet, China), HBR-069, HBR-107 (Sichuan, China). The latter is aawless morphotypically.

The crossing procedure followed that previously
described by Lu and Rothner (1990). Reproductive ability of hybrids between individuals belonging to same or different taxonomic species was studied in two or three generations. Plants of the F₁ and F₂ hybrids were grown in the greenhouse at the Swedish Univ. Agr. Sciences, Sundsvall 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 F₁ plants were analyzed by SDS-PAGE 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 “Intergenome Gene Pool” (IGP). The recombination compatibility of a pair of genotypes (Integrative value C) reflects general homology of parental genomes and is realized in phenotypes through the mediation of hybrid fertility as the reproduction ability A. The pollen formation process is sensitive to only minor aberrations 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 peculiarity is the basis for the proposed approximate scale of recombination (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 of A is less than would be expected theoretically under optimal environmental conditions, suggesting the need for a coefficient of realization k = 1.0.

A level is defined as the Recombination Gene Pool (RGP) and corresponds to the entire gene pool where 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 F₁ is normal. The major difference in A2-level is the potential for F₂ segregation.

B-level refers to the Intergenome Gene Pool (IGP). Within B₁-level, fertility of self-pollinated hybrids is more than zero, with plants in the F₂ 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 are shown in Table 1.

The data suggests that E. sibiricus complex represents an indivisible RGP throughout the Alatac area. Genotype SIC-90-62 from Schuam, 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. nates and SHY genome taxa. However, the process of genome introgression as it relates to speculation is not yet well defined making its

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**Table 1: Seed fertility in hybrids of E. sibiricus in the F₁ and F₂**

<table>
<thead>
<tr>
<th>Cross combination determination</th>
<th>NO</th>
<th>Seed fertility in F₁</th>
<th>Seed fertility in F₂</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Highest value of seed set 1</td>
<td>Highest value of seed set 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pl. 1</td>
<td>Pl. 2</td>
</tr>
<tr>
<td>1. A₁ - 21 X L. 12</td>
<td></td>
<td>75.5</td>
<td>1</td>
</tr>
<tr>
<td>2. A₁ - 01 X L. 12</td>
<td></td>
<td>19.9</td>
<td>1</td>
</tr>
<tr>
<td>3. KJL-31 X L. 26</td>
<td></td>
<td>26.1</td>
<td>1</td>
</tr>
<tr>
<td>4. A₁ - 31 X L. 26</td>
<td></td>
<td>274</td>
<td>1</td>
</tr>
<tr>
<td>5. A₂ - 31 X L. 26</td>
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<td>8. H. 1306 X H. 22M</td>
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<td>18. SH-262 X H. 1249</td>
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<td>23. SH-262 X H. 1249</td>
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<td>29. SH-262 X H. 1249</td>
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<td>30. SH-262 X H. 1249</td>
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</tbody>
</table>

* Determination of crossing: E = electrophoretic, M = morphological

---

**Table 3: Highest values of seed fertility in some hybrids of E. jacea and E. macrourus in generations F₁,F₂**

<table>
<thead>
<tr>
<th>Cross combination determination</th>
<th>NO</th>
<th>Seed fertility in F₁</th>
<th>Seed fertility in F₂</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Highest value of seed set 1</td>
<td>Highest value of seed set 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pl. 1</td>
<td>Pl. 2</td>
</tr>
<tr>
<td>5. JAC-13 X LAC-13</td>
<td></td>
<td>4.1</td>
<td>1</td>
</tr>
<tr>
<td>6. JAC-13 X LAC-13</td>
<td></td>
<td>4.1</td>
<td>1</td>
</tr>
<tr>
<td>7. reciprocal</td>
<td></td>
<td>4.1</td>
<td>1</td>
</tr>
<tr>
<td>8. JAC-13 X LAC-13</td>
<td></td>
<td>4.1</td>
<td>1</td>
</tr>
<tr>
<td>9. reciprocal</td>
<td></td>
<td>4.1</td>
<td>1</td>
</tr>
<tr>
<td>10. JAC-29 X LAC-29</td>
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</tr>
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<td>11. JAC-29 X LAC-29</td>
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<td>15. JAC-29 X LAC-29</td>
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</table>

* Number of plants analyzed
difficult to assign taxonomic rank based on IGP. A segregation of morphological characters in the F₂ 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. silicicus is controlled by two loci with independent inheritance. The phenotypic ratio of awned to awnless in the F₂ population is 15:1. In an attempt to transfer the awnless character from genotypes SH-252 and SH-336 to other genotypes was accompanied by a segregation of a number of Mendelian and polygenic morphological characters.

 Studied genotypes of E. jucundus (#) 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-019 x juc-22* in the F₂). The characters "glaucescent 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 [AC-89-22] for E. macrourus is more or less comparable with all studied genotypes of E. jucundus-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 n₁ = 1.

 Based on F₁:F₂ hybrid fertility all genotypes of E. jucundus 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 subspecies 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 subspecies (Fig. 3).

Little is known about genotypes from the Russian Far East, however, they are known to occur there (Bazal. Rast. Sov. Dal. Vostoka, 1985). It is very likely that E. zapisiz Probat, also belongs to RGP of E. jucundus-E. macrourus complex.

Data regarding seed fertility of infraspecific hybrids in E. caninus (17 combinations) and E. dalchus complex (28 combinations) support the existence of separate RGP and IGP.

E. caninus represents a widespread RGP which includes genotypes from Siberia, Kazakhstan and the South-Eastern part of Russia as a major recombination nucleus (Table 6). The genotypes from Turkey and Yugoslavia form another nucleus. Recombination between the two groups is at the A2-level. The genotype of E. jucundus, GAC-89-21, has probably diverged from the main recombination nucleus of E. caninus recently. This accession differs from E. caninus by having hairy lamina and longer glumes with awns up to 5 mm 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. dalchus represent a single RGP, suggesting that regardless of the geographical region, collections will reflect the basic genotypes worldwide. Within the RGP of the E. dalchus complex, the most distant genotypes were E. eculus from the Far East Russia and E. dalchus from Sichuan China, based on hybrid fertility in the F₁ and F₂ hybrids. A slight decrease in hybrid seed fertility was observed in hybrids along a geographic transect through Siberia, Tian Shan mountains of the USSR, Tibet, and Sichuan Provinces of China.

Genotype IRS-87-06, E. dalchus from Far East Russia, had a low recombination compatibility with any other genotypes within this complex. Its hybrids had very little seed set in the F₁ and F₂ 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. dalchus complex.

The character "long awns" in E. dalchus is controlled by two loci as well as in E. silicicus. The diagnostic character of E. wrosscholdi which has glaucescent stem and leaves, is controlled by a single dominant gene.

The use of RGP and IGP provides an additional tool to better understand phylogenetic relationships in the genus Elymus. A taxonomical species is an artificial category combining one or more RGP 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 phylogenetically. 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 precise one) of parental genotypes and hybrids is an essential part of the RGP-IGP principle. Combined characteristics of stable plant accessions would make up the basis for a precise of recombination compatibility of genotypes that have not been crossed.

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LITERATURE CITED


Agafonova O.V. 1995. Are there three levels of endosperm protein electrophoretic specificity in Elymus species? - This volume.


