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## II. CONTRIBUTIONS

### PRIVATE COMPANIES

#### **AGRIPRO WHEAT**

**Northern Plains Hard Red Spring Wheat, 806 N. Second Street, P.O. Box 30, Berthoud, CO 80513, USA.**

**Southern Plains Hard Winter Wheat, 12167 Hwy 70, P.O. Box 1739, Vernon, TX 76385, USA.**

**Central Plains Hard Winter Wheat, 6515 Ascher Road, Junction City, KS 66441, USA.**

**Northern Soft Red Winter Wheat, P.O. Box 411, 520 E. 1050, South Brookston, IN 47923, USA.**

**Southeastern Soft Red Winter Wheat, P.O. Box 2365, Jonesboro, AR 72402, USA.**

AgriPro Wheat develops wheat varieties for virtually all classes of wheat grown in the United States and Canada. Headquarters are located in Berthoud, CO.

AgriPro Wheat is a business unit of Advanta, BV and is comanaged by Rob Bruns, David Worrall, and Rollin Sears. Rob is the general manager and handles business and strategy. David and Rollin are responsible for research and development and strategy. Bill Kuntz is the national sales manager and is located at Berthoud with Rob Bruns. Rick Novak is in charge of the foundation seed program and also is stationed in Berthoud.

AgriPro Wheat is divided into regional business teams composed of a senior wheat breeder and a regional business manager. These two individuals are responsible for regional product development and market strategies. Presently, we are marketing 48 different wheat varieties adapted to the wheat-growing regions in the U.S. or Canada. AgriPro Wheat is dedicated to creating a successful seed alliance with technology providers, milling companies, and seed associates that ultimately provide wheat growers with significant value derived from wheat seed.

**Southern plains hard winter wheat.** The southern plains hard winter wheat project is headquartered in Vernon, TX. The staff consists of David Worrall, David Graf (regional business manager), and Bradley Burkett.

New facilities were finished in May, 2001, which include offices, seed labs, cold storage, greenhouses, equipment storage, and repair facilities. Approximately 120 acres of irrigated land is available for research work and wheat-breeding nurseries. Breeding efforts will be focused at wheat-growing regions in Texas, Oklahoma, and New Mexico. Primary emphasis will be placed on developing 'dual-use' wheat varieties capable of performing when cattle graze in the autumn and winter and grain production is then required after grazing has been completed. Varieties adapted to this management system require vigorous forage growth during the vegetative phase, excellent disease resistance, and good recovery from grazing allowing for high grain-yield potential.

Two new HRWWs will be released to AgriPro associates in the autumn of 2002. **AgriPro Cutter** is adapted from central Texas to southern Kansas. Cutter has good disease resistance, is medium early, and has a good test weight. AgriPro Cutter has good milling and baking characteristics. **AgriPro Jagalene** is adapted to the high plains of Texas, Oklahoma, Kansas, Colorado, Nebraska, and parts of South Dakota. This variety is short with good straw strength and will be competitive under both dryland and irrigated conditions. Jagalene has good disease resistance, very good drought tolerance, and excellent test-weight patterns. AgriPro Jagalene has excellent milling and baking characteristics.

**Central plains hard winter wheat.** The central plains winter wheat project is located in Junction City, KS. The staff consists of Rollin Sears; Charles Johnson (regional sales manager); and research assistants John Robbens, Jon Rich, and Harold Erichsen.

New facilities were completed in May, 2001, which include an office building, seed and molecular labs, greenhouses, and equipment and seed storage buildings. Approximately 110 acres of highly productive ground is available for wheat research and wheat-breeding nurseries. Breeding efforts will be focused on the Kansas, Colorado, Nebraska, and South Dakota wheat-growing regions. Active programs exist for both HRWW and HWWW. Presently HRWWs are released through our AgriPro associate network and distributed to growers. All HWWWs have been released under contract production and have been grown identity preserved.

AgriPro Cutter and AgriPro Jagalene also will be distributed to central plains associates in the autumn of 2002.

**Northern plains hard red spring wheat.** The HRSW project is headquartered at Berthoud, CO. The staff consists of a senior breeder Joe A. Smith; regional business manager Dennis Tweed; and research assistants Scott Seifert, Linda Sizemore, and Bill Schabinger.

In 2001, we had three sites in central/western North Dakota and five sites in the Red River Valley. Breeding nurseries were located at Casselton and Park River, ND. Cool, early season conditions made the crop lush and taller than normal. Heavy lodging was experienced on early-planted sites in the valley. Data was inconsistent between planting dates. A moderate scab infection at Park River was useful in our screening for this disease. Foliar disease was heavy at most sites.

**Norpro** will be released to farmers in 2002. This variety is a semidwarf with strong straw strength, which many farmers will be looking for in the upcoming season. Norpro has exhibited a good combination of high yield and medium-high protein. It appears well adapted to the entire Northern Plains. With average tolerance to scab, Norpro should be managed accordingly to reduce infections.

We have two new varieties under production, Knudson and Hanna. These will be available to farmers in 2003. **Knudson** offers consistent yield and broad adaptation. **Hanna** has a Canadian background and looks to be a good replacement for Gunner in our AgriPro lineup due to its earlier maturity and more consistent performance. Both of these varieties have above-average tolerance to scab.

We had anticipated the release of two Clearfield® varieties in 2002, but much to our disappointment, the present herbicide tolerance in spring wheats was deemed unsatisfactory. This decision was made jointly by BASF and all of its partners in Clearfield spring wheat development. Presently, we have an accelerated breeding effort to incorporate stronger herbicide tolerance in our spring wheat materials.

**Canadian hard red spring wheat.** This effort is through a joint wheat development agreement between Agricore United of Canada and AgriPro Wheat. The brand name for Agricore United is Proven Seed. Proven Seed research staff consists of Kevin McCallum who is located at the Proven Research Farm in Morden, Manitoba, and Jim Dyck who is located in Saskatoon, Saskatchewan.

Breeding emphasis has been 75 % and 25 % on the CWRS and CPS classes, respectively. We also are developing herbicide tolerant varieties for the Clearfield-production system in both classes.

The breeding nursery is located at the Proven research farm in southern Manitoba. We have had consistent leaf rust and scab infections at this site over the past 8 years. In the 2001 season, we had four sites in Manitoba and three sites in Saskatchewan. The season gave us excess precipitation in Manitoba and excess drought in Saskatchewan. Yield results were mixed. Moderate scab infections were present at most Manitoba sites.

**BW256** (CWRS) and **HY962** (CPS) were supported for registration in 2001. Proven Seed has adopted a numbering system on varietal releases. BW256 has been named 5601HR and HY962 named 5701PR. Other recent varieties, which have been registered, include 5600HR, 5500HR, and 5700PR.

**Northern soft red winter wheat.** The northern SRWW program is located in Brookston, IN. The staff consists of Curtis Beazer, (senior breeder), Don Eckoff (regional sales manager), and research assistants Dayna Scruggs and Eugene Glover.

The AgriPro Wheat northern SRWW program has made several changes to improve variety development for wheat adapted to the eastern corn-belt and Atlantic regions. In the autumn of 2001, AgriPro Wheat northern SRWW

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research relocated to the Brookston, IN, research station. Lab and work area remodeling has been completed and construction of a new wheat greenhouse will be completed for autumn 2002. Along with this move, the main breeding site has been relocated to highly productive soils where varieties such as Twain, Sawyer, and Patton were bred. The new site also has equipment for irrigated nurseries allowing for research on improved sprout and fusarium resistance.

Eugene Glover has joined the team as a research specialist bringing over 15 years of seed breeding experience. He will be responsible for management of all testing sites. Testing will continue to be conducted in the double crop and rotation areas of Missouri, Illinois, Indiana, and Ohio.

**AgriPro Mitchell** was released as registered seed in 2000 to associate marketers and sold as certified seed in the autumn of 2001. AgriPro Mitchell is a high-yielding, medium-maturity variety adapted to the double-crop and rotation areas of the eastern corn belt. AgriPro Mitchell has a unique plant type that works well in an interseeding cropping system. Plant tillering is erect and contained mostly in the drill row allowing for increased light penetration to emerging soybeans in an interseeding program. AgriPro Mitchell has very good test weight and excellent soft wheat milling characteristics.

AgriPro Wheat also has identified two special quality wheat varieties, **AgriPro Hondo** and **AgriPro Charter**, HRWWs with eastern U.S. adaptation. In partnership with ConAgra, an identity-preserved production system has been established for these two varieties.

**Southeastern soft red winter wheat.** The southeastern SRWW program is located in Jonesboro, AR. The staff consists of Barton Fogleman (senior breeder), Gary Moore (regional business manager), and research assistants Michael Montgomery and Christopher DeArmond.

Chris DeArmond has been a welcome addition to our breeding team for the southeast. We are preparing to build a new greenhouse for crossing and other research and are generally upgrading our facilities. The 2000–01 season was marked by a slight decline in overall acres in the southeastern U.S. Dry conditions in the autumn delayed planting in some areas and may be partly responsible for fewer acres being planted. As usual, once it began to rain, soils remained damp and growers who waited for moisture before planting were delayed even further. Although disease pressure was relatively light in the midsouth, grain yields were about 5 bu/acre less than those reported in the previous 2 years.

**AgriPro Natchez** is the name given to D95-7763. This new SRWW has shown adaptation to the midsouth and lower midwest U.S. Natchez has shown moderate resistance or better to many of the foliar diseases (leaf rust, stem rust, stripe rust, and *S. nodorum* and *S. tritici*) and to the soil virus complex (WSSMV/WSBMV). The variety is a medium-maturity wheat that seems to prefer the sandier or loamier soil types.

We are evaluating the release of three new Clearfield SRWWs and should be releasing one or more of these to growers in the autumn of 2002. We have begun an Identity Preserved wheat program in northern Alabama and central Georgia with a major milling company using the new AgriPro Charter special quality wheat. This variety will only be grown by producers who are participating in the IP program.

**Pacific Northwest.** AgriPro Wheat has begun the initial steps necessary to establish a full-scale, varietal-development program for the Pacific Northwest. The staff consists of John Moffatt (senior breeder), Bob Knudson (regional sales manager), and senior assistant breeder Jim Hemerick. Presently, the breeding program is headquartered in Berthoud, CO. During the summer of 2002, a new location will be established in Washington.

Bob Knudson, longtime regional business manager for AgriPro's spring wheat program, has retired from that position and has assumed the regional business manager role for the PNW. Dr. John Moffatt will take on the breeding and be working with Jim Hemerick, longtime AgriPro/Hybritech employee currently living in the Spokane, WA area. Current activities include screening wheats from existing AgriPro hard wheat programs (both spring and winter), germ plasm base building for soft whites, and establishing familiarity with the region's diverse wheat-production requirements, researchers, producers, and seedsmen. Testing for the 2002 crop year consists of 12 locations ranging from Moses Lake, WA, to Blackfoot, ID.

**OR SEED BREEDING COMPANY****Rua João Battisti, 71 – Passo Fundo, RS–CEP, 99050-380, Brazil.**

O.S. Rosa and O. Rosa-Filho.

Wheat production in Brazil in 2001 reached more than 3 million tons for an annual consumption of 10 million tons. Today, Brazil is the country that imports the most wheat.

OR Seeds started breeding activities in 1989. The following cultivars created by the company are now registered for cultivation in various wheat-growing regions of Brazil: OR-1, Rubi, Granito, Taurum, Alcover, Avante, Onix, and Jaspe. The newest cultivar, Jaspe (ORL 91308/Rubi 'S'), was registered for cultivation in the state of Rio Grande do Sul in 2002. The cultivars OR-1, Alcover, and Avante have demonstrated good resistance to BYDV. Rubi, Granito, and Onix are susceptible to BYDV and moderately resistant to WSBMV.

The resistance to FHB in lines ORL 98096 and ORL 98231, which were previously selected because of their excellent resistance to FHB, was confirmed in 2001 in Brazil, Argentina, and in the U.S. Along with scab resistance, these lines have demonstrated good yield potential (7 ton/ha) and will most likely be registered for cultivation in Brazil in 2004.

André C. Rosa finished his Ph.D. degree at Kansas State University and rejoined the company in 2002.

**STOLLER ARGENTINA S.A.****Av. Malagueño s/n-Complejo Industrial U. CO. MA. Ferreyra, C.P. X5020CST, Córdoba, Argentina.*****Effect of foliar fertilization on a wheat crop cultivated under rainfed conditions.***

W. Londero and L.E. Torres and R.H. Maich (Cátedra de Genética, Facultad de Ciencias Agropecuarias–Universidad Nacional de Córdoba).

Stoller Argentina Co., with the technical support of professionals from the College of Agriculture (Córdoba National University), led an experiment to evaluate the effect of three different formulations of foliar fertilizers on the agronomic behavior of a wheat crop cultivated under rainfed conditions.

A production field located at 31°29' S, 64°00' W was planted with the commercial cultivar PROINTA-Puntal (220 seeds/m<sup>2</sup>). An area of 1,200 m<sup>2</sup> was used and subdivided into 12 plots of 100 m<sup>2</sup> each. The plots received four different treatments, applied at tillering and boot developmental stages (Table 1). A complete randomized-block design with three replications was used.

At harvest, four samples of each 1 m<sup>2</sup>/plot were taken and tiller and spike number/m<sup>2</sup>, grain yield (g/m<sup>2</sup>), aerial biomass (g/m<sup>2</sup>), harvest index (%), grain

**Table 1.** Plot, treatment, and application time for material in the experimental plots.

Plot	Treatment	Application time
T <sub>0</sub>	Control	
T <sub>1</sub>	Stimulate (auxins, gibberellins, cytokinins) + Starter (Zn, Cu, Mn, B, S)	start of tillering
T <sub>2</sub>	Starter (Zn, Cu, Mn, B, S) Boro	start of tillering spike in boot
T <sub>3</sub>	Mastermins (N, P, K, Mg, S, Zn, Mn, B, Mo) Mastermins (N, P, K, Mg, S, Zn, Mn, B, Mo)	start of tillering spike in boot

number/m<sup>2</sup>, and 1,000-kernel weight were recorded. From a sample of 20 spikes, plant height (cm), peduncle length (cm), spike length (cm), spikelet number/spike, total weight/spike, grain weight/spike, grain number/spike, and grain number/spikelet were measured. Because of damage caused by a hailstorm, the results were analyzed from a descriptive point of view.

At the plot level, the T<sub>3</sub> mean values for grain yield and its two principal physiological components (aerial biomass and harvest index) were superior than those for the other treatments (included the control). This positive trend also was observed in some physical grain-yield

components such as grain number/spike and grain number/spikelet, and grain number/m<sup>2</sup>; whereas for 1,000-kernel weight, the T<sub>3</sub> mean value did not exceed that of the control (Table 2).

**Table 2.** Values of measured components for each of the treatment regimes.

Variable	Treatment			
	T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>
Grain yield (g/m <sup>2</sup> )	207.08	201.67	195.75	213.83
Aereal biomass (g/m <sup>2</sup> )	1012.19	981.14	962.23	1014.54
Harvest index (%)	20.50	20.59	20.49	21.19
Grains/spike	23	22	22	24
Grains/spikelet	1.44	1.36	1.42	1.52
Grains/m <sup>2</sup>	5,902	5,789	5,828	6,181
1,000-kernel weight (g)	35.12	34.78	33.68	34.58

**Conclusions.** In the future, we plan to study foliar fertilization applied to several bread wheat varieties cultivated in at least two different locations.

## ITEMS FROM ARGENTINA

### CÓRDOBA NATIONAL UNIVERSITY

College of Agriculture, P.O. Box 509, 5000 Córdoba, Argentina.

#### *Recurrent selection for grain yield: morphophysiological changes after four cycles.*

R.H. Maich, Z.A. Gaido, S.P. Gil, G.A. Manera, and M.E. Dubois.

We evaluated four cycles of recurrent selection for grain yield in bread wheat and determined direct and correlated responses. The cycles compared were the C<sub>0</sub> (initial); C<sub>1</sub>, C<sub>2</sub>, and C<sub>3</sub> (intermediate); and C<sub>4</sub> (more advanced).

During 3 consecutive years, 1998, 1999, and 2000, 12 S<sub>1</sub>-derived families/population were sown at the Experimental Farm of the College of Agriculture (31°29' S and 64°00' W), Córdoba, Argentina. Plot data were recorded on grain yield, aerial biomass, harvest index, fertile florets/spikelet, seed number/spikelet, seed number/spike, grain-protein content, test weight, gluten percentage, and mixograms. The information was processed using ANOVA and Duncan's Multiple Range Test. The results indicated that although there was no significant difference between C<sub>0</sub> and C<sub>4</sub> for grain yield and its physiological components, a positive trend was denoted. Statistically significant differences between cycles were observed for the physical components of grain yield including fertile florets/spikelet, seed number/spikelet, and seed number/spike, where the C<sub>4</sub>-derived families had higher mean values than that of the original population (C<sub>0</sub>).

Published results of the physical components of grain yield (i.e., seed number/spike) showed associated changes with respect to improvement in seed production, however, we remain cautiously optimistic about the efficiency of our plant-breeding program. Test weight and bread-making quality measured in the C<sub>4</sub> (protein content, gluten, and mixograms) were similar to the C<sub>0</sub> population with good quality values.



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***Effects of three cycles of recurrent selection on the flag-leaf morphology of bread wheat.***

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M.M. Cerana, S.P. Gil, and R.H. Maich.

We are interested in flag-leaf morphology because it changes throughout different cycles of recurrent selection and it is one of the main organs related to grain filling in bread wheat. We evaluated the effects of three cycles ( $C_0$ ,  $C_1$ ,  $C_2$ , and  $C_3$ ) of recurrent selection for grain yield on the length, width, and flag leaf area of wheat plants.

All material was sown at the Experimental Farm of the College of Agriculture (31°29' S and 64°00' W), Córdoba, Argentina, during 3 consecutive years (1996, 1997, and 1998). Five flag leaves from each experimental unit were studied. Data were processed with ANOVA and Duncan's Multiple Range Test. Significant differences were observed among cycles only for length, the  $C_3$  (the more advanced cycle) plants had shorter leaves than those of the  $C_0$  (the original population), whereas no significant changes were noted in the width and total area of the flag leaves.

**INSTITUTO DE RECURSOS BIOLÓGICOS, INTA CASTELAR**  
**Las Cabañas y Los Reseros s/n, (1712) Villa Udaondo, Pcia Buenos Aires, Argentina.**

***Microsatellite screening of the Rht8 dwarfing gene in Argentinian wheat cultivars.***

M.M. Manifesto and E.Y. Suárez.

Two important dwarfing genes, *Ppd1* and *Rht8*, were introduced in Argentina from the Japanese variety Akakomugi in the 1930s by Professor Nazzareno Strampelli. Studies in different European countries suggest that *Rht8* reduces height by an average of 7 cm (Worland et al. 1988; Worland and Law 1986) with no observable adverse effect on plant yield. Korzun et al. (1998) identified that the microsatellite marker WMS261, tightly linked to *Rht8*, was located on the short arm of chromosome 2D.

Varietal characterization in European wheats shows a high association between the marker and the presence of *Rht8*. Worland et al. (1998) described the role of the 192-bp allele, which came from Akakomugi through the cultivar Ardito, as a reduced-height allelic variant. Similarly, the 165-bp allele would be a height promotor and would be diagnostic for CIMMYT varieties and their descendants. This allele would be present in varieties carrying Norin alleles to counteract extremely low height.

Our study includes 165 bread wheat cultivars from Argentina, released between 1920 and 1998, for microsatellite screening. Modern and key varieties in pedigrees were included. Eight allelic variants were found at the *Rht8* locus with the WMS261 microsatellite. The majority of varieties fell into three major groups. The 165-bp allele was the most numerous and was found in 94 varieties; a 192-bp allele group contains 41 varieties, and the group with a 174-bp allele contains 11 varieties. Other allelic variants were scarce and represented in only one or two, but no more than seven, varieties. Six varieties had two allelic variants simultaneously and were excluded for further analyses.

However, the direct association of each allelic variant and its effect was not possible to establish in this set of varieties. The effect of the 192-bp allele on reduced height in several cases was unclear, because this allele also is present in Chinese Spring and its derivatives 38MA and Sinvalocho that were widely used, and is sometimes found together with Ardito in the pedigree of a single variety. Similarly, the 165-bp allele, diagnostic for CIMMYT varieties (Worland et al. 1998), also was present in old varieties such as Americano 25e, which was largely used in the development of local germ plasm. Apparently, the same SSR has two different alleles of identical weight but with different sequences adjacent to the microsatellite motif (Worland, personal communication). Extensive molecular analyses of the complete pedigrees are required, and more experiments need to be designed for a complete understanding.

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### ***Genotypic characterization of Argentinian wheat varieties at loci related to grain texture.***

M. Bonafede, G. Tranquilli, and E. Suárez.

Grain texture is one of the characters that determines the end-use potential of wheat. This character is inherited as a single genetic factor and is controlled mainly by the hardness (*Ha*) locus on the short arm of chromosome 5D in hexaploid wheat. Recent studies indicate a strong linkage among the *Ha*, *pinaD1*, and *pinbD1* loci. *Pina* and *Pinb* code for puroindoline a and b proteins, respectively.

We wanted to characterize the genetic variability of Argentinian wheat varieties at the *Pina-D1* and *Pinb-D1* loci by using specific PCR molecular markers. One hundred twenty varieties of common wheat from the Base Gene Bank located at our institute were analyzed. We also evaluated were varieties released between 1932 and 1998.

To date, results have shown a low variability for both grain texture-related genes. The germ plasm we evaluated owes its hard texture to two conserved mutations. These allelic variants are the lack of the purindoline a protein, and a glycine to serine mutation in *Pinb*. These variants are represented equally among the germ plasm. These observations are representative of frequency of these alleles to those observed in germ plasm surveys from Europe and the U.S.

### ***Phenotypic characterization of a chlorina mutant of hexaploid wheat***

Adrián E. Bossio, Cecilia Bender, and Alberto Acevedo (also of the Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes, Roque Sáenz Peña 180, B1876BXD Bernal, Buenos Aires, Argentina).

The description of the few chlorina mutants that have been reported in wheat (Pettigrew and Driscoll 1970; Sears and Sears 1968; Williams et al. 1983) share a common feature, relative terms are used to refer to the color of their leaves. To solve this ambiguity, absolute chromatic measurements were determined in a chlorina mutant that was isolated from the bread wheat cultivar Leones INTA (Acevedo et al. 2001). A temporal and spatial study on leaf color was made in plants of the mutant and a mother line that were grown in the greenhouse (Table 1). Color was determined with a Minolta Chroma Meter CR-300 in fully expanded leaves. Color as perceived has three dimensions: hue (a), chroma (b), and lightness (L). Chromaticity includes hue and chroma, specified by two chromaticity coordinates (a and b). Because these two coordinates cannot describe a color completely, a lightness factor also must be included to identify a specimen color precisely. This method is nondestructive.

**Table 1.** Dates of measurement of chromaticity in leaves of the chlorina mutant and the mother line.

Date of measurement	Leaf number
5 May, 2001	4 <sup>a</sup> – 5 – 6
29 May, 2001	6 – 7 – 8
7 July, 2001	8 – 9 – 10 – 11
24 August, 2001	9 – 10 – 11 <sup>b</sup>

<sup>a</sup> lower leaf. <sup>b</sup> upper leaf.

In seedlings, the temporal and spatial patterns of leaf color reveal slight differences between the mutant and the mother line (Table 2, p. 25). Interestingly, these slight differences cannot be detected with the naked eye, and both mutant and mother line genotypes have normal green leaves.

As the plants developed, the temporal and spatial patterns of leaf color in the mutant were increasingly different from those of the mother line. Accordingly, leaves in the mutant turned yellowish, whereas they remained green in the mother line. At the adult-plant stage, dramatic differences were observed between the temporal and spatial patterns of leaf color in the mutant and the mother line (Table 3, p. 25).

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***Engineering catalase levels in wheat for higher tolerance to oxidative stress.***

Cristina A. Kamlofski, Ruben Marrero, and Antonio Diaz Paleo; Instituto de Genética 'Ewald A. Favret', CICV / INTA, B1712WAA Castelar, Buenos Aires, Argentina.

Plant productivity is influenced by environmental stress. Several reports have shown that salt, freezing, and drought stress are accompanied by the formation of reactive oxygen intermediates. These toxic molecules damage membranes, membranes-bound structures, and macromolecules resulting in oxidative stress. Plant cells contain several antioxidant enzyme systems that scavenge these reactive oxygen intermediates. One enzyme system is catalase (CAT), which catalyzes the disassociation of H<sub>2</sub>O<sub>2</sub> into O<sub>2</sub> and H<sub>2</sub>O (Willekens et al. 1995). The protective role of CAT has been examined under several abiotic stress conditions and in plant-pathogen interactions.

One way to increase tolerance to environmental stress can be achieved by over-expressing transgenes encoding protective proteins or enzymes in a target genotype. To evaluate this strategy, we cotransformed bread wheat with the *Cat1* gene from barley (Skadsen et al. 1995), the selectable *hph* (hygromycin resistance) gene, and the marker gene *gusA*. Immature embryos from two Bobwhite lines, one of them the commercial variety ProINTA Federal, were the target for the genetic transformation. The biolistic method was applied using a Particle Inflow Gun (Finer et al. 1992) as the microprojectile accelerator. Approximately 600 immature embryos were bombarded with gold particles coated with plasmid DNA. The cotransformation was performed with three plasmids: pAc-H1, which contains a truncated *hph* coding sequence (Bilang et al. 1991) under the control of rice *actin 1 5'* regulatory signals; pUbiCat, which contains the barley *Cat1* gene under the control of maize *ubi-1 5'* promoter region; and pBPFA 9gus (Gonzalez-Cabrera et al. 1998). Calli growth and selection were made on an MS medium (Murashige and Skoog 1962) with 2 mg/l 2,4-D mg/l. PCR analysis of the nine, T<sub>0</sub> antibiotic-resistant plants revealed that six plants had the *hph* gene. Among the *hph*-positive plants two were *Cat* positive only, four were *gusA* positive only, and one was *Cat* and *gusA* positive. The three plants that were PCR negative for *hph* also were negative for *Cat* and *gusA*. Taken together, these results demonstrate the all transgenic plants showed cointegration of at least two genes, and in one case, cointegration of three genes. Currently, ongoing research is devoted to confirming the number of integration sites by Southern hybridization analysis. Northern blot analysis and CAT activity will be used to study CAT over-expression on the T<sub>1</sub> and subsequent generations.

**Table 2.** Chromaticity coordinates and lightness factor values in leaves of the chlorina mutant and the mother line. Measurements were taken on 5 May, 2001 and represent the average of five measurements/leaf for five plants.

Leaf number	Mother line			Chlorina mutant		
	hue	chroma	lightness	hue	chroma	lightness
4	-15.58	20.75	40.97	-17.84	26.59	44.61
5	-15.34	19.48	39.21	-17.40	23.98	43.26
6	-15.77	17.90	38.45	-18.15	25.39	42.76

**Table 3.** Chromaticity coordinates and lightness factor values in leaves of the chlorina mutant and the mother line. Measurements were taken on 24 August, 2001, and represent the average of five measurements/leaf for five plants.

Leaf number	Mother line			Chlorina mutant		
	hue	chroma	lightness	hue	chroma	lightness
9	-15.50	25.48	46.20	-15.95	34.85	55.43
10	-11.53	14.58	39.26	-16.05	37.45	57.89
11	-10.49	12.55	37.20	-15.87	35.67	52.65



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**ITEMS FROM AUSTRALIA****UNIVERSITY OF ADELAIDE****Waite Campus, Plant Science, Glen Osmond, SA 506, Australia.*****Flour color and Asian noodles.***

Daryl Mares and Anna Campbell (Current address: Animal Genomics, AgResearch, Invermay, Private Bag 50034, Mosgeil, New Zealand).

Flour and noodle color, together with components of color such as xanthophyll content and polyphenol oxidase activity, were examined in a number of DH mapping populations over two seasons. Flour yellowness (b\*) was highly heritable and strongly correlated with xanthophyll content. Highly significant ( $P < 0.001$ ) QTLs for xanthophyll content located on chromosomes 3B and 7A (Sunco/Tasman) also were associated with variation in flour b\* and noodle b\* (white-salted (WSN) and yellow-alkaline (YAN) noodles). Sunco contributed the higher value allele at the 3B locus, whereas Cranbrook contributed the higher value allele at the 7A locus. Within the Sunco/Tasman DH population, there was significant transgressive segregation for flour and noodle b\* on either side of the parents. In part, this was explained by the additive nature of the alleles contributed by the two parents. In the Cranbrook/Halberd and CD87/Katepwa DH populations, QTLs for flour b\* were identified on chromosomes 3B and 7A and 3A and 7B, respectively. A highly significant QTL associated with variation in PPO activity was located on chromosome 2D. PPO and the 2D QTL were not associated with variation in initial flour and noodle brightness but were strongly correlated with noodle darkening. A second, weaker QTL was located on chromosome 2A.

***Preharvest sprouting tolerance.***

Daryl Mares and Kolumbina Mrva.

Sprouting tolerance derived from AUS1408, a white-grained genotype originating in the Transvaal region of South Africa, has been introgressed into locally adapted germ plasm. Improved cultivars should be available to wheat growers in the near future. Some of this material exhibits tolerance equal to the original donor and, at least under Australian conditions, similar to some of the better red-grained wheats and would substantially reduce the incidence and severity of sprouting in Australia. Other material exhibits only an intermediate level of tolerance and appears to have lost one of the two putative genes controlling dormancy in AUS1408. This intermediate level of tolerance, nevertheless, also represents an improvement over most current commercial varieties. Newer sources of sprouting tolerance originating from China have grain dormancy similar to AUS1408 together with other useful disease and agronomic traits. Preliminary results from a half-diallel cross suggest that genetic control of dormancy in the two sources may be very similar. Intermediate dormancy/sprouting tolerance also is characteristic of the older, Australian cultivar Halberd. Variation for grain dor-

mancy in a DH mapping population, Cranbrook (nondormant, very susceptible to sprouting)/Halberd was associated with QTLs on chromosomes 2A, 2D, and 4A. Of particular interest was the QTL on chromosome 4A that appeared to correspond with a QTL reported in other populations, including a red-grained, dormant/nondormant population.

### ***Late-maturity $\alpha$ -amylase (LMA) in wheat.***

Kolumbina Mrva and Daryl Mares.

QTLs controlling the expression of LMA in wheat were detected in a DH population derived from wheat cultivars Cranbrook (LMA source) and Halberd (nonLMA). Cool-temperature treatment of detached tillers was used to induce the expression of LMA in lines carrying the defect. There was a highly significant ( $P < 0.001$ ) QTL on the long arm of chromosome 7B (accounting for 31 % of the variation in the first experiment), with Cranbrook contributing the higher value allele. A second QTL that accounted for 13 % of the variation was found close to the centromere on chromosome 3B. These results indicate that the gene responsible for LMA in Cranbrook is located on the long arm of chromosome 7B and situated distal to the  $\alpha$ -Amy-2 gene that codes for low pI  $\alpha$ -amylase isozymes synthesized in developing grains or later stages of germination.

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Mares DJ and Mrva K. 2001. Mapping quantitative trait loci associated with variation in grain dormancy in Australian wheat. *Aust J Agric Res* **52**:1257-1265.

Mrva K and Mares DJ. 2001. Quantitative trait locus analysis of late maturity  $\alpha$ -amylase in wheat using the doubled haploid population Cranbrook x Halberd. *Aust J Agric Res* **52**:1267-1273.

Mares DJ and Campbell AW. 2001. Mapping components of flour and noodle colour in Australian wheat. *Aust J Agric Res* **52**:1297-1309.

## **VICTORIAN INSTITUTE FOR DRYLAND AGRICULTURE Department of Natural Resources and Environment, Private Bag 260, Horsham, VIC 3401, Australia.**

F.C. Ogonnaya, F. Dreccer, R.F. Eastwood, P.R. Hearnden, E. Martin, J. Oman, D. Rodríguez, and J.S. Brown.

### ***Evaluation of primary synthetic and derived synthetic wheat lines under drought, high temperatures, and saline conditions – a collaboration with CIMMYT.***

Drought and heat stresses are among the most important environmental constraints to extensive wheat production in the Australian wheat belt. Both the amount of rainfall during the cropping season and its reliability are low. For example, the majority of the cropping belt of southern Australia has an average annual rainfall of 250 to 450/500 mm with a 20–30 % annual variation. In addition, large areas are affected by different forms of salinity including sodic or saline subsoils and transient salinity, which occur when salts in the subsoil concentrate as the soil dries out due to high evaporative demand. Rising water tables also can bring salt to the root zone.

The potential to overcome these constraints is through the utilization of novel sources of genetic variation. Considerable variation has been found for resistance and/or tolerance to biotic stresses in wild relatives of wheat (Ogonnaya et al. 2001a). There is a strong likelihood that *Ae. tauschii* and *T. turgidum* will have features of adaptation to marginal conditions that can be recaptured in synthetic wheats. Superior performance under drought and high temperatures has been observed in synthetic-derived wheat lines (Trethowan 2001). We also know that *Ae. tauschii* contributes substantially to salt tolerance by regulating the exclusion of sodium absorption (Ducobsky et al. 1996). In addition, in the process of making synthetic wheat, transgressive segregation for valuable traits may also be obtained.

The Australian Grains Research and Development Corporation (GRDC) is funding (July 2000–June 2005) a project to evaluate the potential of synthetic wheats to increase potential productivity under the drought, high temperature and salinity stresses experienced in Australia. The project involves collaboration between CIMMYT, the Victorian Institute for Dryland Agriculture (VIDA), and the Farming System Institute, Leslie Research Station, Toowoomba, Queensland. This project will evaluate primary synthetic wheats and derived synthetic lines supplied by CIMMYT under Australian conditions. The primary synthetics (~ 50 lines/year) were initially selected at CIMMYT after the imposition of both heat and drought stresses. However, because the physiological mechanism associated with the enhanced performance under such stresses are yet to be elucidated, a physiological approach (Reynolds et al. 2001) will be used to characterize the materials for tolerance to these stresses. This approach will ensure that the adaptive traits detected can be used, independently or in combination, to improve the efficiency of breeding for abiotic stress tolerance.

Physiological characterization will be complemented by an exploratory study with a simulation model (Rodríguez et al. 1999) where the chances of success of different combinations of traits will be evaluated in contrasting environments. This step will help in the definition of crop ideotypes suitable to regions with different stress patterns.

Selected primary synthetics with putative traits contributing to tolerance to the different stresses will be back-crossed to elite Australian lines to develop a pool of wheat germ plasm that can be readily assessed by wheat breeders. In addition to improving tolerance to abiotic stresses, the primary synthetics will be evaluated at VIDA for resistance to a range of biotic and other abiotic stresses limiting wheat productivity in Australia. In collaboration with Dr. Habans Bariana (Plant Breeding Institute, Cobbity, NSW, Australia), they also will be evaluated for seedling and adult-plant rust resistance. Those identified as possessing desirable traits will be used for the development of wheat germ plasm for the Australian wheat breeding entities.

### ***White grained hexaploid wheat with preharvest-sprouting resistance (PHS) derived from *Ae. tauschii*.***

Many Australian white-grained wheats lack adequate PHS resistance, causing sporadic and heavy losses in the high rainfall areas of the Northern Australian wheat belt. Thus, the development of white-grained, sprouting-resistant lines is a high priority for the Australian wheat industry. The D-genome donor of bread wheat, *Ae. tauschii*, contains strong levels of PHS resistance, in which many mechanisms have been implicated including embryo- and glume-based dormancy.

In studies at VIDA, inheritance of embryo-related dormancy was assessed using a cross between accessions of *Ae. tauschii* that differed in levels of seed dormancy. Synthetic hexaploids also were produced using *Ae. tauschii* accessions differing in dormancy and two tetraploid durum parents. The synthetic-derived, hexaploid wheat lines along with their hexaploid and diploid parents were assessed for the expression of embryo-related and seed-related dormancy.

The results indicate that *Ae. tauschii*-derived PHS resistance can be expressed, at least in part, in a white grained background. Significant increases in dormancy were observed in both the naked embryo and mature seed with greater dormancy observed in the mature seed. Despite the increase in dormancy conferred by the presence of the seed coat, all hybrids assessed were shown to be white grained when subjected to an NaOH test (De Pauw and McCaig 1988). The *Ae. tauschii* parent, which is red-grained, expressed complete dormancy for 28 days as mature seed. When assessed as naked embryos, however, germination began by day 5. The increased dormancy expressed by the white-grained, synthetic-derived lines could, therefore, be caused by the presence of pigment precursors in the seedcoat. These precursors have been suggested to be inhibitory to germination and can be present without the full expression of the red pigment.

Analysis of the F<sub>2</sub> data suggests that PHS in the *Ae. tauschii* accession used for this study is controlled by two recessive genes that are complementary. F<sub>3</sub> data is presently being assessed to verify this information. PHS resistance is often expressed as a polygenic trait (Lawson et al. 1997), and, as such, it is likely that more than two genes exist.

A number of mechanisms have been implicated in PHS resistance, with inheritance reported as both simple and complex. Results from the present study concur with previous reports that dormancy is under control of a recessive gene (Han et al. 1999). More specifically, it has previously been shown that *Ae. tauschii*-derived sprouting resistance expressed in an artificial amphiploid is inherited as a recessive trait controlled by one gene (Lan et al. 1997).

The BSA of the F<sub>2</sub> population using RAPD primers has not revealed any variation to date, and a candidate gene approach is being investigated as an alternative approach. The *Vp1* gene, which is necessary for the induction and maintenance of dormancy in maize, has homologues in rice (Hattori et al. 1994), wild oats (Jones, Peters et al. 1997), and wheat (Bailey et al. 1999; Nakamura and Toyama 2001). Homologues of the *Vp1* gene also have been discovered in the noncereal species, tobacco (Phillips and Conrad 1994) and bean (Bobb et al. 1995). A related gene conferring seed dormancy, *Abi3*, also has been identified in *A. thaliana* (Bailey 1999). Using conserved regions among these gene homologues, specific primers will be developed and used to screen for D-genome homologues in the accessions differing for dormancy and possible cosegregation with embryo dormancy.

### ***Evaluation of primary synthetic for common root diseases in Australia.***

Cereal cyst nematode causes significant losses to wheat production in southern Australia. Although a number of resistance genes have been found for CCN resistance (Ogbonnaya et al. 2001b), inadequate resistance levels in current wheat cultivars as well as a wide host range, compound the magnitude of yield losses associated with the incidence of *P. neglectus*.

Primary synthetic hexaploids obtained from CIMMYT were evaluated for resistance to *P. neglectus* and *H. avenae*. Included in the material evaluated were bread wheat lines introgressed with *Ae. ventricosa* chromosomes. Five of the 50 primary synthetic wheats evaluated for resistance to CCN displayed a near complete immune response. Whether this is a different gene than *Cre3* earlier found in *Ae. tauschii* is yet to be determined. Resistance to *P. neglectus* varied among the 100 primary synthetic wheats evaluated (a collaboration with Dr. S.P. Taylor, SARDI, South Australia). Some synthetic wheats displayed higher levels of resistance than those currently available in bread wheat (resistance levels were equivalent to those found in triticale, the resistant control), whereas a limited number had moderate resistance. With the exception of one line with a moderate level of resistance, the bread wheat–*Ae. ventricosa* introgression lines were susceptible.

### ***Marker-assisted selection in wheat breeding.***

The wheat, molecular-marker implementation project at VIDA is part of the Australian Wheat Molecular Marker Program funded by GRDC. The major objective of this project is the utilization of molecular markers to screen and select wheat plants in the wheat breeding populations for a number of traits of interest.

The traits and the loci that are targeted include CCN resistance (*Cre1* and *Cre3*), BYDV resistance (TC14), and the VPM segment conferring resistance to leaf (*Lr37*), stem (*Sr38*), and stripe (*Yr17*) rusts. Other traits that are being investigated are boron tolerance (*Bo1*) and dwarfing (*Rht8*).

All the markers being used are PCR-based markers, requiring smaller amounts of DNA than RFLPs. These markers are mostly dominant SCAR-based markers, which have been developed by CSIRO–Australia. CSIRO and VIDA (Eastwood et al. 1994; Ogbonnaya et al. 1996, 2001b) developed the CCN markers through a collaborative effort). In our program, markers are used to screen early generation BC<sub>1</sub>F<sub>1</sub>s, to select plants for DH production and to screen fixed lines for the desirable loci. Approximately 3,000–4,000 genotypes are analyzed with markers each year for both marker implementation and germ plasm enhancement in wheat.

The major impact of using markers as a selection tool for wheat breeding at VIDA is an increased rate of genetic gain and an increased efficiency in selecting plants with desirable alleles. The application of molecular markers has allowed genotypes fixed for desirable alleles to be identified in earlier generations and reduced the resource expended on material lacking critical alleles. Larger populations with critical alleles can be retained, thus increasing the probability of identifying genotypes that are superior to the recurrent parents.

A set of 75 wheat land races, collected by Dr. Gerald Halloran (retired professor of plant breeding, University of Melbourne), previously characterized for quality traits are being genotyped with 25 SSR markers. We hope to examine the utility of the SSRs reported in the literature for detecting DNA polymorphism and for estimating genetic diversity among these accessions.

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## ITEMS FROM AUSTRIA

### IFA – INTERUNIVERSITY RESEARCH INSTITUTE FOR AGRICULTURAL BIOTECHNOLOGY

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### *Novel tools for developing Fusarium-resistant wheat for Europe.*

H. Buerstmayr, M. Lemmens, U. Scholz, and P. Ruckebauer.

Uwe Scholz joined the IFA team on 1 January, 2002. He previously was working with B. Steffenson on FHB of barley at NDSU.

A new, EU-funded research project began in September 2001 and will continue until August 2005. Eight participants from six states are contributing. The objectives will apply a multidisciplinary approach involving plant pathology, plant breeding, molecular genetics, genomics, and in vitro methods. The overall methodology will involve field and laboratory testing of diverse wheat genotypes and segregating populations for components of FHB resistance, artificial-inoculation methods, molecular-genetic identification, characterization of resistance genes, studies of the host-pathogen interaction for the identification of resistance mechanisms, mycotoxin analyses, and in vitro-selection methods.

The work plan is divided into three workpackages:

**Workpackage 1: Phenotyping of FHB resistance and its components in wheat.** FHB resistance and its components, including resistance to trichothecene mycotoxins of the plant material used in the study, will be evaluated in replicated, multilocation experiments.

**Workpackage 2: Molecular genetic characterization of FHB resistance in wheat.** Three different mapping populations will be used, and two populations for fine mapping will be developed. Additionally, genetic diversity of 96 European and other genotypes with varying degrees of FHB resistance will be elucidated using SSR and candidate-gene markers.

**Workpackage 3: In vitro selection methods and candidate resistance genes for FHB in wheat.** Candidate genes putatively involved in trichothecene resistance will be cloned from wheat, analyzed, and their function in FHB resistance verified by mapping.

***Molecular breeding for FHB resistance in wheat – scientific coöperation between the Republic of Austria and the Islamic Republic of Iran.***

H. Buerstmayr, B. Steiner, and M. Mardi.

This project is a coöperation with the Agricultural Biotechnology Institute and the Seed and Plant Improvement Institute of Iran.

Two Ph.D. students, Mr. Mohsen Mardi from the University of Teheran and Mrs. Barbara Steiner from the University of Agricultural Sciences, Vienna, are working on FHB-resistance mapping and marker verification. The mapping populations are based on the resistance genes in Wangshiubai, Frontana, and Sumai 3. The mapping is using mainly AFLP and SSR markers. A LI-COR DNA analyzer is used for fragment analysis.

***Variation for resistance to head blight caused by *Fusarium graminearum* in wild emmer wheat from Israel.***

H. Buerstmayr, M. Stierschneider, B. Steiner, M. Lemmens, and M. Griesser; and E. Nevo and T. Fahima (Institute of Evolution, University of Haifa, Israel).

In a joint project with the Institute of Evolution, University of Haifa, Israel, wild emmer wheat, previously identified as a rich source for disease resistance genes to several pathogens, was tested for resistance to FHB. Artificial, single-point inoculations were used to evaluate a set of 151 *T. dicoccoides* genotypes from 16 habitats in Israel and one habitat in Turkey for resistance to fungal spread (type-II resistance) in replicated greenhouse experiments. A considerable level of diversity was found among the tested genotypes. The broad-sense heritability for type-II, FHB resistance was 0.71. Among the eight *T. dicoccoides* lines with the lowest relative infection rates, five were from a Mt. Gerizim population, and three were from the Mt. Hermon population. These two habitats are characterized by a relatively cool, semi-wet climate. Thus, it may be possible that *Fusarium* occurrence in these habitats was responsible for natural selection favoring resistance.

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Buerstmayr H, Lemmens M, Hartl L, Doldi L, Steiner B, Stierschneider M, and Ruckenbauer P. 2002. Molecular mapping of QTL for *Fusarium* head blight resistance in spring wheat. I. Resistance to fungal spread (Type II resistance). *Theor Appl Genet* **104**:84-91

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**ITEMS FROM BRAZIL****NATIONAL WHEAT RESEARCH CENTRE — EMBRAPA TRIGO**  
**Centro Nacional de Pesquisa de Trigo (Embrapa Trigo), Rodovia BR 285, Km 174,**  
**99001-970, Passo Fundo, Rio Grande do Sul, Brazil.*****Reaction of wheat cultivars released by Embrapa in relation to aluminum toxicity in acid soils.***

Cantídio N.A. de Sousa.

Acid soils are prevalent in most wheat-growing areas in Brazil. Resistance/tolerance to aluminum toxicity is a general objective in the wheat-breeding program of Embrapa (Brazilian Agricultural Research Corporation) that develops new wheat lines and cultivars in research centers located in Passo Fundo, Pelotas, Londrina, Dourados, and Planaltina. Susceptible cultivars also are released, because there are areas without an aluminum (Al)-toxicity problem. Genotypes are tested in the field and by screening in the laboratory in order to evaluate their reaction to Al toxicity.

Cultivars were evaluated by Embrapa in the field between 1989 and 2000 and the results are presented in Table 1. The methodology was described in *Plant Breed* **117**:217-221, 1998. Trials were grown each year and replicated three times. Plant response to Al in acid soil was evaluated visually on the above-ground parts of plants at maturity and, in some instances, before heading. The susceptibility index to Al toxicity in each year was calculated as the mean of the observations made during the given year. The general index presented in Table 1 (p. 34) is the average of the index of 4 or more years. The most resistant cultivars, with a susceptibility index to aluminum lower than 1.25 were BRS 49, CNT 1, Trigo BR 25, Trigo BR 35, and IAC 5-Maring (resistant check). The most susceptible cultivars were Anahuac 75 (susceptible check) and Trigo BR 36-Ianomami.

***Genetic wheat improvement for durable resistance to disease.***

Leo J.A. Del Duca, Amarilis L. Barcellos, Cantídio N.A. Sousa, Eliana M. Guarienti, Leila M. Costamilan, Márcio Só e Silva, and Pedro L. Scheeren.

Embrapa Trigo has developed activities for the identification of resistance sources and genetic improvement of wheat, with emphasis on leaf rust and powdery mildew. Durable resistance (DR) contributes to a reduction in production costs and for environmental protection, by decreasing the use of chemicals, creating more stable grain yields, and minimizing crop failure by diseases. Besides DR, we strive to incorporate desirable traits for agronomic and industrial-quality characteristics.

Diseases are a great obstacle to wheat production in Brazil, and new cultivars frequently have their commercial cultivation life span shortened by breakdown in resistance. Partial resistance (PR) is an alternative option to the specific resistance for races and may be more durable, although it does not confer immunity. The association between DR and slow rusting with PR and adult-plant resistance has been identified for wheat leaf rust in Brazil (Del Duca et al. 1994). PR is characterized by a reduced rate of development of the disease, despite of the occurrence of susceptible infection. Parameters for its identification are a smaller infection frequency, a longer latent period, a lower rate of production of spores/lesion, and/or a shorter infection period. This resistance type would be controlled by genes of additive action (Parlevliet 1978).

Our emphasis on rusts and powdery mildew is because in biotrophic organisms, new pathogenic races overcome the resistance genes (Parlevliet 1981). In previous DR-breeding strategies, populations were obtained by intercrossing among different sources. The hypothesis was that this resistance, controlled many times by genes of additive action, could be accumulated by intercrossing susceptible cultivars with low infection and selecting derived populations and progenies for transgressive levels of resistance. Progress has been obtained in lines with resistance to a specific disease, but the possibility of its commercial release has been hindered by inherent faults in other important agronomic characteristics.

Our main activities in this research have been to select sources of DR for leaf rust and powdery mildew in different nurseries and select for a more favorable plant type (lower height, better straw, and early cycle) in segregant populations and derived progenies sown in low density, under conditions of artificial inoculation. Grain selection is made after field selection and considering plumpness of grains and lack of disease symptoms. Selection for resistance to powdery mildew, in fields with natural infection and artificial inoculation of leaf rust, has allowed reasonable levels of infection in order to evaluate collections and select plants. Although there is a potential risk for stem rust, we have not been selecting for resistance because of its absence in the field in the last few years and the availability of specific populations with resistance. High levels of leaf rust and powdery mildew have allowed us to distinguish genotypes with low to moderate reactions in crosses, germ plasm, and progenies when compared with highly susceptible check cultivars such as Morocco and OR 1. Considering the difficulties in promoting lines with adequate disease resistance and other important properties for wheat improvement, new

**Table 1.** Cultivar, year of release, susceptibility index in the field, and reaction to aluminum toxicity.

Cultivar	Year of release in Brazil	Al <sup>3+</sup> -susceptibility index	Al <sup>3+</sup> reaction <sup>1</sup>
BRS 49	1996	1.16	R
BRS 119	1997	1.72	MR
BRS 120	1997	2.22	MR
BRS 176	1999	1.60	MR
BRS 177	1999	1.82	MR
BRS 179	1999	1.30	R
BRS 192	2000	1.67	MR
BRS 194	2000	1.34	R
CNT 1	1975	1.08	R
CNT 8	1976	1.84	MR
CNT 10	1977	1.28	R
Embrapa 15	1992	1.26	R
Embrapa 16	1992	1.53	MR
Embrapa 21	1993	2.33	MR
Embrapa 22	1993	2.63	MS
Embrapa 24	1993	1.42	R
Embrapa 27	1994	1.81	MR
Embrapa 40	1995	2.22	MR
Embrapa 41–Ofaié	1995	2.35	MR
Embrapa 42–Nambiquara	1995	2.56	MS
Embrapa 52	1996	1.48	R
Trigo BR 2	1979	2.12	MR
Trigo BR 10–Formosa	1983	2.53	MS
Trigo BR 12–Aruaná	1985	2.81	MS
Trigo BR 15	1985	1.29	R
Trigo BR 16–Rio Verde	1986	1.77	MR
Trigo BR 17–Caiuá	1986	2.75	MS
Trigo BR 18–Terena	1986	2.21	MR
Trigo BR 23	1987	1.39	R
Trigo BR 24	1988	1.79	MR
Trigo BR 25	1988	1.08	R
Trigo BR 26–São Gotardo	1988	3.14	MS
Trigo BR 32	1988	1.43	R
Trigo BR 33–Guará	1989	2.42	MR
Trigo BR 35	1989	1.01	R
Trigo BR 36–Ianomami	1990	3.87	S
Trigo BR 38	1990	1.91	MR
Trigo BR 39–Paraúna	1991	2.44	MR
Trigo BR 40–Tuiúca	1991	2.46	MR
Trigo BR 43	1991	1.50	R
Anahuac 75 <sup>2</sup>	1981	4.31	S
IAC 5–Maringá <sup>3</sup>	1966	1.07	R

<sup>1</sup> R = resistant, MR = moderately resistant; MS = moderately susceptible, and S = susceptible.

<sup>2</sup> Susceptible check cultivar.

<sup>3</sup> Resistant/tolerant check cultivar.

populations are being generated with the aim of associating DR for leaf rust and powdery mildew resistance and desirable agronomic type, for reaction to diseases caused by necrotrophic organisms, for preharvest sprouting, and for industrial quality. As a means to accelerate the selection of new lines, summer generations are being advanced in central Brazil, and the production of DH lines through gynogenesis are obtained from plants selected in the initial generations.

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***Testing early planted wheat in Paraná, 2000–2001.***

L.J.A. Del Duca, O. Rodrigues, G.R. Cunha, R.S. Fontaneli, J. Almeida, N. Antoniazzi, R. Molin, F. Franco, and S.R. Dotto.

To identify wheat lines adapted to early sowing, with greater soil cover, increased grain-yield potential, and greater chances of escaping frost because of a longer emergence and flowering period, 37 lines developed by Embrapa and Coodetec were tested in four locations in the state of Paraná, Brazil, by Embrapa-Soja and Coamo in Campo Mourão, Coodetec in Cascavel, and Fundação ABC in Ponta Grossa and Fundação Agrária in Guarapuava in 2000. In 2001, 26 wheat lines from Embrapa Trigo were tested at Fundação ABC (Castro) and Fundação Agrária (Guarapuava). The trials included Trigo BR 23, Trigo BR 35, CEP 24-Industrial, and Ocepar 21 as early check cultivars. Severe frosts occurred in the state of Paraná in 2000 producing greater damage in the typically early, check wheat cultivars. Such results confirmed the potential advantage of a breeding strategy aimed at obtaining late-early ecoideotypes. High humidity in 2001 in the late winter and spring resulted in a high incidence of head diseases, especially scab, limiting the yield potential of the early planting. Outstanding cultivars in the anticipated-planting trials are shown in Table 2 (p. 36).

***Brazilian wheat production in 1999–2000 and 2000–2001.***

Leo J.A. Del Duca and Eliana M. Guarienti.

Brazilian wheat production has changed from near self-sufficiency (6.1 million ton) in 1987 to a drastic reduction of nearly 1.5 million tons in 1995. However, consumption has increased to about 10 million tons, and Brazil now imports most of the domestically consumed wheat.

Since 1997, Brazilian wheat imports have been growing because of increased demand and stabilized production. During the 1999–2000 season, an increase of 9.7 % relative to the previous period was due to greater consumption by the ration industries and an increase in demand for flour, especially for baking and cookies. In 2000, speculation in the wheat market occurred because of crop failures world wide. Importing 75 % of the annual demand was reflected in price elevation and an increase in foreign exchange. That imports may surpass 8 million tons is a great possibility, at a suspected cost of 1 billion dollars (nupla@conab.gov.br;;paulo.coutinho@conab.gov.br). For the 2000–01 wheat season, 7.6 million tons were imported for a consumption of 10.2 million tons (rubem.alves@conab.gov.br; einge@conab.gov.br). The numbers presented in the Table 3 (p. 37) express the largest harvest of the last 5 years, a result of adequate climatic conditions, technological progress of the national wheat crop, and remunerating prices in the market.

***Main Brazilian wheat cultivars sown in the 1999–2000 season.***

Leo J.A. Del Duca and Eliana M. Guarienti.

Considering the Brazilian 1999–2000 wheat crop, only 23 cultivars made up more than 1 % of the total seed available (Table 4, p. 38). Information about the pedigree and industrial quality of the cultivars is provided. Considering the industrial quality of these 23 cultivars, only five are classified as soft wheats (CEP 27-Missões, FUNDACEP 30, FUNDACEP 32, Trigo BR 23, and FEPAGRO 15). The remaining genotypes, except IAPAR 17-Caeté (very strong gluten), are considered of good bread-making quality. In all Brazilian states, there was a high concentration of the seed



**Table 2.** Outstanding cultivars in anticipated-planting trials in Paraná, Brazil, in 2000–01.

Cultivar	Pedigree	Ponta Grossa Yield (kg/ha)	Guarapuava Yield (kg/ha)
<b>1. 2000 Grain-yield Trial—without fungicide.</b>			
PF 973961	PF 84511/Coker 80.33//CTY/BR 34	3,360	3,749
PF 980416	Coker 80.33/PF 869120//BR 18	3,067	3,782
PF 980441	PF 89261/PF 87373//CEP 24	3,264	3,302
PF 970308	Balkan/PF 79777	3,338	3,121
PF 970346	PEL 73101/BR 5//PF 79777/OASIS	3,221	3,228
PF 980407	PF 89261/BR 32/3/CTY/PF 87107//EMB 16	3,151	2,986
PF 950136	PF 8569/Coker 762	3,022	2,947
PF 980405	CTY/PF 87107//EMBRAPA 16	3,333	2,454
PF 960263	Coker 762*2/CNT 8	2,061	3,644
IPF 55204	Florida 301/Coker 762	2,834	2,759
PF 970332	Coker 762/PAT 7392	2,005	3,413
PF 960262	Coker 762*2/CNT 8	1,826	3,487
CEP 24 (best check)	BR 3/CEP 7887//CEP 7775/CEP 11	1,424	1,904
<b>2. 2000 Double-purpose Trial—without fungicide.</b>			
IPF 55204	Florida 301/Coker 762	3,035	3,840
IPF 64758	Saluda/Coker 762//Coker 80-28/FL 301	3,018	3,210
PF 950136	PF 8569/Coker 762	3,044	3,574
PF 960262	Coker 762*2/CNT 8	3,707	1,924
PF 960263	Coker 762*2/CNT 8	3,317	1,599
PF 970346	PEL 73101/BR 5//PF 79777/Oasis	3,495	3,402
PF 970347	HLN/CNT 7//AMI/CNT 7	3,264	2,953
PF 970349	Coker 762*2/CNT 8	3,668	2,522
PF 980416	Coker 80.33/PF 869120//BR 18	4,048	3,480
CEP 24 (best check)	BR 3/CEP 7887//CEP 7775/CEP 11	2,364	1,307
		Castro	Guarapuava
<b>3. 2001 Grain-yield Trial.</b>			
PF 990446	PF 87107/PF 87451/4/VPM83.11.48/2*BR 14// PF 869120/3/CEP 24	5,978	2,403
PF 990452	Coker 762/PF 905//CTY/BR 34	5,319	3,181
PF 990522	IPF 55204/EMB 16/3/F25950/F30505//PF 88603	4,705	4,063
PF 980417	Coker 762 /PF 89263//EMB 16/3/Coker 762/PF 87373	4,704	1,612
PF 973960	HLN/Coker 80.33//CTY/PF 869120	4,498	3,679
PF 980408	Coker 762/PF 89263//EMB 16/3/Coker 762/PF 87373	4,188	1,397
PF 990498	IPF 55204/PF 88522//CTY/CEP 24	4,096	3,510
PF 990575	Coker 80.33/PF 85202//Coker 762/PF 87107	4,011	2,762
PF 980376	EMB 16/IPF 55204//CEP 24/Coker 762	4,010	2,066
BR 23 (best check)	CC/ALD SIB/3/IAS 54-20/COP//CNT 8	4,244	2,868
<b>4. 2001 Double-purpose Trial.</b>			
BRS 176	HLN/CNT 7//AMIGO/CNT 7	4,989	4,531
BRS 177	PF 83899/PF 813//F27141	4,607	5,066
PF 950136	FLORIDA 301/COKER 762	4,854	4,346
PF 960243	CENTURY/BR 35	4,649	5,241
PF 960262	COKER 762*2/CNT 8	4,948	4,702
PF 960263	COKER 762*2/CNT 8	4,765	4,606
PF 970349	COKER 762*2/CNT 8	4,388	3,902
PF 973961	PF 84511/COKER 80.33//CTY/BR 34	4,220	4,466
PF 980405	CTY/PF 87107//EMBRAPA 16	4,547	3,813
PF 980416	COKER 80.33/PF 869120//BR 18	5,769	4,184
PF 980441	PF 89261/PF 87373//CEP 24	4,652	4,369
BR 23 (best check)	CC/ALD SIB/3/IAS 54-20/COP//CNT 8	5,043	4,544

**Table 3.** Production and grain yield for the 1999–2000 and 2000–2001 Brazilian wheat crops (Source: CONAB).

States	Production (1,000 t)		Grain yield (kg/ha)	
	1999–00	2000–01	1999–00	2000–01
Paraná	575.1	1,690.2	737	2,000
Santa Catarina	57.4	79.1	1,670	1,545
Rio Grande do Sul	891.2	1,022.7	1,600	1,700
<b>Total for southern Brazil</b>	<b>1,523.7</b>	<b>2,792.0</b>	<b>1,111</b>	<b>1,864</b>
Minas Gerais	22.6	21.2	4,100	4,000
São Paulo	27.1	43.1	1,450	2,200
<b>Total for southeast Brazil</b>	<b>49.7</b>	<b>64.3</b>	<b>2,054</b>	<b>2,582</b>
Mato Grosso do Sul	75.3	103.2	1,160	1,700
Goiás	7.4	10.1	1,090	1,300
Distrito Federal	2.3	2.3	4,535	4,535
<b>Total for westcentral Brazil</b>	<b>85.0</b>	<b>115.6</b>	<b>1,177</b>	<b>1,675</b>
<b>Total for all Brazil</b>	<b>1,658.4</b>	<b>2,971.9</b>	<b>1,130</b>	<b>1,867</b>

production of five cultivars (50 %) from a total of 48 wheat cultivars. Considering the size of the cropping area in Brazil in 2000–01 (1.59 million hectares) and the great ecological diversity, a greater cultivar diversity is desirable. The Brazilian wheat crop depends greatly on the climatic conditions, which can cause crop failure every 3 or 4 years (FNP Consultoria & Trade Ltda, Agriannual 2002). Thus, genotype diversification can help to reduce risk. The states of Paraná and Rio Grande do Sul are responsible for most of Brazilian wheat production. This production is widespread over a large number of growing conditions, such as rainfed or irrigated fields, presence or absence of soil aluminum toxicity, and high or low soil fertility. The most important wheat cultivars of each state are given in Table 5 (p. 38), along with information about their pedigree and industrial-quality classification. In 1999–2000, BRS 49 was the most widely grown Brazilian wheat cultivar, having the greatest production area in the three southern states (Rio Grande do Sul, Santa Catarina, and Paraná).

### *Protein and isozyme analyses in Ae. tauschii.*

Sandra Patussi Brammer, Daniela Silva Boscardin, and Caren Regina Cavichioli Lamb.

The species *Ae. tauschii*, donor of the genome D of *T. aestivum*, was evaluated in 2001 using HMW-glutenin and esterase analyses to detect genetic variability in different accessions to compile information that may be useful in wheat-improvement programs. The HMW-glutenins were extracted from 60 seeds, with three replications of each of the 20 accessions analyzed. The presence of 5+10 and 2+12 subunits differentiated the germ plasm lines. For the esterases, 100 plants were analyzed with five replications of the 20 accessions. Wide qualitative and quantitative variability was identified in the esterases. Currently, we also are developing molecular analyses through using microsatellites.

**Table 4.** Seed availability of the prevalent wheat cultivars grown in Brazil in 1999–2000. Industrial quality (W = values from the alveograph method on the deformation energy of the dough: soft (W  $\geq$  50 < 180), bread (W  $\geq$  180 < 300), and strong (W  $\geq$  300). Brazilian states are RS = Rio Grande do Sul; SC = Santa Catarina; PR = Paraná; SP = São Paulo; MS = Mato Grosso do Sul; MG = Minas Gerais; GO = Goiás; DF = Distrito Federal. Percentage for state is the most used cultivars ordered by state. Seed availability is tons available for the 1998–99 season. — incomplete or not available for the 1999–2000 season. Source of seed-availability data are MAPA/Embrapa/ABRASEM and Embrapa-SNT.

Rank	Cultivar	Cross	Seed (t)	%	Quality
1	BRS 49	BR 35/PF 83619//PF 858/PF 8550	29,277.87	13.87	bread
2	OR 1	Embrapa 27/Bagula SIB	23,936.20	11.34	bread
3	IAPAR 53	Sulino/IA 7929	21,951.80	10.40	bread
4	IAPAR 78	VEE SIB/BOW SIB	16,387.55	7.76	bread
5	CEP 27-Missões	CEP 8057/Butuí//CEP 8324	14,469.63	6.86	soft
6	BR 18-Terena	D6301/NAI60//W/RM/3/CIA*2//CHR=ALD45 SEL	11,926.29	5.65	bread
7	CEP 24-Industrial	BR 3/CEP 7887//CEP 7775/CEP 11	11,848.39	5.61	bread
8	FUNDACEP 29	BR 23/CEP 8423//BUC SIB	9,755.74	4.62	bread
9	Trigo BR 23	CC/ALD SIB/3/IAS 54-20/COP//CNT 8	9,260.57	4.39	soft
10	Rubi	PF 869107/KL H 3450 C 3131	7,556.96	3.58	bread
11	Oceoar 22	KLY/BB//CJ SIB/3/ALD SIB/4/S8020	6,073.00	2.88	bread
12	IAPAR 17-Caeté	JUP/BJY SIB	4,888.10	2.32	strong
13	OCEPAR 21	CEP 11/4/KLY/BB//CJ SIB/3/ALD SIB	4,654.25	2.21	bread
14	FUNDACEP 31	BR 8//PVN/ANI SIB	4,530.35	2.15	bread
15	FUNDACEP 32	CEP 85155/3/CEP 7780*2//H499.71A/4*JUP 73/4/BR 23	3,674.12	1.74	soft
16	BRS 119	PF 82252/BR 35//IAPAR 17/PF 8550	3,445.38	1.63	bread
17	IAPAR 28-IGAPÓ	KVZ/BUHO SIB//KAL/BB	2,561.05	1.21	bread
18	FUNDACEP 30	BR 32/CEP 21//CNO 79	2,527.59	1.20	soft
19	CD 101	AU/UP301//OCEPAR 12	2,348.55	1.11	bread
20	FEPAGRO 15	PF 82250/RS 1	2,274.29	1.08	soft
21	EMBRAPA 16	HHN/CNT 7//AMI/CNT 7	2,268.12	1.07	bread
22	EMBRAPA 40	PF 7650/NS 18-78//CNT 8/PF 7577	2,261.53	1.07	bread
23	OCEPAR 16	SIS SIB/VEE SIB	2,230.75	1.06	bread
	Range			6.25	
	Total		211,067.53	100.0	

**Table 5.** Seed availability of the most adopted wheat cultivars in the Brazilian producing states in 1999–2000.

State	Cultivar name	Cross	Quality	Availability	%
RS	BRS49	BR 35/PF 83619//PF 858/PF 8550	bread	15,297.67	18.49
	CEP27-Missões	CEP 8057/Butuí//CEP 8324	soft	13,983.43	16.90
	FUNDACEP 29	BR 23/CEP 8423//BUC SIB	bread	9,214.19	11.14
SC	BRS 49	BR 35/PF 83619//PF 858/PF 8550	bread	1,612.30	34.18
	OR 1	EMBRAPA 27/Bagula SIB	bread	726.00	15.39
	CEP27-Missões	CEP 8057/Butuí//CEP 8324	soft	486.20	10.31
PR	OR 1	EMBRAPA 27/Bagula SIB	bread	22,525.55	18.30
	IAPAR 53	SULINO/IA 7929	bread	21,951.80	17.83
	IAPAR 78	VEE SIB/BOW SIB	bread	16,387.55	13.31
SP	IAC 350–Goiapa	2109-36/SERI	bread	374.42	—
	IAC 24–Tucuruí	IAS 51/IRN 597-70	strong	230.84	—
	IAC 289–Marruá	KVZ/BUHO SIB//KLY/BB, VEE 5, SERI 82	bread	225.00	—
MS	BR 18–Terena	D6301/NAI60//W/RM/3/CIA*2//CHR=ALD45SEL	bread	397.79	89.39
	BR 40–Tuiúca	ANA 75/HUAC SIB	strong	47.20	10.61
MG	Embrapa 22	VEE SIB/3/KLTO SIB/PAT 19//MO/JUP	bread	64.10	—
GO/DF	Embrapa 42	LAP 689/MS 7936	strong	72.00	—

## ITEMS FROM CANADA

**CEREAL RESEARCH CENTRE, AGRICULTURE AND AGRI FOOD CANADA  
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*Some characteristics of AC Elsa spring wheat reselected for improved tolerance (SuperElsa) to wheat streak mosaic virus.*

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As reported here last year (Haber and Seifers 2001), SuperElsa (an unofficial, internal designation) was selected as an apparently true-breeding line from individual AC Elsa plants that had responded with very mild symptoms to seedling infection with WSMV.

Back-assay titrations (Table 1) were used to determine whether improved tolerance or resistance (or both) were associated with superior performance in SuperElsa under WSMV disease pressure. SuperElsa sustained higher infectious virus titres at 18°C than did AC Elsa, even though it had markedly milder symptoms and smaller losses (Fig. 1, p. 41; Haber and Seifers 2001). At 24°C, by contrast, SuperElsa sustained lower infectious virus titres than AC Elsa. Thus, SuperElsa appeared to combine greater tolerance at 18°C with greater resistance at 24°C. Indeed, in contrast to all other lines tested so far (Table 1; Haber and Seifers 2001), SuperElsa appeared to have the unusual trait of being more resistant (sustaining lower titres of infectious virus) at 24°C than at 18°C (Seifers et al. 1995).

**Table 1.** Back-assay titration to susceptible Tomahawk winter wheat test seedlings to determine relative levels of infectious virus.

Dilution of infectious sap	Laura (susceptible check)	Pai Tobarichi (tolerant check)	AC Elsa	SuperElsa (reselected line)	KS96HW103 ( <i>Wsm1</i> resistant)
<b>INFECTIOUS TITRE AT 18°C; MEAN NUMBER (THREE TRIALS) OF INFECTED TEST PLANTS/10.</b>					
25	5.6	10.0	4.3	6.7	0.0
50	6.8	9.7	5.3	7.2	0.0
100	4.2	6.8	4.8	6.9	0.0
200	3.1	4.2	1.7	5.3	0.0
400	1.2	1.4	2.3	4.1	0.0
800	0.9	0.6	1.4	0.6	0.0
1,600	0.3	0.3	0.0	0.3	0.0
3,200	0.3	0.0	0.0	0.3	0.0
<b>INFECTIOUS TITRE AT 24°C; MEAN NUMBER (THREE TRIALS) OF INFECTED TEST PLANTS/10.</b>					
25	9.1	9.0	6.8	5.8	10.0
50	7.8	8.9	7.9	5.7	9.7
100	6.7	8.2	7.0	5.5	9.6
200	3.4	4.5	4.7	2.5	8.9
400	2.8	3.4	4.7	0.9	5.8
800	1.9	0.3	1.3	0.4	4.5
1,600	0.3	0.9	0.3	0.0	2.1
3,200	0.0	0.0	0.0	0.0	1.0

In the first year of agronomic trials (conducted at Swift Current and Indian Head, Saskatchewan, and Morden, Manitoba) SuperElsa performed much like AC Elsa in the absence of WSMV disease pressure (Table 2a, p. 40). Agronomic traits such as height, lodging, maturity, yield, and test weight were similar. Mean seed size (1000-kernel weight) was similar for AC Elsa and SuperElsa when averaged for the three sites but the TKW of SuperElsa varied less among the test sites than did that of AC Elsa or the other tested lines (Table 2b, p. 40). Seed harvested from the Swift Current site was analyzed for cereal-quality parameters, and this initial analysis (Table 2c, p. 40) indicated that SuperElsa had acceptable quality traits.

To determine if SuperElsa had arisen from AC Elsa by a fortuitous out-crossing event or seed mixture, these two lines were compared by an AFLP analysis (Fig. 2, p. 41). The pattern of bands in SuperElsa did not show clear differences from that of AC Elsa with the exception of one band at ~ 130 bp; this band was present in AC Elsa and absent in SuperElsa.

**Table 2a.** Agronomic traits of SuperElsa compared with AC Elsa and selected Canada western red spring wheat checks.

Genotype	Mean relative yield <sup>1</sup> (3 sites)	Relative maturity <sup>2</sup> (3 site mean)	Relative height <sup>3</sup> (3 site mean)
<b>SuperElsa</b>	<b>95.2</b>	<b>3.5</b>	<b>2.8</b>
AC Elsa	96.4	3.5	3.0
AC Barrie	99.6	4.8	3.3
AC Cora	90.6	3.0	3.0
AC Cadillac	90.6	4.5	3.8
AC Superb	101.7	3.8	3.0

<sup>1</sup> Grain yield based on an moving mean and adjusted for control varieties and replication effect.

<sup>2</sup> Time to maturity rated on a scale of 1 (very early) to 6 (very late).

<sup>3</sup> Plant height rated on a scale of 2 (short) to 6 (tall).

**Table 2b.** Characteristics of harvested seed of SuperElsa compared with AC Elsa and selected Canada western red spring wheat checks. TKW = 1,000-kernel weight.

Genotype	Swift Current TKW (g)	Indian Head TKW (g)	Morden TKW (g)	3-site mean TKW (g)	Test weight 3-site mean (kg/hl)
<b>SuperElsa</b>	<b>27.0</b>	<b>27.8</b>	<b>27.5</b>	<b>27.3</b>	<b>74.7</b>
AC Elsa	28.3	34.0	28.0	29.6	74.4
AC Barrie	31.7	35.3	26.7	31.4	75.3
AC Cora	26.9	31.4	23.9	27.2	73.0
AC Cadillac	33.3	37.0	28.6	33.0	77.0
AC Superb	35.1	40.3	29.1	34.9	75.6

**Table 2c.** Quality traits of SuperElsa compared with AC Elsa and selected Canada western red spring wheat checks (data only from Swift Current).

Genotype	Flour yield (%)	Protein (%)	Mixograph dough-quality parameters				
			Mixing development time	Peak height	Peak bandwidth energy	Total energy	Bandwidth energy
<b>SuperElsa</b>	<b>70.7</b>	<b>15.6</b>	<b>2.0</b>	<b>0.199</b>	<b>0.131</b>	<b>55.5</b>	<b>31.0</b>
AC Elsa	69.9	15.3	2.1	0.161	0.097	48.4	30.7
AC Barrie	72.3	15.4	2.1	0.191	0.100	57.0	27.9
AC Cora	68.0	15.0	2.3	0.163	0.089	49.2	26.6
AC Cadillac	71.0	14.8	2.1	0.187	0.111	51.6	28.9
AC Superb	70.7	14.8	2.2	0.188	0.100	55.5	27.1

We now are examining the inheritance of tolerance/resistance in SuperElsa and how it interacts with other sources of resistance. Larger-scale field experiments followed by assessments of grain quality will determine if SuperElsa might provide effective protection in the near term against severe losses from WSMV outbreaks.

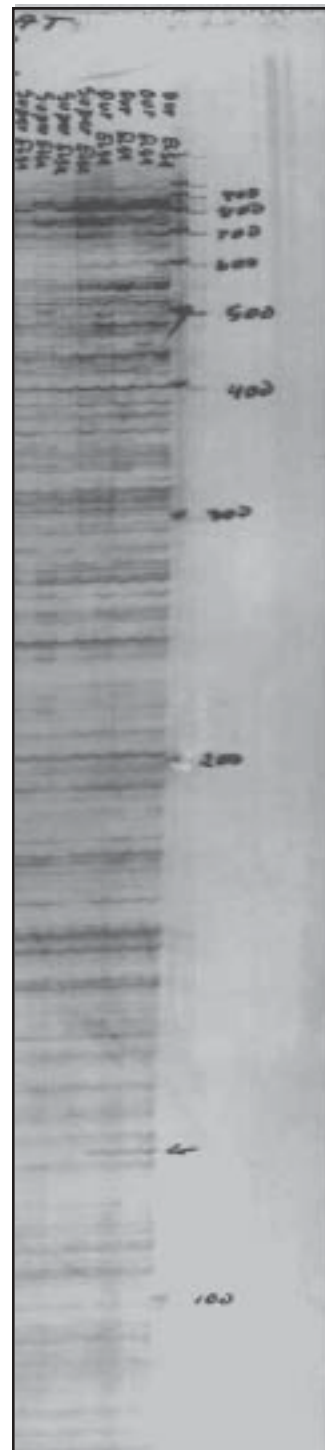


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**Fig. 1.** SuperElsa (right) responds to seedling WSMV infection with milder symptoms than AC Elsa (left).



**Fig. 2.** AFLP analysis comparing SuperElsa (leftmost four lanes) with AC Elsa (labeled ‘Our Elsa’ in next four tracks to the right). Note the similarity of banding patterns between SuperElsa and AC Elsa with the exception of the band highlighted by the arrow (between the 100- and 200-bp ladder bands), which is present in AC Elsa and absent in SuperElsa.

## ITEMS FROM CROATIA

**BC INSTITUTE FOR BREEDING AND PRODUCTION OF FIELD CROPS**  
**d.d. Zagreb, Marulicev trg 5/I, 10 000 Zagreb, Croatia.**

***Achievements in breeding small grains at the Bc Institute–Zagreb.***

S. Tomasovic, R. Mlinar, Z. Martinic-Jercic, and S. Gasperov.

At the Bc Institute, the breeding and genetics of new wheat varieties had three aims, the creation of a scientific research program for establishing an ideotype for wheat varieties, breeding, and building and equipping a newly formed scientific organization at Botinec. The breeding work was in three phases, from 1947–56, 1956–64, and 1964–present.

**Breeding phase one — 1947–56.** The following varieties were released during this period: Vuka, Mura, Kupa, and Zagorka. Compared to the older varieties Ul and Prolific, the new cultivars were shorter, had better lodging resistance, improved resistance to *P. graminis tritici*, a higher yield potential, and production stability.

**Breeding phase two — 1956–1964.** During this period, a new model for wheat varieties was designed based on the following morphological and physiological criteria: early type winter wheats, short or semidwarf growth habit that are resistant to lodging, and a production of 700–750 spikes/m<sup>2</sup>. These wheats were to be resistant to *P. graminis tritici* and *P. triticina* and have improved quality. Varieties registered during this period include Zlatna Dolina and Sanja.

The yield potential, adaptability, and stability in production surpassed the high-yielding foreign varieties from Italy. Their morphology, physiology, and production proved to be very suitable for intensive and mechanized production in Croatia. These varieties have been very successful in Croatia and other parts of the former Yugoslavia and also in Italy and Bulgaria. They have replaced foreign varieties in production in the western part of the Croatia.

**Breeding phase three — 1965–present.** At the start, we breed for varieties similar to Zlatna Dolina, but that are shorter, with improved resistance to lodging, an increase in harvest index, improved flour and grain quality, high-yielding, and resistant or tolerant to powdery mildew, stem and leaf rust, Fusarium, and Septoria. Varieties released during this period include Super Zlatna, Baranjka, Nova Zlatna, Nova Marijana, Zlatoklasa, Moslavka, Miljenka, Dika, Vucedolka, Lonja, Pozezanka, Korana, Podravka, Dakovcanka, and Sana.

A new cycle of breeding work produce high-yielding varieties (over 10 t/ha) with further improvement in grain and flour quality. Varieties released during this period include Marija, Patria, Liberta, Rina, Tina, Zdenka, Mihelca, Aura, Gloria, Concordia, Lana, Laura, Klaudija, Nina, and Adriana. Table 1 lists the total of all grain varieties produced by the Bc Institute.

**Spring wheat breeding (Z. Martinic-Jercic).** Spring wheat breeding produces high yielding, high-quality varieties for the agroecological conditions in hilly terrain. The program was housed at the Bc Institute–Zagreb until the early 1980s and now is in the Department of Plant Breeding, University of Zagreb. The first variety was registered in 1977 under the name Goranka (SW-222). Later releases included Planinka (1977), Brdanka (1980), Livanjka (1980), Anka (1982), and Vidovica (1986).

**Durum wheat breeding (S. Tomasovic).** This program is one of the younger programs at the Bc Institute. Collections of durum wheats were initiated prior to 1985, when it became apparent that Croatia was in need of its own durum wheat varieties. Durum wheats had been collectively ignored in favor of the more widely distributed bread wheats. In Croatia, bread wheats have an advantage over durum wheats in being adapted to the severe winter climate of western Croatia,

**Table 1.** Achievements in breeding at the Bc Institute for Breeding and Production of Field Crops – Zagreb.

Crop	Registered varieties
winter wheat	78
spring wheat	6
durum wheat	2
winter triticale	–
spring oats	4
winter barley	1
spring barley	1

where soft wheat with lower kernel and flour quality not suitable for the pasta industry are grown. In the coastal regions of Istra and Dalmatia, however, a climate similar to Italy and Greece favors growing durum wheats.

Breeding materials were collected first from Hungary and France and were tested in the field at Botinec for economic traits and disease resistance. Additional breeding material was obtained from ICARDA in 1988, CIMMYT in 1989, and their joint cooperation in Turkey in 1990. Collaborations with breeders in Romania and the Russian Federation followed and material was selected based on winter hardiness and spike fertility. Material with good disease resistance and good kernel and flour quality was obtained from Italy, Chile, and Argentina. In 1992, many valuable lines were received from the U.S. This collection of durum wheat is very rich and unique, encompassing the wide genetic and geographical distribution of durum wheats.

Yearly, we select genotypes for our crossing program. Progenies from material originating in Turkey, Romania, and Russia are monitored closely because of their winter hardiness and spike fertility. Preliminary varietal trials were planted in 1991 and 1992 (7.5-m<sup>2</sup> plots) and increased in 1993 in 30 x 60-m<sup>2</sup> plots. Selected lines were planted in an intervarietal trial in 1994 and 1995. Two lines have been submitted for registration with the National Committee of the Republic of Croatia (Bc TD3200/92 and Bc TD3201/92) and three lines have been selected for good agronomic traits (Bc TD3199/92, Bc TD3205/92, and Bc TD3208/92) (see Table 2).

**Winter triticale breeding (S. Tomasovic).** Winter triticale breeding, the newest breeding program at the Bc Institute, was begun in 1990. The concept was to create high-yielding, high-quality varieties for the livestock feeding. From breeding materials collected from Poland, the Russian Federation, Hungary, and Canada, advanced lines with good characteristics have been produced. One line has been sent for registration and release.

**Spring oat breeding (R. Mlinar).** Breeding of spring oats was initiated in the 1950s and focused on selection of material for the climatic conditions of Croatia. The program was discontinued but reintroduced in 1985. Between 1985–95, oat varieties with yields above 7,500 kg/ha have been produced. Two varieties, Istra and Baranja, are distinguished by their high-yield potential and grain quality. The varieties Kupa and Mura, registered in 1995 and 1996, respectively, have been most productive in generating new, improved varieties (see Table 2).

**Winter barley breeding (R. Mlinar).** The winter barley program was initiated in 1965 to create genetic variability by hybridization and induced mutation, breed for resistance to lodging and winter hardiness, and test germ plasm for use as introductions or in crossing. This work was cutback after a reorganization of the institute in 1973 and only varieties of the highest production were grown, including Melior 12, Pegra, Heuters, Dea, Manon, Atlas, and Ager. Ager, a French

**Table 2.** Some varieties, their pedigrees, year of registration, and commercial use registered by the Bc Institute for Breeding and Production of Field Crops – Zagreb.

Variety	Pedigree	Year of registration	Remarks
<b>DURUM WHEATS.</b>			
Primadur (Bc TD3201/92)	Rodur/ <i>T. durum</i> 3152-91-1 (DF 961-83)	1977	in commercial production
Bodur (Bc TD3200/920)	DF 961-83 ( <i>T. durum</i> 3152-01-1/ <i>T. durum</i> 3151-91-1)	1998	not in commercial production
<b>SPRING OATS.</b>			
Istra	Ballad/Flamingsnova	1993	in commercial production
Baranja	Cabana/Condor	1993	in commercial production
Kupa	Ballad/BL-82-187	1995	not in commercial production
Mura	Rollo/ZG-82-5	1996	not in commercial production
<b>BARLEY.</b>			
Favorit (winter, 6-row)	Miss/No. 347	1999	in commercial production
Erih (spring, 2-row)	Carsten INH Erhardt Egr, Germany	1996	in commercial production; for malt and brewing

variety released by INRA in 1963, was the most widespread variety. The program was reinitiated in the 1980 focusing on creating high-yielding varieties that were between the standards of the French variety Plaisant (a six-rowed type) and Rex (a two-rowed type) from the Osijek Institute. Many germ plasm collections were acquired between 1985–92. To date, material from over 580 varieties and lines of winter barley were used to produce 179 new combinations. F1–F10 generations have produced 7,390 genetic populations with 147 lines in preliminary investigation and 13 in comparative yield trials. Four lines are included in the National Committee of the Republic of Croatia registration trials at Botinec and breeder seed production is at 2,944 spikes/row. Four lines are in official trials in Croatia and one in Slovenia (see Table 2).

**Spring barley breeding (R. Mlinar).** In collaboration with the seed-production firm of Carsten INH Erhardt Eger, Germany, we have tested many new varieties of spring barley. One of these lines has been released for use in the malting and beer industries (see Table 2).

### *The most important quality indicators of Bc winter wheat varieties.*

S. Tomasovic, R. Mlinar, I. Ikić, and K. Puskarić.

**A history of Bc wheats.** Winter wheat breeding at the Bc Institute has a long tradition. Initial work began at the Department of Agriculture in Botinec in 1947. Under the leadership of Dr. Josip Potocanac, a program to increase the number of plants per square unit by combining traits from Italian and American varieties was initiated. Intensive breeding work started in 1955 after establishing a genotype adapted to the climate of Croatia. Between 1947–55, Vulka, Mura, Kupa, and Zagorka were released. Vulka, the first registered variety developed by the Bc Institute, was released in 1964. Breeding for yield capacity was the most important objective between 1956–64. Zlatna Dolina, Sanja, and Marijana were released during this period. These varieties initiated the move toward semidwarf wheats. After 1965, our work has focused on improving disease resistance, yield capacity, and grain and flour quality.

Between 1975 and 2000, 70 winter wheat varieties developed by the Bc Institute were registered. We have improved the relevant traits through breeding, including yield, 1,000-kernel weight, test weight, plant height, and length of vegetative period. Material was tested in 50 trials at Botinec between 1995–99 and compared with breeding material developed in the last 20 years. After separating trials into groups according to yield, increases in yield ability were observed. In trials in the 1990s, newly developed varieties had considerably higher yields (6,917 kg/ha) compared with that of the standard check Zitarka (6,192 kg/ha). Expressed as harvest index, grain yield during 1995–99 was superior by 12.7%. At the same time, improvement in 1,000-kernel weight increased from 39.24 g to 44.15 g. Test weights were at similar levels, while maintaining a semidwarf growth type. Length of the vegetative period (days-to-flowering from 1 January) remained similar at 144 compared to 140 days. Most genotypes were similar to Zlatna Dolina (142 days) and Zitarka (137 days). We hope that this new assortment of Bc-developed winter wheats, including material in official testing, possess agronomic traits that will be useful for wheat production both in Croatia and worldwide.

**Bc wheats in production in the year 2000.** Wheat breeding at the Bc Institute is aimed at developing high-yielding varieties with improved quality. Data on yield capacity of Bc varieties tested in large-scale trials at a number of location in 2000 indicate their superiority to both company-released and foreign varieties (Table 3, p. 45). Overall, Bc varieties averaged 7.8 t/ha, compared to all domestic varieties (6.98 t/ha, – 820 kg/ha), all other varieties (7.06 t/ha, – 740 kg/ha), and the all-trial average (7.28 t/ha, – 580 kg/ha). At Belje, seven of the 10 highest yielding varieties were Bc varieties, including Sana (9.49 t/ha), Liberta (9.24 t/ha), Rina (9.17 t/ha), and Marija (9.06 t/ha). The trial mean was 7.96 t/ha. At Osijek, eight Bc varieties were among the top 10 including Andrea (8.27 t/ha), Tina (8.08 t/ha), Patria (7.96 t/ha), and Liberta (7.78 t/ha). At Vinkovci, four varieties were top producers including Tina (7.74 t/ha) and Rina (7.46 t/ha). The trial mean at Vinkovci was 6.47 t/ha. Record yields were produced by Marija (10.0 t/ha) and Rina (9.8 t/ha) at the D. Miholjac (IPK-PZC) location. In the unfavorable conditions at Hana-Nasice, Bc varieties were extremely adaptable (Marija, 9.04 t/ha, and Liberta, 8.01 t/ha). Among the 10 highest ranking varieties at Kutjevo, four were Bc releases (Bc 7031/96, 9.06 t/ha; Patria, 8.94 t/ha; Marija, 8.78 t/ha; and Bc 6171/94, 8.76 t/ha). At Koprivnica, six Bc releases had yields above 8.00 t/ha from a mean trial yield of 7.09 t/ha. High productivity also has been noticed in Slovenian trials. Thus, Bc varieties seem to be meeting the needs of wheat producers and processors.

Newly developed varieties include Zdenka, Aura, Mihelca, Lana, Nina, and Prima. In addition, the variety Adriana was released for the confectionery industry in 1999. Zdenka and Aura are high, A-class varieties released in

**Table 3.** Results of large-scale trials of winter wheats in the Republic of Croatia, 1999–2000.

Location	No. of companies in trial	No of varieties in trial	Varieties in top 10		Mean yield (t/ha)			Yield difference	
			Bc	Other	Bc	Other	Mean	Mean	Other
Belje	5	38	7	3	8.49	7.51	7.96	530	980
Osijek (IPK)	6	49	8	1	7.38	6.28	6.77	610	1,100
Miholjac (IPK)	6	37	5	3	8.68	7.63	8.14	540	1,050
Pik Vinkov	12	78	4	1	6.97	6.64	6.47	500	330
Hana	4	22	3	3	7.99	7.38	7.76	230	610
Kutjevo	8	52	4	3	8.57	8.18	8.61	40	390
PG-Kopriv	7	44	6	2	7.46	7.07	7.09	370	390
Mean			5.3 53 %	2.3 23 %	7.93	7.24	7.54	391	693
Mean of first four locations			60 %	20 %	7.88	7.02	7.34	545	865

**Table 4.** Yields (t/ha) of winter wheat varieties in 2000 and 2001. All varieties were released from the Bc Institute for Breeding and Production of Field Crops–Zagreb.

Established varieties	Yields (t/ha)		New varieties	Yields (t/ha)	
	2000	2001		2000	2001
Marija	8.65	7.13	Zdenka	7.23	6.42
Sana	8.14	7.37	Aura	6.43	5.92
Tina	7.80	7.15	Nina	7.05	7.44
Patria	8.07	7.36	Lana	7.61	7.10
Liberta	8.03	7.12	Prima	7.94	7.81

**Table 5.** Quality traits of winter wheat varieties developed by the Bc Institute–Zagreb.

Variety	Proteins (%)	Sedimentation (ml)	Milling percentage	Wet gluten (%)	Falling number	Water absorption (%)
Marija	13.0	41.5	70.0	24.8	207	54.3
Sana	13.0	35.0	70.3	23.9	190	60.6
Tina	12.0	39.1	72.2	27.9	210	56.5
Patria	13.7	40.0	69.7	29.9	198	57.0
Liberta	13.9	40.2	71.0	27.7	292	60.0
Adriana	9.1	16.2	67.5	25.8	169	52.1
<b>NEW VARIETIES.</b>						
Mihelca	13.6	46.6	71.3	26.4	237	54.2
Zdenka	13.2	45.4	73.0	31.0	372	59.3
Aura	13.0	46.0	71.9	29.7	329	58.2
Lana	13.3	44.7	72.8	30.7	317	61.6
Nina	14.8	54.0	71.1	23.9	170	55.3
Prima	13.4	45.0	71.5	26.7	255	57.5

1999. Zdenka and Divana have particularly good extensogram measurements. Tina, Lana, Nina, and Prima are typical bread wheats with good quality, extensibility, and elasticity of gluten. Mihelca and Prima have superior grain yield (8.02 t/ha and 10.06 t/ha, respectively) and are of high quality. Yields of these winter wheat varieties, as well as results from relevant quality testing are listed in Tables 3–5 and Table 6, p. 46.

To date, 83 winter wheat varieties developed by the Bc Institute have been registered. Eighty-one of these varieties are bread wheats and two are durum wheats.



**Table 6.** Quality traits of winter wheat varieties from the Bc Institute for Breeding and Production of Field Crops–Zagreb in large-scale varietal trials.

Variety	Farinogram							Extensogram					Falling number
	Wet gluten (%)	Water absorption (%)	Dough development	Stabilization (min)	Resistance (min)	Softness degree (FJ)	Quality No.	Quality group	Energy (cm)	Elasticity (mm)	Resistance (EJ)	E/R	
Marija	27.99	53.20	1.3	1.5	2.8	60	56.4	B1	48.10	110	300	2.72	341
Patria	32.62	56.60	1.8	2.5	4.3	95	55.8	B1	32.30	110	205	1.86	216
Tina	30.04	50.30	1.3	1.7	3.0	105	52.4	B2	74.70	152	270	1.77	238
Liverta	28.43	57.80	1.6	1.4	3.0	90	52.6	B2	49.70	132	230	1.74	315
Adriana	25.80	52.10	1.5	0.7	2.2	115	26.4	C2	50.80	150	220	1.46	169
Mihelca	30.30	52.30	1.2	1.8	3.0	70	56.6	B1	109.7	188	280	1.48	362
Adenka	33.06	56.90	1.9	1.7	3.6	70	62.9	B1	116.1	140	270	1.93	333
Aura	30.04	50.30	1.7	1.8	3.5	70	61.7	B1	111.3	150	270	1.80	307
Lana	30.70	61.60	2.5	0.8	3.3	50	67.8	B1	76.7	217	150	1.69	317
Nina	30.19	54.70	1.3	1.2	2.5	100	51.2	B2	74.2	155	265	1.70	295
Prima	28.30	55.30	1.1	1.6	2.7	100	51.4	B2	77.9	152	270	1.77	284

## ITEMS FROM THE CZECH REPUBLIC

### RESEARCH INSTITUTE OF CROP PRODUCTION Drnovsk· 507, 161 06 Prague 6 - Ruzyně, Czech Republic.

#### *Collection of triticale in the Czech gene bank.*

Z. Stehno and L. Cejka.

As a relatively new crop, the growing area of triticale in the Czech Republic is increasing. The area of triticale grown as grain crop increased from 15,000 ha in 1997 to 49,500 ha in 2001. Grain yield was relatively stable and ranged between 3.74 t/ha (2000) and 4.04 t/ha (1999) (Table 1).

Winter cultivars are more productive and were grown on most of the triticale growing area. Among the released cultivars are one spring and seven winter types. Two winter cultivars, Kolor and Ring, were bred by Czech company Selgen, a.s. Most of the other winter and spring cultivars registered in the Czech Republic come from central Europe.

The collection of winter triticale was established in 1980s, and spring triticale accessions have been collected since 1992. At present, the collection consists of 398 winter and 214 spring cultivars and advanced lines. Triticale accessions are regularly evaluated in field trials on 10-m<sup>2</sup> plots in four replications. The winter wheat Samanta is used as a long-term check cultivar and enables us to compare of results from different years. Resistance to lodging and powdery mildew, rusts, and Septoria diseases are emphasized during evaluation.

**Table 1.** Growing area and average yield of triticale in the Czech Republic between 1997–2001.

Year	Growing area (ha)	Yield (t/ha)
1997	14,912	3.83
1998	20,308	3.90
1999	25,972	4.04
2000	37,001	3.74
2001	49,499	3.87

Table 2. Results of the winter triticale evaluations in 2001, Research Institute of Crop Production, Czech Republic.

Cultivar	Plant height (cm)	Lodging	Powdery mildew	Septoria	Rusts	Yield (t/ha)
Samanta (winter wheat)	102	6.2	5.2	3.5	3.5	8.56
Eldorado	132	5.5	9.0	6.0	8.0	9.46
Origo	135	5.2	9.0	4.5	2.7	8.97
Binova	121	3.7	9.0	5.2	9.0	10.12
Angus	124	4.0	9.0	5.5	5.5	8.45
Mundo	134	6.2	9.0	5.0	9.0	11.65
Lamberto	132	4.5	9.0	6.7	9.0	11.69
Nemo	132	6.7	9.0	5.0	8.7	9.65
Fidelio	123	7.5	9.0	5.7	9.0	8.94
Typo	137	5.0	9.0	5.0	4.5	9.11
Countri	123	4.0	9.0	4.5	9.0	10.03
Piano	129	6.2	9.0	4.7	5.0	9.58
Zolder	109	5.2	9.0	3.7	2.2	7.92
Sirius	131	3.7	9.0	4.0	9.0	10.37
Ticino	139	4.7	9.0	6.0	9.0	11.78
Boreas	117	7.2	9.0	4.5	9.0	11.17
Santop	132	5.7	9.0	5.5	9.0	11.86
Trinidad	134	4.7	9.0	4.7	8.7	11.24
Prado	135	5.0	9.0	4.7	6.7	10.41
Marko	129	4.2	9.0	4.2	9.0	8.32

In 2001, we confirmed that triticale plants are usually higher (7–37 cm) than check wheat plants (Table 2). Consequently, resistance of triticale to lodging was lower in general; only Boreas, Nemo, Piano, and Mundo reached or had better lodging resistance than that of Samanta.

The situation with powdery mildew is very clear. No symptoms of this disease appeared on any of triticale cultivars. Samanta was medium susceptible to the disease. All triticale accessions have higher resistance to *Septoria* than the check wheat. Similarly, all triticale cultivars had higher resistance to rust infection, except Origo and Zolder.

All triticale cultivars except Zolder, Marko, and Angus overcame check winter wheat cultivar Samanta in grain yield.

### ***Announcement of the on-line catalog of Wheat Pedigrees, Genes, and Identified Alleles.***

I. Faberova and S.P. Martynov (N.I. Vavilov Research Institute of Plant Industry (VIR), St. Petersburg, Russian Federation).

An internet catalog of pedigrees and identified alleles, developed in collaboration between the N.I. Vavilov Research Institute of Plant Industry (VIR), St. Petersburg, and the N.I. Vavilov Institute of General Genetics RAS, Moscow (Russian Federation), and RICP Prague (Czech Republic), is now available at <<http://genbank.vurv.cz/wheat/pedigree/>>. The application includes genealogies, genes, and identified alleles of 69,632 wheat accessions, linked to 2,529 bibliographical references. This internet catalog follows the *Catalogue, Genealogies and Gene Alleles Identified in 31 000 Cultivars and Lines of Wheat* (Vols. I and II, 1992) and *Genealogies and Gene Alleles of Wheat, 15 000 Cultivars and Lines* (Vol. III, 1996) published as hard copies and the well-known electronic Catalogue GRIP versions I and II at <<http://www.psu.missouri.edu/grip/>>.

This new on-line catalogue allows searches of ancestors in multilevel pedigree trees and the location of genes and their combinations among the wide wheat assortment. Graphic forms for genealogical trees, basic passport data, and

bibliographical references can be displayed as accompanying information. We expect that the catalogue will be a useful tool for wheat breeders and researchers. All data were collected and compiled by S.P. Martynov, VIR, St. Petersburg, and T.V. Dobrotvorskaya, VIGG, Moscow. The Internet application was designed at RICP Prague in collaboration with Dr. Martynov.

### *The presence of Norin 10 dwarfing genes in winter wheat cultivars.*

J. Chrpova, M. Skorpik, V. Síp, and L. Bobkova (SELGEN a.s., Breeding Station, Uhretice, Czech Republic).

Of 57 winter wheat cultivars registered in the Czech Republic, 20 cultivars (35 %) were insensitive to applied gibberellic acid (GA), which indicates the presence of Norin 10 dwarfing (*Rht*) genes. By hybridization analyses in F<sub>2</sub> generation, the presence of the *Rht1* gene was detected in six cultivars (Astella, Elpa, Iona, Solara, Vlada, and Rheia), and 14 cultivars were found to carry *Rht2* gene (Athlet, Contra, Corsaire, Record, Rialto, Ritmo, Sepstra, Sarka, Versailles, Vlasta, Windsor, Clever, Trend, and Mladka) (Table 3).

**Table 3.** Response to gibberellic acid (GA; S = sensitive) and the presence of *Rht1* or *Rht2* genes in GA-insensitive genotypes.

Cultivar	Response/ <i>Rht</i> gene	Cultivar	Response/ <i>Rht</i> gene	Cultivar	Response/ <i>Rht</i> gene
Alana	S	Ebi	S	Samara	S
Alka	S	Elpa	<i>Rht1</i>	Saskia	S
Apache	S	Estica	S	Semper	S
Asta	S	Hana	S	Sepstra	<i>Rht2</i>
Astella	<i>Rht1</i>	Iona	<i>Rht1</i>	Sida	S
Athlet	<i>Rht2</i>	Koötka	S	Síria	S
Banquet	S	Livia	S	Solara	<i>Rht1</i>
Batis	S	Ludwig	S	Sulamit	S
Blava	S	Mladka	<i>Rht2</i>	Svitava	S
Bill	S	Mona	S	Sarka	<i>Rht2</i>
Boka	S	Nela	S	Torysa	S
Brea	S	Niagara	S	Tower	S
Bruneta	S	Record	<i>Rht2</i>	Trane	S
Bruta	S	Regina	S	Trend	<i>Rht2</i>
Clever	<i>Rht2</i>	Rexia	S	Versailles	<i>Rht2</i>
Complet	S	Rheia	<i>Rht1</i>	Viginta	S
Contra	<i>Rht2</i>	Rialto	<i>Rht2</i>	Vlada	<i>Rht1</i>
Corsaire	<i>Rht2</i>	Ritmo	<i>Rht2</i>	Vlasta	<i>Rht2</i>
Drifter	S	Samanta	S	Windsor	<i>Rht2</i>

In coöperation with the Breeding Station at Uhretice, SELGEN, a.s., selection for taller GA-insensitive plants resulted in development of an advanced breeding line SG-RU 24, which was tested for 3 years in the Official Trials of the Czech Republic. This new cultivar, carrying *Rht1* (transferred from the parental cultivar Vlada), was registered in 2002 under the name Rheia. Mladka (registered in 2002), bred in the Breeding Station Uhretice, SELGEN a.s., is another new very high yielding Czech winter cultivar with the Norin 10 gene *Rht2* (transferred from the cultivar Contra).

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**Resistance to *Fusarium* head blight infection and DON accumulation in winter wheat.**

V. Síp, S. Sykorová, J. Chrpová, and L. Papoušková.

The resistance to FHB infection and accumulation of DON was evaluated in 10 selected winter wheat cultivars after inoculation with two isolates of *F. culmorum* at two experimental sites for 3 years. In the trials with infected and uninfected (control) variants and two replicates, we determined a visual symptom score (VSS), grain weight/spike (GWS), grain number/spike (GNS), 1,000-kernel weight (TKW), heading date, and DON content (using a quantitative ELISA method).

On average, the pathogen caused reductions (R) in GNS, TJW, and GWS of 24 %, 36 %, and 51 %, respectively. The average DON content was 30 mg/kg. ANOVA showed highly significant genotypic differences in the characters measuring resistance/tolerance to FHB and also in DON content, but relatively larger were the effects of the isolate and conditions of year and experimental site. Cultivars did not differ in their response to the isolate, but the aggressiveness of isolates was different in different years. Resistant or moderately resistant cultivars Arina, SG-U-466 (Bona), and Sparta ranked 1–4 for VSS, GWS-R, TKW-R, and DON. To the contrary, the early cultivar Hana ranked tenth for VSS, ninth for GWS-R, fifth for TKW-R, and fourth for DON, suggesting a high affection of grain yield due to infection and relatively lower effect on grain size and DON content.

Predicting DON content was better using reduction of TKW and TKW after infection than by symptom scoring. Variability in DON content was high with the susceptible response (high infection severity). Cultivars resistant or moderately resistant to FHB showed a stable reaction (regression coefficient  $b < 1$ ). Variation in DON content using correlation analysis and principal component analysis showed the positive effects of conditions that caused high reductions of grain size and number and led to high performance of these traits in the control, an uninfected variant at later heading. High variation in DON content evidently also was due to factors that influenced the beginning of infection in particular year (location) and variety. These results stress the importance of reaching high infection severity in resistance tests.

**Reference.**

Síp V, Sykorová S, Papoušková L, Stuchlíková E, and Chrpová J. 2001. The influence of *Fusarium culmorum* L. infection on mycotoxin content in grain of selected wheat varieties. **In:** Proc Internat Conf Sustainable Systems of Cereal Crop Protection against Fungal Diseases as the Way of Reduction of Toxin Occurrence in Food Webs. Kromeriz, Czech Rep, 2–6 July, 2001. pp. 225-229.

**ITEMS FROM ESTONIA**

**INSTITUTE OF EXPERIMENTAL BIOLOGY AT THE ESTONIAN  
AGRICULTURAL UNIVERSITY  
Department of Plant Genetics, 76902, Harku, Harjumaa, Estonia.**

***Monosomic analysis of powdery mildew resistance in common wheat cultivar Sunnan.***

T. Enno, H. Peusha, and O. Priilinn.

Knowledge of the interactions between the genetic systems of host plants and pathogens is very important for successful breeding programs and the development of resistant cultivars. Powdery mildew is one of the most destructive diseases of common wheat and, 28 genes for resistance against this disease have been described to date (McIntosh et al. 1998; Järve et al. 2000; Peusha et al. 2000). Some wheat cultivars contain only one gene for powdery mildew resistance, but others have two or three, and combinations of two or more effective genes may afford better genetic control (Szunics and Szunics 1999).

The common spring-wheat cultivar Sunnan was bred in Sweden (Weibullsholm Breeding Station) and has the pedigree 'Pompe B2//Sappo/Drabent'.

We attempted to locate the resistance genes for powdery mildew in Sunnan by monosomic analysis and determined if they were new resistance genes. The monosomic plants of Chinese Spring were identified cytologically and crossed as females with disomic plants of Sunnan. All  $F_1$  hybrids were screened cytologically and monosomic lines were grown in the greenhouse to obtain an  $F_2$  population. Powdery mildew isolate No. 6, known to be avirulent on Sunnan, was used to test the segregating  $F_2$  population. The detailed methods for inoculation of leaf segments and disease assessments were described in Hsam and Zeller (1997).

Segregation for resistant and susceptible plants in the  $F_2$  populations fit the ratio of 243:13, except for chromosomes 2A, 7A, 6B, and 5D, which were clearly different, indicating that resistance in Sunnan is controlled by four duplicate complementary genes located on these chromosomes (Table 1). Assessment of resistance in the  $F_3$  families demonstrated that all plants in the progenies of two crosses, CS-M 2A/Sunnan (244 plants) and CS-M5D/Sunnan (492 plants), were resistant to the pathogen.

Powdery mildew genes *Pm4b*, *Pm1* + *Pm9*, and *Pm2* are located on the chromosomes 2A, 7AL, and 5DS, respectively (Briggle 1969; Sears and Briggle 1969; McIntosh and Baker 1970). Transfers of resistance genes from cultivated and wild relatives of *Triticum* to commercial common wheat cultivars have been successful. For example, *Pm4b* was transferred from the tetraploid *T. carthlicum* to hexaploid wheat (The et al. 1979), and *Pm2* was transferred from *Ae. tauschii* to common wheat (McIntosh and Baker 1970; Tosa and Sakai 1991; Lutz et al. 1995). The resistance genes located on the 2A and 5D chromosomes in Sunnan are the effective genes *Pm4b* and *Pm2*, inherited from tetraploid wheat. In future experiments, we intend to test allelism to verify our suspicions. The source and origin of genes located on chromosomes 7A and 6B could not be deduced now.

Monosomic and disomic hybrids  $F_1$  CS/Sunnan were analyzed cytologically for chromosome behavior at metaphase of the first meiotic division. Cytogenetical analysis of meiotic associations revealed two reciprocal translocations involving chromosomes 1A/1D and 7B/6D (Table 2, p. 51). We found that the genes that cause a decrease in chromosome pairing in  $F_1$  hybrids between the CS monosomics and Sunnan were on chromosomes 3A, 6A, 2D, and 4D, and genes that enhance pairing are on chromosomes 5A, 7A, 5B, and 7D.

This work was supported by the Estonian Science Foundation (Grant N 4720).

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**Table 1.** Segregation of  $F_2$  hybrids from crosses of Chinese Spring monosomic lines with cultivar Sunnan.

Monosomic lines	No. of plants	Powdery mildew isolate N6		$X^2$ 243:13
		Resistant	Susceptible	
1A	125	119	6	0.019
2A	92	91	1	3.039*
3A	148	143	5	0.883
4A	121	115	6	0.003
5A	257	244	13	0.000
6A	63	60	3	0.012
7A	92	91	1	3.039*
1B	89	85	4	0.060
2B	81	77	4	0.003
3B	29	28	1	0.158
4B	125	120	5	0.327
5B	55	52	3	0.014
6B	127	125	2	3.234*
7B	147	140	7	0.029
1D	111	106	5	0.073
2D	13	12	1	0.184
3D	113	108	5	0.098
4D	130	123	7	0.025
5D	66	66	0	—
6D	89	83	6	0.517
7D	112	107	5	0.085
CS <sub>dis</sub> / Sunnan	316	304	12	1.071

\*P = 0.05



**Table 2.** Chromosome pairing in monosomic F<sub>1</sub> hybrids (Chinese Spring/Sunnan).

Mono- somic line	No. of PMCs	Mean number per cell						% of PMCs with triva- valents but no univalents	No. of PMCs with multivalents	
		Bivalents			Univalents	Chias- mata	Multi- valents		1 <sup>III</sup>	1 <sup>IV</sup>
		Ring	Rod	Total						
1A	137	15.74	3.39	19.13	2.51	35.03	0.06	1.46	3	5
2A	109	16.67	2.76	19.43	2.08	36.24	0.05	—	—	5
3A	143	15.38	3.99	19.37	2.09	34.86	0.04	—	3	3
4A	73	16.26	3.44	19.69	1.55	36.00	0.01	—	—	1
5A	51	16.76	2.98	19.74	1.43	36.56	0.02	—	—	1
6A	102	14.53	4.74	19.27	2.26	33.95	0.05	—	1	4
7A	118	16.92	3.17	20.09	1.41	37.05	0.02	—	1	1
1B	79	16.76	2.94	19.72	1.47	36.55	0.02	—	1	1
2B	58	16.60	2.86	19.46	1.93	36.17	0.03	—	—	2
3B	82	15.90	3.50	19.40	2.12	35.35	0.02	—	2	—
4B	96	15.68	3.93	19.61	1.69	35.35	0.02	—	1	1
5B	92	17.25	2.51	19.76	1.34	37.11	0.03	—	—	3
6B	157	15.94	3.48	19.42	2.01	35.48	0.04	—	1	5
7B	99	16.79	2.89	19.68	1.51	36.57	0.03	1.01	—	2
1D	65	16.20	3.09	19.29	2.09	35.63	0.08	1.54	3	1
2D	136	15.01	4.33	19.34	2.22	34.41	0.02	—	1	2
3D	94	16.54	3.06	19.60	1.71	36.20	0.02	—	1	1
4D	97	14.60	4.29	18.89	1.94	33.62	0.04	—	—	4
5D	127	16.57	3.08	19.65	1.55	35.32	0.04	—	2	3
6D	98	16.64	3.04	19.68	1.60	36.34	0.01	1.02	1	—
7D	136	17.74	2.09	19.83	1.30	37.59	0.01	—	—	1
CS <sub>dis</sub> / Sunnan	140	16.76	3.67	20.43	1.08	37.24	0.01	—	1	1

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***The electrophoretic spectra of storage proteins of cereal cultivars and breeding lines proposed for cultivation in Estonia.***

M. Tohver, R. Koppel<sup>1</sup>, A. Kann<sup>2</sup>, A. Mihhalevski<sup>2</sup>, I. Rahnu<sup>2</sup>, and R. Täht<sup>2</sup>.

<sup>1</sup>Jõgeva Plant Breeding Institute, EE 48309 Jõgeva, Estonia and <sup>2</sup>Tallinn Technical University, Ehitajate tee 5, EE 19086 Tallinn, Estonia.

In 2000–01, the polymorphism of gliadin and HMW-glutenin subunits of wheat, triticale, and rye cultivars and breeding lines was examined with the aid of one-dimensional A- and SDS-PAGE. Our aim was to identify new breeding lines, test the authenticity of cultivars, and study the influence of the *Glu-1* loci on bread-making quality.

Plant stocks were obtained from comparative variety trials, the Estonian Control Center of Plant Production, and the Jõgeva Plant Breeding Institute, and comprise of cultivars from Estonia, Finland, Norway, Sweden, Lithuania, Poland, and Germany.

A total of 20 spring (including Heta, Bastian, Laari, and Tähti), 46 winter (including Portal, Otto, Residence, Bercy, Sani, Eka, Kalvi, Linna, Aura, Nisu, Pitko, Vakka, Ramiro, and Virvinta) wheat, 14 triticale (including Vision, Moreno, Tewo, Lupus, Dagro, Modus, Dato Prego, Pinokio, Lasko, Presto, SV 92280, and SW 98578), 2 rye (Sangaste and Apart) cultivars, and numerous breeding lines were investigated.

The electrophoreses were performed on the 10 % polyacrylamide gels in acid (A-PAGE) (Metakovsky, Novoselskaya 1991) for gliadin proteins and in SDS-PAGE (D'Ovidio 1996) for HMW-glutenin subunits. Gliadins were extracted from crushed kernel with 70 % ethyl alcohol at 40°C for 1 h. The electrophoresis was performed for 20 min at 200 V and 1 h at 500 V. HMW-glutenin subunits were extracted using a buffer containing 0.125 M Tris-HCl, pH 6.8, 2.75 % (w/v) SDS, 10 % (v/v) glycerol, and 1 % (w/v) dithiothreitol (DTT) for 1 h at 70°C. Electrophoresis was by SDS-PAGE (T = 10 %, C = 1.28 %). After electrophoreses, the gels were stained with Coomassie Brilliant Blue R-250, destained, and photographed. Gliadins were analyzed according to the nomenclature of Metakovsky and Novoselskaya (1991) and Jackson et al. (1996); HMW-glutenin bands were analyzed according to the nomenclature of Payne and Lawrence (1983).

The most represented *Gli-1* alleles in wheat cultivars and breeding lines were *Gli-A1a*, *Gli-B1b*, and *Gli-D1a*. The most frequent alleles for the HMW-glutenin subunits were *Glu-A1b*, *Glu-B1b*, and *Glu-D1d*. In some cases, we detected secalin bands in breeding lines of winter wheat. The T1BL·1RS translocation is easily detectable in A-PAGE. Many cultivars from neighboring countries were analyzed earlier and data were published (Johansson et al. 1995; Sontag-Strohm 1997; Ruzgas and Liutkevicius 2000). These cultivars were grown in our environment, and we verified their authenticity.

The baking quality of wheat was positively influenced by HMW-glutenin subunits 1 (*Glu-A1*), 7+9 (*Glu-B1*), 14+15 (*Glu-B1*), and 5+10 (*Glu-D1*).

Recent years have seen an increased interest in growing triticale in Estonia. The most common HMW-glutenin (*Glu-1*) alleles in hexaploid triticale (AABBRR) were subunits 0 or 2\* (*Glu-A1*) and 7+19 or 13+16 (*Glu-B1*). Some cultivars were heterogeneous, representing different alleles in kernels. We found two variations of *Glu-B1* in Moreno (bands 7+26 or 6+8), Presto (bands 7+9 or 7+26), Pinokio (7+8 or 7+9), and SW 98578 (7+8 or 7+9). Some HMW-glutenin subunit patterns in the triticale cultivars (Dato) were more similar to the typical pattern of rye, whereas the glutenin-subunit patterns of most of the triticale cultivars were closer to that of wheat. As for chemical composition, triticale is more similar to wheat, whereas for free sugars, it is closer to rye, and this is the reason of the low baking quality of triticale. Considering those indices characterizing bread-making properties (falling number, protein content, Zeleny number, water absorption capacity, and bread volume), the best cultivars were Moreno, Presto, Tewo, Dato, and SV 92280, which agrees with HMW-glutenin subunit composition.

The winter rye is traditional crop in Estonia, well adapted for local soil and weather conditions. Rye is highly heterozygous crop. This species usually cross-pollinates, consequently most cultivars are the mixtures of different genotypes. We observed this on the electrophorograms. Nearly every kernel had a different protein pattern.

The protein pattern of rye (AARR) lacks many of the bands that are found in wheat (AABBDD) or triticale (AABBRR). The absence of  $\alpha$ -gliadins is characteristic for rye.

The rye proteins do not be able to form gluten because of the structure of proteins. The suitability of rye for bread making is defined with reading the viscosity of amylograms, which cannot be less than 200 BU with Falling Numbers between 90–140. Falling Numbers of Estonian-grown rye have been in the range of 113–241 (Veskus and Kann 1997).

Two Master of Science theses, The relationship between fractional composition of proteins and bread-making quality of triticale varieties, by I. Rahnu, and The investigation of bread-making quality of triticale cultivars proposed for cultivation in Estonia, by A. Mihhalevski, were defended in 2000 and 2002, respectively.

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**ITEMS FROM ETHIOPIA****INSTITUTE OF BIODIVERSITY CONSERVATION AND RESEARCH  
P.O. Box 30726, Addis Ababa, Ethiopia.*****The significance of in situ conservation: a comparative analysis of durum wheat diversity between in situ (Bale and eastern Shewa Zones) and ex situ (Ethiopia) conservation sites.***

Yemane Tsehaye.

**Introduction.** The loss of crop genetic resources in countries like Ethiopia can be linked to the spread of modern agriculture in two major ways. First, the introduction of high-yielding, genetically narrow, uniform cultivars (often less dependable than the landraces they replaced when grown under the traditional agricultural management), resulted in abandonment of the broad genetic base in farmers' varieties. Second, the planting of vast areas to uniform cultivars with a narrow genetic base made agricultural productivity extremely vulnerable to yield-limiting factors such as disease and pests. According to Loutte et al. (1997), farmers also were given several socioeconomic incentives to replace varieties that evolved within their agro-ecosystem with improved/introduced varieties in many regions of the world.

Efforts to conserve crop diversity and cope with the rapid loss of genetic resources to date have focused on maintaining genetic diversity in static *ex situ* gene banks. However, a variety of problems with reliance on *ex situ* conservation strategies have been acknowledged, such as inadequate sampling procedures during field collection and the lack of representation in gene banks of the entire range of diversity of a given crop and its close genetic relatives (Altieri et al. 1987). The complex interaction of genetically diverse, indigenous varieties (farmers' varieties) with their associated pests, predators, and pathogens has been arrested. The traditional farmer fails to retain knowledge associated with farmers' varieties.

Recognizing this situation was the basis for a conservation and enhancement strategy for *in situ* (on-farm) landraces/farmers' varieties in 1989 in Ethiopia through the project 'A Dynamic Farmer Based Approach to the Conservation of Ethiopia's Plant Genetic Resources' funded by Global Environment Facility (GEF). Through a novel strategy of establishing community gene banks, the Institute of Biodiversity Conservation and Research (IBCR) in Ethiopia has played a leadership role internationally in establishing an on-farm conservation mechanism to complement *ex situ* conservation. The project was executed in six zones in the country including the Bale and eastern Shewa zones. Durum wheat is one of the crops under the conservation program in both of these sites.

The uniqueness of the Ethiopian durum wheat germ plasm was observed by Vavilov (1957) and later confirmed by others (Pecetti et al. 1992). Important features for crop improvement have been reported in the Ethiopian wheats of farmers' varieties, including resistance to leaf rust, powdery mildew, and glume blotch; long coleoptile short culm, early ripening, high protein content, and adaptation to low soil fertility and waterlogged conditions; and resistance to drought and Hessian fly (Proceddu et al. 1975; Amri et al. 1990; Pecetti et al. 1992; Bechere and Tesemma 1997).

Determining the magnitude of genetic variability and the pattern of distribution in different *in situ* crop conservation sites and comparing it with *ex situ* collection is essential for the successful conservation and sustainable utilization of the genetic materials. Such a study also will contribute tremendously to *ex situ* gene bank management, the identification of sites for *in situ* conservation, and serve as a benchmark for future assessment of the extent of genetic erosion. The present study is, therefore, aimed at assessing the level of genetic diversity in durum wheat by comparing two *in situ* crop conservation sites and populations from *ex situ* collections.

**Material and Methods.** Ten and 19 durum wheat farmers' varieties (locally named varieties) currently under the *in situ* conservation program in the Bale and eastern Shewa sites, respectively, and 48 durum wheat accessions (representative samples of the original materials that were collected in the Bale zone where the current *in situ* site is located) were used.

A randomized, complete-block design with two replicates was used. Each of the farmers' varieties were grown in a replicate in two 4-m rows with a 0.2-m row spacing. Seeds were sown at a rate of 125 kg/ha. Altogether, a total of 77 populations (2,816 individuals) were used for this study.

Five qualitative traits, beak awn length (short (1), intermediate (2), or long (3)), glume color (white to yellow (1), red to brown (2), or purple to black (3)), glume hairiness (absent (1) or present (2)), seed color (white to yellow (1), red to brown (2), or purple to black (3)), and spike density (lax (1), intermediate (2), or dense (3)) were used to compare the two *in situ* and one *ex situ* sites. These characters were chosen because their expression is little or unaffected by environment, which makes them reliable morphological markers for characterization of wheat germ plasm. Farmers who use folk taxonomy to identify their varieties also use most of the traits selected in this study. These traits attract the eye of the wheat germ plasm curator and signal the presence and/or absence of phenotypic variation in the field according to Belay (1997).

Percentage frequencies of each phenotypic class of each character was used to estimate the Shannon-Weaver diversity index,  $H$ , which is defined as:

$$H = \sum_{i=1}^n p_i \ln p_i$$

where  $n$  is the number of phenotypic classes per character and  $p_i$  is the proportion of the total number of plants in the  $i^{\text{th}}$  class.  $H$  was standardized by converting to the relative index,  $H' = H / H_{\text{max}}$ . A correspondence analysis (on multivariate basis) was used to plot the populations in a low-dimensional graphic presentation. Correspondence analysis is a weighted principal component analysis of a contingency table. The computations were conducted using SAS version 8.002 (1999).

**Result and Discussion. Estimates and analysis of diversity.** Table 1 gives the estimation of  $H'$  for each of the five characters in the two *in situ* conservation sites and the *ex situ* gene bank. The highest mean-diversity index ( $\bar{H}'$ ) pooled over characters was obtained for the eastern Shewa and Bale *in situ* conservation sites ( $\bar{H}' = 0.81$  and  $0.64$ , respectively), whereas populations from the *ex situ* had the lowest level of diversity ( $\bar{H}' = 0.49$ ). The overall mean diversity index for both the *in situ* sites and the population from the gene bank was  $0.73 \pm 0.09$ . The correspondence analysis (on multivariate basis) also was very accurate in discriminating between the populations. Plotting the first two dimensions obtained from the correspondence analysis indicated that the populations from the gene bank and the *in situ* had distinct morphological attributes (Fig. 1, p. 56). Populations from the two *in situ* sites seemed to be more diverse than their *ex situ* counterpart. Most of the populations sampled from the gene bank were negative in the first dimension and showed less variability. We also observed that the materials from the gene bank have poor germination potential and vigor in the field.

The relative contribution of the five qualitative characters for  $H'$  varied considerably (Table 1), with high variation for seed color ( $H' = 0.99$ ), glume color ( $H' = 0.91$ ) and beak length ( $H' = 0.66$ ). Seed color was the most diverse character in both *in situ*

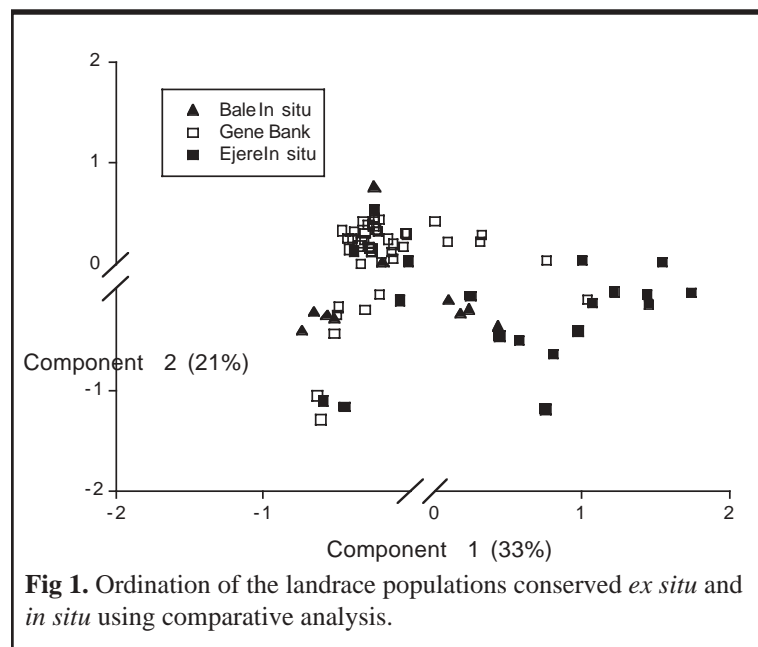
sites and the gene bank populations. A high level of diversity for seed color also has been reported in a world collection of Ethiopian tetraploid wheat (Jain et al. 1975) and in collections from the central highlands (Tesemma and Belay 1991) and northern and northcentral regions of Ethiopia (Bechere et al. 1995). A possible reason for the occurrence of such a high level of diversity of seed color could be the utilization of different seed-color types in traditional consumption for different purposes in Ethiopia.

ANOVA revealed significant differences between the conservation strategies (*in situ* and *ex situ* conservation, see Table 2, p. 56). Traits such as spike density, glume hairiness, and seed color played major roles in distinguishing populations.

**Table 1.** Estimates of the Shannon-Weaver and Simpson's diversity indices,  $H'$ , for five qualitative characters in wheat from *ex situ* and *in situ* collections.

Seed source	Beak length	Glume color	Glume hairiness	Seed color	Seed density	$\bar{H}' \pm \text{SE}$
Bale ( <i>in situ</i> )	0.76	0.82	0.40	0.95	0.28	$0.64 \pm 0.13$
E. Shewa ( <i>in situ</i> )	0.74	0.95	0.92	0.84	0.62	$0.81 \pm 0.06$
Gene bank ( <i>ex situ</i> )	0.48	0.84	0	0.93	0.18	$0.49 \pm 0.18$
Total	0.66	0.91	0.55	0.99	0.52	$0.73 \pm 0.09$





These results are further supported by partitioning of the phenotypic diversity within and between the conservation strategies (Table 3). Eighty-seven percent of the total variation was found between the conservation strategies, whereas 13 % was found within the conservation strategies. Spike density, glume hairiness, and seed color contribute relatively more (30 %, 20 %, and 8 %, respectively) to the differentiation between the *in situ* and *ex situ* conservation sites. These traits are more useful in discriminating the two *in situ* sites from the *ex situ* in this particular study.

According to Belay et al. (1991), spike density is highly associated with environmental conditions especially with altitude. They noted that lax spike is a dominant trait in areas at high altitude and high rainfall. In these conditions, lax spikes may confer resistance to diseases that attack the spike and susceptibility may be associated with the degree of compact-

ness (Belay et al. 1991; Parry et al. 1995). In the arid condition in Syria, dense spikes were reported to be dominant (Ellings and Nachit 1991). The association of glume hairiness with resistance to Karnal bunt (Warham 1988) and powdery mildew (Negassa 1986) also have been reported. Among the seed-color groups, the purple-black type was reported to have an earlier maturity and higher tillering capacity than the other groups, making them better suited to water-logged soil conditions in areas of high altitude (Belay et al. 1995).

These results clearly show that traits, especially the spike density, glume hairiness, and seed color, are highly associated with environmental factors such as temperature and rainfall. This, in turn, indicates the traits have an adaptive significance.

Considerable evidence indicated that damage to chromosomes, some of it resulting in heritable changes, takes place as seeds loose their viability. Studies in barley

**Table 2.** Mean squares for variation between *in situ* (Both Bale and Eastern Shewa sites) and the *ex situ* (gene bank).

Characters	Between <i>in situ</i> and <i>ex situ</i> sites DF = 2	Within <i>in situ</i> and <i>ex situ</i> sites DF = 74
Beak length	0.14	0.09
Glume color	0.09	0.09
Glume hairiness	2.07**	0.05
Seed color	0.31*	0.09
Spike density	0.66**	0.03

\*=P<0.05, \*\*=P<0.01

**Table 3.** Partitioning of the phenotypic diversity into within and between conservation strategies.

Characters	H <sub>di</sub>	H <sub>c</sub>	H <sub>c</sub> / H <sub>di</sub>	(H <sub>di</sub> - H <sub>c</sub> ) / H <sub>di</sub>
Beak length	0.66	0.66	1	0
Glume color	0.91	0.87	0.96	0.04
Glume hairiness	0.55	0.44	0.80	0.20
Seed color	0.99	0.91	0.92	0.08
Spike density	0.52	0.36	0.69	0.31
Mean	0.73	0.65	0.87	0.13

H<sub>di</sub> = diversity index for each character calculated from the entire data set; H<sub>c</sub> = average diversity index of each character for the two *in situ* and the *ex situ* sites; H<sub>c</sub> / H<sub>di</sub> = Proportion of diversity within the two conservation strategies; (H<sub>di</sub> - H<sub>c</sub>) / H<sub>di</sub> = Proportion of diversity between the two conservation strategies in relation to the total variation.

and wheat showed that as storage age increases, chromosome aberrations (per cell) increases (Gundhardt et al. 1953). Changes in the properties of DNA associated with loss of viability in rye seeds, namely the loss of DNA-template activity (Holden and Williams 1984) and decreases in the molecular size of extractable DNA (Cheah and Osborne 1978), also have been observed.

In conclusion, conservation at the on-farm level allows for continuing farmer selection, interaction with the environment, and gene exchange with wild species so that evolution of landraces may continue. Under this system, many cultivated crops species have coexisted with local environmental factors, and the flow of genes among genotypes has taken place with minimum interference. The development of new variations and increasing the diversity within the crop genotypes has been aided. Therefore, *ex situ* conservation needs to be complemented in a way that will maximize the retention and continued evolution of the adaptive qualities of landraces, which will avoid the loss of variation that occurs in sampling and maintenance. The present study indicates the necessity of strengthening and expanding the *in situ* conservation programs in a broad range of agroecological conditions to obtain maximum diversity and utility as source materials in crop-improvement programs.

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## ITEMS FROM GERMANY

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#### *Sixty years of disease-resistance screening.*

During a 60-year period, 10,348 accessions belonging to 21 species (hexaploid, tetraploid, and diploid) of the genus *Triticum* and 489 accessions of the genus *Aegilops* were scored for disease resistance. Tests were made at the seedling stage for powdery mildew, leaf rust, stripe rust, and eyespot, and at the adult-plant stage for powdery mildew, leaf rust, stripe rust, eyespot, and *S. nodorum*. Not all accessions were analyzed for all diseases at all different growth stages, however, for most of the material, scoring data for several diseases are available. For a certain number of accessions, repeated tests were made in different years. About 125,000 disease scores, recorded on index cards using different scoring scales, were computerized, converted into a 1–9 scale, and used to summarize the results. About 20 % of the material analyzed was classified as heterogeneous. For accessions without detectable segregation, a large variability for resistance/susceptibility was detected. At the adult-plant stage, resistant accessions without visible infections were identified for all diseases. The percentage of resistant accessions at that growth stage always was higher than those found in the material tested at the seedling stage. About 90 % of the accessions identified to be resistant at the seedlings stage were resistant or only slightly infected as adult plants. The probability for finding resistant material was shown to be highest in *Aegilops* and diploid species of the genus *Triticum* (> 50 %) but decreased with increasing ploidy level to about 10 % in the hexaploid wheats. Highly resistant accessions can be identified within the hexaploid gene pool but with a much lower efficiency. The higher input needed here for screening will be compensated by a lower effort necessary for using this material in the breeding process. The data obtained for the individual accessions are available via the internet at <http://www.ipk-gatersleben.de>.

#### *Stem-reserve mobilization.*

A selection of 28 Egyptian wheat varieties was grown in the greenhouse and evaluated for the ability to mobilize stored stem reserves under drought stress. For the investigation, we used a method for chemical desiccation of the plant canopy. Two weeks after anthesis in one replication of the experiment, the canopies were sprayed with potassium iodide (0.5 %). In order to calculate the rate of reduction in grain weight caused by the treatment, the 1,000-kernel weight of the treated plants was compared with that of controls after harvest. The percentages of reduction ranged between 16.97 % for Seds 3 and 33.83 % for Sakha 8 (Table 1, p. 59). Sakha 8 was already known to be drought resistant. Further candidates for increased drought insensitivity are the varieties Gemmiza 7 and Gemmiza 9.

#### *Geographical distribution of red-coleoptile color genes.*

A collection of 254 wheat accessions of the Gatersleben gene bank was scored for coleoptile color determined by anthocyanin pigmentation. Fifteen seeds/accession were placed on filter paper and kept for 36 hours at 4°C to synchronize germination. The temperature was increased to 15°C and the color of the coleoptiles was scored after 5–8 days of growth at a photoperiod of 14-h light/10-h darkness. The results obtained are given in Table 2 (pp. 60–65). About 71 % (181) of all tested varieties had noncolored coleoptiles. Red and dark red coleoptiles were detected in 22 % (56) and 7 %

(17) of the plants, respectively, One accession was heterogeneous. A high percentage of red coleoptiles was detected in *T. macha* lines from Georgia and *T. spelta* from Germany and Switzerland.

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**Table 1.** Percentage of grain weight reduction after chemical desiccation of the plant canopy of wheats originated from Egypt.

Variety	% grain weight reduction
Sakha 8	33.83
Gemmiza 7	31.98
Gemmiza 9	31.76
Seds 4	29.19
Giza 155	28.11
Sakha 92	27.54
Seds 7	27.52
Seds 9	26.69
Seds 5	25.19
Sahel 1	24.59
Sakha 61	24.28
Seds 8	23.75
Seds 6	23.73
Giza 139	23.05
Gemmiza 5	22.96
Seds 2	22.41
Giza 164	21.29
Gemmiza 3	21.09
Sakha 69	21.02
Giza 163	20.49
Gemmiza 1	20.40
Sahel 6	19.15
Giza 144	17.48
Giza 160	18.85
Giza 157	18.66
Seds 3	16.97
Giza 167	15.68

**Table 2.** Coleoptile color of 254 selected wheat accessions from the Gatersleben gene bank. The countries of origin are listed in alphabetical order; accession numbers are given in brackets. *T. sp.* = no taxonomic classification of accessions of the genus *Triticum*; – not colored; + red; ++ dark red; and +/- segregating.

Country of origin	Species/subspecies, variety name, accession number	Coleoptile color
AFGHANISTAN	<i>T. aestivum</i> L. (TRI2638)	–
	<i>T. aestivum</i> L. (TRI4054)	–
	<i>T. compactum</i> Host var. <i>echinoideum</i> (TRI18691)	+/-
	<i>T. compactum</i> Host var. <i>erinaceum</i> (TRI18692)	–
	<i>T. compactum</i> Host var. <i>subfetissovii</i> (TRI18693)	+
	<i>T. compactum</i> Host var. <i>suberinaceum</i> (TRI18694)	++
	<i>T. compactum</i> Host var. <i>sericeum</i> (TRI18695)	–
	<i>T. compactum</i> Host var. <i>sericeum</i> (TRI18696)	+
	<i>T. compactum</i> Host var. <i>suberinaceum infalt</i> (TRI18697)	–
	<i>T. compactum</i> Host var. <i>albiceps</i> (TRI18698)	+
	<i>T. compactum</i> Host var. <i>erinaceum</i> (TRI18699)	–
	<i>T. compactum</i> Host var. <i>sericeum</i> (TRI18700)	+
	<i>T. compactum</i> Host (TRI18701)	–
	<i>T. compactum</i> Host var. <i>fetissovii</i> (TRI18702)	–
	<i>T. compactum</i> Host (TRI18703)	–
	<i>T. compactum</i> Host var. <i>rubriceps</i> (TRI18704)	–
	<i>T. compactum</i> Host (TRI18705)	–
	<i>T. compactum</i> Host var. <i>sericeum</i> (TRI18707)	–
	<i>T. compactum</i> Host (TRI18708)	–
	<i>T. compactum</i> Host var. <i>sericeum</i> (TRI18709)	++
<i>T. compactum</i> Host var. <i>echinoideum</i> (TRI18710)	–	
<i>T. compactum</i> Host var. <i>fetissovii</i> ‘Lyuchak’ (TRI18727)	–	
ALBANIA	<i>T. sp.</i> (TRI17941A)	–
	<i>T. sp.</i> (TRI17941B)	–
	<i>T. sp.</i> (TRI18784)	–
ARMENIA	<i>T. sp.</i> (TRI18785)	–
	<i>T. sp.</i> (TRI18601)	–
	<i>T. sp.</i> (TRI18602)	–
	<i>T. sp.</i> (TRI18604)	–
	<i>T. sp.</i> (TRI18605)	–
	<i>T. sp.</i> (TRI18607)	–
	<i>T. sp.</i> (TRI18610A)	–
	<i>T. sp.</i> (TRI18610B)	–
	<i>T. sp.</i> (TRI18612)	–
	<i>T. sp.</i> (TRI18618A)	–
	<i>T. sp.</i> (TRI18618B)	–
	<i>T. sp.</i> (TRI18619)	–
	<i>T. sp.</i> (TRI18623A)	–
	<i>T. sp.</i> (TRI18623B)	–
	<i>T. sp.</i> (TRI18649)	–
	<i>T. sp.</i> (TRI18654)	–
	<i>T. sp.</i> (TRI18656)	–
	<i>T. sp.</i> (TRI18657)	–
	<i>T. vavilovii</i> Thum. (TRI18758)	–
	<i>T. vavilovii</i> Thum. var. <i>vavilovii</i> (TRI18760)	–
<i>T. sp.</i> (TRI18810)	–	
<i>T. sp.</i> (TRI18811)	+	
<i>T. sp.</i> (TRI18834)	–	
<i>T. sp.</i> (TRI18837)	–	
<i>T. sp.</i> (TRI18838)	–	
<i>T. sp.</i> (TRI18839)	–	



**Table 2 (continued).** Coleoptile color of 254 selected wheat accessions from the Gatersleben gene bank. The countries of origin are listed in alphabetical order; accession numbers are given in brackets. *T. sp.* = no taxonomic classification of accessions of the genus *Triticum*; – not colored; + red; ++ dark red; and +/- segregating.

Country of origin	Species/subspecies, variety name, accession number	Coleoptile color
AUSTRIA	<i>T. aestivum</i> L. (TRI8629)	–
	<i>T. spelta</i> L. var. <i>duhamelianum</i> ‘Tiroler spelz’ (TRI18728)	++
	<i>T. spelta</i> L. var. <i>duhamelianum</i> (TRI18729)	+
	<i>T. spelta</i> L. var. <i>duhamelianum</i> (TRI18767)	++
AZERBAIJAN	<i>T. compactum</i> Host var. <i>humboldtii</i> (TRI18690)	+
BULGARIA	<i>T. sp.</i> (TRI18321A)	–
	<i>T. sp.</i> (TRI18321B)	–
	<i>T. sp.</i> (TRI18325)	–
	<i>T. sp.</i> (TRI18326)	–
CANADA	<i>T. aestivum</i> L. ‘Lee’ (TRI7738)	–
CECHOSLOVAKIA	<i>T. aestivum</i> L. ‘Draga’ (TRI8036)	–
CHINA	<i>T. aestivum</i> L. (TRI4093)	–
	<i>T. aestivum</i> L. (TRI4420)	–
	<i>T. aestivum</i> L. (TRI4421)	+
	<i>T. aestivum</i> L. (TRI5272)	–
	<i>T. aestivum</i> L. ‘Chua–Bej 672’ (TRI8052)	–
	<i>T. aestivum</i> L. ‘Chun–Man–Mai’ (TRI8054)	–
	<i>T. aestivum</i> L. ‘Hua–Bej 187’ (TRI8086)	–
	<i>T. aestivum</i> L. ‘Yinchuan Nr. 1’ (TRI18252A)	–
	<i>T. aestivum</i> L. ‘Yinchuan Nr. 1’ (TRI18252B)	–
	<i>T. aestivum</i> L. (TRI18452)	–
	<i>T. aestivum</i> L. (TRI18453)	–
	<i>T. compactum</i> Host var. <i>wernerianum</i> (TRI18714)	–
	<i>T. compactum</i> Host var. <i>erinaceum</i> J 20 (TRI18754)	–
	<i>T. compactum</i> Host var. <i>erinaceum</i> ‘I–Tczin–Hun–Man–Chi–Mai’ (TRI18774)	–
	<i>T. sp.</i> (TRI18843)	–
	<i>T. sp.</i> (TRI18845)	–
<i>T. sp.</i> (TRI18882)	+	
CYPRUS	<i>T. durum</i> Desf. (TRI10594)	–
	<i>T. sp.</i> (TRI18828)	–
	<i>T. sp.</i> (TRI18830)	–
ETHIOPIA	<i>T. sp.</i> (TRI18847)	–
FINLAND	<i>T. sp.</i> (TRI18555)	–
	<i>T. sp.</i> (TRI18557)	–
	<i>T. sp.</i> (TRI18559)	–
	<i>T. sp.</i> (TRI18561)	–
	<i>T. sp.</i> (TRI18564)	–
	<i>T. sp.</i> (TRI18572)	–
	<i>T. sp.</i> (TRI18578)	–
	<i>T. sp.</i> (TRI18591)	–
	<i>T. sp.</i> ‘Chernaya Persidskaya’ (TRI18669)	–
FRANCE	<i>T. aestivum</i> L. ‘Primepi’ (TRI7893)	–
	<i>T. aestivum</i> L. ‘Blevoy’ (TRI9449)	–
	<i>T. spelta</i> L. var. <i>duhamelianum</i> ‘Epeautre Brune Ordinaire’ (TRI18724)	+
	<i>T. spelta</i> L. var. <i>album</i> ‘Epeautre Ordinaire Sans Barbes’ (TRI18725)	–
	<i>T. spelta</i> L. ‘Epeautre Blond Ohdore’ (TRI18726)	–
GEORGIA	<i>T. aestivum</i> L. (TRI13618)	+
	<i>T. macha</i> Dekarp. et (TRI18743)	+
	<i>T. macha</i> Dekarp. et var. <i>colchicum</i> (TRI18744)	+

**Table 2 (continued).** Coleoptile color of 254 selected wheat accessions from the Gatersleben gene bank. The countries of origin are listed in alphabetical order; accession numbers are given in brackets. *T. sp.* = no taxonomic classification of accessions of the genus *Triticum*; – not colored; + red; ++ dark red; and +/- segregating.

Country of origin	Species/subspecies, variety name, accession number	Coleoptile color	
GEORGIA	<i>T. macha</i> Dekarp. et (TRI18745)	+	
	<i>T. macha</i> Dekarp. et var. <i>macha</i> (TRI18746)	+	
	<i>T. macha</i> Dekarp. et var. <i>palaeomereticum</i> (TRI18747)	+	
	<i>T. macha</i> Dekarp. et var. <i>palaeomereticum</i> (TRI18748)	+	
	<i>T. macha</i> Dekarp. et var. <i>subletshchumicum</i> (TRI18749)	++	
	<i>T. macha</i> Dekarp. et var. <i>palaeomereticum</i> (TRI18750)	+	
	<i>T. macha</i> Dekarp. et (TRI18751)	+	
	<i>T. macha</i> Dekarp. et (TRI18752)	+	
	<i>T. macha</i> Dekarp. et var. <i>submegrelicum</i> (TRI18756)	+	
	<i>T. macha</i> Dekarp. et (TRI18757)	+	
	<i>T. macha</i> Dekarp. et (TRI18759)	+	
	<i>T. macha</i> Dekarp. et (TRI18765)	+	
	<i>T. compactum</i> Host var. <i>rubriceps</i> (TRI18769)	–	
	<i>T. sp.</i> (TRI18832)	–	
	<i>T. sp.</i> (TRI18873)	–	
	<i>T. sp.</i> (TRI18874)	–	
	<i>T. sp.</i> (TRI18875)	–	
	<i>T. sp.</i> (TRI18876)	–	
	<i>T. sp.</i> (TRI18877)	–	
	GERMANY	<i>T. spelta</i> L. var. <i>album</i> (TRI18716)	+
		<i>T. spelta</i> L. var. <i>neglectum</i> (TRI18717)	–
		<i>T. spelta</i> L. var. <i>duhamelianum</i> (TRI18718)	++
<i>T. spelta</i> L. var. <i>album</i> (TRI18719)		+	
<i>T. spelta</i> L. var. <i>duhamelianum</i> (TRI18720)		++	
<i>T. spelta</i> L. var. <i>caeruleum</i> (TRI18721)		+	
<i>T. spelta</i> L. var. <i>arduini</i> (TRI18722)		+	
<i>T. spelta</i> L. var. <i>duhamelianum</i> (TRI18723)		+	
<i>T. compactum</i> Host var. <i>Griceositerinum</i> 'Igel Unbehaart Rot' (TRI18766)		+	
<i>T. compactum</i> Host var. <i>kanaschii</i> (TRI18770)		–	
INDONESIA	<i>T. sp.</i> (TRI18853)	–	
	<i>T. sp.</i> (TRI18854)	+	
	<i>T. sp.</i> (TRI18543)	–	
	<i>T. sp.</i> (TRI18547A)	+	
	<i>T. sp.</i> (TRI18547B)	+	
	<i>T. sp.</i> (TRI18584)	–	
	<i>T. sp.</i> (TRI18585)	–	
	<i>T. sp.</i> (TRI18586)	++	
	<i>T. sp.</i> (TRI18587)	+	
	<i>T. sp.</i> (TRI18588)	+	
	<i>T. sp.</i> (TRI18590)	–	
	<i>T. sp.</i> (TRI18664)	+	
	<i>T. sp.</i> (TRI18665)	–	
	<i>T. sp.</i> (TRI18860)	+	
<i>T. sp.</i> (TRI18862)	–		
IRAN	<i>T. aestivum</i> L. (TRI5592)	–	
	<i>T. aestivum</i> L. (TRI5987)	–	
	<i>T. aestivum</i> L. (TRI6126)	–	
	<i>T. aestivum</i> L. (TRI6186)	–	
	<i>T. aestivum</i> L. (TRI6431)	++	

**Table 2 (continued).** Coleoptile color of 254 selected wheat accessions from the Gatersleben gene bank. The countries of origin are listed in alphabetical order; accession numbers are given in brackets. *T. sp.* = no taxonomic classification of accessions of the genus *Triticum*; – not colored; + red; ++ dark red; and +/- segregating.

Country of origin	Species/subspecies, variety name, accession number	Coleoptile color	
IRAN	<i>T. aestivum</i> L. (TRI6561)	–	
	<i>T. sp.</i> ‘Gendum Dondoni Shutur’ (TRI18625)	–	
ISRAEL	<i>T. sp.</i> (TRI18277)	–	
	<i>T. sp.</i> (TRI18827)	–	
ITALY	<i>T. dicoccon</i> S. (TRI14233)	–	
	<i>T. aestivum</i> L. (TRI14856)	–	
	<i>T. aestivum</i> L. (TRI14860)	–	
	<i>T. sp.</i> ‘Salome’ (TRI17716)	–	
	<i>T. sp.</i> ‘Carusella’ (TRI17996A)	–	
	<i>T. sp.</i> ‘Carusella’ (TRI18782)	–	
	<i>T. sp.</i> ‘Carusella’ (TRI18783)	–	
JAPAN	<i>T. sp.</i> (TRI18819)	++	
KAZAKSTAN	<i>T. sp.</i> (TRI18823)	+	
	<i>T. sp.</i> (TRI18868)	–	
CROATIA	<i>T. sp.</i> (TRI17981)	+	
MAROCCO	<i>T. sp.</i> (TRI18282A)	–	
	<i>T. sp.</i> (TRI18282B)	–	
	<i>T. sp.</i> (TRI18282C)	–	
	<i>T. sp.</i> (TRI18283A)	–	
	<i>T. sp.</i> (TRI18283C)	–	
	<i>T. sp.</i> (TRI18287A)	–	
	<i>T. sp.</i> (TRI18287B)	–	
	<i>T. sp.</i> (TRI18288A)	–	
	<i>T. sp.</i> (TRI18288B)	–	
	<i>T. sp.</i> (TRI18288C)	–	
	<i>T. sp.</i> (TRI18840)	–	
	MEXICO	<i>T. sp.</i> (TRI18644A)	–
		<i>T. sp.</i> (TRI18644B)	–
MONGOLIA	<i>T. sp.</i> (TRI18567)	++	
	<i>T. sp.</i> (TRI18569)	–	
NEPAL	<i>T. sp.</i> (TRI10845)	+	
PAKISTAN	<i>T. sp.</i> (TRI18662)	–	
	<i>T. sp.</i> (TRI18663)	+	
	<i>T. sp.</i> (TRI18667)	–	
	<i>T. sp.</i> (TRI18668)	+	
	<i>T. sp.</i> (TRI18857)	–	
	<i>T. sp.</i> (TRI18859)	–	
	<i>T. sp.</i> (TRI18863)	–	
	<i>T. sp.</i> (TRI18864)	+	
	<i>T. sp.</i> (TRI18849)	++	
POLAND	<i>T. sp.</i> (TRI18643)	–	
	<i>T. sp.</i> (TRI18849)	++	
	<i>T. sp.</i> (TRI18849)	++	
PORTUGAL	<i>T. aestivum</i> L. ‘Indiano Mocho’ (TRI3017)	–	
	<i>T. aestivum</i> L. ‘Molle I’ (TRI3104)	–	
	<i>T. aestivum</i> L. ‘Galego Barbado x Rieti’ (TRI3905)	–	
ROMANIA	<i>T. aestivum</i> L. ‘F 158–69’ (TRI10410)	–	
RUSSIA	<i>T. durum</i> Desf. ‘Sary–Bugda’ (TRI18730)	–	
	<i>T. compactum</i> Host var. <i>erinaceum</i> (TRI18742)	–	
	<i>T. sp.</i> (TRI18833)	–	
	<i>T. sp.</i> (TRI18865)	–	
SPAIN	<i>T. sp.</i> ‘Trigo’ (TRI18671)	–	

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Country of origin	Species/subspecies, variety name, accession number	Coleoptile color
SPAIN	<i>T. sp.</i> (TRI18672)	–
	<i>T. sp.</i> ‘Trigo’ (TRI18674)	+
	<i>T. sp.</i> ‘Grandal’ (TRI18677)	–
	<i>T. sp.</i> ‘Trigo’ (TRI18684)	–
	<i>T. sp.</i> (TRI18824)	–
	<i>T. sp.</i> (TRI18855)	–
SWEDEN	<i>T. compactum</i> Host var. <i>wittmackianum</i> (TRI18712)	–
SWITZERLAND	<i>T. spelta</i> L. var. <i>album</i> ‘Elsenegg Weisskorn’ (TRI18731)	+
	<i>T. spelta</i> L. var. <i>album</i> ‘Riniker Weisskorn’ (TRI18732)	+
	<i>T. spelta</i> L. var. <i>album</i> ‘Riniker Weisskorn’ (TRI18733)	+
	<i>T. spelta</i> L. var. <i>album</i> ‘Rufenach Weisskorn’ (TRI18734)	+
	<i>T. spelta</i> L. var. <i>album</i> ‘Rufenach Weisskorn’ (TRI18735)	+
	<i>T. spelta</i> L. var. <i>album</i> ‘Lenzburger Weisskorn’ (TRI18736)	+
	<i>T. spelta</i> L. var. <i>duhamelianum</i> ‘Muri Rotkorn’ (TRI18737)	++
	<i>T. spelta</i> L. var. <i>duhamelianum</i> ‘Battig Rotkorn’ (TRI18738)	+
	<i>T. spelta</i> L. var. <i>duhamelianum</i> ‘Oberkulmer Rotkorn’ (TRI18739)	+
	<i>T. spelta</i> L. var. <i>duhamelianum</i> ‘Oberkulmer Rotkorn’ (TRI18740)	++
	<i>T. spelta</i> L. var. <i>duhamelianum</i> ‘Liestaler Rotkorn’ (TRI18741)	++
	<i>T. sp.</i> (TRI18852)	–
	SYRIA	<i>T. sp.</i> (TRI18826)
TAJIKISTAN	<i>T. sp.</i> (TRI18573)	–
	<i>T. sp.</i> (TRI18574)	–
	<i>T. sp.</i> (TRI18813)	++
	<i>T. sp.</i> (TRI18869)	–
	<i>T. sp.</i> (TRI18871)	–
	<i>T. sp.</i> (TRI18872)	–
TIBET	<i>T. sp.</i> (TRI18246A)	–
	<i>T. sp.</i> (TRI18786)	–
	<i>T. sp.</i> (TRI18787)	–
	<i>T. sp.</i> ‘Karpotok’ (TRI18788)	–
	<i>T. sp.</i> (TRI18790)	–
TUNISIA	<i>T. durum</i> Desf. (TRI17309A)	–
	<i>T. durum</i> Desf. (TRI17309B)	–
	<i>T. sp.</i> (TRI18799)	–
TURKEY	<i>T. sp.</i> (TRI18628)	–
	<i>T. sp.</i> (TRI18635)	–
	<i>T. sp.</i> ‘Yazlyk’ (TRI 18660)	–
	<i>T. sp.</i> (TRI18812)	–
	<i>T. sp.</i> (TRI18817)	++
	<i>T. sp.</i> (TRI18821)	–
	<i>T. sp.</i> (TRI18822)	–
	<i>T. sp.</i> (TRI18825)	–
	<i>T. sp.</i> (TRI18842)	–
	TURKMENISTAN	<i>T. sp.</i> ‘Misri–Bugdai’ (TRI18614)
<i>T. compactum</i> Host var. <i>kerkianum</i> (TRI18762)		–
<i>T. compactum</i> Host var. <i>pseudorubriceps</i> (TRI18763)		+
<i>T. compactum</i> Host var. <i>fetissovii</i> (TRI18772)		–
UKRAINE	<i>T. spelta</i> L. var. <i>alefeldii</i> (TRI18715)	+
	<i>T. aestivum</i> L. var. <i>erythrosperrum</i> ‘Krymka Odesskaya’ (TRI18764)	–

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Country of origin	Species/subspecies, variety name, accession number	Coleoptile color
UZBEKISTAN	<i>T. aestivum</i> L. (TRI17891A)	–
	<i>T. aestivum</i> L. (TRI17891B)	–
	<i>T. aestivum</i> L. (TRI17895A)	–
	<i>T. aestivum</i> L. (TRI17895B)	–
	<i>T. sp.</i> (TRI18866)	–
YUGOSLAVIA	<i>T. aestivum</i> L. 'Mura' (TRI9848)	–

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## ITEMS FROM HUNGARY

### AGRICULTURAL RESEARCH INSTITUTE OF THE HUNGARIAN ACADEMY OF SCIENCES

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**The wheat season.** A severe drought in autumn 2000 was followed by a much warmer winter than usual. Because of the drought in spring and early summer and an epidemic of yellow rust, which was the first ever experienced in Hungary, flag leaves withered very early in many locations. Despite the unfavorable weather, a fairly good yield was harvested, though the protein content of the grain was lower than in previous years. Persistent rain experienced during harvest led to a further deterioration in the quality. Good bread-making quality was achieved most frequently with varieties such as Mv Magdaléna, Mv Cseardás, and Mv Magvas, which have genetically high protein content, resistance to yellow rust, and a stable falling number.



**Breeding.**

Z. Bedő, L. Szunics, L. Láng, O. Veisz, I. Karsai, A. Juhász, M. Rakszegi, Gy. Vida, P. Szücs, Cs. Kuti, M. Megyeri, and M. Gál.

**Breeding.** Five Martonvásár-bred winter wheat varieties were registered in 2001.

**Mv Verbunkos** (Mv 218-98) is a new, top-quality wheat with good agronomic properties. Selected from the cross 'GT2412/Mv15/Fatima', the new variety has 14.5–15 % protein and a 37–41 % wet-gluten content,  $A_2$  farinograph value, very good sprouting resistance, and high water uptake under Hungarian conditions. Mv Verbunkos outyielded Mv Emma, an older variety with similar quality, by 10–12 %. The outstanding quality is accompanied by good stability. The variety has reliable frost resistance and good field resistance to powdery mildew, stem rust, and yellow rust.

**Mv Mambo** (Mv 28-98) is an extra early maturing, hard red, awned wheat (pedigree: GK Kalaka/Mv16//F2076) characterized by very good baking quality. Mv Mambo has excellent frost and lodging resistance; good resistance to powdery mildew, leaf rust and stem rust; and very good resistance to yellow rust.

**Mv Marsall** (Mv 29-987) is an early, hard red wheat variety. Selected from the cross 'MvC410-90/GK Kalaka//MvC410-90/Fatima', the variety has high yield, good bread-making quality, and very good sprouting resistance and water uptake. Mv Marsall is frost resistant, has good lodging resistance, moderately resistant to powdery mildew and stem rust, and has a high level of resistance to leaf rust.

**Mv Amanda** (Mv 213-98) is a very attractive, high-yielding awnless variety (pedigree: Mv Palma/GK Kalaka//Mv Palma/Fatima). Baking quality is  $A_2$ -B<sub>1</sub>, with a 31–32 % wet-gluten content. Mv Amanda is moderately resistant to leaf rust and powdery mildew, and has medium frost resistance.

**Mv Panna** (Mv 25-98) is an early, hard red bread wheat (pedigree: Mv213-88/F2076) with a wet-gluten content of 31–32 % and farinograph quality of  $A_2$ . The variety has good field resistance to powdery mildew and yellow rust.

**Computerized management system for cereal breeding.** A program for wheat breeding was compiled in Martonvásár in 1983 and was used for over 10 years with minor modifications to satisfy breeding requirements. Due to new ideas and advances made in the field of computer and other technologies, the data structure and program system have been redesigned. The chief emphasis in developing the Martonvásár Wheat Breeding Software was because of the handling of genealogical and observation data and on the classifications required for well-based selection decisions. The widely-used Windows 9x/NT/2000 operational system, MSAccess data base-handling system, and Visual Basic 5.0 programming language were chosen for this purpose. The subprograms controlling major breeding tasks include the transfer of the data of the selected lines with the necessary modifications to the data base of the new experimental year, the handling of new crosses and seed shipment data, the preparation of the field plan, various output (labels, fieldbooks), and input (manual, online) possibilities and a simple statistical module.

**Transgenic wheat studies.** Transgenic wheat seedlings have been produced containing the gene used for selection and the gene of the Dx5 HMW-glutenin protein subunit chosen for transfer. In the course of this work, 47 transformed, regenerated wheat plants were obtained, in 12 of which the presence of the incorporated gene could be clearly demonstrated.

In a joint British–Australian experiment, the transgenic wheat line B73-6-1, developed from the Australian spring wheat L88-6, which contains extra copies of the 1Dx5 HMW-glutenin gene, was retested in field experiments. A field comparison of the transformant and the original genotype indicated that the protein content, wet-gluten content, grain hardness, and Zeleny, SDS and RMT indexes were greater than those of the control under central European conditions; whereas the extensibility and elasticity of the dough were poorer. The over-expression of the 1Dx5 HMW-glutenin subunit thus appears to result in extra-strong dough, making it suitable for blending with poorer quality flours or for novel end-uses.

**Storage protein studies in old Hungarian wheat germ plasm.** On the basis of its HMW and LMW glutenin and gliadin allele composition, the quantitative traits of its storage proteins, and the quality traits of the lines, the population

of the old Hungarian wheat variety Bánkúti 1201 can be divided into two subgroups based on the 7+8 or 7+9 alleles coded at the *Glu-B1* locus. The differences found in these two types mask the effects of the other loci (e.g., the 2\*<sup>B</sup> allele first identified in Bánkúti 1201, which contains an extra cysteine and is coded at *Glu-A1*), or allows them to be expressed only through their interactions. Lines possessing alleles *Glu-B3i* and *Gli-A1m*, which are characteristic of the population, have strong extensibility. In approximately 60 % of the lines tested, the *Glu-B1* 7+9 allele was detected in association with *Glu-B3i* and *Gli-A1m*. The joint presence of these alleles and the interactions between them ensure the high protein and gluten contents and the good extensibility characteristic of one type in the population. The other group of lines, which have alleles 7+8 at the *Glu-B1* locus, have a greater *Glu-B1x* subunit content, greater insoluble polymeric glutenin content, and, thus, a more stable, stronger dough structure, and smaller extensibility than the previous group. The complex high quality of the variety, i.e., the balance between strong and stable, but nevertheless extensible gluten, is thought to be attributed to a satisfactory proportion of these two types (~ 60 % 7+9 and ~ 40 % 7+8).

**Vernalization studies.** The optimum and minimum vernalization requirements of the 50 winter wheat varieties grown over the last 50 years or currently grown on large areas in Hungary were tested under controlled conditions in the phytotron and in the field and expressed as the time required to heading. The close, significant ( $r = 0.961^{***}$ ) correlation observed between the heading dates in the phytotron and field proved that, with the exception of Mironovskaya 808, a 55-day vernalization treatment was sufficient to completely satisfy the cold requirements of the tested varieties. The vernalization requirements demonstrated in the experiment, which ranged from 35 to 55 days, were in line with those characteristic of the winter wheat species. The minimum vernalization requirements ranged from 0–30 days, depending on the variety. The wheat varieties Skorospelka 3b, Mv 10, Korona, Fatima 2, and Alföld 90 represented a special type, since they were capable of heading, though protractedly, even without vernalization. Without exception, the pedigrees of the varieties with the shortest optimum vernalization requirements included parents of southern origin or of spring type. We found that under experimental plant growth conditions (in the greenhouse or phytotron) the majority of the varieties headed at the date characteristic of the genotype, after 35–45 days of vernalization.

**Winter durum frost-resistance studies.** Data from the phytotron and overwintering data recorded in field showed that on average *T. durum* genotypes achieve a lower level of hardening that lasted for a shorter period than that of the winter *T. aestivum* varieties. The frost resistance of durum varieties and the moisture content of the soil indicated a negative correlation. Using RAPD primers, polymorphisms were demonstrated between the durum genotypes despite the relatively restricted genetic basis. Tests with RFLP probes located on barley chromosome 5H failed to demonstrate any substantial differences between the genotypes. We used a bulked-segregant analysis and RAPD primers on the  $F_2$  generation of a spring/winter durum cross. The sensitive genotypes gave an extra amplification product with OPS04 primer. On the basis of regression analysis, the presence of this product reduced the frost resistance by an average of 37 %. The segregation of the OPS04 marker explained 40 % of the phenotypic distribution in the population.

**Sprouting resistance studies.** Tests were made on the falling number stability of the grain of triticale and wheat plants grown in the phytotron. By applying artificial precipitation and increased humidity treatments during the second half of grain filling and in the over-ripe stage, genotypic differences could be detected in the decline in the falling number, but the date and duration of treatment also had a significant influence on changes in quality.

**Winter durum wheat breeding.** In winter durum wheat breeding the most important aim continues to be an improvement in the frost resistance and winter hardiness and in the pasta-making quality and productivity. The winter durum wheat varieties **Mv Maxi** and **Mv Makaróni** were state registered in 2001. Mv Maxi is a high-yielding variety with good technological quality and excellent adaptability. Mv Makaróni had excellent cold tolerance in phytotron tests and has one of the highest yellow pigment contents of all the winter durum wheat varieties bred in Hungary. Last autumn, two high-yielding, cold-tolerant lines with satisfactory technological quality were entered for state trials. The molecular genetics laboratory continues to work on the elaboration of a marker-assisted selection method to improve the efficiency of selection for higher yellow pigment content.

Polymorphism was detected using RAPD primers between the durum genotypes, which have a relatively restricted genetic basis. The durum genotypes examined did not exhibit any significant difference with RFLP probes localized on the 5H chromosome of barley. A bulk-segregant analysis was made in the  $F_2$  generation of a spring/winter durum cross using RAPD primers. Frost-sensitive plants gave an extra amplification product with the OPS04 primer. Regression analysis revealed that the presence of this product reduced the frost resistance of the plants by an average of 37 %. The segregation of the OPS04 marker explained 40 % of the phenotypic segregation in the test population.

**Disease resistance studies.** The degree of infection in genotypes carrying known leaf and stem rust resistance genes was investigated in artificially inoculated nurseries. We found that in 2001 the resistance genes *Lr9*, *Lr19*, *Lr24*, *Lr25*, *Lr29*, and *Lr35* gave complete protection against leaf rust. Near-isogenic lines for genes *Lr23* and *Lr37* had 10 % or less infection. As observed last year, certain resistance genes which were previously effective against leaf rust exhibited a greater degree of infection in 2001 (*Lr13*, *Lr18*, *Lr21*, *Lr34*, *Lr38*, *Lr44*, *LrB*, and *LrW*). Varieties with the genes *Sr5+6+8a+17*, *Sr7a*, *Sr9b*, *Sr9d+12+24*, *Sr11*, *Sr27*, *Sr31*, *Sr36*, *Sr37*, *SrDr+1*, *SrTi3*, and *SrGT* had little infection with stem rust.

As in many other parts of the country, a local yellow rust epidemic of natural origin developed in Martonvásár in 2001. Although no effort to breed for yellow rust resistance is made at the institute, a large proportion of the varieties exhibited a greater or lesser extent of resistance to the pathogen. Among the varieties state registered in recent years, Mv Madrigál, Mv Palotás, Mv Mezőföld, Mv Optima, Mv Emma, Mv Magvas, Mambo, Mv Panna, and Mv Csárdás had excellent resistance.

The race composition and virulence of the natural powdery mildew population found in the Martonvásár area and the efficiency of known resistance genes were examined in the greenhouse under controlled conditions. In 2001, the dominant races of wheat powdery mildew (and their frequency) were as follows: 63 (22.37 %), 90 (14.47 %), 72 (13.2 %), 77 (12.5 %), and 70 (7.24 %). The number of virulence genes in the pathogen population was 4.35. The resistance genes *Pm4a* (Khapli), *Pm1+2+9*, *Pm2+Mld*, *Pm4b+*, and *Pm4a+* provided satisfactory protection against the wheat powdery mildew pathotypes identified.

The resistance of 216 *T. aestivum* and 34 *T. durum* varieties and breeding lines and 63 foreign resistance sources to spike Fusarium was tested in an irrigated experiment with artificial inoculation. The intensity of infection (spike infection, grain mass changes, visible grain infection and grain infection apparent during germination) was evaluated in the field and in the laboratory. The variety Mv Palotás was again outstandingly resistant in 2001 on the basis of infection determined after incubation. An additional seven breeding lines were found with an average Fusarium infection of less than 10 %. Among the sources of resistance, Frontana, Nobeokabozukomugi, BVAL213064, and Praag8 showed the least infection.

The team also is involved in the development of resistant varieties (*T. aestivum*) to serve as the biological basis for environment-friendly, cost-saving plant protection and production technologies. The majority of the newly registered wheat varieties (Mv Panna, Mv Marsall, Mv Amanda, Mv Verbunkos, and Mambo) have good powdery mildew resistance, whereas some (Mv Marsall and Mv Amanda) are resistant to leaf rust and several (Mv Verbunkos, Mv Panna, and Mv Marsall) have good stem resistance. Complex resistance, or slight susceptibility to several pathogens, also provides the varieties with satisfactory protection.

**Abiotic stress resistance studies.** Various components of the antioxidant defence system were studied to determine the effect of various nitrogen sources ( $\text{NH}_4^+$ ,  $\text{NO}_3^-$ , and  $\text{NH}_4\text{NO}_3$ ) and salt treatment (NaCl) on Martonvásár wheat varieties grown in liquid culture. As a response to various stress factors, there is a rise in the concentration of free radicals and active oxygen species in living organisms. The antioxidant defence system provides protection against the ensuing damage. Changes in the environmental conditions (such as different nitrogen sources or salt treatment) may also act as stress factors. Changes were recorded in the activities of the glutathione reductase, superoxide dismutase, guaiacol peroxidase, catalase, and glutathione-S-transferase enzymes, and it was observed that in some of the enzymes ammonium treatment induced an increase in activity similar to that caused by salt treatment.

The investigations also covered the effect of salt treatment and different nitrogen sources on the isoforms of the antioxidant enzymes. We concluded from the results that ammonium ions act as a stress signal, which induces the activation of certain enzymes responsible for early stress adaptation processes. Some of these enzymes, such as guaiacol peroxidase, and catalase, respond with a change in activity, whereas in others (glutathione reductase and superoxide dismutase) a new isoform appears.

In connection with cold-tolerance studies, changes in the activity of antioxidant enzymes were examined on a total of 13 varieties. Leaf samples were taken for analysis in December, January, and February. The enzyme activity was highest in December for glutathione reductase and in January for ascorbate peroxidase, guaiacol peroxidase, catalase, and glutathione-S-transferase. The varieties could be divided into two groups; one with poor cold tolerance and

one with good frost resistance. The differences in enzyme activities indicate that the different groups follow different strategies to counteract the oxidative stress caused by the cold.

In phytotron tests to investigate the effects of possible global climate changes, a substantial increase in biomass was observed in the early stages of development as the result of increased atmospheric CO<sub>2</sub> concentration. This increase in atmospheric CO<sub>2</sub> concentration had a greater effect on the vegetative biomass than on the grain yield.

The effect of increased atmospheric CO<sub>2</sub> concentration differed not only for different cereal species, but also within the species for varieties with different genetic backgrounds. The yield-increasing effect of higher atmospheric CO<sub>2</sub> concentration was able to compensate in part for the yield-reducing effect of rainfall deficiency, so the grain yield losses were lower than in drought-stressed plants grown at normal atmospheric CO<sub>2</sub> concentration.

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### Genetic studies.

J. Sutka, G. Galiba, M. Molnár-Láng, G. Kocsy, G. Kovács, G. Linc, A. Vágújfalvi, E.D. Nagy, A.F. Bálint, B. Tóth, and I. Molnár.

**Cold-hardening studies.** Using some of the chromosome substitution lines developed from the crosses of the donor Cheyenne to Chinese Spring, it was shown that the accumulation of water-soluble carbohydrates during different stages of hardening was time dependent. Moreover, there was a significant correlation between the rate of carbohydrate accumulation and the frost tolerance. The expression and regulation of a wheat gene homologous to the cold-regulated barley *cor14b* gene was compared in frost-sensitive and frost-tolerant wheat genotypes at different temperatures. Studies made with chromosome substitution lines showed that the threshold induction temperature polymorphism of the *cor14b* wheat homologous gene was controlled by loci located on chromosome 5A of wheat, whereas in *T. monococcum*, the *cor14b* gene was mapped on the long arm of chromosome 2A<sup>m</sup>. A study on the effect of cold hardening on the glutathione (GSH) metabolism showed that chromosome 5A of wheat has an influence on the GSH accumulation and on the ratio of reduced and oxidized glutathione as part of a complex regulatory function during cold hardening. In addition, the level of increase in the GSH content during hardening may indicate the degree of frost tolerance in wheat.

**Drought-tolerance analysis.** To evaluate the genetic background of quantitative criteria of drought tolerance in wheat, six generations of a cross between the varieties Plainsman and Cappelle-Desprez were grown in a randomized complete block design with three replications in the greenhouse of the College of Agriculture, University of Tehran, in 1997. Genetic variation was found for yield potential (Yp), stressed yield (Ys), excised-leaf water retention (ELWR), relative water loss (RWL), relative water content (RWC), and harvest index (HI) under water stress conditions. High heterosis and heterosis were observed in the F<sub>1</sub> hybrid for Ys, HI, and spike-yield index (SYI). Genetic analysis exhibited overdominance in the inheritance of Ys, RWL, ELWR, HI, biomass, and SYI, whereas RWC and Yp were controlled by the additive type of gene action. High narrow-sense heritability estimates were found for ELWR, biomass, and SYI. The high genetic advance for ELWR, RWC, HI, and SYI indicated that direct selection could be effective for these traits. The epistatic effects (additive x additive = [i] for Yp, Ys, and RWL, additive x dominance = [j] for ELWR, and dominance x dominance = [l] for RWL) were found to be exceedingly large.

**Nutrient composition studies.** Cereals, especially common wheat, are the most important staple food; however, their grains often contain very low amounts of available iron (Fe), zinc (Zn), copper (Cu), and manganese (Mn). The aim of the present research was to study the existing variability in the seed inorganic nutrient composition of various *Triticum* and *Aegilops* species and investigate the hypothesized correlation between the ploidy level and the seed mineral nutrient concentrations. The results showed that higher copper (Cu), zinc (Zn), calcium (Ca), and magnesium (Mg) contents were generally observed in the caryopsis of *Aegilops* species, whereas higher iron (Fe) concentration was found in the grains of *Triticum* accessions. The results do not confirm the hypothesis that the grains of the ancient wheat species (einkorn, emmer, and spelt) generally have higher mineral nutrient contents than the recently cultivated varieties, except for Fe, which is contained in a higher amount in the seeds of diploid wheat species. No correlation was found between the ploidy level and genome types (A, AB, AG, ABD, S, D, UM, and DMS) and the mineral nutrient content of the



grains. Similar results were found using hierarchical cluster analysis. Because the 1,000-kernel weight of wild species is generally smaller than that of cultivated species, the correlation between seed weight and mineral-nutrient concentrations also was studied. The results showed that the increase in seed weights correlated significantly with the decrease in Ca and Mg concentrations. The results showed that to improve the human nutritional quality of wheat varieties, the best sources of higher Cu, Zn, Ca, and Mg content could be found in the *Aegilops* genus, whereas genotypes with higher Fe content could be found in the *Triticum* genus.

**Cereal Gene Bank activity.** The Cereal Gene Bank in Martonvásár was founded in 1992 and contains not only 3,150 common wheat and maize varieties and genotypes but also samples belonging to other cereal species, including numerous accessions of wild wheat relatives and other valuable genetic stocks. Besides classical gene bank activities, several research programs have been initiated for the evaluation and exploitation of the genetic diversity of various wheats, wheat-related species, and their hybrids. These programs cover the use of classical genetic methods, *in situ* hybridization, and other molecular-genetic techniques. Recently, special attention has been given to the diploid *Triticum* species, especially to einkorn. Based on a relatively large collection (about 160 einkorn accessions), a new prebreeding method was developed to produce agronomically useful lines of einkorn.

**Identification of wheat-barley translocations.** Five wheat-barley translocations in a wheat background were characterized through a combination of cytogenetic and molecular genetic approaches. The wheat chromosome segments involved in the translocations were identified using sequential GISH and two-color FISH with the probes pSc119.2 and pAs1. The barley chromatin in these lines was identified using SSR markers. A total of 45 markers distributed over the total barley genome were selected from a recently published linkage map of barley and screened on the translocation lines. The following translocations were identified: T2DS-2DL-1HS, T3HS-3BL, T6BS-6BL-4HL, T4D-5HS, and T7DL-7DS-5HS. Wheat-barley disomic and ditelosomic addition lines for the chromosomes 3HS, 4H, 4HL, 5H, 5HL, and 6HS were used to determine the correct localization of 21 markers and the position of the centromere. An ancient intragenomic rearrangement between chromosome arms 1HL and 5HS was detected in barley. Physical mapping of the SSR markers on chromosomes 1H and 5H was made using the intragenomic and interspecific translocation breakpoints and the centromere as physical landmarks.

**Diploid pollen production in in vitro floret cultures of cereals using colchicine treatment.** Colchicine, the well-known antimitotic alkaloid, has long been used for the reduplication of the genomes of animal and plant cells. In the present experiments spikelet cultures of one *T. monococcum* and one *T. turgidum* subsp. *carthlicum* genotype, four rye (Amilo, Lovászpatonai, Merkator, and Motto), and two barley (Igri and Mv 50) varieties were exposed to colchicine treatment in various stages of development.

In each variety, seed set only occurred when the anthers of the cultured spikelets contained microspores in the binuclear stage. On nutrient medium with a pH of 5.8, seed set was only recorded in the barley variety Mv 50 cultured on control medium. When the pH of the medium was adjusted to 4.5, the barley variety Igri performed well. This variety also set seeds on control medium containing 0.02 % or 0.04 % colchicine. In the case of rye, seed set only was observed in the variety Merkátor. Seedlings were successfully grown on germinating medium by excising the embryos from the endosperm-deficient seeds.

From the microscopic analyses, we could see that a colchicine treatment led to a drastic reduction in the number of viable microspores, but the vast majority of those that survived developed into trinuclear pollen. In some of the pollen grains the cell nuclei were considerably bigger than in those developed on control medium, suggesting that they were diploid cell nuclei. In *T. turgidum* subsp. *carthlicum*, MK cytological analysis of the offspring revealed a triploid karyotype ( $3n = 42$ ), indicating the fusion of gametes with different chromosome numbers.

Based on the preliminary results, the colchicine treatment of floret cultures would appear to be an efficient alternative method in polyploid research.

### **Cell biology studies.**

An isolated microspore-culture system was optimized in wheat for cocultivation with *in vivo* fertilized zygotes. However, the media requirements of microspores and isolated zygotes are different. An MMS3 medium (Hu et al. 1997. Plant Cell Rep. 16: 520-525) with 90 g/l maltose was suitable for growing zygotes isolated 18–22 hours after pollination

into plants (14 fully fertile plants from 76 isolated zygotes). For microspore cultures and zygotes, the cultivars Mv Pálma and Siete Cerros, respectively, were used. Applying these genotypes the matured plants originated from the microspores and zygotes are easily discernible. This is the first report about growing zygotes with microspores, as nurse cells, from the same species.

**Characterization of aluminium tolerance of wheat genotypes selected by microspores.** Fertile dihaploid plants were regenerated from microspores selected under conditions of aluminium stress. The selection did not cause alteration in the fertility. However, under stress conditions (i.e., in hydroculture system containing aluminium at low pH), these genotypes accumulate lower amount of aluminium and represent a higher root growth than the Al-sensitive plants. Aluminium accumulation could not be observed in leaves.

In vitro fertilization of isolated egg cells of wheat was made and the dynamics of actin filaments during in situ egg-cell development, and postfertilization was analyzed by using fluorescence-labeled, microinjected F-actin-specific phalloidin. Based on these results, a scenario addressing the potential role of the actin filaments in governing zygotic cell cleavage was drawn. By capitalizing on the ratio approach and microinjecting ER-specific fluorochromes and ER Ca-ATPase-specific inhibitors, the signal transduction of egg cell activation was followed up in in vitro fertilized female gametes isolated from wheat.

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**ITEMS FROM INDIA****BHABHA ATOMIC RESEARCH CENTRE****Nuclear Agriculture and Biotechnology Division, Mumbai-400085, India.*****Current activities: combining quality traits with durable rust resistance and molecular studies in wheat.***

B.K. Das and S.G. Bhagwat (Nuclear Agriculture and Biotechnology Division), and N. Eswaran, A. Saini, and N. Jawali (Molecular Biology and Agriculture Division).

For combining desirable HMW-glutenin subunits and durable rust-resistance genes, intervarietal crosses involving recent bread wheat cultivars and some of our experimental lines were made. The cultivars and experimental lines used in the crosses were PBW-343, HUW-206, WH-542, HD-2385, HD-2285, Vidisha, KS-1, and SK-1. The  $F_2$  populations were scored for morphology, flowering time, and rust reaction after artificial inoculation. A study of genetic diversity of Indian cultivars using PCR-based markers is being investigated. The association of molecular markers with quality traits, agronomically important traits, and rust resistance genes also are being studied.

***Use of a 1-gram, SDS-sedimentation test for bread-making quality in bread wheat.***

B.K. Das and S.G. Bhagwat.

In breeding for improvement of bread-making quality in wheat, small-scale tests for assessing gluten strength or bread-making quality is required in order to select in early generations where sample size is the limiting factor. An SDS-sedimentation volume test using 6 g of whole meal (Axford et al. 1979) is a small-scale test that is used routinely to assess bread-making quality. A sedimentation test using 1 g of whole meal was reported for durum wheat (Dick and Quick 1983). We used the 1-g test on 19 experimental lines of bread wheat. The protein percentage in these lines ranged from 12.9 to 15.4. A sedimentation test was performed using both 6-g and 1-g samples. Results from the 6-g test volume was recorded in milliliters and 1-g test the sedimentation height in millimeters. A significant positive correlation ( $r = +0.753^{**}$ ) exists between the sedimentation values obtained by both the methods. We therefore conclude that the 1-g sedimentation test may be useful for selecting early generation bread wheat lines.

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***Effect of sphaerococcum locus on plant morphology.***

S.G. Bhagwat.

The sphaerococcum trait previously was transferred to an agronomically suitable background by backcrossing. Among the  $BC_3F_4$  segregants, crosses were made between sphaerococcum trait carriers and noncarriers. The parents and  $F_1$  plants were raised in pots. At anthesis, culm length of the tallest tiller, spike length, spikelet number, flag leaf blade length, and maximum width were measured. Spikelet number/cm of spike length was calculated. Stomatal impressions were made from the middle of the upper surface of the flag-leaf blade and stomatal frequency/mm<sup>2</sup> was estimated.

Among the parents, sphaerococcum morphology was associated with a 29 % reduction in the culm length and a 23 % reduction in the spike length. The number of spikelets remained unchanged. Number of spikelets/cm spike length increased by about 30 %. Flag-leaf blades were 11 % wider and 3.6 % longer.

The  $F_1$ s were intermediate in culm and spike length, but the flag-leaf blades were larger. Flag-leaf blade area estimates for the sphaerococcum-type parent, nonsphaerococcum-type parent and the  $F_1$  were  $26.0 \pm 0.95$ ,  $21.4 \pm 1.32$ , and  $29.6 \pm 0.61$ , respectively, and stomata/mm<sup>2</sup> were  $70.5 \pm 2.52$ ,  $67.4 \pm 1.12$ , and  $59.9 \pm 2.71$ , respectively. These results indicate that the sphaerococcum morphology is associated with higher stomatal frequency for the same leaf area. The shortening of the culm in the sphaerococcum type appears to be related to the shortening of parenchymatous cells in the stem.

### ***Grain morphometry studies in wheat.***

S.G. Bhagwat, J.K. Sainis, and S.P. Shouche; and R. Rastogi (Computer Division).

Three selections derived from three backcrosses and the recurrent parent were used to study grain morphometry. Grains were imaged in a downward position using a scanner in the transparency mode. Image analysis was made using the Comprehensive Image Processing Software, which was developed at the Bhabha Atomic Research Centre. Significant differences were detected in geometric parameters and moments. Using 45 parameters, Euclidean distances were calculated when it was possible to differentiate between the partners in four out of the six pair combinations. Previously, we had observed that the system was able to distinguish between wheat varieties, and our recent results show that this system can distinguish between closely resembling wheat samples.

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## **BHARATHIAR UNIVERSITY**

**Cytogenetics Laboratory, Department of Botany, Coimbatore – 641 046, India.**

### ***Genetics and breeding studies in wheat: transfer of rust resistance genes into Indian wheat cultivars.***

V.R.K. Reddy, K.M. Gothandam, and G. Kalaiselvi.

Specific genes for resistance to leaf, stem and stripe rusts present singly or in combination were transferred from hexaploid wheat stocks to seven Indian hexaploid wheat cultivars, HD 2687, PBW 343, MACS 2496, HW 2034, HW 4001, RAJ 1555, and K 8962. The genes include five leaf rust-resistance genes (*Lr19*, *Lr24*, *Lr28*, *Lr32*, and *Lr37*), three stem rust-resistance genes (*Sr24*, *Sr25*, and *Sr38*), and one stripe rust-resistance gene (*Yr17*). A simple backcross method was used to transfer these genes; NILs of each in  $BC_2F_5$  and  $BC_5F_5$  were made from all the 35 cross combinations. The lines were screened for resistance to individual rust races as seedlings in the glasshouse and with a mixture of races at adult plant stage in natural/artificial epiphytic conditions in the field. Immune to moderately resistant reactions at seedling stage and highly resistant reactions at adult plant stage provided by these genes strongly advocate the use of specific rust resistance gene (gene complex) for durable resistance. The lines with *Sr24* and *Sr25* showed a variable

reaction pattern, probably due to interactive effects with genes already present in the recurrent parent. Lines with the leaf rust-resistance genes *Lr28* showed resistance also to stem and stripe rust indicating the presence in the donor parents of additional resistance genes.

### ***Allelic variation of HMW-glutenin subunits in Indian hexaploid wheats.***

Thirty-two cultivars of *T. aestivum* were analyzed for their allelic variations of HMW-glutenin subunits by SDS-PAGE. A numeration system was followed for designating the HMW-glutenin subunits. The *Glu-1* quality score was calculated according to the composition of HMW-glutenin subunits. A total of 10 alleles were identified, three (a, b, and c) at the *Glu-A1* locus, four (a, b, c, and d) at the *Glu-B1* locus, and three (a, b, and d) at the *Glu-D1* locus. The most frequent HMW-glutenin subunits were 2\* at *Glu-A1*, 7 at *Glu-B1*, and 5+10 at *Glu-D1*. The most frequent protein combinations are 2\*, 7+8, 2+12 and 2\*, 7, 5+10. The *Glu-1* quality score ranged from 5–10. The *Glu-1* quality score 8 is present in a large number of cultivars.

### ***Genetic divergence in hexaploid wheat.***

One hundred twenty cultivars of bread wheat were evaluated for 16 yield and associated traits. All the genotypes were grouped into 11 clusters. Cluster I was the largest and included 62 cultivars followed by cluster IV with 20, cluster VI with 13, and cluster XI with 11. The remaining cultivars were distributed in seven clusters with two cultivars in each cluster. Analysis of variance for each individual character showed highly significant differences among the cultivars for all the sixteen characters. The mean performance of each cluster showed appreciable differences for all the characters. The characters of harvest index, protein content, flag leaf area, and straw strength contributed maximum to genetic divergence. Characters such as days-to-heading, days-to-maturity, plant height, and threshability contributed a minimum to the genetic divergence. The phenotypic, genotypic, and environmental correlation coefficients studies revealed that days-to-heading, days-to-maturity, spikelets/ear, straw strength, and protein content were positively correlated with grain yield. Harvest index exhibited a positive correlation with 1,000-kernel weight, texture, and biological yield; and a negative correlation with the other characters. Protein content exhibited positive correlation with spike length, 1,000-kernel weight, and grain yield. Based on genetic divergence and the mean performance of yield and other traits, six diverse and superior genotypes were selected. These genotypes may be involved in a multiple-crossing program to recover transgressive segregates with high genetic yield potential.

### ***Hybrid necrosis and hybrid chlorosis in 4x and 6x Indian wheats.***

Eighty-five bread, 11 emmer, and 23 durum wheats were crossed to three hexaploid testers to determine their genotypic status with reference to necrosis and chlorosis genes. The testers are *T. aestivum* subsp. *macha* var. *subletschchumicum* (*Ne1ne2 Ch1ch2*) and *T. aestivum* cultivars C 306 (*Ne1ne2 ch1Ch2*) and Sonalika (*ne1Ne2 ch1Ch2*).

In *aestivum* wheats, 52 out of 85 varieties have *Ne2* and *Ch2* (*ne1Ne2 ch1Ch2*) and 12 have *Ne1* and *Ch2* (*Ne1ne2 ch1Ch2*). The remaining 21 varieties are noncarriers (*ne1ne2 ch1ch2*) for both genes. In dicoccum wheat, six of the 11 varieties have *Ne1* and *Ch1* (*Ne1ne2 Ch1ch2*), two have *Ne1* (*Ne1ne2 ch1ch2*), and the remaining three are noncarriers. In the durum wheats, 19 of 23 varieties have *Ne1* and *Ch1* (*Ne1ne2 Ch1ch2*) and the remaining four are noncarriers for both genes. Allelic variation at the *Ch* (*Chs*, *Chm*, and *Chw*) and *Ne* (*Nes*, *Nem*, and *New*) loci were observed.

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### *Development and use of molecular markers for genetic mapping and marker-assisted selection in wheat.*

**Construction of molecular genetic map using SSRs with International Wheat Microsatellite Mapping Network (IWMMN).** Ten centers (including Meerut, India) of the IWMMN collaborated in extending the SSR genetic map. As part of the Wheat Microsatellite Consortium (WMC), large numbers of SSR primer pairs (~ 400) were developed. Fifty-eight of 176 primer pairs tested were polymorphic between the parents of ITMI mapping population 'W7984/Opata 85' that was used earlier for the construction of RFLP maps in bread wheat. Using this population and the framework map, a total of 66 microsatellite loci were mapped, which were distributed on 20 of the 21 chromosomes (except on chromosome 6D). These 66 SSR loci are added to the existing 384 SSR loci earlier mapped in bread wheat. This important study with 20 authors has been accepted for publication and will appear in the forthcoming issue of the journal *Theoretical and Applied Genetics*.

**QTL mapping for different economic traits in bread wheat — QTL interval-mapping using the ITMI mapping population and the ITMI map for QTL analysis for yield and yield-contributing traits.** Using QTL Cartographer software, QTL interval mapping for seven yield and yield-contributing traits (tillers/plant, spike length, spikelets/spike, grains/spike, biological yield, harvest index, and grain yield) was undertaken in bread wheat using a set of 110 RILs of the ITMI mapping population. For each trait, values were averaged from 15 plants/genotype (five from each of the three replications). Data on a set of 358 mapped molecular markers (both genotype data and genetic distances) were provided by M.S. Röder, IPK, Gatersleben, Germany, and were used for QTL analysis. CIM resolved 29 QTLs (LOD = 2.1 to 5.5) distributed on 16 chromosomes (six from the A genome and five each from B and D genomes). Seven molecular markers associated with QTLs also showed significant marker-trait association, both in regression and t-tests and can be used for MAS. QTL effects ( $R^2$ ) ranged from 5.3 to 50.6 %, and, when measured irrespective of LOD score, gave a characteristic L-shaped distribution suggesting that there are many minor QTLs that should also be taken into account during MAS. Multitrait, composite-interval mapping (MCIM) for two groups of correlated traits (tillers/plant, biological yield, harvest index, and grain yield; and spike length, spikelets/spike, and grains/spike) detected 41 QTLs for seven individual traits. CIM also may give both false positives and false negatives and several QTL may be pleiotropic, influencing more than one trait. In some cases, for the same QTL, LOD scores in MCIM were generally higher than those in CIM, thus placing higher level of confidence and reducing the possibility of false positives in these QTL identified both in CIM and MCIM.

*QTL analysis for growth and leaf characters.*

QTL interval mapping for four growth characters (early growth habit, days-to-heading, days-to-maturity, and plant height), and association studies for two leaf characters (leaf color, scored as dark and pale green; and leaf waxiness scored as waxy and nonwaxy) also were conducted utilizing the ITMI mapping population. Using QTL Cartographer software, the CIM for all the four growth characters and MCIM for three correlated traits (excluding plant height), were made. For the growth characters, CIM suggested the presence of 14 QTLs (LOD = 2.0–12.7), of which only six were common with those among the 18 QTLs identified by MCIM. Some false positives among QTLs identified by CIM is possible. The 14 molecular markers that were closest, one for each of the 14 QTLs identified by CIM, also were tested for marker-trait association using regression and t-tests. Five markers showed significant association and, therefore, are recommended for MAS. Incidentally, the QTLs associated with these five markers were identified by both CIM and MCIM, thus placing a higher level of confidence in these markers. Some of the QTLs identified by CIM and joint MCIM also affected more than one trait. During CIM for individual traits, the phenotypic variation explained by all QTLs that were identified at LOD score  $\geq 2.0$  or above together accounted for approximately 17–91 % of the phenotypic variation. However, QTL effects, when measured irrespective of LOD score, exhibited a characteristic L-shaped distribution. The two leaf characters had a 100 % correlation. Tests of independence of attributes involving each of the 358 molecular markers with the two leaf traits identified 14 molecular markers spread over seven different chromosomes, each showing significant association with both leaf color and leaf waxiness. Some of these markers are the same and also exhibited association with some growth and yield traits studied earlier, thus adding to their utility in wheat breeding through MAS.

*QTL analysis for preharvest sprouting tolerance.*

QTL interval mapping for preharvest-sprouting tolerance (PHST) was also conducted using the ITMI mapping population. At crop maturity, data for PHST were recorded on each of 110 RILs belonging to the population. At the time of physiological maturity, 15 spikes from each of the 110 RILs (five each per genotype per replication) were harvested separately and scored for tolerance to preharvest sprouting following Baier (Ann Wheat Newslet 1987; 33:40). Observations on sprouting were recorded after 10 days. The data on PHST were scored on the scale of 1–9. Although there was a narrow range of variability between parents of ITMI population for PHST, a wide range of variability was available among the RILs derived from the cross. This encouraged us to conduct composite-interval mapping leading to identification of five QTLs on four chromosomes (1D, 2D, 4B, and 6A). Individual QTL effects ( $R^2$ ) ranged from 5.91–21.07 %. The total  $R^2$  value calculated after summing individual QTL effects was 55.02 %. Individual QTL effects exhibited an L-shaped distribution, again explaining presence of large number of QTLs with moderate to small effects.

**QTL interval mapping using trait-specific mapping populations — preparation of framework map and QTL analysis for grain protein content.**

In a continuation of earlier studies, QTL interval mapping for grain protein content (GPC) using a set of 100 RILs derived from a cross between the parents WL711 (low GPC) and PH132 (high GPC) was conducted. From the GPC data on RILs evaluated in five different environments at three different locations, QTLs were mapped by single marker analysis (SMA), interval mapping (IM), and composite interval mapping (CIM) using QTL-Cartographer V. 1.21. For this purpose a framework genetic map was prepared using the above population. The map had 173 loci involving 171 SSR primer pairs. As many as 13 QTLs with a LOD score  $\geq 2.5$  were detected on eight different chromosomes. The maximum number of QTLs (10) were detected by CIM, followed by SMA and IM (five each). Of the 13 QTLs, seven were identified by CIM at a LOD score higher than the threshold LOD calculated for each environment after 1,000 permutations. Two of these seven QTLs also were identified by all the three methods. The present map is being saturated using SAMPL and AFLP markers. Genotyping of RILs with 13 primer combinations using SAMPL primers 6 and 7 with one AFLP primer pair has already been completed.

*Preparation of framework maps for preharvest sprouting tolerance and grain weight and their proposed use in QTL interval mapping.*

The ongoing project Marker assisted selection for some quality traits in bread wheat, funded by National Agricultural Technology Project (NATP) of Indian Council of Agricultural Research (ICAR), also involves preparation of the framework linkage maps for other two grain quality traits of PHST and grain weight (GW). We have a mapping population of 100 RILs for each of these traits. The data on these traits at three different locations were recorded in the year 2001 and also will be recorded in 2002. Data also are being collected on other traits including early growth habit, days-to-heading, days-to-maturity, leaf color, leaf waxiness, plant height, tillers/plant, spike length, spikelets/spike, grains/spike, grain yield, harvest index, preharvest sprouting tolerance, and grain weight. This data collected over environments will be used for finding 'genotype x environment' interactions. At present, RILs of PHST are being genotyped using SAMPL markers; genotyping has already been completed using four primer combinations with SAMPL primer 6.

After completing the genotyping of PHST RILs with SAMPL primers 6 and 7, and AFLP primers, genotyping of GW RILs will be done. Using the molecular genotyping data, framework maps will be prepared that will be used for QTL interval mapping.

**Association analysis identifies molecular markers for different traits in bread wheat.** Molecular markers linked with QTLs/major genes for traits of interest are being routinely developed in several crops using material derived from planned crosses such as  $F_2$ , RILs, and DH populations. However, the unavailability of mapping populations and the substantial time needed to develop such populations are sometimes major limitations in the development of molecular markers for specific traits. To overcome this difficulty, association studies involving the use of germ plasm collections for the development of molecular markers has been conducted. During the present study, binary data for SSR, AFLP, and SAMPL markers used earlier for diversity studies were utilized for a study of marker-trait associations involving data scored on 14 phenotypic traits in 55 elite and exotic wheat genotypes. Using both simple linear regression and multiple regression methods, a total of 351 molecular markers (131 SSR, 166 AFLP, and 54 SAMPL) were identified, each of which showed significant association with at least one of the 14 traits. Out of the above 351 markers, 47 were common in both the regression methods (23 SSR, 21 AFLP, and 3 SAMPL). The 47 markers that were common in both analyses are important and can be used for MAS in wheat breeding after conducting the necessary validation studies.

**Development and use of EST-derived SSR and SNP markers in bread wheat.** SSRs and SNPs are the second and third generation markers of choice and will be used extensively in the future for a variety of purposes. These markers are preferred because of their ubiquity and uniform distribution in nuclear and organellar genomes of plants and animals. Because their development is labor-intensive and expensive, their large-scale use in plant genotyping and MAS may take time. To reduce the development cost of these markers in bread wheat, we used the ever-expanding EST database. EST-derived SSRs/cSSRs and SNPs/cSNPs have many intrinsic advantages over genomic SSRs and SNPs. They are free by-products of the EST database and can be quickly obtained by electronic search of the database. Furthermore, because they are present in expressed regions of genome, which are generally conserved, these markers also have high transferability, and therefore also are useful in comparative genomics. Their presence in the expressed regions of genome also make them particularly useful for studies involving marker-trait association. We developed two, user-friendly tools, MSL and SNPL, which can be easily used by the beginners to mine SNPs and SSRs, respectively. With the help of these tools, we detected 1,083 SSRs (1–7 motif length) and 19 SNPs, using EST databases (dbEST and EMBL located at NCBI and EBI sites, respectively). We have designed primers for 36 cSSRs. Four of these primer pairs also were synthesized and used successfully for PCR amplification of SSRs. These primers also were tested on two accessions of barley to elucidate their functionality beyond genera (i.e., to test their transferability). All the four primer-pairs tested are functional; they amplified the products of accepted sizes both in wheat and barley. Generally these primer-pairs are intraspecifically monomorphic, but one of the primer-pairs was polymorphic between the two genotypes of bread wheat that represent parents of GPC mapping population. All the four primer-pairs tested showed polymorphism between barley and wheat.

**Mapping of SAMPL markers and their conversion into SCAR markers for MAS.** SAMPL markers can be used for mapping. Those markers associated with a trait of interest can be converted into PCR-based markers, SCARs, which can be easily used for MAS. Cloned SAMPL fragments also can be used as probes in RFLP and FISH to study their genomic distribution. We used SAMPL markers in bread wheat for the first time to study genetic diversity and possible marker-trait associations (Theor Appl Genet **104**:465-472. 2002). The polymorphic SAMPL bands are now being used for preparation of framework maps using three mapping populations developed for individual traits (grain protein content, preharvest sprouting tolerance, and seed weight), and the SAMPL bands identified to be associated with these traits are being converted into SCARs through sequencing of the corresponding amplified products.

#### **Study of ribosomal DNA in wild barley (*Hordeum spontaneum*).**

*Intergenic spacer (IGS) length polymorphism in wild barley.*

A total of 112 accessions of wild barley belonging to three different microsites (Evolution Canyon, Tabigha, and Neve Yaar) from Israel having contrasting ecogeographical conditions were analyzed for IGS length polymorphism. Genomic DNA was digested with *SacI* and, after DNA hybridization, was probed with rDNA probe pTA71. Repeat-unit lengths varied from 9.2–11.0 kb. We observed 12 lines constituting 34 different rDNA phenotypes, which were largely correlated with different ecogeographic conditions prevailing at the microsites. In the accessions from Neve Yaar, homogenization of IGS length at the two loci located on two different chromosomes were observed. FISH analysis ruled out the possibility of loss of one of the two NOR loci.

*Methylation status of rDNA.*

For analyzing methylation status at rDNA repeat units, we used a pair of isochizomers, *HpaII* (CG methylation-sensitive) and *MspI* (methylation insensitive), and also the methylation-sensitive restriction enzyme *BamHI*. *HpaII* cleaved the ribosomal-repeat unit at a solitary site giving a single band of the size of repeat unit, whereas *MspI* cleaved it at several sites giving multiple bands and suggesting that rDNA repeat units in barley are heavily methylated.

*BamHI* also gave multiple bands of variable densities, suggesting that the site for this enzyme is not uniformly methylated in all ribosomal-repeat units. A combination of *BamHI* bands in a genotype was designated as the phenotype of that accession, and seven such phenotypes were available. Out of a total of 11 *BamHI* bands, four (1.8, 3.8, 7.2, and 8.9 kb) were present in all the genotypes and the remaining seven (2.4, 3.0, 3.6, 4.1, 4.6, 5.0, and 5.9 kb) were polymorphic by presence or absence in different genotypes giving rise to seven different phenotypes. Out of seven phenotypes, six are microsite specific. Thus, the present study resolves microsite-specific methylation of different sites within the intergenic spacer, suggesting that natural selection also plays an important role in methylation of these different sites that may perhaps have a role in regulation of gene expression.

**Identification of a T1BL-1RS translocation in some wheat genotypes using three different approaches.** The short arm of chromosome 1 of rye is known to have genes for resistance to important fungal diseases and insect pests and high yield. Chromosome arm 1RS also is one of the most widely utilized sources of alien chromatin in wheat improvement. Therefore, knowledge of wheat varieties containing the T1BL-1RS translocation will allow judicious selection of material to be used in a crossing program by the plant breeders for wheat improvement. In view of the above, a total of 19 genotypes/important Indian wheat variety were studied for the identification of the T1BL-1RS translocation using three different approaches including study of satellite chromosomes in mitosis, GISH, and STMS-marker analysis. The results suggested that eight wheat varieties (PBW373, PBW343, PBW175, UP2338, UP2425, UP2418, UP2382, and CPAN3004) of the 19 examined have the T1BL-1RS translocation. These varieties may be considered as a useful source of germ plasm in wheat breeding.

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***Deformation of spikelets and spike sterility in wheat caused by winter frost in the high altitude of the Nilgiri Hills in Tamilnadu, India.***

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The damage caused to tea plantations and hilly vegetables due to winter frost at high altitudes of the Nilgiri hills in Tamilnadu, India, is a common phenomenon. However, there was no such report on wheat until now. Winter or ground frost usually occurs every year in the high-altitude, open lands of the Nilgiri Hills during the winter months (from 15 November–15 February). On a frosty night, room temperature can be 0–2°C and soil temperature below 0°C. The dew on the leaf surface of grass, crops, bushes, and tea plants growing in the open lands at high altitudes will crystallize to form ice. Temperatures the following day can reach 18–22°C quickly in the bright sunshine. Because of the high diurnal variation, extreme low temperatures result in the drying and scorching of the leaves. Plants of the grasslands and tea plantations and other crops may wither totally. These cold conditions usually occur for 3–10 consecutive days, followed by a warm period, and again by frosty nights. In a particular year, 1–3 cold periods can occur. The frosts do not affect the vegetative stages of wheat, barley, rye, oats, and *Brassica*.

At the IARI Regional Station, wheat is sown year round, generation after generation, to reduce the time for varietal improvement by taking advantage of favorable weather conditions. However, farmers follow the normal sowing times, kharif (June–July) and Rabi (November–December). From our observations, it is now evident that both bread and dicoccum wheat are damaged by frost at flowering. We confirmed that flowering during frosty conditions resulted in the deformation of the lower spikelets and, subsequently, no spikelets were found on the lower portion of the spike. The remaining spikelets on the upper portion were sterile or the fertilized embryos aborted. The spikes of the lower surface were male sterile and entirely open. The spikes that emerged after a frost period had perfect seed set. This type of crop damage was noticed mainly on wheat grown in open fields rather than on slopes or near tree plantations. The crop loss varied between 50–100 %.

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***HW 2045, a rust-resistant wheat variety for late-sown conditions of northeastern India.***

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An early maturing, high-yielding wheat variety coupled with resistance to rusts and blight is needed for the northeastern Plains Zone of India (comprising the states of Bihar, Jarkand, parts of Uttar Pradesh, West Bengal, and Assam) as more area is used for late-sown crops in the predominantly rice–wheat cropping system. The early maturing variety **HW 2045** was bred at the IARI-Regional Station, Wellington, through a backcross method using HD 2402, which has the *Th. ponticum*-derived, linked genes *Lr19* and *Sr25* and the mutant line Sunstar\* 6/C 80-1 developed by Knott and McIntosh, in a white-seeded background with highly reduced yellow pigmentation in the endosperm. HW 2045 was released for cultivation in the northeast India during 2001–02. This variety possesses remarkable resistance to all existing pathotypes of stem, leaf, and stripe rusts and moderate resistance against the foliar blight. This variety also exhibited slow senescence contributing to increased grain yield under the late-sown conditions. In the All-India Coordinated Yield Trial, the variety HW 2045 has an average yield of 41.2 q/ha when compared to the best check NW 1014, 39.7 q/ha. This is the first time a variety with *Lr19* and *Sr25* has been released for commercial cultivation in Gangetic Plains of India. HW 2045 will act as genetic barrier against any fresh epidemic of rusts in the plains of North Eastern India.

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***Development of new plant type (NPT) wheats with increased yield potential: methodology and response to various levels of fertility.***

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India has achieved spectacular progress in wheat production due to conceptual wheat breeding and now ranks as the second largest wheat-producing nation after China. The replacement of tall wheats susceptible to lodging by semidwarf, high-yielding, input-responsive and rust resistant wheats in the early 1960s ushered in the green revolution. The national average for wheat yields has increased from about one t/ha in the 1960s to nearly 2.7 t/ha in the later half of 1990s. Wheat production rose from 11 million tons in 1960–61 to 75.4 million tons in 1999–2000 with a buffer stock of 26.5 million tons. World wheat production is 570 million tons, and trade in 2001–02 is estimated to be 107.3 million tons, which is 4.3 million tons more than last year despite a decline in wheat production. India's share of exports is expected to be 3 million tons in 2001–02 because of several limitations, including grain quality. The world price of high-grade wheat is \$124 USD/t, whereas Indian wheat is worth around \$105 USD/t. Therefore, the cost of wheat production must be reduced and grain quality improved to make the Indian wheat internationally competitive (Nagarajan 2001).

The 1 % annual genetic gain in productivity gain during the last 25 years was due to newer generations of high-yielding varieties, but the yield potential has slowed considerably in recent decades. In addition, the cost of cultivation has increased; fertilizer contributed nearly 26 % followed by labor (18.67 %), harvesting (13.91 %), threshing (12.19 %), and land preparation (10.02 %) (Nagarajan 2001). Therefore, the competitiveness of wheat farming will depend on opportunities for dramatically reducing unit costs of production, which can be achieved by shifting the yield frontiers and/or increasing the efficient use of inputs (Pingali 1999).

For achieving the breakthrough in yield potential breeding at CIMMYT Mexico have developed a new wheat type called Buitre through 20 years of pre-breeding, genetic manipulation and countless recombinations. This unique ideotype has robust stem, a long spike (> 30 cm), multiple spikelets and florets, large leaf area and broad leaves. This change should increase yields by improving the harvest index and input use efficiency. However, due to some unknown physiological imbalance or disorder the spikes remains largely sterile and resulting grains are mostly shriveled in



addition, the plants are generally highly susceptible to rust, specially leaf rust and stripe rust (Rajaram and van Ginkel 1996).

The Indian Agricultural Research Institute, New Delhi, initiated strategic research in 1994 to further enhance wheat productivity by designing an NPT utilizing some local types characterized by very long spikes but with shriveled grains. These local types have low tillering and are highly susceptible to rusts. The NPT has combined the three yield components (grain weight, grain number/spike, and tillers/plant) along with dark-green, thick and broad leaves, thick stem, higher biomass and resistance to leaf and stem rusts. The NPT is the first of its kind in the country and in the world. Several NPT lines exhibiting increased yield potential at low levels of fertilizer application due to improved physiological efficiency. These lines also are resistant to leaf and stem rusts and a better grain quality because of high nitrate-reductase activity (high NR type) and grain protein content near 12.5 %.

**Materials and Methods.** *Parental lines.* The material involved as parental lines for the development of NPT genotypes with increased yield potential were local germ plasm Sirsa Farm Wheat (SFW) and two released wheats (Vaishali and Vidisha) with bold, lustrous grains and the tightly linked resistance genes (*Lr24/Sr24*) for leaf and stem rusts derived from *Th. elongatum*.

*Breeding method.* A variant of pedigree method of selection was used to handle segregating populations of the  $F_2$ - $F_5$  for developing the NPT combining desirable yield components and resistance to rusts. The breeding scheme was as follows:

1st year	$P_1/P_2$	Parents include SFW (a local type) and Vaishali and Vidisha (released varieties).
1st year (off-season nursery)	$F_1$	25–35 seeds, space-planted, harvested in bulk.
2nd year	$F_2$	<ol style="list-style-type: none"> <li>1. Space-plant more than 2,000 plants.</li> <li>2. Screen for leaf rust resistance in artificial epidemics.</li> <li>3. Select and harvest superior plants showing NPT characteristics along with resistance to rusts.</li> <li>4. Select and carry forward plants with plump grains.</li> </ol>
3rd year	$F_3$	<ol style="list-style-type: none"> <li>1. Plant row progenies at commercial seeding rate</li> <li>2. Screen for leaf rust resistance in artificial epidemics.</li> <li>3. Select 100 spikes expressing NPT characteristics and resistance to rusts from the families.</li> <li>4. Reselect spikes with plump grains and bulk seed.</li> </ol>
4th year	$F_4$	<ol style="list-style-type: none"> <li>1. Plant bulk progenies at commercial seeding rate.</li> <li>2. Screen for leaf rust resistance in artificial epidemics.</li> <li>3. Select 100 spikes expressing NPT characteristics and resistance to rusts from the families.</li> <li>4. Thresh ears individually and screen for plump grain.</li> </ol>
5th year	$F_5$	<ol style="list-style-type: none"> <li>1. Plant head-row progenies; screen for leaf rust resistance in artificial epidemics.</li> <li>2. Bulk superior rows.</li> <li>3. Select grain.</li> </ol>
6th year	$F_6$	<ol style="list-style-type: none"> <li>1. Plant yield trials.</li> <li>2. Grow off-season, summer nursery.</li> <li>3. Screen for stem rust resistance in artificial epidemics.</li> <li>4. Quality test.</li> </ol>
	$F_7$	<ol style="list-style-type: none"> <li>1. Agronomic trials include seeding rate/variety, different nutrient levels, date of sowing/variety, and growing in a furrow-irrigated, raised-bed system.</li> <li>2. Quality test samples from different nutrient levels.</li> <li>3. Fingerprint of selected lines.</li> </ol>

*Methodology for physiological studies.* The high-yielding NPTs, DL 1266-5 and DL 1266-2, along with best standard check PBW 343 were grown in the field. Phosphorus in the form of  $P_2O_5$  and potassium in the form of Muriate of potash were added at the rate of 60 and 40 kg/hectare at the time of sowing. Nitrogen at 100, 150, and 200 kg/hectare in the form of urea was added in three equal splits. The first split was applied as a basal dose, and the 2nd and 3rd splits were applied at crown-root initiation and booting stage, respectively. Each fertilizer treatment was replicated three times, and all the genotypes were used for each of the treatment.

A large number of main shoots were tagged and data was collected on yield attributes including grain weight/spike, grain number/spike, and 1,000-kernel weight. In the present study, assay of nitrate-reductase activity was restricted to the laminae of the main shoot of the tagged plants only. Previous studies indicate that the laminae reduce the nitrate taken up by the plant by more than 75 % (Nair and Chatterjee 1990). Nitrate-reductase activity was assayed in vivo as suggested by Hagman and Hucklesby (1971) and Nair and Abrol (1977). The reduced N content in the grain of the final harvest was determined by the alkaline-phenol-sodium hypochloride method using a Technicon Autoanalyser (Anonymous 1971). Biomass, grain yield, and number of ears/m<sup>2</sup> were sampled randomly.

An analysis of variance and the least significant difference was made for all the varieties, however, our results and discussion shall be confined to the comparison between the check PBW 343 and the sister lines DL 1266-2 and DL 1266-5.

*DNA fingerprinting.* Seven lines, DL 1266-1, DL 1266-2, DL 1266-5, DL 1266-6, DL 1266-10, DL 1266-16, and DL 1266-17, derived from the cross 'SFW/Vaishali'; two other unrelated breeding lines, DL 1337-1 and DL 1396-11; the two parent genotypes, SFW and Vaishali; and two commercial varieties, PBW 343 and HD 2329 were used. Approximately 100 seeds/sample were germinated under aseptic conditions. Seedlings were harvested, bulked, frozen in liquid nitrogen, and used for DNA isolation. DNA was isolated by the standard CTAB method of Doyle and Doyle (1990), purified by RNase treatment followed by phenol chloroform extraction, dissolved in 10 mm Tris-Cl buffer, quantified by analyzing gels using uncut lambda DNA as standard, diluted to 5 ng/ml, and used in PCR.

Thirty-three STMS markers already mapped on the hexaploid wheat genome, one per chromosome arm, were selected. Custom-synthesized primers were used in the PCR amplification. The reaction mixture contained 10 ng of template DNA, 20 ng of each primer, 250  $\mu$ m of each dNTP, 1x PCR assay buffer, and 0.2 unit of *Taq* DNA polymerase. Amplification was in a thermal cycler (Perkin-Elmer model 9600) with the following specifications: 94°C for 5 min, 35 cycles at 94°C for 1 min, 55°C for 1 min, 72°C for 2 min, and at 72°C for 7 min. The amplified products were separated on a 3 % metaphor agarose gel (FMC, USA). The samples were electrophoresed using 1x TBE buffer, stained using gelstar dye, and photographed using polaroid photographic system. The bands were scored and used to make a binary data matrix. Jaccard's similarity coefficient was calculated and used to establish genetic relationship based on clustering. The computer package NTSYS.PC was used for cluster analysis.

**Results and Discussion.** Breeding work on changing plant architecture was initiated in 1994 at the Indian Agricultural Research Institute, New Delhi, with the hybridization of the released varieties Vaishali and Vidisha with a local wheat, SFW. In this endeavor, several strains were successfully designed of which DL 1266-1, DL 1266-2, DL 1266-5, DL 1266-6, DL 1267-2, DL 1267-3, and DL 1267-4 were selected to represent the NPT of wheat (Table 1). These NPT genotypes possess a high 1,000-kernel weight, a higher number of grains/spike, a higher biomass, dark green, broad leaves, thick stems, a plant height between 85–100 cm, and good root system. Thus, the negative correlation between yield components has been broken,

**Table 1.** Performance range of the new plant type wheat lines for yield and yield-contributing factors in comparison to best checks during 1998–99 and 1999–2000.

Cross	Biological yield (g/m <sup>2</sup> )	Grain yield (g/m <sup>2</sup> )	Tillers/m <sup>2</sup>	Grains/spike	1,000-kernel weight (g)
SFW/Vaishali and SFW/Vidisha	1,883–2,160	667–707	274–541	41–56	45–52
Checks PBW 343 HD 2329, UP 2338	1,920–2,036	553–625	446–514	36–42	36–39

leading to a positive correlation between grain weight and grain number/spike with optimum productive tillering capacity. In these genotypes, the physiological efficiency of partitioning of dry matter to economic yield has increased. Better synchronization (post-anthesis) of assimilate source, path, and the grain sink, resulting in the increased availability of assimilate for proper development and grainfilling may lead to high grain weight because of proper filling of all grains in all spikelets resulting in higher number of grains/spike.

Success in achieving high productivity on a sustained basis will depend upon our ability to develop new methods of feeding the plants. Research on breeding and feeding should be made concurrently by a team of breeders, physiologists, agronomists, and soil scientists. At the same time, wheat scientists, including breeders, physiologists, agronomists, and biotechnologists are working together to harness the maximum yield potential of these NPT wheats (Swaminathan 2000).

*Exploitable yield potential.* In order to know the exploitable yield potential of these NPT wheats, DL 1266-5, a newly designed wheat genotype (pedigree: SFW/Vaishali) was planted (6 rows/5 beds of 5.5 m) adjacent to an irrigation channel 2.5-m width at commercial seeding rate of 100 kg/hectare. Fertilizer was applied at 100 N:60 P:40 K and five irrigations were provided to these plots.

We observed that plants in the border row towards the irrigation channel of five beds (each 5.5 m) expressed the full potential of the NPT compared to inner rows where there was interplant competition. Table 2 indicates a highly significant increase in all yield components in the border rows when compared to the inner rows. The mean yield of one row is 1.656 kg (90.66 q/ha), which is an overall increase of 218.5 % of border rows over the inner rows. These findings strongly suggest that the NPT wheat DL 1266-5 has a very high yield potential, if grown with suitable technology such as FIRB, which utilizes and exploits the border effect. The exploitation of very high yield potential of NPT wheats will not only save the land but also reduce the cost of cultivation, which is presently very high.

**Table 2.** Performance of border rows and inner rows for yield and yield components of DL 1266-5, a wheat line from new plant type. Row items are B = border row; I = inner row.

Trait	Row	Bed 1 (R1)	Bed 2 (R2)	Bed 3 (R3)	Bed 4 (R4)	Bed 5 (R5)	Total	Mean	Advantage to border row (%)
Mean no. of grains/spike	B	92.0	93.2	91.2	92.8	90.8	460.0	92.0	69.4
	I	54.8	55.0	53.8	54.2	53.6	271.4	54.3	
Mean spike weight (g)	B	5.12	5.16	5.13	5.14	5.09	25.64	5.13	120.2
	I	2.34	2.40	2.30	2.37	2.24	11.65	2.33	
1,000-kernel weight (g)	B	55.6	55.3	56.3	55.4	56.1	278.7	55.7	29.8
	I	42.7	43.6	42.8	43.7	41.8	214.6	42.9	
Yield of 1 row of 5.5 m (kg)	B	1.670	1.830	1.550	1.720	1.510	8.280	1.656	218.5
	I	0.550	0.550	0.560	0.540	0.430	2.600	0.520	
Estimated number of tillers in a 5.5-m row	B	326	355	302	335	297	1,616	323	44.8
	I	222	229	243	228	192	1,114	223	

Further efforts will be needed to increase the yield potential of the NPT wheats along with incorporation of diverse genes for resistance to rusts for durable resistance. These genotypes have been crossed with indigenous and exotic germ plasm with the objective to increase the number of productive tillers/plant while keeping 1,000-kernel weight and grain number/spike constant. A large number of segregating and fixed materials were generated, which are in testing at various levels. Advanced material is in testing at different levels of inputs and FIRB in collaboration with wheat agronomists at our institute. These lines also are being analyzed for quality parameters.

*Durable resistance to rusts.* From the first generation, we have proceeded with the objective that *Lr24* will provide high level of resistance not only in India, but also in whole Indian subcontinent including Pakistan, Bangladesh, Nepal, and Bhutan. After 1998, resistance to all the three rust pathogens was initiated with the aim to develop derivatives of NPT lines with enhanced resistance leading to durable resistance along with enhanced yield by increasing tillering capacity without affecting grain weight and number of grains/spike. A large number of cultivars/exotic and indigenous germ plasms (including PBW 343, PBW 373, UP 2338, UP 2425, HD 2687, HD 2733, NI 1202, HS 295, HS 365, HW series, WH 542, HD2009, Oasis, Milan, Pios, Opata, and Kauz) having genes for durable resistance to three rusts (*Lr34*, along with *Lr13*, *Lr26*, *Lr23*, *Lr10*; *Sr2*, *Sr5*, *Sr31*; and *Yr9* and *Yr18*) were involved in generating NPT wheats with further increases in yield potential and durable resistance.

*Physiological and biochemical studies of the NPT genotypes DL 1266-2 and DL 1266-5.* Biomass tends to increase with increasing nitrogen levels, i.e., 100, 150, and 200 kg N/hectare in the standard check cultivar PBW 343, whereas in DL 1266-2 and DL 1266-5, the maximum biomass was achieved at 150 kg N/hectare. Thus, the new lines are more efficient N utilizers at lower N fertility levels, although the grain yield/m<sup>2</sup> was not significantly higher at different nitrogen levels and between the three genotypes (Table 3). The over all harvest index was superior in DL 1266-2 and DL 1266-5 as compared to PBW 343 check, particularly at low fertility levels. We concluded from the harvest index data that the NPT wheats are more efficient mobilizers of the assimilates to the developing grain sink. The number of productive tillers/m<sup>2</sup> is always lower in the NPT genotypes as is evident from number of spikes/m<sup>2</sup>. This means that DL 1266-2 and DL 1266-5 also are nutrient efficient, as they produce limited number of synchronous tillers with thick stems and compact, long spikes. The superior harvest index also supports this fact and indicates a superior assimilate reserve accumulation and its mobilization to the developing grain sink so that a very high number and improved grain weight result in significantly higher grain weight/spike are sustained.

Such improved ideotypes predicted earlier also are expected to be nutrient-efficient based on a comparative study of pre and post-green revolution plant types (Pande et al. 1983). Analysis of grain weight/spike confirms the superiority of NPT genotypes over the check at all the three fertility levels. We may conclude that NPT lines have evolved a superior spike architecture that ensures improved transportation of assimilates to the grain. This proposal becomes obvious as remarkable increases in grain weight/spike, even at low fertility levels, is achieved in these NPT genotypes. Thus, the need to develop new

**Table 3.** Influence of different nitrogen fertility levels on yield components of DL 1266-5. A C indicates the check cultivar.

Character	Genotype	Nitrogen levels (kg per hectare N)			
		100	150	200	Mean
Biomass/m <sup>2</sup> (g)	DL 1266-2	1,280	1,585	1,566	1,477
	DL 1266-5	1,541	1,741	1,632	1,638
	PBW 343 (C)	1,491	1,665	1,784	1,647
Grain yield/m <sup>2</sup> (g)	DL 1266-2	589	691	653	644
	DL 1266-5	681	790	729	733
	PBW 343 (C)	650	759	700	703
Number of spikes/m <sup>2</sup>	DL 1266-2	261	285	310	285
	DL 1266-5	308	335	331	325
	PBW 343 (C)	456	462	496	471
Grain weight/spike (g)	DL 1266-2	4.66	4.92	4.80	4.79
	DL 1266-5	5.70	5.79	5.32	5.60
	PBW 343 (C)	2.69	2.71	2.86	2.75
Number of grains/spike (g)	DL 1266-2	76	85	89	83.33
	DL 1266-5	100	100	98	99.33
	PBW 343 (C)	58	60	66	61.33
1,000-kernel weight (g)	DL 1266-2	58.99	57.73	54.39	57.00
	DL 1266-5	56.77	57.84	54.06	56.22
	PBW 343 (C)	46.81	45.75	43.26	45.27
Protein %	DL 1266-2	12.06	13.10	14.41	13.19
	DL 1266-5	12.17	13.01	13.70	12.96
	PBW 343 (C)	11.27	12.06	11.76	11.70

and more efficient and improved plant type to meet the future demands particularly under low fertility conditions is met. As for the analysis of grain-yield components, the grain number/spike, grain weight/spike, and 1,000-kernel weight show remarkable superiority even at lower fertility levels in these NPT lines compared to the check cultivar.

Grain protein is an important quality attribute in wheat that also determines its export potential. In general, when compared to the check, the grain protein in the NPT lines was more than 1 % at 100 and 150 kg N/ha, more than 2 % at 200 kg N/ha. DL 1266-5 had the highest grain protein/m<sup>2</sup> followed by check, irrespective of fertility levels. These results indicate that DL 1266-5 was the most efficient nitrogen utilizer irrespective of nitrogen fertility levels and harvested maximum grain protein/m<sup>2</sup>. Shera (a post-green revolution variety), which also produced synchronous and limited number of tillers, also was efficient utilizer of applied nitrogen (Pande et al. 1983; Abrol and Nair 1976; Abrol et al. 1976), however it did not have the earliness, thick stem, or heavy and compact spikes that the NPT genotype DL 1266-5 possesses. Among the NPTs from the mid 1980s, Shera was considered the most efficient utilizer of applied N and showed maximum nitrate-reductase activity at different nitrogen fertility levels (Nair and Abrol 1977). The newly designed plant type, although much superior to existing cultivars, possesses tremendous scope for further improvement in both quantitative and qualitative yields.

*Finger printing for protection of NPT genotypes.* The markers amplified a maximum of two alleles. Of the 33 markers used, 20 detected polymorphism among the genotypes used in the study. The polymorphic markers in combination precisely identified each genotype. The lines derived from the cross 'SFW/Vaishali' were closer genetically to the parents than the other lines and the varieties used in the analysis. Maximum similarity was observed between sister lines DL 1266-2 and DL 1266-10. The lines DL 1396-11 was most divergent from others. The line DL 1266-1, DL 1266-2, DL 1266-5, DL 1266-10, DL 1266-16, and DL 1266-17 were more close to the parent SFW, whereas DL 1266-6 was closer to the Vaishali parent.

STMS-based DNA fingerprints of the NPT wheat lines can be used for protection of these lines from any unauthorized use. STMS markers already were mapped and the polymorphism was in the nonoverlapping genomic regions. Moreover, STMS is PCR based and highly reproducible between laboratories. Therefore, the STMS-based fingerprints developed for the wheat lines can be used with high degree of confidence.

### Conclusion.

The NPT lines with increased yield potential and better grain quality are highly suitable for export purposes and will put India on the export map of the world in addition to eliminating the problem of malnutrition in poorer section of society.

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## ITEMS FROM ITALY

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***Reactions of cultivars of durum wheat to WSBMV during 2000–01.***

C. Rubies-Autonell, C. Ratti, and V. Vallega.

Wheat soilborne mosaic virus is widespread in the northern and central regions of Italy, causing severe economic losses on both common and durum wheat crops. Yield losses of up to 50 % and 70 % have been recorded on susceptible cultivars of common wheat and durum wheat, respectively. WSBMV also has been detected recently in a number of farms in southern Italy and in Sicily. During the 2000–01 season, 31 durum wheat cultivars were assayed in a severely WSBMV-infested field situated near Minerbio (Bologna) to study their reaction to this virus. The cultivars were grown in 10-m<sup>2</sup> plots distributed in the field according to a randomized block design with three replicates. As in previous years, resistance to WSBMV was evaluated on the basis of DAS-ELISA readings, symptom severity (on a 0–4 scale), and agronomic performance. Symptom severity (mean score = 1.1; range = 0.1–3.0) and ELISA values (mean = 0.585; range 0.0–1.159) were moderately high. Foliar samples from cultivars Neodur and Colorado had null ELISA values, remained symptomless throughout the season, and produced relatively high grain yields. Cultivars Lloyd and Nefer showed moderately high levels of resistance, confirming their good performance in previous trials, and the same was observed for cultivars Giotto, Meridiano, and Vitron, tested for the first time. The cultivar Provenzal, classified as susceptible in a previous trial, showed a high degree of resistance during the 2000–01 season. All the other cultivars assayed proved at least moderately susceptible to WSBMV. Simple correlation coefficients between agronomic data, ELISA values, and symptom scores were relatively high and mostly statistically significant (Table 1). Regression analysis indicated that the five cultivars representing the highest disease scores (i.e., Vesuvio, Simeto, Cirillo, Claudio, and Portorico) suffered grain losses attributable to WSBMV of about 50 % and a mean plant height reduction of about 10 %.

**Table 1.** Simple correlation coefficients between disease ratings, ELISA values, and various plant characters for 31 cultivars of durum wheat grown in the 2000–01 season in a field infested by wheat soilborne mosaic virus. ELISA values with \* are significant at P = 0.05; \*\* are significant at P = 0.01.

	Disease severity	ELISA values
Grain yield	– 0.621**	– 0.570**
Test weight	– 0.315	– 0.371*
Plant height	– 0.645**	– 0.404*
1,000-kernel weight	– 0.288	– 0.079
ELISA values	0.799**	—

***Reactions of cultivars of common wheat to WSBMV during 2000–01.***

V. Vallega, C. Ratti, and C. Rubies-Autonell.

Thirty-five cultivars of common wheat were grown in a severely WSBMV-infested field near Minerbio (Bologna) during the 2000–01 season. Entries were grown in 10-m<sup>2</sup> plots distributed in the field according to a randomized block design with three replicates. Resistance to WSBMV was evaluated only on the basis of DAS-ELISA readings and symptom severity (on a 0–4 scale). Agronomic data also were collected, but could not be used with sufficient confidence as a resistance parameter because various cultivars were very severely damaged by yellow rust. Symptom severity (mean score = 0.5; range = 0.0–1.7) and ELISA values (mean = 0.656) were relatively low, especially if compared with those observed on the 33 durum wheats grown in an adjacent field. Eight of the common wheat cultivars analyzed, Belfiore, Colfiorito, Enesco, Etecho, Genio, Pandas, Tremie, and Victo, had null ELISA values and remained nearly



asymptomatic throughout the entire season. Belfiore, Etecho, and Victo had not been assayed before, whereas the other five cultivars also had been classed as highly resistant in previous trials. The cultivars Cranklin, Faro, Guadalupe, Marvao, Positano, Ravenna, and Tibet, tested in 2000–01 for the first time, proved susceptible to WSBMV. As might be expected because of the effects of the concomitant yellow rust epidemic, the agronomic data collected were not significantly correlated with either ELISA values or symptom scores (Table 2). Therefore, the damage caused by WSBMV could not be estimated.

**Table 2.** Simple correlation coefficients between disease ratings, ELISA values, and various plant characters for 35 cultivars of common wheat grown in the 2000–01 season in a field infested by wheat soilborne mosaic virus. ELISA values with \* are significant at  $P = 0.05$ ; \*\* are significant at  $P = 0.01$ .

	Disease severity	ELISA values
Grain yield	– 0.246	– 0.304
Test weight	– 0.005	– 0.058
Plant height	– 0.352	– 0.225
1,000-kernel weight	– 0.050	– 0.204
ELISA values	0.834**	—

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## EXPERIMENTAL INSTITUTE FOR CEREAL RESEARCH via Mulino, 3 – 26866 S. Angelo Lodigiano (LO), Italy.

### *Bread wheat genotypes for sustainable cropping systems.*

M. Perenzin, M. Corbellini, and G. Boggini.

In the present economic and political context where farmers are forced to reduce inputs, bread wheat cultivars able to tolerate a moderate reduction in inputs are needed. The old Italian varieties are not useful for this purpose, mainly because of their low-yielding ability and very poor bread-making quality. Consequently, attention is on the selection of genotypes that can sustain high productivity at reduced rates of chemical applications (fertilizer, herbicides, and pesticides). Field trials in an organic-cropping system allowed the identification of modern genotypes that can provide a satisfactory yield without a high reduction in bread-making quality.

### *Allelic variability at the waxy loci in Italian wheat germ plasm.*

G. Boggini, M. Cattaneo, S. Empilli, and P. Vaccino.

Waxy wheats, characterized by a reduction or an absence of amylose in the starch, may find a use in the production of modified food starch, and their flour may be used to extend the shelf life of baked products. The primary enzyme responsible for the synthesis of amylose in amyloplast, granule-bound starch synthase (GBSS), is present in bread wheat in three isoforms encoded by the structural genes *Wx-A1*, *Wx-B1*, and *Wx-D1*. We analyzed 288 cultivars of bread wheat,

139 cultivars of durum wheat, and about 200 accessions from other *Triticum* species in order to find genetic variability for the waxy trait. Electrophoretic separation of GBSS allowed the identification of 63 bread wheats deficient in the *Wx-B1*, one in the *Wx-A1*, and one in the *Wx-D1* protein isoforms, and a *T. turgidum* subsp. *dicoccum* lacking the *Wx-A1* isoform. The wheat accession with *Wx* mutations were evaluated with a Rapid Visco Analyser to investigate starch properties. All the analyzed cultivars showed peak viscosity and final viscosity different from that of normal wheat. Other analyses to evaluate the amylose-amylopectin ratio and the rheological characteristics of the partial-waxy genotypes are under way. A crossing program to select double and null waxy wheat mutants is in progress.

### ***Puroindoline and kernel hardness in Triticum aestivum and Triticum monococcum.***

N.E. Pogna, L. Gazza, G. Boggini, and M. Corbellini.

Puroindolines a (pin A) and b (pin B), two lipid-binding proteins affecting grain texture, were investigated in diploid and hexaploid wheat species by A-PAGE fractionating and PCR amplification. A-PAGE provided a clear separation of pin A and pin B, which occurred as two or four major bands in *T. aestivum* and *T. monococcum*, respectively. By A-PAGE analyses, four different puroindoline patterns were identified among the 67 diploid wheat accessions, all of them exhibiting a very soft grain texture as determined by the single-kernel characterization system. Among the 66 bread wheat cultivars analyzed, four A-PAGE patterns, two alleles coding for pin A and four alleles coding for pin B, were identified. Grain softness proved to be associated with the presence of alleles *Pina-D1a* and *Pinb-D1a* coding for wild-type pin A and pin B, respectively. On the other hand, medium to hard grain texture was associated with either the absence of pin A (allele *Pina-D1b*) or the occurrence of a single amino acid substitution in wild type pin B (alleles *Pinb-D1b* and *Pinb-D1d*). Bread wheat cultivars with the same puroindoline composition showed a remarkable variation in grain hardness, suggesting that factors other than pin A and pin B may effect grain texture.

### ***Hybrid wheat development.***

M. Perenzin, M. Corbellini, and M. Cattaneo.

The agronomic and quality performance of the best F<sub>1</sub> hybrids produced in Italy in the last years clearly indicate that although a positive trend was observed in yield potential, the level of standard heterosis did not increase because high-yielding cultivars are continuously produced by conventional breeding. Thus, there is concern about the need to develop specific strategies for identifying parental lines characterized by high general-combining ability and specific-combining ability. Among 200 hybrids previously evaluated, one hybrid (AxB) with the highest SCA effects for grain yield was identified. In order to substitute the two parents used with two superior lines, ten cross combinations involving five cultivars with high specific-combining ability effects for parent A and five for parent B were identified. From these ten crosses, a total of 142 lines were selected, crossed with A and B according to a topcross design and fingerprinted with AFLPs. The hybrids obtained were evaluated in replicated plot trials in two locations. Ten lines were identified which, in turn, were crossed according to a diallel mating design. Work is in progress to evaluate the agronomic and quality of the hybrids obtained compared to the best commercial varieties and exploit the use of genetic distances based on molecular markers for the selection of superior parental lines.

### ***Breeding for resistance to powdery mildew.***

A. Brandolini, M. Corbellini, and M. Perenzin.

The breeding program is based on backcrossing and MAS selection and aimed at the introgression of powdery mildew-resistance gene *Pm13* in bread wheat cultivars is currently at BC<sub>5</sub> stage. Evaluation of lines similar to the recurrent parents also is under way.

**Genetic analysis of einkorn wheat quality traits.**

A. Brandolini, P. Vaccino, S. Empilli, and M. Corbellini.

An einkorn wheat consensus map, obtained in collaboration with the Max-Planck Institut of Cologne, is completed and in press. The map was used to localize QTLs for quality and agronomic traits. A major QTL for bread-making quality was detected on chromosome arm 1S, and the gene responsible for free threshing was positioned on 2S, in a position compatible with *Tg* genes of polyploid wheats. Progenies of two-, three- and four-way crosses of lines with good agronomic and quality traits (earliness, free-threshing, short straw, gluten quality, and large kernel) are in evaluation.

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**ITEMS FROM JAPAN**

**GIFU UNIVERSITY – FACULTY OF AGRICULTURE**  
**1-1 Yanagido, Gifu 501-1193, Japan.**

Nobuyoshi Watanabe.

**Near-isogenic lines of tetraploid and hexaploid wheat for brittle rachis.**

Brittle rachis, which causes spontaneous shattering of kernels, is an adaptive character of wild species. Major genes located on chromosomes 3A, 3B, and 3D determine brittle rachis. The brittle-rachis phenotype is nearly lost in domesticated wheats. Near-isogenic lines for brittle rachis have been developed for considering domestication of wheat plants. For tetraploid wheat, the brittle-rachis genes were introduced from Langdon-*T. turgidum* subsp. *dicoccoides* chromosome substitution lines, LDN(DIC 3A) and LDN(DIC 3B). The BC<sub>6</sub> plants are growing. For hexaploid wheat, the

brittle-rachis genes were derived from the Chinese endemic species, *T. tibetana* and *T. yunnanense*, where the brittle-rachis trait is determined by a gene on chromosome 3D. These genes were incorporated into the cultivar Novosibirskaya 67. The BC<sub>4</sub>-BC<sub>6</sub> plants are growing. They will become available soon for the experiments.

The spelt gene *q* is known as an alternative to the brittle-rachis gene. Near-isogenic lines for spelt also have been developed. The sources for *q* were *T. turgidum* subsps. *dicoccoides*, *T. dicoccum*, *T. paleocolchicum*, European *T. spelta*, Iranian *T. spelta*, *T. macha*, and speltoid mutants. The BC<sub>2</sub> plants are growing. These NILs will be utilized to assess the effects of the gene for domestication of wheat.

**NATIONAL AGRICULTURAL RESEARCH CENTER FOR TOHOKU REGION  
Fukushima campus, Arai, Fukushima 960-2156, Japan.**

***Quality evaluation of common wheat endosperm protein fingerprints as indices of Japanese white-salted noodle (udon) eating quality.***

Hiro Nakamura.

**Summary.** Protein fingerprints of common wheat endosperm were used to determine the indices of Japanese soft Udon-noodle quality. The endosperm proteins of Japanese udon wheat lines were fractionated by SDS-PAGE to determine protein-composition differences in two soil environments. The differences between the lines included differences in the composition of the endosperm with regard to a 53-kD protein band or HMW-glutenin subunit 2\* and in the sensory viscoelasticity score of cooked noodles, which is related to the eating quality in Japanese udon wheats. Kanto 107 showed variation for the presence of the 53 kD and HMW-glutenin subunit 2\* bands between the soil environments.

Nitrogen fertilization is a main factor affecting wheat protein and quality (Jia et al. 1996; Toyokawa et al. 1989a). Many studies have shown that the increased or decreased flour protein contents can lead to changes in wheat endosperm protein components (Scheromm et al. 1992; Zhu et al. 1999). Therefore, it would be of interest to explore the relationship between endosperm protein and udon noodle-making quality in this study. The flour protein content of wheat is usually 9–16 % of the dry weight, and world production of wheat grain protein is vast. In addition to being of great importance nutritionally, grain protein plays a fundamental part in food processing, for instance, in the manufacture of bread, biscuits, breakfast cereals, pasta, and Japanese udon-noodle products. However, most studies are applicable to the quality of wheat flour for bread, cakes, and cookies rather than for udon noodles. Improvement of end-use quality in wheat depends on a thorough understanding of the influences of environment, genotype, and their interaction (Peterson et al. 1992).

Endosperm protein composition is determined mainly by the genotype (Payne et al. 1987). However, it is largely accepted that nitrogen nutrition affects the wheat protein content and composition and directly influences the technological quality of wheat samples (Scheromm et al. 1992). Several recent reports suggest that wheat nitrogen content conditions could quantitatively affect wheat endosperm protein components (Jia et al. 1996; Zhu et al. 1999). The quantitative and qualitative differences in endosperm-protein composition observed upon exposure of the same genotype to different field conditions and wheat flour nitrogen contents under similar climatic conditions have not been previously described. Udon-making quality from paddy field is not much better than that of udon wheat grown in an upland field due to a lower protein content of wheat grains in Japan (Yamashita 1994). Recently, this has been an important problem in udon wheat production in Japan. Due to paddy-rice overproduction in Japan, udon wheat has been grown in paddy fields instead of cultivating paddy rice. Therefore, improving the quality of udon wheat from paddy fields has been a most important research component of wheat breeding in Japan.

Statistically significant correlations were found in the present study for the Japanese udon wheat 53-kD protein band, HMW-glutenin subunit 2\*, flour-protein content, sensory viscoelasticity score of cooked soft salted-noodles, flour-amylose content, and different field types. Significant differences in the band pattern of endosperm proteins from various genotypes between upland and paddy fields were observed in this study. All the differences observed in this research were demonstrated by simple 1-dimensional SDS-PAGE. Therefore, most protein effects (quantitative and

qualitative) presented on the gel are based on differences in the HMW-glutenin subunit 2\* and 53-kD protein band (LMW-glutenin or gliadin).

In the present study, the nitrogen deficiency of wheat in the paddy fields for the first two wheat-growing seasons may have prevented the synthesis in only Kanto 107 of the 53-kD protein band and instead produced the HMW-glutenin subunit 2\* protein band and may explain the low-protein content in the first and second growing seasons, when there was an absence of the 53-kD protein band and an accompanying presence of HMW-glutenin subunit 2\* for Kanto 107. However, further work using a greater number of pure varieties or lines with different genetic backgrounds is needed to confirm these results. Climatic conditions between seasons could not explain the observed differences in protein composition, because all plants of each genotype were exposed to the same amount of water and the same temperature during the wheat-growing season in the same field, and the 53-kD protein band and HMW-glutenin subunit 2\* of Kankei lines or Norin 61 were stable. Although the nitrogen availability caused variations in endosperm protein components by affecting the nitrogen nutrient supply, genotype was a more important factor in determining the final protein composition. The quantitative and qualitative differences in endosperm protein composition were observed under field conditions of wheat nitrogen deficiency. This work also demonstrated that under nitrogen-deficient field conditions, the use of endosperm-protein fingerprints from naturally growing wheat plants can be misleading as a breeding tool. Cereals grown in nitrogen-deficient soils that are different from those in which they were bred could produce grains with different amounts of each gluten component (Scherommet al. 1992; Jia et al. 1996; Zhu et al. 1999), which would have implications if the affected protein is a determinant of flour quality. Caution should be exercised when identifying the field source of seeds used for wheat breeding, especially from genetic resources with high-protein content. However, the most important texture of udon-noodle acceptability is the sensory viscoelasticity score related to eating-quality of cooked noodles (National Food Research Institute 1984; Nakamura 2001).

From these studies, we concluded that the primary protein 53-kD band of endosperm protein appeared to be most responsible for the desirable viscoelastic texture of cooked udon-noodle. In contrast, the primary protein HMW-glutenin subunit 2\* of endosperm protein appeared to be most responsible for reduced viscoelastic texture of cooked udon-noodle. Kanto 107 and the four Kankei lines possessed the 53-kD protein band related to good sensory viscoelasticity score and eating quality of cooked noodles. These two protein bands of Kanto 107 were sensitive to different protein contents between different field conditions, but this sensitivity was not evident in Norin 61 and the Kankei lines. No relatively variability in protein components such as the 53-kD protein band and HMW-glutenin subunit 2\* were observed, and their sensory viscoelasticity scores of cooked noodle were likewise stable.

Therefore, it is of interest that Norin 61 do not have the 53-kD protein band that is correlated to high sensory viscoelasticity in cooked udon noodles. Jia et al. (1996) reported that accumulation of gliadins becomes more important than that of glutenin, and albumins and globulins are less represented as the flour protein increases. The protein content may not only affect the quantitative, but also qualitative, characters.

In wheat breeding programs aimed at changing the endosperm-protein band pattern, the fingerprint has been considered a reliable wheat breeding tool. The information may also be of interest to plant breeders, because breeders are now taking endosperm-protein composition into account when choosing patterns for intended crosses to produce good udon wheat lines. The apparent importance of amylose and the influence of amylose/amylopectin ratios on udon quality has been studied (Takada 1987; Toyokawa et al. 1989). We know that a low amylose content (a high amylopectin content) is correlated with good udon-noodle viscoelasticity scores of 21.0–25.0 (full score) and is correlated with high udon-noodle making quality in Japan. Starches of varied amylose/amylopectin ratios have been selected and incorporated into Japanese udon-wheat breeding programs. In our study, flour amylose content influenced the sensory viscoelasticity score of cooled noodles; however, the two primary endosperm protein bands had a greater effect on the sensory viscoelasticity score of cooked noodles. In the present study, amylose content does not seem to play the most major role in increasingly the sensory viscoelasticity score of cooled noodles. However, it is probable that the 53-kD protein band and HMW-glutenin subunit 2\* strongly affect to the sensory viscoelasticity scores of udon noodles.

Nagao et al. (1977) reported that selected Australian wheats and American soft white and white club wheats showed favorable characteristics for Japanese udon-type noodles and also that Australian standard white is superior to Japanese wheat in udon-making quality (Takada 1987). This approach to the selection of early-generation udon wheat lines in the Japanese udon-wheat breeding program is very effective, particularly because the procedure is simple, and experimental equipment can be obtained at reasonable price. Good udon wheat lines, such as Kanto 107, may give poor udon-noodle making quality when used in nutrient-deficient conditions.



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**Acknowledgments.**

The author wishes to thank Mrs. Shinko Kawakami for her assistance with this research work. Thanks are due to National Agriculture Research Center for providing wheat samples.

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**Publications.**

- Nakamura H. 2001. Evaluation of wheat endosperm protein fingerprints as indices of Udon-noodle making quality. *Aus J Agric Res* **52**:919-923.

**ITEMS FROM MEXICO**

**INTERNATIONAL MAIZE AND WHEAT IMPROVEMENT CENTER — CIMMYT  
Lisboa 27, Colonia Juárez, Apdo. Postal 6-641, 06600, D.F., México.**

***Free threshing bread wheat/synthetic hexaploid derivatives resistant to *Fusarium graminearum*.***

A. Mujeeb-Kazi and R. Delgado.

Synthetic hexaploid wheats that are the products of crosses between *T. turgidum* and *Ae. tauschii* are the basic germ plasm sources for screening of entries with resistance/tolerance to biotic and abiotic stresses. Once desired germ plasm lines are identified, the next step is introgressing the trait into elite but stress-susceptible bread wheat cultivars. Head scab in bread wheat is one stress for which genetic diversity is limited and is a disease of major concern in the high-rainfall mega-environments. We have identified several synthetic hexaploids that express good, type-II resistance and

many of these were crossed into leading bread wheat cultivars several crop cycles ago. The resulting progenies of these crosses were advanced by the pedigree method, and selections were advanced if they possessed a level of scab resistance similar to or better than Sumai 3 and also had resistance to leaf and stripe rusts. In addition, all lines had stem rust resistance, with resistance to *S. tritici*, Karnal bunt, and *H. sativum* considered a bonus.

Scab screening was done in Toluca, Mexico, and the germ plasm was tested for several years. Selections were directed towards free-threshing, agronomically suitable plant types in order to facilitate further breeding options. Selections focused on scab type-II (spread) resistance data plus other complimentary traits. The yearly crop harvest in October in Toluca was taken to Obregon for a November planting and May harvest, after which the advanced generation materials were retested in Toluca during the June–October crop cycle. This shuttle breeding, adopted from our main wheat-breeding program protocol, allowed for desirable outputs as to quality disease-resistant plants that were photoperiod insensitive. We currently maintain 143 elite lines for scab resistance and several from later crosses that are in the  $F_4$  generation. The involvement of various *Ae. tauschii* accessions in the pedigrees of the derivatives indicate a wide array of genetic diversity that should be beneficial for imparting durability of scab resistance to bread wheat germ plasm.

Some of the best of the 143 lines over multiyear testing are included in Table 1 (p. 95) and the most promising, less-advanced  $F_4$  materials are listed in Table 2 (p. 96). The inoculation and scoring protocols were standard and are described in detail by Mujeeb-Kazi and Delgado on page 97 of this issue.

### ***D-genome contribution to Fusarium type-II resistance in synthetic-hexaploid wheats (SH; $2n = 6x = 42, AABBDD$ ).***

A. Mujeeb-Kazi and R. Delgado.

Our wide crosses program has given FHB (type II, spread) screening in Toluca, Mexico, a high priority since 1998. Two germ plasm categories have been candidates for evaluation that are broadly classified under interspecific and intergeneric hybridization areas. Within these two categories, the initial emphasis was placed on interspecific hybrids with the focus on contributions of *Ae. tauschii* accessions towards wheat improvement. The diversity in this species was exploited by hybridizing the diploid grass with elite durum wheat cultivars, which yielded SH wheats. Over 800 such synthetics have been produced so far and comprise the germ plasm for various stress-constraint screening evaluations.

Evaluation for scab resistance involves several categories or types. Our wide-crosses program conducts type-II (spread) tests. Promising entries are identified and transferred to the main pathology program for retesting of type-II infection data and also for extending the tests for other scab categories (types I, III, and IV). During these independent tests, we also monitor the type-II data generated, and when matches are observed, data is consolidated and reported (Table 3, p. 97).

From the several SH wheats produced and tested, the first-year evaluation allowed us to select 36 entries with high resistance levels for type-II resistance based upon their similarity to Sumai 3 or their superiority to this resistant check cultivar. These 36 SH wheats were then evaluated between 1998 and 2001. Of these lines, entries with consistently better data than Sumai 3 for type-II resistance are presented in Table 3 (p. 97). A very high level of stringency testing was maintained over each cropping cycle in Toluca, Mexico, in that 10 fully extruded spikes from each SH entry were inoculated when the anthers became visible in the central spike region. Cotton swabs soaked in the inoculum were placed within the lemma and palea of the central floret of each spike under field conditions, covered with a glassine bag, and evaluated after 35 days for symptom development. The spore concentration was about 50,000. The data in Table 3 reports the mean percentage of infected florets for each SH entry across years. Susceptible and resistant check cultivar spikes also were inoculated daily. Emphasis was given to each individual spike within the 10 of each test entry. If a single spike within the 10 tested for an entry exceeded the 15 % type infection level (considered as the resistant score from the performance of Sumai 3 over several years of data) the entry was classified as susceptible. Additional stringency was provided by using the same three personnel to inoculate the entire wide-cross material tested. The SH subset reported here was handled by the same person each year (R. Delgado).

Each of the 15 SH wheats identified as resistant have a unique *Ae. tauschii* accession in the pedigree. The SH wheats are generally later flowering than elite bread wheats, later in maturity, taller, and exhibit a spring to mild facultative habit. The 1,000-kernel weight in a majority exceeds that of bread wheat (approximately 40 g) with a range from

**Table 1.** Fusarium head scab data (type II – spread) of some superior advanced bread wheat/synthetic hexaploid derivatives from multiyear evaluations in Toluca, Mexico. *Ae. tauschii* accession number in wheat wide crosses working collection are in parentheses. Bread wheat cultivars in pedigrees are indicated by the superscript <sup>BW</sup> the first time they occur in one of the pedigrees.

2001 Entry No.	Pedigree	Infection Score (%)			
		1998	1999	2000	2001
1	Mayoor (resistant bread wheat)	11.3	12.9	8.5	13.5
4	Flycatcher (susceptible bread wheat)	30.7	40.5	28.5	31.3
10	Mayoor//TKSN1081/ <i>Ae. tauschii</i> (222)	8.2	11.9	6.2	9.5
14	Mayoor//TKSN1081/ <i>Ae. tauschii</i> (222)	11.0	13.5	10.3	10.0
19	Mayoor//TKSN1081/ <i>Ae. tauschii</i> (222)	12.6	7.0	7.9	7.6
23	Mayoor//TKSN1081/ <i>Ae. tauschii</i> (222)	12.6	11.9	12.0	9.8
26	Mayoor//TKSN1081/ <i>Ae. tauschii</i> (222)	13.1	9.3	9.6	12.6
37	Mayoor//TKSN1081/ <i>Ae. tauschii</i> (222)	10.2	7.7	9.6	13.6
50	Mayoor//TKSN1081/ <i>Ae. tauschii</i> (222)	11.8	9.6	12.7	8.7
53	Mayoor//TKSN1081/ <i>Ae. tauschii</i> (222)	9.2	12.0	11.2	13.4
65	BCN <sup>BW</sup> //Ceta/ <i>Ae. tauschii</i> (954)	13.0	14.8	6.3	9.1
67	BCN//Croc1/ <i>Ae. tauschii</i> (662)	12.4	12.0	11.4	10.0
68	BCN//Doy1/ <i>Ae. tauschii</i> (447)	12.0	12.0	11.0	8.9
69	BCN//Doy1/ <i>Ae. tauschii</i> (447)	13.0	11.4	5.8	7.3
72	Altar 84/ <i>Ae. tauschii</i> (224)//2*Yaco <sup>BW</sup>	10.2	10.5	8.8	8.9
78	Croc1/ <i>Ae. tauschii</i> (224)//2*Oyata <sup>BW</sup>	11.2	11.9	11.4	10.8
79	Oyata/6/68.111/RGB-u//Ward/3/FGO/4/Rabi/5/ <i>Ae. tauschii</i> (878)	9.2	8.7	12.0	8.2
80	Oyata//Croc1/ <i>Ae. tauschii</i> (879)	12.5	14.2	9.0	12.8
82	Sabuf <sup>BW</sup> /5/BCN/4/Rabi//GS/CRA/3/ <i>Ae. tauschii</i> (190)	6.9	13.1	9.8	10.4
83	Sabuf/3/BCN//Ceta/ <i>Ae. tauschii</i> (895)	11.0	14.0	13.1	12.6
84	Sabuf/3/BCN//Ceta/ <i>Ae. tauschii</i> (895)	7.8	14.0	8.4	11.2
90	Chir3*/5/CS/ <i>Th. curvifolium</i> //Glen/3/ALD/PVN/4/CS/ <i>L. racemosus</i> //2*CS/3/CNO79 <sup>BW</sup>	11.0	13.4	11.7	8.6
98	Mayoor/5/CS/ <i>Th. curvifolium</i> //Glen/3/ALD/PVN/4/CS/ <i>L. racemosus</i> //2*CS/3/CNO79	8.9	10.1	9.1	11.9
101	Chirya.1 <sup>BW</sup>	6.9	9.7	9.0	7.9
102	Chirya.3 <sup>BW</sup>	12.2	12.7	6.1	10.2
103	Chirya.3	6.0	13.3	—	10.2
107	CNO79//Ruff/ <i>Ae. tauschii</i> /3/Maize	8.4	14.8	12.7	9.1
113	PJN <sup>BW</sup> /BOW <sup>BW</sup> //Oyata*2/5/YAV-3/SCO//Jo69/CRA/3/YAV79/4/ <i>Ae. tauschii</i> (498)	10.2	9.8	7.5	11.7
117	PJN/BOW//Oyata*2/3/GAN/ <i>Ae. tauschii</i> (437)	9.8	10.9	12.8	11.3
130	Rabi//GS/CRA/3/ <i>Ae. tauschii</i> (190)/4/Mirlo <sup>BW</sup> /BUC <sup>BW</sup> //VEE#7 <sup>BW</sup>	10.1	11.8	13.4	9.4
138	Dverd 2/ <i>Ae. tauschii</i> (1026)/3/Mirlo/BUC//VEE#7	8.3	9.0	9.2	8.3
141	Rabi//GS/CRA/3/ <i>Ae. tauschii</i> (190)/4/PJN/BOW//Oyata	9.2	9.6	10.8	12.0

47.7–62.4 g. Furthermore, most of these SHs with type-II resistance over 3 years of testing also have superior Karnal bunt resistance, where a similar long-term test across years in Obregon, Mexico, under artificial inoculation identified several entries with immunity to the pathogen (Table 3, p. 97). This new batch of 15 synthetic hexaploid entries now forms the new base for current prebreeding activities that involve elite, but scab-susceptible, bread wheat cultivars as recipient parents.

**Table 2.** Fusarium head scab (type II – spread) data of 14 advanced bread wheat/synthetic hexaploid derivatives following 2 years of evaluations in Toluca, Mexico. *Ae. tauschii* accession number in wheat wide crosses working collection are in parentheses. Bread wheat cultivars in pedigrees are indicated by the superscript <sup>BW</sup> the first time they occur in one of the pedigrees.

2001 Entry No.	Pedigree	Days-to- flowering	Height (cms)	Infection Score (%)	
				2000	2001
6	BCN <sup>BW</sup> /Doy1/ <i>Ae. tauschii</i> (447)	90	115	12.5	10.4
7	Altar 84 <sup>BW</sup> / <i>Ae. tauschii</i> (224)//2*Yaco <sup>BW</sup>	82	120	5.1	10.4
8	Sabuf <sup>BW</sup> /3/BCN//Ceta/ <i>Ae. tauschii</i> (894)	90	125	6.3	11.0
9	Sabuf/5/Bcn/4/Rabi//GS/CRA/3/ <i>Ae. tauschii</i> (190)	85	125	10.7	10.8
11	PJN*/BOW//Opata*2/3/GAN/ <i>Ae. tauschii</i> (437)	82	125	10.7	13.2
12	Rabi//GS/Cra/3/ <i>Ae. tauschii</i> (190)/4/PJN/BOW//Opata <sup>BW</sup>	82	125	11.9	11.6
1131	BCN//Doy1/ <i>Ae. tauschii</i> (447)/3/Mayoor/TKSN1081/ <i>Ae. tauschii</i> (222)	80	120	7.0	7.6
1139	Altar 84/ <i>Ae. tauschii</i> (224)//2*Yaco/3/Mayoor/TKSN1081/ <i>Ae. tauschii</i> (222)	80	120	7.1	7.3
1211	Opata/6/68.111/RGB-u/Ward/3/FGO/4/Rabi/5/ <i>Ae. tauschii</i> (878)/7/ Mayoor <sup>BW</sup> /TKSN1081/ <i>Ae. tauschii</i> (222)	82	120	7.8	7.2
1227	BCN//Doy1/ <i>Ae. tauschii</i> (447)/3/Altar 84/ <i>Ae. tauschii</i> (224)//2*Yaco	80	105	6.4	5.3
1232	BCN//Doy1/ <i>Ae. tauschii</i> (447)/7/Opata/6/68.111/RGB-u/Ward/3/FGO/4/Rabi	80	100	6.8	5.4
1272	Mayoor/5/CS/ <i>Th. curvifolium</i> //Glen†/3/ALD/PVN/4/CS/ <i>L. racemosus</i> //2*CS/3/CNO79/6/Altar 84	85	115	7.0	6.2
1299	BCN//Ceta/ <i>Ae. tauschii</i> (895)/3//Altar 84/ <i>Ae. tauschii</i> (224)//2*Yaco	80	105	6.8	7.5
1315	BCN//Ceta/ <i>Ae. tauschii</i> (895)/3//Alta r84/ <i>Ae. tauschii</i> (224)//2*Yaco	80	100	6.5	6.3

### ***New, free-threshing, bread wheat/D genome synthetic hexaploid derivatives with Septoria tritici resistance.***

A. Mujeeb-Kazi and R. Delgado.

Synthetic hexaploids derived from durum wheat/*Ae. tauschii* crosses have become a potent source of diverse resistance/tolerance to biotic/abiotic stresses. Several such synthetics were identified with superior resistance to leaf blotch. The resistance has been attributed to at least two phenomenon (a) an extended latency period and (b) a lack of pycnidia formation. After identifying resistance in the synthetics, the traits are transferred into elite but *S. tritici*-susceptible bread wheat cultivars. This effort requires first the transfer, followed by selection of derivatives with a good agronomic-plant type that are free threshing in habit and preferably resistant to leaf, stem, and yellow rusts.

Ten bread wheat/*Ae. tauschii*/bread wheat advanced derivatives that were *S. tritici*-resistant and free-threshing were registered previously (Mujeeb-Kazi et al. 2001) and eight more subsequently identified (Delgado and Mujeeb-Kazi 2001) in a preliminary screening evaluation. We currently report on seven, new advanced lines and include more supportive data on the eight lines from screening in the year 2001 (Table 4, p. 98). These 15 lines represent new genetic diversity contributed by several *Ae. tauschii* accessions for resistance to *S. tritici* and agronomic contributions of the durum and elite wheat cultivars in each resistant lines pedigree. The progressive, double-digit scoring scale (Eyal et al. 1987) used limited all selected lines to a stringent 3–2 score (Table 4) versus the susceptible bread wheat controls that reached levels of 8–7 to 9–9 at maturity.

All the 15 lines are homozygous through the use of a wheat/maize haploidy induction protocol (Mujeeb-Kazi 2000). These double haploids are current candidates for germ plasm registration after seed increase.

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**Haploidy in bread wheat: the current protocol.**

A. Mujeeb-Kazi, S. Cano, A.A. Vahidy, T. Razzaki, J.L. Diaz-de-León, and R. Delgado.

Haploid production in bread wheat using the sexual, bread wheat/maize procedure has developed into an efficient tool that is addressing several research areas (Mujeeb-Kazi 2000). Most significant, is the use in wheat breeding as the protocol enhances the work/cost efficiency associated with eventual varietal release. Other applications cover genetics, cytogenetics, molecular biology, pathology, and transformant stability. A global price per doubled haploid (DH) production ranges from approximately USD \$5–60, with a majority of cost between UDS \$ 25–40. Our costs are USD \$10 during the normal cycle for spring habit wheats (June to October) or USD \$ 15 at other times for spring wheats plus winter/facultative. We describe below our current DH protocol stages after summarizing data of a decades of investigation across a range of bread wheat-based materials of various habits. We contend that if our program only focused on DH production, the service, if performed in Mexico with its well-established infrastructure in El Batán, CIMMYT, Int., would be UDS \$5/DH for spring wheats and USD \$7.50 for winter/facultative germ plasm during the normal June to October cycle. The protocol steps are as follows over a calendar year.

**Table 3.** Promising D genome synthetic hexaploid wheats (2n = 6x = 42, AABBDD) for scab resistance (type II – spread) following 3 years of evaluation at Toluca, Mexico. *Ae. tauschii* accession number in wheat wide crosses working collection are in parentheses.

2001 Entry No.	Pedigree	Days-to-flowering	Days-to-physiological maturity	Plant height (cm)	Awn color	1,000-kernel weight (g)	Karnal bunt (%)	Scab type II	
								1999	2000
1	68.111/RGB-U//WARD/3/FGO/4/RABI/5/ <i>Ae. tauschii</i> (629)	103	145	145	Brown	42.8	0.0	11.9	13.0
2	YAR/ <i>Ae. tauschii</i> (783)	96	137	140	Brown	54.4	1.5	12.3	12.8
3	68.111/RGB-U//Ward/3/FGO/4/Rabi/5/ <i>Ae. tauschii</i> (878)	96	142	140	Brown	54.4	0.0	12.4	12.0
4	GAN/ <i>Ae. tauschii</i> (180)	103	145	145	Brown	60.0	0.0	10.7	11.4
5	LCK59.61/ <i>Ae. tauschii</i> (313)	110	145	135	Brown	55.6	0.0	11.5	7.3
6	Scoop 1/ <i>Ae. tauschii</i> (358)	103	145	140	Brown	59.6	0.0	12.0	11.4
7	Botno/ <i>Ae. tauschii</i> (625)	122	165	145	Brown	53.2	0.0	15.0	6.0
8	CPI/Gediz/3/GOO//JO69/CRA/4/ <i>Ae. tauschii</i> (409)	103	145	140	Brown	62.4	0.0	15.0	11.7
9	Dverd 2/ <i>Ae. tauschii</i> (1027)	114	145	140	Brown	48.4	0.0	14.6	10.1
10	CETA/ <i>Ae. tauschii</i> (172)	100	137	140	Brown	53.3	0.0	12.7	9.6
11	CETA/ <i>Ae. tauschii</i> (306)	110	145	140	Brown	50.9	0.0	14.7	13.9
12	CETA/ <i>Ae. tauschii</i> (445)	103	145	140	Brown	47.7	0.0	13.4	15.3
13	CPI/Gediz/3/GOO//JO/CRA/4/ <i>Ae. tauschii</i> (1018)	103	145	150	Brown	59.6	1.8	14.9	11.1
14	CETA/ <i>Ae. tauschii</i> (1031)	103	145	150	Brown	50.0	0.0	14.9	9.4
15	<i>Ae. tauschii</i> (1026)/DOY1	103	145	150	Brown	55.5	0.0	13.7	10.2
16	Sumai 3 (resistant check)	85	125	105	Brown	—	—	10.8	11.1
17	Mayoor/TKSN1081/ <i>Ae. tauschii</i> (222)	81	120	90	Brown	40.2	2.8	9.0	5.7



**Table 4.** Progressive evaluation of *Septoria tritici* (days-from-planting) of advanced, free-threshing derivatives from bread wheat/*Ae. tauschii* synthetic combinations conducted in Toluca, Mexico, during 2001. *Ae. tauschii* accession number in wheat wide-crosses working collection are in parentheses. Bread wheat cultivars in pedigrees are indicated by the superscript <sup>BW</sup> the first time they occur in one of the pedigrees.

Pedigree	Progressive scoring dates (days)						Plant height (cm)
	81	88	95	105	120	135	
Altar 84/ <i>Ae. tauschii</i> (Bangor)†//Esda <sup>BW</sup>	0-0	1-1	1-1	1-1	1-1	2-1	105
Croc 1/ <i>Ae. tauschii</i> (205)//2*FCT <sup>BW</sup>	0-0	1-1	1-1	2-2	2-2	2-2	85
Croc 1/ <i>Ae. tauschii</i> (205)//Kauz <sup>BW</sup>	0-0	1-1	1-1	1-1	1-1	2-2	90
Croc 1/ <i>Ae. tauschii</i> (213)//PGO <sup>BW</sup>	0-0	1-1	1-1	2-1	2-1	2-2	80
Altar 84/ <i>Ae. tauschii</i> (219)//Opata <sup>BW</sup>	0-0	1-1	1-1	1-1	1-1	2-1	100
Altar 84/ <i>Ae. tauschii</i> (221)//Siren <sup>BW</sup>	0-0	1-1	1-1	1-1	1-1	1-1	110
Altar 84/ <i>Ae. tauschii</i> (221)//PGO	1-1	1-1	1-1	2-1	2-1	3-2	105
Croc 1/ <i>Ae. tauschii</i> (224)//Opata	1-1	1-1	1-1	1-1	2-1	3-2	100
Altar 84/ <i>Ae. tauschii</i> (224)//2*Yaco <sup>BW</sup>	1-1	1-1	1-1	1-1	1-1	2-2	110
Altar 84/ <i>Ae. tauschii</i> (224)//2*Yaco	1-1	1-1	1-1	1-1	1-1	2-1	100
Opata*2//Sora/ <i>Ae. tauschii</i> (323)	1-1	1-1	1-1	1-1	1-1	2-1	100
BCN <sup>BW</sup> //YUK/ <i>Ae. tauschii</i> (434)	0-0	1-1	1-1	1-1	1-1	2-1	110
BCN/3/FGO/Usa 2111// <i>Ae. tauschii</i> (658)	1-1	1-1	1-1	1-1	3-2	3-2	100
BCN//Croc 1/ <i>Ae. tauschii</i> (662)	0-0	1-1	1-1	1-1	1-1	1-1	100
Opata/6/68.111/RGB-u//Ward/3/FGO/4/Rabi/5/ <i>Ae. tauschii</i> (878)	0-0	1-1	1-1	2 1	2-1	2-1	100

## Winter/facultative bread wheats

1. Germinate/vernalize (2 months).
2. Plant (June).
3. Grow to flowering (June–15 August).
4. Crossing (15 August–30 September).
5. Embryo rescue (5 September–15 October).
6. Seedling differentiation (25 September–5 November).
7. Transplant (25 September–5 November).
8. Grow haploids (20 October–30 November).
9. Colchicine treatment (20 October–November 30).
10. Vernalize for 6 weeks (1 November–10 December).
11. Bag all spikes in glassine envelopes.
12. Harvest DH seed (all by 15 April).

## Spring bread wheats

1. Not applicable.
2. Plant (June).
3. Grow to flowering (June–15 August).
4. Crossing (15 August–30 September).
5. Embryo rescue (5 September–15 October).
6. Seedling differentiation (25 September–5 Nov).
7. Transplant (25 September–5 November).
8. Grow haploids (20 October–30 November).
9. Colchicine treatment (20 October–30 November).
10. Not applicable.
11. Bag all spikes in glassine envelopes.
12. Harvest DH seed (all by 1 March).

The experimental details of methodology are now fine tuned since our report in the *Annual Wheat Newsletter* (47:116, 2001) and are highlighted here for facilitating ready access.

1. Cut spikes from selected plants and keep in tap water in plastic jars under greenhouse regimes of 24°C day/14°C night, 65 % RH, and 14-h natural light.
2. Hand emasculate each spike 2 days after cutting according to the conventional breeding procedure. Cover with a plastic bag holding about 15 spikes to promote humidity.
3. Pollinate with fresh maize pollen (9:30–10:30 a.m.) 3 days after emasculation. Spikes are left uncovered after pollination. 2,4 dichlorophenoxy-acetic acid, at 100 ppm, is added to each plastic container.
4. Treat with 2, 4 dichlorophenoxy-acetic acid for 3 days (a variation from 6 days used earlier). The reduced duration time promotes speedier germination of embryos in the media.
5. Culture media, seedling differentiation, transplanting, colchicine treatment, and management aspects are all similar to those reported earlier (Mujeeb-Kazi 2001).

The data from close to 7,000 DHs produced between June 2001 and March 2002 suggest an embryo-recovery frequency of 25–35 %, differentiation into seedlings between 90–100 %, and a doubling rate between 95–100 %. Some researchers have achieved an embryo-recovery frequency between 50–70 %, but the final DH output is low because of lower rates for differentiation and doubling. We are exploring if the use of gibberellic acid (75 ppm) in the crossing stage and the early (bud) pollinations used earlier in complex intergeneric hybridizations (Mujeeb-Kazi et al. 1987) can augment our present 25–35 % embryo-recovery rate. Currently, this sexual haploid induction route is a favorite tool for bread wheat germ plasm. The promise of the newer microspore technology is an upcoming alternative that will revolutionize the working scenario if genotype specificity does not exist. This development is keenly awaited in the public sector.

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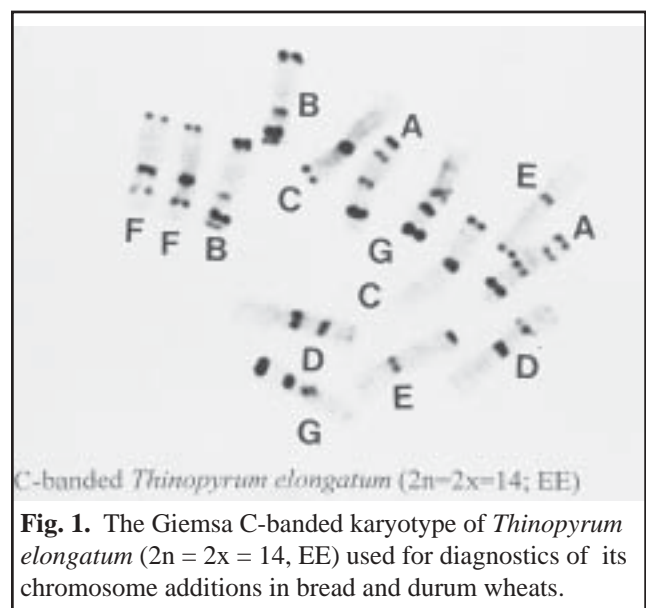
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### *Disomic chromosome additions of Thinopyrum elongatum (2n = 2x = 14) in bread and durum wheat.*

A. Mujeeb-Kazi, A. Cortés, V. Rosas, J.L. Diaz-de-León, A.A. Vahidy, and T. Razzaki.

*Thinopyrum elongatum* is a grass noted for its tolerance to salinity and resistance to FHB, two constraints that limit bread and durum wheat production. The species was hybridized with the bread wheat cultivar Chinese Spring (Dvorak and Knott 1974) that subsequently led to production of addition lines. Dvorak et al. (1988) reported homoeologous additions of chromosomes belonging to groups 3, 4, and 7 as being positive contributors to salt tolerance. We decided to remake this hybrid combination with two significant modifications. First, *Th. elongatum* was used as the female parent in the hybrid in order to capture the cytoplasmic influence if any should exist and, second, use a commercial bread wheat cultivar other than Chinese Spring so the derivatives would be agronomically superior and better adapted to global field testing conditions.

Consequently, a hybrid between *Th. elongatum* and the *T. aestivum* cultivar Goshawk 'S' was made and its amphiploid ( $2n = 8x = 56$ , AABBDD $\overline{EE}$ ) produced. One strategy was to screen the amphiploid for salt tolerance and resistance to FHB (type II). If the observations were positive for either stress, the seven possible *Th. elongatum* chromosome addition lines would be produced. The amphiploid was tolerant/resistant to both stresses (Mujeeb-Kazi 2001), and by adopting an  $F_1$  topcross breeding protocol, we have produced the seven disomic, chromosome addition lines in the topcross bread wheat cultivar Prinia, which is superior to Goshawk. The C-banded karyotype of *Th. elongatum* (Fig. 1) was used to validate the addition lines because each alien chromosome had a unique banding pattern and the bread wheat chromosomes. The addition lines are highly fertile and are the basis for delineating their associations with both stress constraints being investigated. We expect that more than one chromosome will contribute to the resistance to each stress. The *ph*-gene-mediated protocol (Mujeeb-Kazi 2001) has already been set in place.



C-banded *Thinopyrum elongatum* ( $2n=2x=14$ ; EE)

**Fig. 1.** The Giemsa C-banded karyotype of *Thinopyrum elongatum* ( $2n = 2x = 14$ , EE) used for diagnostics of its chromosome additions in bread and durum wheats.

For durum wheats, salinity tolerance is of some concern but the lack of scab resistance currently is of greater significance. The contributions of alien species for durum wheat improvement are not too extensive and based on the response of *Th. elongatum* in a bread wheat background for superior type-II scab resistance, we initiated crosses between this diploid (pollen parent) and an elite durum cultivar. An amphiploid was produced ( $2n = 6x = 42$ , AABBEE) from the  $F_1$  hybrid ( $2n = 3x = 21$ , ABE) that gave a promising type-II scab resistance score. Using a conventional topcross breeding strategy, we have produced single to multiple monosomic additions. Jauhar and Peterson (2000) observed difficulty in stabilizing *Th. elongatum* additions in durum wheat and, as a result, the successful transfer of scab resistance to yield a stable resistant euploid ( $2n = 4x = 28$ ) durum from any of the *Th. elongatum* chromosomes contributing to scab resistance has not been achieved. Our strategy, in addition to selfing the single/multiple monosomic additions to extract various disomics with  $2n = 4x = 28$  plus a unique *Th. elongatum* chromosome pair from the selfed progeny, has been to use those durum cultivars in the top cross and in the original hybrid cross that respond to haploid generation via the maize procedure. Consequently, we produced a large number of unique monosomic *Th. elongatum* additions with  $n = 2x = 14$  plus one alien chromosome, where each of the seven *Th. elongatum* chromosomes were represented. These haploids have been treated with colchicine and have set seed. The harvest and cytological confirmation, coupled with seed increase of the  $2n = 4x = 28 + 2$ , covering all unique *Th. elongatum* chromosome pairs is now close at hand.

This study was targeted towards the production of the complete set of *Th. elongatum* disomic addition lines in an elite spring durum wheat cultivar. The next step will be scab screening in Toluca, Mexico. Based upon our observations with *Th. bessarabicum*, scab resistance may be present on several of the *Th. elongatum* addition lines. As a precautionary step, we already have crossed the durum/*Th. elongatum* amphiploid with the durum *ph1c* genetic stock to enforce homoeologous exchanges that have been described by Mujeeb-Kazi (2001) for the bread wheat germ plasm. This approach also should address the salinity tolerance objective where several *Th. elongatum* chromosomes are known to be contributors.

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#### ***A complete set of Thinopyrum bessarabicum disomic chromosome addition lines in the bread wheat cultivar Prinia.***

A. Mujeeb-Kazi, A. Cortés, A.A. Vahidy, T. Razzaki, J.L. Diaz-de-León, and R. Delgado.

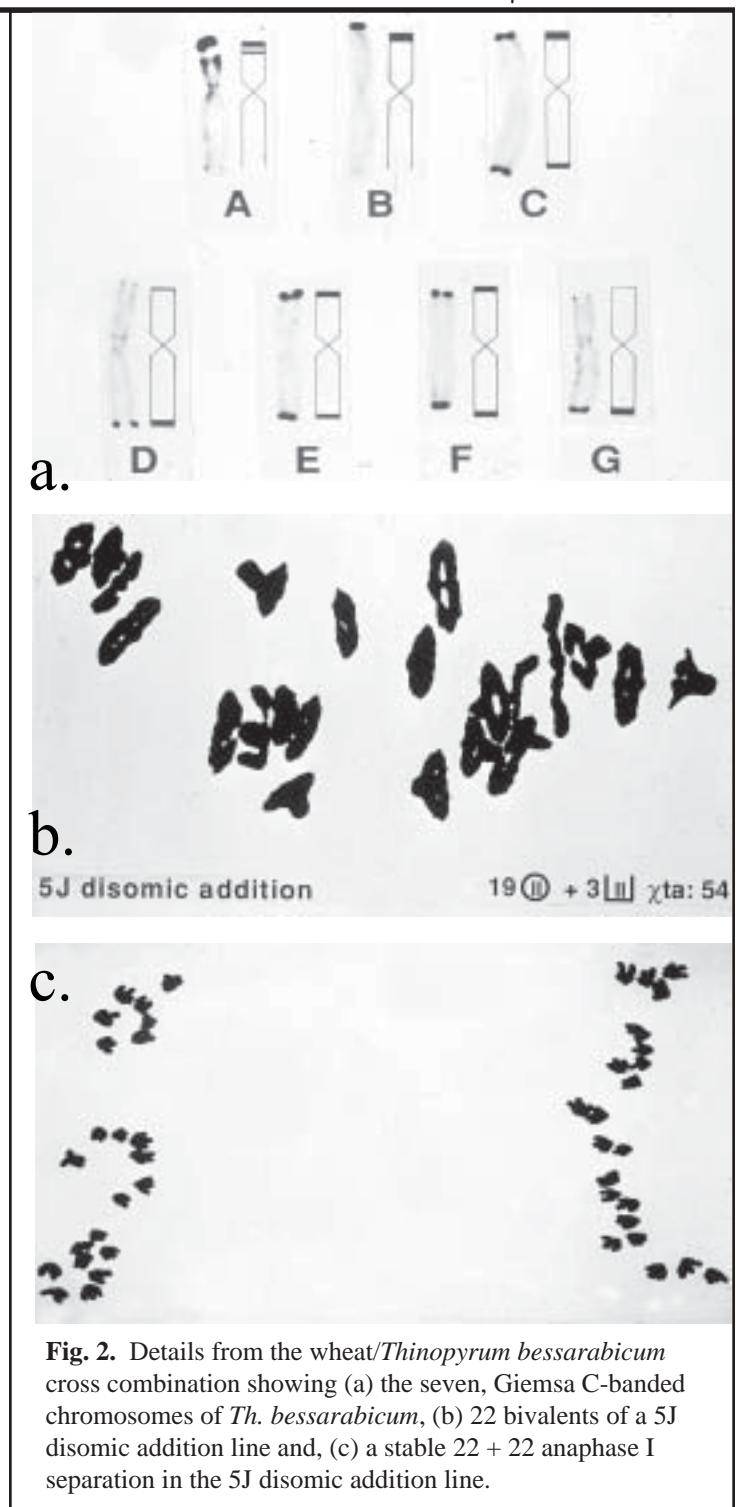
*Thinopyrum bessarabicum* is a self-fertile, maritime grass species possessing salt tolerance (Gorham et al. 1985), root-knot nematode resistance (Jensen and Griffin 1994), and resistance to FHB (Mujeeb-Kazi 2001). Currently in our wide cross program, breeding for resistance to FHB is of high priority for bread wheat. One utilization strategy is to produce alien chromosome addition lines in bread wheat, screen these for stress resistance, and cytogenetically manipulate to them to introgress the beneficial alien contribution from the target disomic addition lines. Our initial step was to produce a complete addition line set of the seven disomic *Th. bessarabicum* chromosomes. Six disomic additions were initially reported by Mujeeb-Kazi et al. 2000 (1J, 2J, 3J, 4J, 5J, and 7J). These lines subsequently were analyzed by FISH and AFLP diagnostics (Zhang et al. 2002) and the status of five (1J, 2J, 4J, 5J, and 7J) were confirmed. Line 3J was not a disomic addition but a translocation product. Thus, additions for 3J and 6J were missing that we have now produced from a  $BC_1$ , self-fertile, 50-chromosome derivative possessing 21 bivalents of wheat and four *Th. bessarabicum* bivalent chromosomes including the 3J and 6J pairs.

Backcross derivatives of the 50-chromosome  $BC_1$  plant with the cultivar *Prinia* gave 46-chromosome progeny; 42 wheat plus four of *Th. bessarabicum*. An additional backcross gave progeny that was either a 3J or a 6J single monosomic (43 chromosomes) or was a double 3J + 6J monosomic (44 chromosomes). Each of these derivatives were

crossed with maize to yield 22-chromosome haploids, which were validated by C-banding as 21 + 3J and 21 + 6J and treated with colchicine to yield the respective 3J and 6J disomic addition lines. Thus, the disomic set of the seven *Th. bessarabicum* chromosomes was completed. Interdisomic addition line crosses were made involving each disomic addition line (i.e., 1J/2J, 1J/3J, 1J/4J, 1J/5J, 1J/6J, 1J/7J). The  $F_1$  progeny was cytologically analyzed by chromosome counts ( $2n + 6x = 42 + 2$ ), Giemsa C-banding, and meiotic metaphase association, where 21 II+2 I indicated the validity of each addition line. Each addition line also was checked by FISH and C-banding (mitosis) to ascertain structural integrity. All seven addition lines are in a spring bread wheat cultivar Prinia background (93.75 %) and have good fertility. These lines will be more suitable for biotic/abiotic stress screening because of their superior agronomical background. The karyotype in Fig. 2a is for the seven alien *Th. bessarabicum* chromosomes that are associated with disomic addition lines 1J to 7J. These lines are partially characterized by their respective biochemical markers (William and Mujeeb-Kazi 1995). Each disomic addition line has a stable meiotic association with 22 bivalents (Fig. 2b) and normal anaphase I separation (Fig. 2c) with high seed set.

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**Fig. 2.** Details from the wheat/*Thinopyrum bessarabicum* cross combination showing (a) the seven, Giemsa C-banded chromosomes of *Th. bessarabicum*, (b) 22 bivalents of a 5J disomic addition line and, (c) a stable 22 + 22 anaphase I separation in the 5J disomic addition line.



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***Origin and cytogenetics of a bread wheat/Aegilops ovata intergeneric hybrid and its complete/partial amphiploids.***

A.A. Vahidy, F. Shafiq, A. Cortés, V. Rosas, and A. Mujeeb-Kazi.

The wild wheat relatives of the tribe Triticeae are distributed in three gene pools; primary, secondary, and tertiary. Utilization of these pools (other than conventional) forms the area of wide hybridization that includes both interspecific and intergeneric hybridization. In general, wide hybridization has become an important tool for widening the genetic variability of cultivated species. It has been particularly useful in transferring desirable genes into cultivated wheat from their wild relatives for addressing biotic and abiotic stress constraints. These wild species represent a valuable source of genetic variability for wheat breeding, with *Aegilops* species being a rich source of desirable genes for wheat improvement. In such alien-diversity introgression programs, the production of F<sub>1</sub> hybrids is a critical starting point from which advanced derivatives are developed. The use of the alien source is an important factor and in general the primary gene pool species have priority since homologous exchanges facilitate rapid end-products to emerge. However, more distant sources residing in the tertiary gene pool also can provide superior resistance and do have a significant place in wide-crossing programs targeted for wheat improvement. Products from the use of these distant species are slow to obtain and often require complex cytogenetic manipulation strategies, but their exploitation is justified when one gauges the need for diverse genes that can add to durable stress resistances.

*Aegilops geniculata* is one such tertiary gene pool species that has superb resistance to Karnal bunt, BYDV, leaf rust, and cereal cyst nematode, coupled with a high level of salinity tolerance in a majority of its accessions. Of these, our major wheat production constraints in Pakistan are for Karnal bunt, leaf rust, and salinity, for which any possible introgressions into the lead cultivars of our region form a viable research objective. We decided to hybridize bread wheat cultivar Sarsabz with an *Ae. geniculata* accession and have developed some stable genetic stocks from this F<sub>1</sub> hybrid that will form the basis of additional introgression studies. Intergeneric hybrids and addition lines were produced earlier and reported upon by Friebe et al. (1999) who produced 13 disomic addition and one monosomic addition lines, thus getting the complete set of 14 additions. This is the first time that such an attempt has been made in an elite wheat cultivar from Pakistan.

The F<sub>1</sub> hybrid plants obtained from crossing Sarsabz with *Ae. geniculata* were all mitotically regular with 2n = 5x = 35, ABDUM chromosomes, and expressed a mean meiotic association of 30.3 univalents plus 2.32 rod bivalents. It was a low frequency cross. F<sub>1</sub> management and colchicine treatment protocols were similar to those described by Mujeeb-Kazi et al. (1987). Three amphiploid seed were obtained; each with 2n = 10x = 70, AABBDDUUMM chromosomes, which were ascertained by orcein-stained, root-tip chromosome counts and Giemsa C-banding. The mean meiotic association of the amphiploid was 4.6 univalents + 18.7 ring bivalents + 10.8 rod bivalents (total 29.5 bivalents) + 1.4 trivalents + 0.2 quadrivalents. The C<sub>1</sub> progeny from these 70-chromosome amphiploids was highly aneuploid and less than 1.0 % of the plants possessed the expected normal chromosome number. However, there was an abundance of C<sub>1</sub> progeny with 56 chromosomes. These plants were designated as partial amphiploids. Ganeva et al. (1992) also had observed similar aneuploid trends in the original amphiploid and chromosome number reductions. These partial amphiploids were highly fertile and produced derivatives from C<sub>2</sub> to C<sub>6</sub> that maintained 56 chromosomes (Fig. 3a, p. 103). The expectation was that all the 42 wheat chromosomes would be present and 14 (A to N) would represent the contributions from *Ae. geniculata*; U, M, or a mixture of U- and M-genome chromosomes.

The partial amphiploid has remained stable over six generations of selfing and maintains the 56 mitotic chromosomes that are meiotically associated as 28 bivalents either as perfect rings or a mixture of rods and rings (Figs. 3b and 3c, p. 103). The chromosome complement, however, deviates from the expected 42 chromosomes of wheat and 14 of *Ae. geniculata* because Giemsa C-banding identified 30 wheat and 26 *Ae. geniculata* chromosomes. The missing wheat chromosomes were 3A, 4A, 1B, 2D, 5D, and 7D (six pairs), and the *Ae. geniculata* chromosomes present were representative of 13 pairs and given arbitrary designations of A to M with N assigned to the missing pair. The BC<sub>1</sub> progeny from this amphiploid with Sarsabz gave stable plants with 49 chromosomes and all 13 *Ae. geniculata* chromosomes in the amphiploid were represented in a single dose. Thus, it would be possible to develop 13 disomic chromosome addition lines (A to M), and the missing line would be for chromosome N. The meiosis of the BC<sub>1</sub> plant had a majority of the meiocytes with 15 bivalents (all of wheat) + 19 univalents that were composed of 13 *Ae. geniculata* (A to M) and six wheat (3A, 4A, 1B, 2D, 5D, and 7D) chromosomes. Subsequent backcrossing of these 49-chromosome BC<sub>1</sub> plants has led to the production of several derivatives where euploidy for the 42 chromosomes of wheat has been achieved and single to triple monosomic addition chromosomes are present. Upon selfing, these lines have led to the isolation of 44-



chromosome progeny in which a disomic *Ae. geniculata* chromosome pair is present. The additions completed so far are for 11 chromosomes alphabetically designated as A, B, D, E, F, G, H, I, J, L, and M. Addition lines of two of the *Ae. geniculata* chromosomes are not available and additional backcross progenies are needed for the isolation of these chromosomes designated as C and K. To speed up this process, we have generated haploid plants from each target BC derivative that represent the missing chromosomes C and K in the population. The selfed progeny also will be used to complement this haploid strategy. If the two missing chromosome addition lines are not produced, then the selfed germ plasm of the specific backcross will lead to another round of haploid generation and selfing.

The hybridization of *Ae. geniculata* with the elite wheat Sarsabz coupled with additional backcrosses to the same cultivar has enabled us to produce derivatives in a superior wheat plant type that will facilitate testing of these genetic stocks for the necessary stress constraints being addressed. We have modified the cytogenetic manipulation strategy of Mujeeb-Kazi (2001) slightly in that the use of the *ph* Chinese Spring genetic stock will be incorporated only on each of those 44-chromosome disomic addition lines that indicate a positive value. The progeny derived from this *ph*-manipulation strategy will be critically analyzed for wheat/alien exchanges.

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**Fig. 3.** The partial bread wheat/*Aegilops geniculata* amphiploid showing (a)  $2n = 8x = 56$  chromosomes in a mitotic cell, (b) a meocyte with 28 ring bivalents, and (c) a meocyte with 6 rod bivalents + 22 ring bivalents.

**Scab resistance of some partial amphiploid derivatives of bread wheat/*Thinopyrum intermedium*.**

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The Crop Breeding Institute of Heilongjiang Academy of Agricultural Sciences has been engaged in a bread wheat/*Th. intermedium* crossing program since the 1970s. Partial amphiploids were developed by backcross breeding and named Yuanzhong 1, Yuanzhong 2, Yuanzhong 3, Yuanzhong 4, Yuanzhong 5, Yuanzhong 6, and Yuanzhong 7 (syn. Zhong 1 to 7). These lines are known to possess resistance or tolerance to various biotic and abiotic stresses and are widely used as important germ plasm for wheat improvement. In Heilongjiang, the Academy of Agricultural Sciences developed three wheat varieties called Longmai 8, 9 and 10; the Northeast Normal University in Jilin province released Xiaobing 32 and 33 derived from Yuanzhong 1 and 3; and the Shannxi Academy of Agricultural Sciences obtained Shanmai 89150, 897, and 611 derived from Yuanzhong 4 and 5. Some of the parental partial amphiploid lines also have been used in Australia for BYDV resistance.

Cumulative results from the use of the Yuanzhong partial amphiploids indicate that these germ plasm lines are highly resistant to leaf rust, stem rust, stripe rust, spot blotch, powdery mildew, WSMV (except Yuanzhong 1), take-all (except Yangzhong 1), and BYDV. In 2001, these materials were inoculated with the FHB pathogen to evaluate type-II resistance in Heilongjiang, China. At CIMMYT, we examined their DON content (type III) using the FLUOROQUANT method and determined their somatic chromosome number. These results are listed in Table 5.

The data in Table 5 shows the mitotic chromosome number and range, the number of infected spikelets/spike,

the percent of infected spikelets, and the DON content of the partial amphiploids. Yuanzhong 2 and 5 have the lowest values among the tested materials. Both these partial amphiploids could be potential and valuable resistant germ plasm for incorporating scab resistance into bread wheat. The data of Table 5 are from one cycle of tests and additional testing is necessary. This testing is planned for a field study in China and laboratory studies at CIMMYT, Mexico, during 2002.

Currently, we conclude that Yuanzhong 2 and 5, show satisfactory type-I and type-II resistance to FHB. Yuanzhong 6 and Yuanzhong 7 are from the same cross as Yuanzhong 5 and are similar to Yuanzhong 5 for many characteristics. Thus, these two (6 and 7) were not evaluated for scab resistance in this study. Both of the resistant partial amphiploids (2 and 5) have 56 chromosomes with normal bivalent meiosis indicative of stability. The stability is reflected in the BC<sub>1</sub> progeny, which have a normal 49 chromosomes. We are using two strategies of resistance introgression. The conventional strategy relates to the production of disomic addition lines, screening, and the cytogenetic manipulation of the resistant entry to permit genetic introgression. The second option uses is *ph*-gene mediated at the 56-chromosome level and also later when the addition line/s with scab resistance have been identified. The delayed involvement of the *ph* gene holds priority as this route will be more targeted. The protocol being utilized for the *ph*-based manipulation is that of Mujeeb- Kazi (2001).

**Reference.**

Mujeeb-Kazi A. 2001. Intergeneric Hybrids in Wheat: current status. **In:** Proc IV Internat Triticeae Symp (Hernandez P, Moreno MT, Cubero JI, and Martin A eds). 10–12 September 2001, Córdoba, Spain. pp. 261-164.

**Table 5.** Scab resistance in a set of partial amphiploids from bread wheat and *Thinopyrum intermedium*.

Material	Chromosome		Infected spikelets number/spike	Infected spikelets (%)	DON (ppm)
	composition	number			
Yuanzhong 1	ABDX	50–52	0.9	6.2	3.2
Yuanzhong 2	ABDX	56	0.5	3.1	0.0
Yuanzhong 3	ABDE	56	2.5	15.0	1.5
Yuanzhong 4	ABDE	56	1.0	6.0	3.6
Yuanzhong 5	ABDE	56	1.1	6.1	0.9
<i>Th. intermedium</i>	XXE1E1E2E2	42	0.5	2.5	—

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***Cytogenetics of wheat and alien Triticeae species amphiploids.***

A. Cortés, A.A. Vahidy, T. Razzaki, V. Rosas, R. Delgado, and A. Mujeeb-Kazi.

Intergeneric hybrids within the Triticeae are a potent source of exploiting alien genetic diversity for wheat improvement. Perennial sources yield perennial hybrids that can be maintained as a living herbarium only requiring meticulous maintenance via cloning each combination twice a year with a cytological check to ensure their valid hybrid status. Hybrids with the annual Triticeae species tend to be annual and producing an amphiploid is necessary for their maintenance. The other option is to pollinate the self-sterile  $F_1$  with a wheat cultivar to generate a  $BC_1$  progeny, utilizing the fusion of an unreduced egg-cell with the pollen gamete. Our technique has been very productive for yielding doubled products in the maize-induced, DH program where over the past several years the doubling frequency has ranged between 90.0–100.0 % across many bread wheat germ plasms. However, amphiploid production using colchicine and dimethylsulfoxide (DMSO) for intergeneric hybrids been an extremely low frequency event for us over a span of two decades and has never exceeded more than 5.0 % at any one time; generally being less than 1.0 %. Despite the low doubling rate with intergeneric hybrids, we have been able to produce a large number of amphiploids (Tables 6 and 7, p. 106–107) and still continue to expand the list by varying the colchicine concentration and treatment times from those reported by Mujeeb-Kazi et al. (1987). The significance of amphiploids was emphasized by Gill (1987), and the importance of such genetic stocks since then is even further recognized.

Rosas et al. (1996) reported on the pedigree, expected  $C_0$  chromosome and plant number, the generations of advance of each amphiploid, total amphiploid plants observed in each combination, the mitotic chromosome range, and seed amount available. We are now updating the salient aspects for these and other amphiploid combinations. The meiotic association data for all aneuploid combinations also is in our database. Indications (\*) also are provided that may allow researchers to target their programs around some combinations to address various biotic/abiotic stresses that constrain wheat production. In those cases, we have made an advances by top-crossing the desired amphiploids by a quality spring bread wheat and also by the Chinese Spring *ph*-mutant genetic stock for the swift incorporation of these materials in wheat developmental programs as required by collaborators globally. The use of the *ph* stock follows the strategy that Mujeeb-Kazi (2001) proposed for facilitating the production of wheat/alien chromosome translocation products. Amphiploids with a durum base also have been exploited similarly by crossing the combinations with an elite, durum, spring wheat cultivar and selecting a few combinations by the durum *ph1c* genetic stock.

In general, hybrids that have given us fertile amphiploids (Tables 6 and 7, p. 106–107) have wheat as the female parent with the alien male donors ranging from diploid ( $2n = 2x = 14$ ) to hexaploid ( $2n = 6x = 42$ ). The resulting amphiploids thus range from  $2n = 8x = 56$  to  $2n = 12x = 84$  for bread wheat and from  $2x = 6x = 42$  to  $2n = 10x = 70$  for durum wheat. Hyper- and hypoploidy existed in all of the combinations but greater mitotic stability was prevalent in those combinations where the amphiploid chromosome number was either 42 or 56. These germ plasms also were high in fertility and produced sufficient plump seed in larger numbers per plant than the rest of the amphiploids with 70 to 84 chromosomes.

Cytological analyses of the various amphiploids over several ( $C_n$ ) generations have indicated that even when the starting plant has a stable chromosome number, it rarely produces progeny that maintains this stability particularly when the amphiploid is of 70 or 84 chromosomes. The progenies of amphiploids with 42 or 56 chromosomes are comparatively more stable and very few derivatives are aneuploid. We have maintained one sample from three individual plants of each amphiploid that have the normal chromosome number or very near the expected number. In addition, for each amphiploid, there is a bulk sample from several other plants that had some aneuploidy. These comprise the initial sources for stress screening and, if a combination is identified as being of positive value for a trait, then a stable individual plant sample can be utilized for prebreeding via addition line production or by *ph*-based chromosome manipulation.

Stresses of greater current significance for wheat production in the various global megaenvironments are related to biotic and abiotic areas. Some of these are the resistance to the three rusts, FHB, *Cochiobolus sativus*; Karnal bunt, *S. tritici*, *S. nodorum*, powdery mildew, BYDV, cereal cyst nematode, general root rots, RWA; tolerance to salinity, drought, heat, cold, sprouting, waterlogging, aluminum, micronutrients; and yield. All stress constraints are influenced by quality. For sustainable agriculture, a blend of genes representing maximized genetic diversity is advantageous and in this context, and the role of the amphiploid stocks reported here becomes highly significant. The practical value of these amphiploids is genetically assured because their  $F_1$  hybrids all exhibited a codominant phenotype and so do their

**Table 6.** Details of some annual/perennial Triticeae species amphiploids with various bread wheat cultivars. Combinations useful for practical agricultural are indicated with an asterisk (\*).

Pedigree	Expected chromosome number	Current generation	Chromosome range observed	Seed amount (g)
<b>ANNUAL TRITICEAE</b>				
CS/ <i>Aegilops variabilis</i>	2n = 10x = 70	C-11	64-69	10
Alondra/Pavon// <i>Ae. variabilis</i>	2n = 10x = 70	C-11	65-70	10
Faisalabad/ <i>Ae. variabilis</i>	2n = 10x = 70	C-11	66-70	10
Jauhar/ <i>Ae. variabilis</i>	2n = 10x = 70	C-11	64-71	10
Asakazekomugi/ <i>Ae. variabilis</i>	2n = 10x = 70	C-11	65-69	10
Fukohokomugi/ <i>Ae. variabilis</i>	2n = 10x = 70	C-11	66-70	10
Lu 26/ <i>Ae. variabilis</i>	2n = 10x = 70	C-11	68-70	15
Pak 81/ <i>Ae. variabilis</i> *	2n = 10x = 70	C-11	69-71	15
Punjab/ <i>Ae. variabilis</i>	2n = 10x = 70	C-11	68-70	15
Sarsabz/ <i>Ae. ovata</i> *	2n = 8x = 56	C-8	55-57	25
<b>PERENNIAL TRITICEAE</b>				
Fremont/ <i>Thinopyrum scythicum</i>	2n = 10x = 70	C-9	64-68	10
Chinese Spring (CS)/ <i>Th. podperae</i>	2n = 12x = 84	C-9	72-77	10
CS/ <i>Th. bessarabicum</i> *	2n = 8x = 56	C-9	54-57	25
CS/ <i>Th. intermedium</i>	2n = 12x = 84	C-9	74-78	15
CS/ <i>Th. trichophorum</i>	2n = 12x = 84	C-9	72-76	15
CS/ <i>Th. junceiforme</i> *	2n = 10x = 70	C-9	65-70	10
Tobari 66/ <i>Th. junceum</i>	2n = 12x = 84	C-9	70-78	5
CS// <i>Th. repens/A. desertorum</i>	2n = 10x = 70	C-9	64-70	20
CS/ <i>Th. scirpeum</i> *	2n = 10x = 70	C-9	68-71	10
Pavon/ <i>Th. rechingeri</i>	2n = 10x = 70	C-9	69-71	25
CS/ <i>Th. elongatum</i> *	2n = 8x = 56	C-10	54-57	25
<i>Th. elongatum</i> /Goshawk *	2n = 8x = 56	C-12	55-56	30

**Table 7.** Details of some annual/perennial Triticeae species amphiploids with various durum wheat cultivars. Combinations useful for practical agricultural are indicated with an asterisk (\*).

Pedigree	Expected chromosome number	Current generation	Chromosome range observed	Seed amount (g)
<b>ANNUAL TRITICEAE</b>				
Laru/ <i>Aegilops variabilis</i>	2n = 8x = 56	C-7	54-57	15
Arlin/ <i>Ae. variabilis</i>	2n = 8x = 56	C-7	55-56	15
Altar/ <i>Ae. variabilis</i> *	2n = 8x = 56	C-7	55-56	15
Bia/ <i>Ae. variabilis</i>	2n = 8x = 56	C-7	55-56	15
Ceta/ <i>Ae. ventricosa</i>	2n = 8x = 56	C-7	52-57	10
Capelli/ <i>Ae. ovata</i> *	2n = 8x = 56	C-7	55-56	25
Capelli/ <i>Ae. triuncialis</i>	2n = 8x = 56	C-7	53-57	10
Capelli/ <i>Ae. speltoides</i>	2n = 6x = 42	C-7	36-43	5

respective amphiploids. We infer the codominance as an indicator of the genetic expression of the alien species in a bread or durum wheat background. From the total F<sub>1</sub> hybrids that we have produced, several still have not responded to doubling and still remain candidates of a continuously on-going exercise that is carried out twice a year after each physical cloning of the F<sub>1</sub>s. In addition, a program to further enrich the genetic pool targets producing new hybrid combinations since several annual and perennial Triticeae species have still not been hybridized with wheat.

**Table 7 (continued).** Details of some annual/perennial Triticeae species amphiploids with various durum wheat cultivars. Combinations useful for practical agricultural are indicated with an asterisk (\*).

Pedigree	Expected chromosome number	Current generation	Chromosome range observed	Seed amount (g)
<b>PERENNIAL TRITICEAE</b>				
<i>Elymus fibrosus</i> /Cocorit 71	2n = 8x = 56	C-9	55-56	10
Altar 84/ <i>Thinopyrum scirpeum</i>	2n = 8x = 56	C-9	55-56	10
Capelli/ <i>Th. acutum</i>	2n = 10x = 70	C-9	55-70	20
Yavaros/ <i>Th. acutum</i>	2n = 10x = 70	C-9	55-70	20
Cocorit 71/ <i>Th. acutum</i>	2n = 10x = 70	C-9	55-70	20
Yavaros 79/ <i>Th. intermedium</i>	2n = 10x = 70	C-9	55-71	15
Cocorit 71/ <i>Th. intermedium</i>	2n = 10x = 70	C-9	55-68	15
Mexicali 75/ <i>Th. intermedium</i>	2n = 10x = 70	C-9	55-67	15
Capelli/ <i>Th. intermedium</i>	2n = 10x = 70	C-9	55-68	15
Cocorit 71/ <i>Th. junceiforme</i> *	2n = 8x = 56	C-9	55-57	10
Cocorit 71/ <i>Th. pulcherrimum</i>	2n = 10x = 70	C-9	58-68	10
Mexicali 75/ <i>Th. pulcherrimum</i>	2n = 10x = 70	C-9	59-71	10
Mexicali 75/ <i>Th. podperae</i>	2n = 10x = 70	C-9	64-71	10
Mexicali 75/ <i>Th. trichophorum</i>	2n = 10x = 70	C-9	58-70	10
Mexicali 75/ <i>Th. varnense</i>	2n = 10x = 70	C-9	58-70	10
Yavaros 79/ <i>Th. varnense</i>	2n = 10x = 70	C-9	58-70	10
Capelli/ <i>Th. varnense</i>	2n = 10x = 70	C-9	67-70	10
Arlin/ <i>Th. glaucum</i>	2n = 10x = 70	C-9	65-70	15
Croc 1/ <i>Th. glaucum</i>	2n = 10x = 70	C-9	65-70	10
Yavaros 79/ <i>Th. glaucum</i>	2n = 10x = 70	C-9	64-71	10
Dverd 2/ <i>Th. glaucum</i>	2n = 10x = 70	C-9	65-69	10
Arlin/ <i>Th. acutum</i>	2n = 10x = 70	C-9	65-69	10
Altar 84// <i>Th. acutum</i> / <i>Th. intermedium</i>	2n = 10x = 70	C-9	64-72	15
Croc 1// <i>Th. acutum</i> / <i>Th. intermedium</i>	2n = 10x = 70	C-9	63-70	15
Laru// <i>Th. acutum</i> / <i>Th. intermedium</i>	2n = 10x = 70	C-9	65-68	15
Arlin// <i>Th. acutum</i> / <i>Th. intermedium</i>	2n = 10x = 70	C-9	66-69	15
Arlin 1/ <i>Th. junceiforme</i>	2n = 8x = 56	C-9	55-57	15
Altar 84/ <i>Th. junceiforme</i> *	2n = 8x = 56	C-9	54-56	15
Croc 1/ <i>Th. junceiforme</i> *	2n = 8x = 56	C-9	54-56	15
Altar 84/ <i>Elytrigia pungens</i>	2n = 10x = 70	C-9	66-68	15
Yavaros 79/ <i>Th. scirpeum</i> *	2n = 8x = 56	C-9	55-56	15
Laru/ <i>Pascopyrum spicatum</i>	2n = 10x = 70	C-9	65-71	5
Dverd/ <i>Th. trichophorum</i>	2n = 10x = 70	C-9	65-71	10
Croc 1/ <i>Th. trichophorum</i>	2n = 10x = 70	C-9	65-71	10
Rok/Kml// <i>Th. trichophorum</i>	2n = 10x = 70	C-9	66-70	10
Laru/ <i>Th. trichophorum</i>	2n = 10x = 70	C-9	65-70	10
Altar 84/ <i>Th. varnense</i>	2n = 10x = 70	C-9	67-71	10
Altar 84// <i>Th. acutum</i> / <i>Th. intermedium</i>	2n = 10x = 70	C-9	68-72	10
Laru/ <i>Th. varnense</i>	2n = 10x = 70	C-9	67-71	10
Dverd_2/ <i>Psathyrostachys juncea</i> *	2n = 6x = 42	C-9	41-42	25
Yavaros 79/ <i>Th. elongatum</i> *	2n = 6x = 42	C-3	41-42	25

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**Identification of specific *Aegilops geniculata* microsatellite markers potentially useful for detecting introgressions into bread wheat.**

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*Aegilops geniculata* is a valuable source of genes for improving wheat resistance to some biotic and abiotic stresses. Promising *Ae. geniculata* accessions with resistance to BYDV and CCN were selected and crossed with susceptible, high-yielding bread and durum wheat cultivars and the Chinese Spring *ph*-mutant line using conventional protocols (Mujeeb-Kazi et al. 1987).

For each cross, some F<sub>1</sub> hybrids were treated with colchicine to produce amphiploids. The remaining F<sub>1</sub> hybrids were backcrossed to their wheat parents to produce BC<sub>1</sub> derivatives. The BC<sub>1</sub> plants with complete chromosome set (2n = 8x = 42, AABBMU) were crossed with bread or durum wheat parents (BC<sub>2</sub>) or selfed to produce a BC<sub>1</sub>F<sub>2</sub>. This seed will be used to advance desired combinations for applied purposes via addition, substitution, and recombination lines.

To facilitate the identification of the introgressed alien material in a wheat background, we made a search for *Ae. geniculata* M- and U-genome-specific molecular markers. Microsatellite SSRs, which show adequate levels of polymorphism and are known to be widely distributed in the cereal genome, have been used in a number of studies involving genotype identification, genetic diversity, mapping, and identification of marker-trait associations. We used a set of SSRs to analyze the chromosomal constitution in the progenies of *Triticum/Ae. geniculata* crosses, avoiding the conventional time-consuming, cytological analyses.

*Ae. geniculata* is presumed to be an amphiploid of two diploid species *Ae. comosa* (M genome) and *Ae. umbellulata* (U genome) (Kimber et al. 1988). Friebe et al. (1999) confirmed the chromosomes similarities between the U and M genomes of *Ae. geniculata* and the diploid progenitors,

**Table 8.** *Triticum* and *Aegilops* genotypes used in the analysis. Genotype items include the accession number in the wide-crosses working collection (MZ) and country of origin. Some lines are resistant to barley yellow dwarf virus (\*) or cereal-cyst nematode (\*\*).

Genotype	Species	Genome
Sooty 9/Rascon 37	<i>T. durum</i>	AB
Kucuk	<i>T. durum</i>	AB
Altar 84	<i>T. durum</i>	AB
Prinia	<i>T. aestivum</i>	ABD
Chinese Spring ph	<i>T. aestivum</i>	ABD
Baviacora	<i>T. aestivum</i>	ABD
MZ 21 (France) *	<i>Ae. geniculata</i>	MU
MZ 97 (Cyprus) *	<i>Ae. geniculata</i>	MU
MZ 149 (Greece) *	<i>Ae. geniculata</i>	MU
MZ 1 (Bulgaria) **	<i>Ae. geniculata</i>	MU
MZ 61 (Tunisia) **	<i>Ae. geniculata</i>	MU
MZ 63 (Libya) **	<i>Ae. geniculata</i>	MU
MZ 77 (Jordan) **	<i>Ae. geniculata</i>	MU
MZ 96 (Cyprus) **	<i>Ae. geniculata</i>	MU
MZ 124 (Spain) **	<i>Ae. geniculata</i>	MU
MZ 161 (Bulgaria)	<i>Ae. umbellulata</i>	U
MZ 162 (Turkey)	<i>Ae. umbellulata</i>	U
MZ 163 (Iran)	<i>Ae. umbellulata</i>	U
MZ 164 (Bulgaria)	<i>Ae. comosa</i>	M
MZ 165 (Grece)	<i>Ae. comosa</i>	M
MZ 166 (Greece)	<i>Ae. comosa</i>	M
Prinia/ <i>Ae. geniculata</i> (MZ 77)	F <sub>1</sub> <i>T. aestivum/Ae. geniculata</i>	ABDMU
Altar/ <i>Ae. geniculata</i> (MZ 97)	F <sub>1</sub> <i>T. durum/Ae. geniculata</i>	ABMU
Kucuk/ <i>Ae. geniculata</i> (MZ 96)	F <sub>1</sub> <i>T. durum/Ae. geniculata</i>	ABMU

developed a complete set of *T. aestivum*/*Ae. geniculata* addition lines and assigned all *Ae. geniculata* U and M chromosomes to their homoeologous groups. Using C-banding, Fernandez-Calvin and Orellana (1992) analyzed the pairing affinities between *Ae. geniculata* and wheat genomes in an *Ae. geniculata*/*T. aestivum* hybrid with the *ph1b* mutation. They revealed that the A- and D-genome chromosomes more frequently associated with the M- and U-genome chromosomes of *Ae. geniculata* than did the wheat A or D or *Ae. geniculata* M or U chromosomes with wheat B-genome chromosomes.

Consequently, our priority was to study D-genome microsatellites derived from hexaploid wheat or *Ae. tauschii*. Wheat microsatellites mapped on A genome will be tested further in order to extend the possibility of detecting the alien material in potential A-U- and A-M-recombinations.

A set of 24 genotypes involving nine accessions of *Ae. geniculata* with resistance traits, three *T. durum* and three *T. aestivum* cultivars with high-yield potential but susceptible to diseases and pests, three *Ae. comosa*, three *Ae. umbellulata*, and three *Triticum*/*Ae. geniculata* hybrids were used in this study (Table 8. p.108). DNA extraction, PCR amplification, and gel electrophoresis were made according to standard established protocols of the CIMMYT Molecular Genetics Laboratory (Hoisington et al. 1994).

Sixty-six wheat and *Ae. tauschii* microsatellites (GWM and GDM), provided by M. Röder (IPK, Gatersleben, Germany), were tested (Table 9). All primer pairs used gave amplification products on hexaploid wheat. Among them, 53 (80.3 %) also amplified products on *Ae. geniculata*, the *Triticum*/*Ae. geniculata* hybrids, and at least one of the diploid species. Only five microsatellites revealed monomorphic bands between *Triticum* and *Ae. geniculata* genotypes. From the remaining 48 primers, 24 (50 %) showed useful polymorphisms (strong bands that easily detected size differences between *Triticum* and *Ae. geniculata* alleles) and could be employed as molecular markers for *Ae. geniculata* chromosomes. Eight of these clearly distinguished all *T. durum* and *T. aestivum* cultivars from all *Ae. geniculata* accessions tested (Table 9). The other 16 only differentiated *T. durum* from *Ae. geniculata* genotypes, only *T. aestivum* from *Ae. geniculata* genotypes, or only some specific *Triticum*/*Aegilops* combinations.

The eight selected microsatellites were mapped on chromosomes 1D (GWM848), 2D (WGM455, GDM35, and GDM148), 4D (GDM34), 5D (GWM205), and 7D (WGM974 and GDM37) (Röder et al. 1998, unpublished; Pestsova et al. 2000). They are good candidates for *Ae. geniculata* chromosome identification. These primers and those giving good differentiation between *Ae. geniculata* and *T. durum* or *T. aestivum* will be tested on a set of Chinese Spring nullisomic-tetrasomic lines and on the *T. aestivum*/*Ae. geniculata* chromosome addition lines (developed and described by Friebe et al. 1999) for assigning appropriate location of the detected loci. Once the chromosomal location of these markers is verified using cytogenetic stocks, they can be used to identify wheat lines with introgressions from *Ae. geniculata*.

**Table 9.** Number of SSRs tested and selected for their capacity to differentiate *Triticum* and *Aegilops geniculata* and their chromosomal location according Röder et al. (1998) and Pestsova et al. (2000). Items with an asterisk (\*) are from M. Röder (unpublished).

Wheat chromosome group	SSRs tested	SSRs selected	<i>Triticum</i> / <i>Ae. geniculata</i>	<i>T. durum</i> / <i>Ae. geniculata</i>	<i>T. aestivum</i> / <i>Ae. geniculata</i>	Specific combinations
1D	10	3	GWM 848 *	GWM 642	—	GWM 903 *
2D	12	5	GWM 455 GDM 35 GDM 148	—	GWM 157	GDM 93
3D	10	3	—	GWM114	GWM 161	GDM 128
4D	9	5	GDM 34	GDM 129	GDM 61 GDM 125	GWM 165
5D	9	5	GWM 205	GWM 159 GWM 192	—	GDM 99 GDM 68
6D	6	1	—	GDM 108	—	—
7D	10	2	GWM 974 * GDM 37	—	—	—
Total	66	24	8	6	4	6

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***Cytogenetics of wheat and alien Triticeae species self-fertile BC<sub>1</sub> derivatives.***

A. Cortés, V. Rosas, R. Delgado, and A. Mujeeb-Kazi.

Bread wheat cultivars when hybridized with alien Triticeae species yield perennial intergeneric hybrids that are generally of a normal cytogenetic make-up and possess half the chromosomes of each parent involved. The F<sub>1</sub> hybrid may produce amphiploids when treated with colchicine. After emasculation and pollination by a bread wheat cultivar, these hybrids are the source of the BC<sub>1</sub> progeny. When an amphiploid is not obtained, the self-sterile but female-fertile perennial F<sub>1</sub> hybrid can be directly pollinated by bread wheat to yield BC<sub>1</sub> progeny similar to that from the amphiploid route. This alternate route extends the range of alien diversity for agricultural utility. In either case, the BC<sub>1</sub> germ plasm can be screened for resistance to biotic/abiotic stresses.

A unique advantage of almost all the BC<sub>1</sub>s we have produced is their self-fertility and codominance of the expressed phenotype (Table 10, p. 111). All bread wheat/hexaploid alien species F<sub>1</sub> hybrids (2n = 6x = 42) yield BC<sub>1</sub> derivatives that are cytologically normal with 2n = 9x = 63 chromosomes. When grown and allowed to self, all are self-fertile except that the BC<sub>1</sub>F<sub>2</sub> derivatives are either 2n = 9x = 63 or 2n = 8x = 56. In the latter case, a single genome is eliminated and the remaining two are closely related as evidenced by the predominant normal meiotic relationships of bivalent associations (Delgado et al. 1996).

Intergeneric hybrids with 2n = 5x = 35 chromosomes all lead to BC<sub>1</sub> self-fertile and stable progeny of 2n = 8x = 56 chromosomes that pair as bivalents during meiosis. The 56-chromosome BC<sub>1</sub> combinations are of great cytogenetic and applied interest. Continued selfing for seed increase enables recombination to occur and be perpetuated due to the bivalent associations of the 14 chromosomes of the two closely related alien genomes. Hence, one could expect the alien addition lines produced directly from the F<sub>1</sub> hybrid and those from the BC<sub>1</sub>F<sub>n</sub> material to be structurally different. In the F<sub>1</sub> hybrid, the two related genomes (e.g. E<sub>1</sub>E<sub>2</sub> designations for *Th. curvifolium*) do not pair, and so the structural entity is unaltered. In BC<sub>1</sub>F<sub>1</sub> with 56 chromosomes, 28<sub>II</sub> are common, which indicates a E<sub>1</sub>- and E<sub>2</sub>-genomic association. This suggests structural modifications in the E<sub>1</sub> and E<sub>2</sub> chromosomes as a consequence of recombination, which could significantly alter the content of alien addition lines produced from such a BC<sub>1</sub>F<sub>n</sub> source as compared to the minimally altered chromosome addition lines produced from the BC<sub>1</sub>F<sub>1</sub> source where a single recombinational opportunity prevails. The practical advantage is that recombination may facilitate gene pyramiding. The scheme in Fig. 4 (p. 111) elucidates these crucial steps based upon a bread wheat and *Th. curvifolium* F<sub>1</sub> hybrid combination.

**Maintenance.** For each combination in Table 10, BC<sub>1</sub>F<sub>2</sub> selfed seed was obtained and 8–10 seed/entry were planted. These plants were cytologically analyzed and two plants for each combination with normal/near normal mitotic counts, vigorous growth, and near perfect bivalent meiosis were selected for further advance. From these two plants, those with the highest and plumpest seed were chosen as the BC<sub>1</sub>F<sub>3</sub> candidate for a similar process to give a BC<sub>1</sub>F<sub>4</sub> candidate. BC<sub>1</sub>F<sub>10</sub> plants with normal or near normal cytology produced seed that was bulked to give the seed number (g) indicated in Table 10.

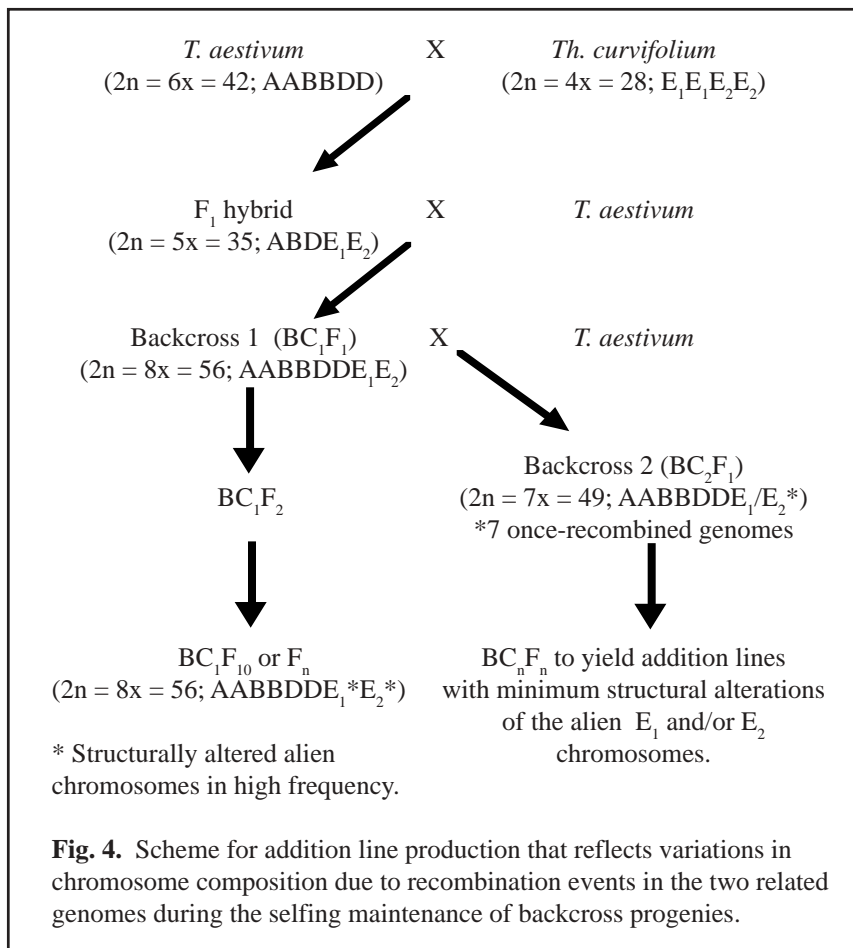
**Table 10.** Backcross 1, self-fertile bread wheat/alien Triticeae combinations elucidating their somatic mitotic detail, selfed status, and seed availability. Bread wheat cultivars in pedigrees are abbreviated as NAC = Nacozari, PVN = Pavon, CS = Chinese Spring, GLEN = Glennson, FRE = Fremont, GEN = Genaro, CNO = Ciano, FLD = Fielder. For somatic-chromosome range, the number in parentheses indicates the number of plants with the normal, expected, mitotic-chromosome number.

BC <sub>1</sub> combination (BC <sub>1</sub> F <sub>1</sub> )	Somatic chromosome number	BC <sub>1</sub> F <sub>1</sub> selfed status			Seed (g)	
		BC <sub>1</sub> F <sub>n</sub>	Total plants observed (F <sub>2</sub> -F <sub>9</sub> )	Plants in F <sub>10</sub>		Somatic range
NAC/ <i>Thinopyrum acutum</i> //PVN	56	F10	90	15	54-57 (12)	15
CS/ <i>Th. intermedium</i> //BUC	56	F10	90	15	53-56 (11)	15
CS/ <i>Th. intermedium</i> //CS	63	F10	54	18	54-63 ( 6)	7
CS/ <i>Th. pulcherrimum</i> //GLEN	56	F10	90	15	55-57 (12)	15
CS/ <i>Th. pulcherrimum</i> //PVN	63	F10	72	18	53-62 ( 0)	9
CS/ <i>Th. trichophorum</i> //GLEN	56	F10	90	15	54-57 (13)	15
NAC/ <i>Th. varnense</i> //FRE	59	F10	90	15	55-59 ( 9)	15
NAC/ <i>Th. varnense</i> //FRE	63	F10	72	18	57-63 ( 5)	5
CS/ <i>Th. bessarabicum</i> //GEN	49	F10	180	25	46-52 (18)	30
CS/ <i>Th. curvifolium</i> //CNO	56	F10	90	15	54-57 (10)	15
FLD/ <i>Th. junceiforme</i> //CNO	56	F10	90	15	55-57 (11)	15
CS// <i>Th. repens</i> /Ag. <i>desertorum</i> /3/CNO	56	F10	90	15	54-56 ( 8)	8
CS/ <i>Th. scirpeum</i> //CNO	56	F10	90	15	55-56 (12)	20
CS/ <i>Th. scirpeum</i> //PVN	56	F10	90	15	54-57 (10)	20
CS/ <i>Th. scythicum</i> //FRE	56	F10	90	15	53-56 (10)	15

From each BC<sub>1</sub>F<sub>10</sub> group of plants, one candidate plant also was selected and its progeny maintained as an individual plant source for further advance if needed and for critical study if the altered chromosomal structure by recombination within related genomes would warrant further investigation. Each such selected plant across each combination had a vigorous growth habit, was mitotically near normal or normal, had stable meiotic associations, and set abundant well-filled seed.

**Reference.**

Delgado, R., A. Cortés, V. Rosas, and A. Mujeeb-Kazi. 1996. *Triticum aestivum* x perennial Triticeae BC<sub>1</sub> self-fertile derivatives with complete and partial synthetic genomes. Ann Wheat Newslet 42:146-147.



**Fig. 4.** Scheme for addition line production that reflects variations in chromosome composition due to recombination events in the two related genomes during the selfing maintenance of backcross progenies.