ITEMS FROM HUNGARY

AGRICULTURAL RESEARCH INSTITUTE OF THE HUNGARIAN ACADEMY OF SCIENCES

Brunszvik u. 2, H-2462 Martonvásár, Hungary. www.mgki.hu, www.martonvasar.eu

Wheat season. The 2007–08 season was favorable for the winter cereals. The winter was milder than average, and neither real drought or heat stress, nor severe disease attack endangered the good yield. National wheat average reached 5.02 t/ha, that was the highest yield in the last 5 years. Quality of wheat harvested before mid July was very good, than the long rainy period reduced the quality in some regions.

Breeding.

Z. Bedö, L. Láng, O. Veisz, G. Vida, M. Rakszegi, K. Mészáros, and S. Bencze.

Breeding. Two winter wheat cultivars were registered in Hungary in 2008; the winter wheats Mv Toborzo, Mv Marsall, Mv Suba, Mv Kolo, Mv Beres, and the winter durum Mv Makaroni were registered in Kosovo.

Mv Bodri (Mv 18-05) is an early maturing short straw cultivar with high yield potential, selected from the cross 'GT6687-12R/ERYT162'. The frost resistance level determined in phytotron tests is very good. Mv Bodri has average protein content, with good gluten quality. The HMW-glutenin composition is 1,7+9,5+10, and the cultivar does not carry the T1B·1R translocation. Mv Bodri is moderately resistant to powdery mildew and leaf rust and resistant to stem rust.

Mv Toldi (Mv 19-05) is an early maturing, top quality wheat. The head type is awned, the plant height is optimal (95-100 cm), with good lodging resistance. mv Toldi has good frost tolerance and winter hardiness. The cultivar was selected from the cross 'Mironovskaya-Ostistaya/Atay85//Alfold'. Mv Toldi has high gluten content and very good baking quality; the dough strength and stability, especially, are excellent. The HMW composition is 2*, 7+9, 5+10.

Disease resistance studies.

Marker-assisted selection. Within the framework of international projects (Bioexploit-EU FP6 and NAP-BIO-NEWSEED), molecular marker-assisted selection is being used to incorporate known resistance genes (*Lr9*, *Lr24*, *Lr25*, *Lr29*, *Lr37*, and *Lr47*) into four Martonvásár cultivars (Mv Emma, Mv Madrigál, Mv Magvas, and Mv Pálma). The presence of the resistance genes is detected using public PCR-based (STS and SCAR) markers in various backcross generations.

Effective *Lr* **genes.** The degree of infection exhibited by genotypes carrying known leaf rust resistance genes was tested in artificially inoculated nursery. Genes *Lr9*, *Lr19*, *Lr24*, *Lr25*, *Lr28*, *Lr29*, *Lr35*, *Lr37*, and *Lr47* continued to provide effective protection against leaf rust in Martonvásár in 2008.

Powdery mildew race survey. Powdery mildew isolates collected in the Martonvásár area were used to determine the race composition of the pathogen population, the degree of virulence, and the efficiency of known resistance genes. The races dominant in 2008 (and their frequency) were 51 (43.5%), 76 (25.8%), and 77 (9.7%). The virulence complexity in the pathogen population was calculated as 6.18, which was higher than in any previous year. Almost complete protection against the tested wheat powdery mildew isolates was provided by the resistance gene combination Pm4a+ (Khapli).

Abiotic stress resistance studies. Work has begun on the collection of genetic materials and the development of various types of population for an analysis of the genetic regulation of heat tolerance. Wheat cultivars with various types of ad-

aptation were identified in preliminary studies, after which a cross was made between the heat-tolerant, drought-sensitive cultivar Mv Magma and the heat-sensitive, drought-tolerant cultivar Plainsman V. Homozygous lines were developed either from anther culture or using the SSD method. A total of 11,424 anthers were cultured from the 'Plainsman V/Mv Magma' cross (STR3 line), 39.3% plants of which were successfully regenerated, with 12.4% green plant regeneration. At present, 222 DH₀ plants of microspore origin are being raised from this line in the phytotron. In the case of the 'Mv Magma/Plainsman V' cross, 6,250 anthers were cultured. The plant regeneration ratio was 28.3%, and the green plant regeneration ratio 11%. A total of 281 plants have been planted out from this cross.

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A N N U A L W H & A T N & W S L & T T & R Department of Plant Genetic Resources and Organic Breeding.

M. Molnár-Láng, G. Kovács, É. Szakács, A. Schneider, I. Molnár, and A. Sepsi.

Characterization of a leaf rust-resistant wheat-Thinopyrum ponticum partial amphiploid BE-1 using sequential multicolor GISH and FISH. In situ hybridization (multicolor GISH and FISH) was used to characterize the genomic composition of the wheat-Th. ponticum partial amphiploid BE-1. The amphiploid is a high-protein line having resistance to leaf rust and powdery mildew and has a total of 56 chromosomes per cell. Multicolor GISH using J., A., and D-genomic probes showed 16 chromosomes originating from Th. ponticum and 14 A-genome, 14 B-genome, and 12 D-genome chromosomes. Six of the Th. ponticum chromosomes carried segments different from the J genome in their centromeric regions. We demonstrated that these alien chromosome segments did not originate from the A, B, or D genomes of wheat, so the translocation chromosomes were considered to be J^s-type chromosomes carrying segments similar to the S genome near the centromeres. Rearrangements between the A and D genomes of wheat were detected. FISH, using Afa family, pSc119.2, and pTa71 probes, allowed the identification of all the wheat chromosomes present and the determination of the chromosomes involved in the translocations. The 4A and 7A chromosomes were identified as being involved in intergenomic translocations. The replaced wheat chromosome was identified as 7D. The localization of these repetitive DNA clones on the Th. ponticum chromosomes of the amphiploid was described in the present study. On the basis of their multicolor FISH patterns, the alien chromosomes could be arranged in eight pairs and could also be differentiated unequivocally from each other.

Polymorphism analysis using 1RS-specific molecular markers in rye cultivars of various origin. Six different 1RS-specific molecular markers (RMS13, Bmac213, GPI, 5S, SCM9, and IAG95) were tested in 20 rye cultivars of various origin. The aim of the experiments was to choose rye cultivars which give polymorphic PCR products with these 1RS-specific markers compared to the wheat cultivar Mv Magdaléna, which contains the T1BL·1RS translocation. The polymorphic rye cultivars can be presumed to differ from the T1BL·1RS translocation originating from the Petkus rye cultivar and will hopefully carry effective resistance genes that can be incorporated into the T1BL·1RS translocation in wheat. Twenty rye cultivars (at least two plants/cultivar) were analyzed with these markers. Of the 52 rye samples analyzed, three plants were polymorphic, one (Kisvárdai Alacsony from Hungary) for the 5S marker, one (Kriszta from Hungary) for the RMS13 marker, and one (Porto from Portugal) for the SCM9 marker. The polymorphic plants were grown to maturity in the phytotron.

Fluorescent in situ hybridization polymorphism on the 1RS chromosome arms of cultivated Secale cereale species. The study was focused on the selection of S. cereale cultivars of different geographic origin showing polymorphism detectable by FISH on their 1RS chromosome arms. One perennial and four annual genotypes were tested. FISH with the DNA probes pSc119.2 and (AAC)_s. The pSc119.2 probe gave hybridization signals different from that of the rye 'Petkus' on the 1RS arms of all five rye cultivars examined. Differences were manifested mainly in the intensity of the labelling, but the complete lack of FISH signals and double signals were also observed. The other chromosomes of the five rye cultivars could also be identified, and polymorphism for both DNA probes was detected on them.

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Genetic and physiological studies.

A. Vágújfalvi, A. Soltész, I. Vashegyi, G. Kocsy, and G. Galiba.

Verification of candidate genes for wheat frost tolerance by transformation. *Cbf* genes are the most likely candidate genes for frost tolerance in cereals. Recently, we proved that out of the 13 *Cbf* genes encoded in the wheat genome, *Cbf14* and *Cbf15* are the most important in the control of low temperature tolerance in wheat. In a collaborative work with Dr. Wendy Harwood (Department of Crop Genetics, John Innes Centre, Norwich UK), barley plants were transformed with these candidate genes via an *Agrobacterium*-mediated transformation method. The presence of the transgene was confirmed by PCR. Transgenic lines will be tested for improved abiotic stress tolerance.

Identification and characterization of stress-responsive candidate genes in wheat. A wheat oligonucleotide microarray (15,000 oligos) was developed in a collaboration with the Biological Research Center in Szeged (Dr. János Györgyey). Cold-hardened wheat genotypes with different freezing tolerance were compared for the selection of cold-responsive genes which may be involved in the hardening process. The earlier identified cold-responsive candidate genes were further characterized. Their expression was examined following various abiotic stress treatments in different organs.

Publications.

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Cell Biology Department.

B. Barnabás, K. Jäger, H. Ambrus, and A. Fábián.

Cryopreservation of wheat gametic cells. The cryopreservation method of wheat egg cells using a simple one-step vitrification procedure was elaborated. The procedure involved loading the cells with 25% of a vitrification solution, dehydration, droplet vitrification, and storage in liquid nitrogen, unloading, and rehydration of the cells. Supplementation with 120 mM ascorbic acid significantly increased the proportion of viable egg cells after de- and rehydration. During the early phase of rehydration, ascorbic acid reduced the probability of membrane damage caused by rapid water uptake. Maintaining the temperature of the cells at 0°C during the de- and rehydration processes increased cell survival by 29%. Wheat egg cells dehydrated and vitrified in vitrification solution VS4A, consisting of 30% glycerol, 10% sucrose, 120 mM ascorbic acid, and 5% propylene glycol, subsequently thawed, unloaded in BVSA, and rehydrated, showed post-thaw cell viability of 12.7%.

Abiotic stress tolerance. The effects of meiotic water deficit and combined heat and drought stress were studied on microsporogenesis and fertility of wheat. Among normal pollen, 12% of the drought-stressed, tolerant Plainsman V and

34% of the sensitive Cappelle Desprez pollen were arrested at early stages of gametogenesis. Drought stress manifested in significant reduction of the mean fertility in both sensitive (41%) and tolerant (33%) genotypes. Combined stress applied during meiosis among developmental arrests caused serious morphological anomalies in the sensitive genotype. When plants of the Planisman V were subjected to simultaneous drought and heat, an additional 24% significant decrease occurred in the ratio of normal pollen. The fertility of the basal part of the spikes was similar to the control in both genotypes, but the seed set in the middle and on the top of the spikes decreased significantly as a consequence of combined drought and heat stress.

Wheat plants of drought-tolerant Plainsman V and drought-sensitive Cappelle Desprez genotypes were subjected to drought and combined drought and elevated, 34/24°C day/night temperature at three various phases of reproductive development; at meiosis, from the 1st to the 5th day after pollination; and from the 5th to the 9th day of seed development. After seed maturation, the germination frequency of the grains was calculated, and the seminal root number of the seedlings was determined. Neither of the stress treatments affected the germination percentage of the genotypes significantly. Neither of the treatments reduced the number of seminal roots in the case of the tolerant genotype. However, in the sensitive cultivar, the ratio of seedlings with only one root increased to 42% after drought stress applied 5–9 days after pollination (DAP). In this genotype, the combined stress increased the proportion of one-rooted seedlings up to 56% and 78% when applied during 1–5 DAP and 5–9 DAP, respectively.

Cytological alterations caused by aluminum (Al) were examined in anther cultures of the commercial wheat Mv Pálma, and the efficiency of in vitro selection was demonstrated. Although the anther walls retarded the appearance of toxicity symptoms, cytological changes similar to those observed in root cells were detected in the microspores. The severity of Al toxicity and the efficiency of selection depended on the Al concentration and the mode of treatment. Single Al treatments (0.6 and, especially, 1.6 mM) gave DH lines with increased Al tolerance. Repeated Al treatment severely inhibited the cell division of the microspores, and it was lethal even at a concentration as low as 0.6 mM. The results show that microspore embryogenesis can be exploited for studying the cytological effect of Al and for increasing the Al tolerance of wheat.

Paraquat and cold tolerance of DH maize plants selected and regenerated from microspores using paraquat as ROS progenitor were compared to those of a nonselected DH line and the original hybrid. Three of five paraquat-tolerant DH lines possess higher cold tolerance than the control DH line and the original hybrid during the germination period. On the other hand, plants exposed to low-temperature stress (8°C) at the early autotrophic phase of developments resulted in a higher cold tolerance in all of the five paraquat-selected DH lines. The results demonstrated that the microspore-selected DH lines using paraquat as a ROS progenitor resulted not only in higher tolerance against the paraquat-mediated oxidative damage but helped in the protection against the low-temperature stress.

Embryo development in wheat. Currently, great interest is shown in understanding the process of embryogenesis and, due to the relative inaccessibility of these structures *in planta*, extended studies are carried out in various *in vitro* systems. Embryos developing *in vitro* closely followed the morphology of their *in planta* counterparts, and their cell types and tissues also were similar, demonstrating the applicability of the present culture system for studying the process of zygotic embryogenesis. However, some important differences also were detected in the case of *in vitro* development; the disturbance of or lack of initial polarity led to changes in the division symmetry of the zygotes and subsequently to the formation of uniform cells in the globular structures. Presumably, differences between the *in vitro* and *in planta* environments resulted in a lower level of differentiation and maturation in *in vitro* embryos and in abundant starch and protein accumulation in the scutellum.

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ITEMS FROM INDIA

BHABHA ATOMIC RESEARCH CENTRE

Nuclear Agriculture & Biotechnology Division, Mumbai-400085, India.

Current activities: Improvement of wheat quality and rust resistance in Indian wheat.

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

Improvement of wheat for quality in an Indian wheat background is being carried out by using HMW-glutenin subunits as a selection criterion. The rust resistance genes Sr31/Lr26/Yr9 and Sr26, Sr24/Lr24 are being combined with high yielding ability and specific HMW subunits. Selected lines from several intervarietal crosses in different generations (F,, F_3 , and F_4) are being evaluated.

Radiation-induced mutations in wheat.

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

Earlier, the cultivar C306, known for its good chapati-making quality was treated with gamma rays, and mutants with early flowering were isolated in the M₂ generation. The parent showed anthesis in about 75 days, whereas the mutants showed anthesis in 50 to 63 days. Seven mutant lines were analysed for quality traits. Grain-protein content ranged from 11.9 to 14.9% compared to 13.1% in the parent. SDS-PAGE of total grain protein showed that the mutants had an unaltered HMW-glutenin subunit pattern. Rheological properties estimated using a Brabender Farinograph showed that the mutant lines had comparable water absorption, dough-development time, dough stability, degree of softening, and quality number. The early mutants are being carried forward.

MP3054 and Hindi 62 were treated earlier with gamma rays. M,-generation plants were grown in 2008-09. Plants that flowered early and had reduced culm length were identified as mutants and harvested individually.

A bread wheat genetic stock with morphological markers for dark glumes, hairy glumes, hairy leaf, purple culm, and red grain was mutagenized with gamma rays. In the M, generation, plants with altered morphology were identified and individually harvested. The M₂ was grown as plant-to-row progeny. Although variations for the extent of glume pigmentation or hairiness, spike morphology, and culm length were observed, lines were found to segregate for the mutant traits.

Validation and marker-assisted selection for rust resistance and quality-related genes in Indian wheat.

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

Validation of SCAR marker SCS1302₆₀₉ for gene Sr24. Molecular markers developed for traits such as disease resistance using a specific genotype may not necessarily work in others. Hence, validating markers in diverse genotypes is important. In this study, marker SCS1302₆₀₉ (Gupta et al. 2006) reported for Lr24/Sr24 was validated by analyzing