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ITEMS FROM HUNGARY

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Wheat season. After the extremely dry 2011 (263 mm), the drought continued in 2012. The total yearly precipitation in 2012 reached only 337 mm, compared to the average 550 mm. Due to the dry conditions, no disease epidemics occurred. The national wheat average reached only 3.7 t/ha, which is lower than the last 20-year average. Wheat still performed relatively much better than corn, which had only half the yield compared to the potential. The quality of the harvested wheat was good.

Breeding.

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

New releases. Four Martonvásár winter wheat cultivars were registered in Hungary in 2012.

Mv Pengő (Mv 10-09) is a very high-yielding, early maturing, hard red winter wheat. The origin of the cultivar is 'Marsall/CF621'. Mv Pengő is supposed to replace Mv Marshall, the highest yielding Martonvásár wheat present on the market at the moment. The cultivar has good winter hardiness and good lodging resistance thanks to the presence of Rht1. Mv Pengő has better bread-making quality than that of Mv Marsall; its wet gluten content is 30–32%, Farinograph quality B1(A2), Alveograph W value 220–300, with a low P/L ratio. Mv Pengő is moderately resistant to powdery mildew and leaf rust.

Mv Nádor (Mv15-09) is a very high-yielding, early maturing, dwarf cultivar designed for farmers applying high doses of fertilizer and seeking high yield. Under high-input conditions, Mv Nádor is capable of achieving record yields. Selected from the cross 'DI9812/Vekni', Mv Nádor inherited a high wet gluten content (34–36%) from Mv Vekni, with a dough quality characterized as B1 Farinograph category. Mv Nádor carries the T1B·1R translocation, and possess medium Alveograph and Extensograph quality. Mv Nádor has reliable winter hardiness, excellent lodging resistance, and is moderately resistant to leaf diseases.

Mv Kokárda (Mv09-09) is a high-yielding, soft red winter wheat with excellent agronomical properties from the cross 'Fleming/Amanda'. The cultivar has been selected for industrial utilization. Mv Kokárda has a low (23–26%) wet gluten content, flour water uptake lower than 50%, and very low Farinograph stability. Mv Kokárda has excellent resistance to lodging, leaf rust, and powdery mildew. Because of good adaptability, Mv Kokárda can be successfully grown under different yield levels.

Mv Pántlika (Mv24-09) is a medium-early, quality hard red winter wheat selected from the cross 'MV10-2000/Marsall', with a maturity time is similar to that of Mv Suba. In spite of its excellent quality, the productivity of Mv Pántlika is comparable to that of medium-quality, high-yielding cultivars. The cultivar has a high gluten content (34–37%), very good rheological quality, a A1–A2 Farinograph category, and 320–390 Alveograph W value.

Breeding for quality traits. β -glucan content of wheat. The effects of heat (H), drought (D), and the combination of the two stresses (H+D) on the dietary fiber content and composition (arabinoxylan and β -glucan) of three winter wheat cultivars (Plainsman V, Mv Magma, and Fatima 2) were determined. The stress treatments were applied on the 12th day after heading and continued for 15 days. We used the 1,000-kernel weight and protein content, β -glucan and arabinoxylan (AX), and the structure of AX β -glucan to determine the bt enzymatic fingerprint. Principal component analysis showed that heat and drought stress decreased the 1,000-kernel weight and the β -glucan contents of the seeds relative to the untreated control samples, whereas the protein and AX contents of the same samples increased when calculated on a dry weight basis. The highest amounts of AX and protein were in the H+D-stressed samples, with heat stress also increasing the water extractability of the AX. All the stresses decreased the β -glucan content of the seeds as well as the quantity of the DP3 and DP4 unit in all cultivars. However, whereas the content of AX content generally increased under all stresses, drought stress had negative effect on the AX content of the drought-tolerant Plainsman V. Fatima 2 behaved similar to Plainsman V with regard to drought tolerance, but was very sensitive to heat stress, whereas Mv Magma was the most resistant to heat stress.

Breeding for high arabinoxylan content. Cereals contain some promoting bioactive components, but their advantages have not or only partly been utilized. Our goal is to develop different cereal-based, functional products for the bakery, pasta-, flour, and the ready-to-eat food market keeping in mind the requirements of regional Hungarian tradition and easy utilization. The first step is to develop an excellent raw/plant material for the processing industry. During the 'Healthgrain' project, the exotic, Chinese wheat cultivar Yumai-34 was identified to contain extremely high quantities of water-extractable arabinoxylans (WE-AX) in the flour compared to wheat cultivars now in commercial worldwide. This property was utilized to start a breeding program to produce wheat cultivars with good adaptability and a high content of dietary fiber. We studied whether arabinoxylans could effectively be used for breeding purposes, and the improvement of the nutritional properties could have an influence on the yielding potential and processing quality of wheat. The F₇–F₈ generations of Yumai-34 with Mv-Emese, Mv-Mambo, Ukrainka, Lupus, or Courtot had already been developed. The greatest number of crossing lines was produced with Ukrainka, whereas the highest WE-AX content, similar to that

of the control (~1% d.m.), was analyzed in lines from the 'Lupus/Yumai-34' cross. The increase in WE-AX content had a negative effect on the yield ($r\ 1\% = -0.427$), heading date ($r\ 1\% = -0.510$), and the Zeleny sedimentation ($r\ 5\% = -0.299$) of the breeding lines in all the three years of this study. These results will help to develop the selection strategy of high dietary fiber lines by focusing on the early maturing breeding lines. Recently, 36 lines were planted in three replications to check the homogeneity and stability of the selected lines, especially with regard to arabinoxylan content.

Breeding for starch properties. The ratio of amylose to amylopectin in starch is decisive for determining the physico-chemical properties of the starch, susceptibility to enzymatic hydrolysis, gelling, and pasting behavior, which could be of technological quality importance. Both low- and high-amylose wheat are useful for the different effects they have on flour or semolina functionalities. High-amylose starches are used widely as thickeners and strong gelling agents, but they also have a positive effect on the dietary fiber content of the flour by increasing the level of starch resistant to human enzymatic digestion after consumption. The resistant starch plays significant role inside the intestine, protecting against diseases such as colon cancer, type-II diabetes, and obesity.

In order to produce healthier wheat genotypes with high amylose content (40%) and good agronomical properties, triple mutant lines (*Sgp-A1*, *Sgp-B1*, and *Sgp-D1*) have been created (Lafiandra et al.). These lines were multiplied and crossed with five current commercial varieties of bread wheat (Solstice, Lona, Koreli, Ukrainka, and Yumai-34) to introgress the three mutations. Three backcrosses were carried out, during which marker-assisted selection was made in each generation in order to select the possible triple mutant lines for backcrossing. Finally, in the BC₃ generation, 0.82% of the 380 studied lines were found to be triple mutants, 13% mutant and/or heterozygote for a given allele, and 30.79% of the lines were possible double mutants. Forty-seven possible triple mutant lines were analyzed in details for amylose content, and 17 genotypes were found to have nearly 40% of amylose content (37–44%) comparable to the mutant control. From these high-amylose lines, three had already been mutants for all the three alleles. Seeds of these lines will be multiplied and studied for processing quality by measuring starch viscosity properties.

Pannon wheat R&D system. The Pannon wheat R&D system is a complex, regional program in which internationally compatible quality criteria were developed in order to improve the exportability of the Hungarian wheat cultivars by taking into consideration the expectations of the processing industry and the consumers. As a next step, drought-resistant, hard wheat cultivars with high protein and gluten content were developed that are sufficient to the quality criteria. To reach the appropriate quality, a plant management and consulting system also was developed for supporting the work of the farmers, and a fast screening and transfer system was developed to help millers and the processing industry to identify the appropriate seed resources.

Based on these results, critical points setting back the realization of the whole system were identified. These are the questions of extra production costs, the quality stability of the cultivars, the different scaling of the real seed production, or the realization of raw material quality in the processing industry.

In overcoming these problems, the development of a new, reconsidered, national strategy was begun in 2009. In the frame of this project, new breeding lines with stable quality were developed with special attention relating to the kernel type and the rheology. Cultivars and breeding lines were tested in large-scale experiments, agrotechniques optimized, and the stability of the quality monitored. Confirmation of the benefits of the Pannon wheat quality and its realization in the processing technologies has already begun.

Disease resistance studies. Stem rust resistance. Among the genotypes carrying designated *Sr* genes, lines with *Sr28*, *Sr30*, *Sr34*, or *Sr36* were highly resistant to stem rust, and additionally some more major resistance genes (*Sr6*, *Sr9e*, *Sr17*, and *Sr31*) provided effective resistance against the spread of the pathogen.

Powdery mildew virulence survey. Studies on the composition of the wheat powdery mildew population showed that race 77 was present even in higher ratio (81.5%), than in 2011, followed by the race 76 (11.2%). Race 51, which infected all the differentials of Nover's set, appeared in even lower frequency than in the previous year (2.1%). The virulence complexity of the pathogen population was 5.16, which represented a further decline compared to previous years.

Fusarium head blight resistance. The molecular marker linkage map, based on a population with Ning 8331 (resistant parent) and Martonvásári 17 (moderately susceptible parent) origin, was constructed. Two major QTL linked with FHB resistance were identified in Ning 8331. One of them, with a putative effect on type-II FHB resistance, is located on the 2DL chromosome arm. The effect of 3BS QTL (*Fhb1*) was proven both in sprayed and in single floret inoculated experiments.

Abiotic stress resistance studies. Investigations on the osmotic stress tolerance of the dihaploid population in the seedling stage showed variation in the sensitivity of DH lines to the 15% PEG-6000 solution. The mean value of shoot length and shoot and root fresh and dry weight of the population decreased considerably in response to PEG treatment compared to control values.

The DArT analysis of the RIL population originating from the cross between the heat stress tolerant Mv Magma and the sensitive Plainsman V wheat cultivars has been completed resulting in 967 DArT markers with known chromosomal locations. Significant differences in the marker coverage of the three wheat genomes were noted; more than 50% of the markers are located on the B genome, 29% on the A genome, and only 20% on the D genome. The marker coverage of the homologous chromosome groups was the highest for groups 7 and 3 and the lowest for groups 4 and 5. Chromosome 1B had the highest number of markers (153), whereas chromosome 6D had only two DArT markers.

Investigations on the water use efficiency of the small-grain cereals revealed that water uptake was most intense during the heading. A connection also was found between the length of the vegetation period and the developmental stage in which plants were most sensitive to water shortages. Water withdrawal during the stage of the first node appearance resulted in a significant yield loss of the cultivars, which had a relatively rapid rate of development. For genotypes with a longer vegetation period, however, the highest sensitivity to water shortage was recorded at the grain-filling stage. In a greenhouse experiment, the water use efficiency (grain yield/total water use) of the winter wheat genotypes was found to be between 0.7 and 1.6 at the optimum water supply level. Drought stress treatment resulted in a decline of the water use efficiency in different phenological stages. The WUE values were between 0.56 and 1.39 at the stage of the first node appearance, 0.28–1.28 at heading, and 0.16–1.18 at the grain-filling period.

Growth of wheat plants at elevated CO₂ level (EC, 750 µmol/mol) was found to result in a more relaxed state of the antioxidant enzyme system in the plants of all three cultivars. The very low, uniform activity values of glutathione reductase, glutathione-S-transferase (GST), ascorbate peroxidase (APX), and guaiacol peroxidase were caused by activity decreases in cultivars with higher ambient values. A parallel increase in catalase (CAT) was recorded in two cultivars. Despite the fact, that the sensitive cultivar had better tolerance to drought at elevated CO₂ levels, drought triggered a more pronounced, general response in the antioxidant enzyme system at EC, leading to very high values of APX, CAT, and GST that were very similar in all the cultivars. These high values were due to a higher level of oxidative stress under drought at EC; because the decline in net photosynthesis was faster compared to ambient values, while there was no change in the rate of electron transport.

Molecular breeding. Using diagnostic molecular markers, the allele compositions of the *Vrn-A1*, *Vrn-B1*, *Vrn-D1*, *Ppd-B1*, and *Ppd-D1* genes were determined in a wheat collection consisting of 683 genotypes. On the basis of these five genes, the cultivar collection (521 European, 62 Asian, 6 African, 90 American, and 4 Australian cultivars and lines) could be divided into 24 combination groups. The distribution frequencies of the allele groups differed not only from one continent to the other, but also between various geographic regions of Europe and between the countries of Central Europe. The dominant (spring) *VRN-A1*, *VRN-B1*, and *VRN-D1* alleles were present at a frequency of 6–7% in the total set of the genotypes. The *VRN-A1* and *VRN-B1* alleles were more frequent in cultivars from Australia, Africa, and America, whereas the *VRN-D1* allele was more common in Asian cultivars. The semi-dominant *Ppd-D1a* photoperiod-insensitive allele was present in 57% of the cultivars and most frequently observed in Asian, Australian, and European cultivars. Within Europe, the photoperiod-insensitive allele was dominant in the eastern, southern, and southeastern regions (93%, 85%, and 93%, respectively), and the photoperiod-sensitive allele was more frequent (63%) in western Europe. The two alleles of *Ppd-D1* gene were present in almost equal proportions (52% vs. 48%) in central Europe. The *Ppd-B1* photoperiod-insensitive allele was present in 22% (151) of the genotypes, all representing breeding materials from the Asian, American, and European continents; 38 genotypes from Asia (61%), 23 from America (26%), and 88 from Europe (17%). The vast majority of the European genotypes bearing this allele type originated from central and southeastern Europe.

The molecular marker linkage map of the 'MvTD10-98/PWD1216' population was constructed using AFLP (six primer combinations) and DArT (~2,000 probes) markers. The map contains 462 markers arranged in 21 linkage groups and extends over 1,784 cM. The map is based on 347 DArT markers, which assigned the 21 linkage groups to 14 chromosomes. Four different QTL linked with yellow index (Minolta b*) were identified on chromosome 1B, 3B, 5B, and 7A. The strongest QTL, located on the distal part of the short arm of chromosome 7A, explained 17.3% of the phenotypic variation, and the remaining three QTL explained 7.3–11.9% of the variation of the yellow index. The significant effect of the QTL located on the chromosome 3B and 7A could be detected in five consecutive years (2007–11). The alleles with positive effect were in all cases originated from the PWD1216 parent.

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Identification and phenotypic description of new wheat – six-rowed, winter barley disomic additions. To increase the allelic variation in wheat–barley introgression lines, new wheat–barley disomic addition lines were developed containing the 2H, 3H, 4H, 6H, and 7H chromosomes of the six-rowed Ukrainian winter barley Manas. This cultivar is agronomically much better adapted to the central European environment than the two-rowed spring barley Betzes previously used. A single ‘Asakaze/Manas’ wheat × barley hybrid plant was multiplied in vitro and one backcross plant was obtained after pollinating 354 regenerated hybrids with wheat. The addition lines were selected from the self-fertilized seeds of the 16 BC₂ plants using genomic in situ hybridization. The addition lines were identified by fluorescence in situ hybridization (FISH) using repetitive DNA probes (HvT01, GAA, pTa71, and Afa family), followed by confirmation with barley SSR markers. The addition lines were grown in the phytotron and in the field, and morphological parameters (plant height, fertility, tillering, and spike characteristics) were measured. The production of the disomic additions will make it possible to incorporate the DNA of six-rowed winter barley into the wheat genome. Addition lines are useful for genetic studies on the traits of six-rowed winter barley and for producing new barley dissection lines.

High-resolution, molecular cytogenetic techniques in plants: pachytene- and fiber-FISH. FISH is the most versatile and accurate molecular cytogenetic technique for determining euchromatic-heterochromatic boundaries and the locations of repetitive and single-copy DNA sequences and of chromosome-specific BAC clones on chromosomes. The combination of cytogenetic and genetic methods yields a high-resolution physical map. FISH allows direct mapping of specific DNA sequences inside the cell (interphase nuclei), along meiotic pachytene chromosomes and isolated chromatin (DNA fibers). The increased sensitivity of the technique, and its ability to detect gene locations provide a powerful research tool for genetic and prebreeding studies. FISH-based, physical mapping plays an important role and is increasingly

used for studies at the cytological level on the chromatin organization that controls gene expression and regulation. The mini-review describes some of the benefits of alternative FISH-based techniques and their application for studying plant chromosomes and genomes.

Karyotypic analysis of *Triticum monococcum* using standard repetitive DNA probes and simple-sequence repeats.

Triticum monococcum represents an important source of useful genes and alleles that it would be desirable to use in wheat breeding programs. The well-defined landmarks on the A^m chromosomes could accelerate the targeted introgression of *T. monococcum* chromatin into the wheat genome. FISH, using the repetitive DNA probes pSc119.2, the Afa family, and pTa71, showed that the pSc119.2 probe was not suitable for the identification of A^m chromosomes. In contrast, the whole set of A^m chromosomes (especially chromosomes 1, 4, 5, and 7) could be discriminated based on the hybridization pattern of pTa71 and the Afa family. In situ hybridization with microsatellite motifs (GAA, CAG, AAC, and AGG) proved that SSRs represent additional landmarks for the identification of A^m chromosomes. The most promising SSR probes were the GAA and CAG motifs, which clearly discriminated the 6A^m chromosome and, when used in combination with the Afa family and pTa71 probes, allowed the whole set of A^m chromosomes to be reliably identified. In conclusion, FISH using the repetitive DNA probes of the Afa family and pTa71, combined with SSR probes, makes it possible to identify the A^m chromosomes of *T. monococcum* and discriminate them from A^u chromosomes in the polyploid wheat background.

Detection of various U and M chromosomes in wheat–*Aegilops biuncialis* hybrids and derivatives using FISH and molecular markers.

The aim of the study was to select wheat–*Ae. biuncialis* addition lines with *Ae. biuncialis* chromosomes differing from those that were introgressed into the wheat–*Ae. biuncialis* addition lines produced earlier in Martonvásár, Hungary. During the experiments, new wheat–*Ae. biuncialis* addition lines with chromosomes 2U^b, 3U^b, 5U^b, 6U^b, 7U^b, 5M^b, 6M^b, and 7M^b were selected. The 2U^b disomic addition line is relatively stable; 91% of the progenies contain this chromosome pair. The 6M^b disomic addition line proved to be dwarf and sterile, but still exists as a monosomic addition line. Progenies analyzed from the 6U^b monosomic addition line did not carry the 6U^b chromosome. One plant containing the 5U^b, 3U^b, and 7U^b chromosomes and one plant with chromosomes 5M^b, 6M^b, and 7M^b showed very low fertility. Each of the plants produced a single seed, but seeds of the parent plants are still available. Line 49/00 carried a submetacentric *Ae. biuncialis* chromosome pair and the chromosome number 44 has been constant for several generations. After FISH, no hybridization site was observed on the *Ae. biuncialis* chromosome pair using the pSc119.2 and Afa family repetitive DNA probes, so it was not possible to identify the *Ae. biuncialis* chromosome pair. However, using wheat SSR markers and the (GAA)_n microsatellite DNA probe allowed it to be characterized more accurately. These new lines facilitate gene transfer from *Ae. biuncialis* into cultivated wheat and the selection of U- and M-genome-specific wheat SSR markers.

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ITEMS FROM INDIA

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Molecular breeding, induced mutagenesis, and transcriptome analysis in wheat.

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Deployment of molecular markers for the improvement of some important quality traits and drought tolerance in bread wheat. We are attempting to pyramid one or more QTL/genes for grain quality traits along with the *Lr24* gene for leaf rust resistance into a number of high-yielding Indian wheat cultivars with a view to develop wheat cultivars with improved grain protein content (GPC), leaf rust resistance, and preharvest sprouting tolerance (PHST) using marker-assisted selection (MAS). QTL for grain yield under water stress are also being introgressed into high-yielding Indian wheat cultivars, which are otherwise drought sensitive. The progress made in relation to the above objectives is summarized below.

MAS for grain protein content (GPC) and leaf rust resistance. To introgress two genes, one for high GPC (*Gpc-B1*) and the other for leaf rust resistance (*Lr24*), two high-yielding recipient Indian bread wheat cultivars (Lok1 and HD2967) were crossed with a donor genotype PBW343 (*Gpc-B1+Lr24*). The initial crosses were made at the Research Farm, CCSU, Meerut, during 2009–10 crop season. The two resulting F_1 s were grown and backcrossed with the respective recipient genotype in an off-season nursery at the Keylong Research Station during the summer of 2010. Seeds of two BC_1F_1 s were planted at Research Farm CCSU, Meerut, during 2010–11. Foreground MAS in the two BC_1F_1 s was carried out in two steps; the two BC_1F_1 populations included 938 plants for the recipient parents Lok1 and 1,015 plants for the recipient parent HD2967. These BC_1F_1 plants were screened using a SSR marker (ucw108) specific to the *Gpc-B1* gene for high GPC. As a result, 340 plants involving Lok1 and 149 plants involving HD2967 were selected from the two backcross populations. Foreground selection for leaf rust resistance gene *Lr24* was carried out on the GPC positive plants using SCAR marker SCS73719; 147 BC_1F_1 plants involving Lok1 and 39 BC_1F_1 plants involving HD2967 each carried both the *Gpc-B1* and *Lr24* genes, and were selected for background selection.

For background selection, a set of 127 SSRs were used for plants involving Lok1 and 118 SSRs were used for plants involving HD2967. The SSR markers for background selection were chosen such that these were evenly distributed on all the 21 wheat chromosomes. In this manner, eight BC_1F_1 plants (involving Lok1) with >80% RPG recovery were selected and these plants were backcrossed with the recipient genotype Lok1 again to obtain BC_2F_1 seeds. However, no BC_1F_1 plant involving HD2967 could be selected for further backcrossing, because each of the selected plants contained donor parent allele in homozygous condition for one or more SSR markers. This observation was unexpected and could not be explained without further studies, which we propose to undertake in future.

The BC_2F_1 population involving Lok1 was raised in an off-season nursery at Keylong Research Station during 2011. Foreground selection for *Gpc-B1* and *Lr24* was in a set of 248 BC_2F_1 plants; 43 positive plants with the two genes were selected. Background selection of these 43 plants with the help of SSR markers that were in heterozygous state in BC_1F_1 plants led to the selection of eight plants with >93% RPG recovery, and BC_2F_1 seed was harvested. Eight BC_2F_2 progenies were planted during the 2011–12 crop season at the Research Farm at CCSU, Meerut. Out of 380 BC_2F_2