

ITEMS FROM HUNGARY

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The wheat season. A rainy spring and summer characterized the 2009–10 wheat season. Precipitation exceeded 1,000 mm compared to the average of 550 mm. Due to the wet conditions, heavy *Septoria*, *Helminthosporium*, head blight, and leaf rust epidemics occurred. The national wheat average reached only 3.72 t/ha, slightly lower than the 3.85 t/ha harvested in the dry year 2009. Quality of wheat harvested was average.

Breeding.

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Breeding. Four Martonvásár winter wheat cultivars were registered in Hungary in 2010.

Mv Tallér (Mv 10-07) is an early maturing cultivar with outstanding yield potential and medium breadmaking quality, selected from the cross 'Andy-2/F15//Gore/3/Mambo'. The cultivar has very good winter hardiness, good lodging resistance. Mv Tallér is a medium quality milling wheat with a wet gluten content of 30–32%, Farinograph quality B1, and Alveograph W value of 180–190. Mv Tallér has good field resistance to powdery mildew and leaf rust, and moderate resistant to stem rust.

Mv Apród (Mv 17-07) is a medium, early maturing semi dwarf hard red winter wheat. The advantage of Mv Apród compared to the previous cultivars is the combination of the semidwarf plant height with the high gluten (protein) content and high yield. The average wet gluten content of Mv Apród is 34–37% and the gluten quality is medium (B1 Farinograph quality and 160 Alveograph W). With reliable winter hardiness, Mv Apród is resistant to powdery mildew and leaf rust, and moderately resistant to stem rust.

Mv Kikelet (Mv 07-07) is a facultative wheat with medium winter hardiness. Mv Kikelet was tested together with real winter types and outyielded the standards. In spring sowings, the yield potential is competitive with spring cultivars. The quality of Mv Kikelet is good in autumn sowings and has higher gluten content and even better gluten quality in spring sowings. Mv Kikelet is moderately resistant to the major leaf diseases.

Mv Melodia (Mv09-07) is a hard red winter wheat with good breadmaking quality. Mv Melodia was selected from the cross 'Lone/OD162//Ukrainka'. Wet gluten content is typically between 30–34% and the Farinograph quality is A2-B1. Mv Melodia has good field resistance against leaf diseases and moderate susceptibility to head blight.

Breeding for quality traits.

Breeding of wheat with high arabinoxylan and protein content. Analyses carried out in the framework of the HEALTHGRAIN FP6 EU project headed by Peter Shewry demonstrated that the Yumai-34, an exotic Chinese wheat cultivar, contains large quantities of water-extractable arabinoxylan (WE-AX). A crossing program was aimed at breeding wheat genotypes with good agronomic adaptation and high dietary fiber content. The protein content also was analyzed in this population in order to select for genotypes with several advantageous traits. Lines with WE-AX contents similar to that of Yumai-34 and improved protein content were identified in the F₅ generation. These will be further analysed in the next generation.

Selection of durum wheat with high tocol content. Tocol content was determined in 36 durum wheat cultivars and breeding lines and was found to vary widely. Extreme values were 29.1 and 52.7 g/g in the first year and 28.5 and 56.9

g/g in the second. The fact that the maximum values were almost double the minimum values justified the initiation of a breeding program aimed at improving the tocol content. Total tocol contents in excess of 50 g/g were recorded for seven of the 36 durum cultivars and lines investigated. Three of these were chosen as crossing partners on the basis of their agronomic and chemical quality traits. One line, MvTD37-08, is now in its second year of state variety trials, whereas the other two lines are still being tested in field and laboratory trials. The compound with the most beneficial physiological effect, tocopherol, made up 11.3–22.8% of the total tocol content, with values of over 8 g/g in the best lines. In Mv Makaróni, 22.77% of the over 50 g/g total tocol content consisted of tocopherol (11.69 g/g). The second highest tocopherol content (9.33 g/g) was observed for the advanced line MvTD07-09. The results achieved in the second year confirmed observations made in the first year; the same two genotypes had extraordinarily high tocopherol content compared to the other lines.

Disease resistance studies.

The weather in Hungary in 2010 was favorable for the pathogens responsible for *Fusarium* head blight. In order to determine the species composition, damaged spikes were collected from several regions of the country and pure cultures were developed from a total of 114 infected grains. Morphological species determination was carried out in Tulln (Austria). The results showed that *Fusarium graminearum* was the dominant pathogen (present on 110 of the 114 samples), two isolates were identified as *F. sambucinum*, and one each as *F. culmorum* and *F. verticillioides*.

The QTL mapping of the 'Bánkúti 1201-9086/Mv Magvas' population developed to analyze the FHB resistance of old Hungarian wheat cultivars was continued by testing the parental lines with a total of 140 microsatellite markers. Eighty of the primers examined gave products, 55 of which were polymorphic. The entire population was tested with 33 SSR markers and with 24 different AFLP combinations. Data analysis resulted in the identification of 286 polymorphic markers suitable for mapping. The 319 polymorphic markers now available are sufficient to initiate the analysis of linkage groups.

An artificial inoculation and evaluation method was elaborated under greenhouse conditions to investigate resistance to *Mycosphaerella graminicola*, the fungal species responsible for the Septoria leaf spot disease. Mv Kolo, Mv Regiment, and three breeding lines proved to have outstanding resistance.

Genotypes with designated leaf rust resistance genes were tested for infection in an artificially inoculated nursery. Genes *Lr9*, *Lr19*, *Lr24*, *Lr25*, *Lr28*, *Lr29*, and *Lr35* provided effective protection against leaf rust in Martonvásár in 2010.

Studies on the composition of the wheat powdery mildew population showed that race 76 was present in the highest ratio (42.6%) in 2010, followed by race 77 (29.6%). Race 51, which infected all the cultivars used for differentiation, appeared with lower frequency than in previous years (16.0%). The virulence complexity of the pathogen population was 5.79, which represented a decline compared with previous years, mainly due to the reduced frequency of race 51.

In the framework of international and Hungarian projects (Bioexploit-EU FP6 and DTR_2007), resistance genes *Lr9*, *Lr24*, *Lr25*, *Lr29*, *Lr35*, *Lr37*, *Pm21*, and *Stb2* were introduced into wheat cultivars adapted to Hungarian conditions. By the joint incorporation of several resistance genes (pyramiding), winter wheat genotypes carrying new *Lr* gene combinations were developed.

Abiotic stress resistance studies.

To investigate the genetic regulation of heat tolerance, the responses of lines from a two-parental, doubled-haploid population to heat stress during the early stages of embryo development were analyzed on the basis of yield components and phenological parameters. Averaged over the population, high temperature was found to cause the greatest reduction in the grain number, which ranged from 3–75% compared with the control. In order to compile a molecular marker map of the population, the application of two molecular marker systems was begun: 16 AFLP reactions were carried out using AFLP primers exhibiting great polymorphism, and mapping was performed using 29 SSR markers with known chromosome localization.

The genetic diversity of active wheat breeding programs in southeastern Europe was analyzed using AFLP and SSR markers on a collection of 114 winter wheat cultivars. Considerable differences were found between cultivars originating from different countries, with the greatest similarity between those from Hungary and Romania and Serbia and Macedonia. When the phenotypic traits of the same cultivars were investigated, the powdery mildew and leaf rust resistance was found to be closely correlated with genetic diversity, whereas heading date, plant height, and a number of yield components were completely independent of genetic diversity. The parallel determination of genotypic and phenotypic diversity made it possible to distinguish groups of wheat cultivars with a similar genotype but a different phenotype, and with a different genotype but a similar phenotype. This information can be put to direct use in breeding during the selection of crossing partners.

Fourteen wheat genotypes (Ukrainka, two lines of Mv Hombár, M3, Glenlea, Mv Verbunkos, Mv Toborzó, Mv 4, Mv Mezőföld, Mv Magvas, the 9086 line of Bánkúti 1201, Plainsman, Mv Magma, and Tommi) and two durum genotypes (PWD1216 and MvTD10-98) were tested using 84 SSR markers. These genotypes are the parental pairs of mapping populations. After evaluating the polymorphism identified, work will begin on the detailed testing of the various populations.

Experiments proved that, among the yield components of wheat, grain mass decreased to the greatest extent in response to water withholding at first node appearance. The yield-increasing effect of enhanced atmospheric CO₂ concentration was greatest when applied at first node appearance. The grain mass and grain number only decreased as the result of drought during the ripening period at the higher carbon dioxide level, but the values recorded were still higher than at normal concentration.

When examining the joint effect of drought and enhanced atmospheric CO₂ concentration, a relative increase in the protein content of wheat grains was found when water deficit occurred at first node appearance, and this increase was greater at normal atmospheric CO₂ concentration. Drought during heading caused a similar increase in grain protein content at both carbon dioxide levels. Higher atmospheric CO₂ concentration caused a reduction in the grain protein content. This reduction was greatest when the treatment was applied at heading. An increase in the duration of drought stress had the most significant effect on the protein content when the stress occurred at first node appearance.

Photosynthesis was stimulated by higher atmospheric CO₂ concentration, so that in response to water withholding, the decrease in the carbon fixation of the wheat plants proceeded at a slower rate and only reached a significant level at lower values of soil moisture. The assimilation of the plants also remained at a more intensive level when an enhanced atmospheric CO₂ concentration was applied during heading or ripening, which could be attributed to the slower plant development at a higher atmospheric CO₂ concentration. The photosynthetic activity declined to a lesser extent during ripening than in the earlier developmental phases.

Considerable genotypic differences were found in the responses of the wheat varieties to water withholding and enhanced atmospheric CO₂ concentration. Mv Mambó proved to be a drought-tolerant cultivar, with smaller changes in yield parameters in response to stress, whereas the yield and physiological parameters of Mv Regiment decreased substantially when subjected to drought. However, Mv Regiment made better use of surplus CO₂, so it produced outstanding yields in a CO₂-enhanced environment.

Publications.

- Balla K, Rakszegi M, Bencze Sz, Karsai I, and Veisz O. 2010. Effect of high temperature and drought on the composition of gluten proteins in Martonvásár wheat varieties. *Acta Agron Hung* 58(4):343-353.
- Benkovics AH, Vida G, Nelson D, Veisz O, Bedford I, Silhavy D, and Boulton MI. 2010. Partial resistance to wheat dwarf virus in winter wheat cultivars. *Plant Path* 59(6):1144-1151.
- Ficsor A, Bakonyi J, Tóth B, Tomcsányi A, Palágyi A, Csősz M, Károlyi-Cséplő M, Mészáros K, and Vida G. 2010. First report of spot form of net blotch of barley caused by *Pyrenophora teres* f. *maculata* in Hungary. *Plant Dis* 94(8):1062.
- Curtis TY, Powers SJ, Balagiannis D, Elmore JS, Mottram DS, Parry MAK, Rakszegi M, Bedő Z, Shewry PR, and Halford NG. 2010. Free amino acids and sugars in rye grain: implications for acrylamide formation. *J Agric Food Chem* 58(3):1959-1969.

- Gebruers K, Dornez E, Bedo Z, Rakszegi M, Frás A, Boros D, Courtin CM, and Delcour JA. 2010. Environment and genotype effects on the content of dietary fiber and its components in wheat in the HEALTHGRAIN diversity screen. *J Agric Food Chem* 58(17):9353-9361.
- Gebruers K, Dornez E, Bedo Z, Rakszegi M, Courtin CM, and Delcour JA. 2010. Variability in xylanase and xylanase inhibition activities in different cereals in the HEALTHGRAIN diversity screen and contribution of environment and genotype to this variability in common wheat. *J Agric Food Chem* 58(17):9362-9371.
- Kuti Cs, Láng L, Gulyás G, Karsai I, Mészáros K, Vida Gy, and Bedő Z. 2010. Integration of molecular genomic data into the Martonvásár breeding information system. *Acta Agron Hung* 58(4):333-342.
- Rakszegi M, Kisgyörgy BN, Tearall K, Shewry PR, Lang L, Phillips A, and Bedő Z. 2010. Diversity of agronomic and morphological traits in a mutant population of bread wheat studied in the Healthgrain program. *Euphytica* 174(3):409-442.
- Rakszegi M, Bognár Z, Li Z, Békés F, Láng L, and Bedő Z. 2010. Effect of milling on the starch properties of winter wheat genotypes. *Starch-Starje* 62:115-122.
- Sestili F, Botticella E, Bedő Z, Philips A, and Lafiandra D. 2010. Production of novel allelic variation for genes involved in starch biosynthesis through mutagenesis. *Mol Breed* 25(1):145-154.
- Shewry PR, Piironen V, Lampi AM, Edelmann M, Kariluoto S, Nurmi T, Fernandez-Orozco R, Ravel C, Charmet G, Andersson AAM, Aman P, Boros D, Gebruers K, Dornez E, Courtin CM, Delcour JA, Rakszegi M, Bedő Z, and Ward JL. 2010. The HEALTHGRAIN wheat diversity screen: effects of genotype and environment on phytochemicals and dietary fiber components. *J Agric Food Chem* 58(17):9291-9298.
- Varga B, Bencze Sz, Balla K, and Veisz O. 2010. Combined effect of the drought duration and elevated atmospheric CO₂ level on physiological and yield parameters of winter wheat. *Acta Agron Hung* 58(4):323-331.
- Vida G, Gál M, Uhrin A, Veisz O, Wang Z, Kiss T, Karsai I, and Bedő Z. 2010. Application of molecular markers in breeding for leaf rust resistance in wheat. *In: 60. Tagung, Züchtung und genressourcen gegen abiotische stressfaktoren. 2010.* pp. 65-71.

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Development of a wheat genotype combining the recessive crossability alleles *kr1kr1kr2kr2* and the T1BL·1RS translocation for the rapid enrichment of IRS with new allelic variation. The main objective of this work was to develop a wheat genotype containing both the recessive crossability alleles (*kr1kr1kr2kr2*), allowing high crossability between 6x wheat and diploid rye, and the T1BL·1RS wheat-rye translocation chromosome. This wheat genotype could be used as a recipient partner in wheat-rye crosses for the efficient introduction of new allelic variation into IRS in translocation wheats. After crossing the wheat cultivars Mv Magdaléna and Mv Béres, which have the T1BL·1RS translocation involving chromosome arm 1RS from Petkus rye, with the line Mv9 *kr1*, 117 F₂ plants were analyzed for crossability, 10 of which had higher than 50% seed set with rye and, thus, presumably carried the *kr1kr1kr2kr2* alleles. Four of the 10 plants contained the T1BL·1RS translocation in the disomic condition as detected by GISH. The wheat-rye F₁ hybrids produced between these lines and the rye cultivar Kriszta were analyzed in meiosis using GISH. T1BL·1RS/1R chromosome pairing was detected in 62.4% of the pollen mother cells. The use of FISH with the repetitive DNA probes pSc119.2, Afa family, and pTa71, allowed the 1R and T1BL·1RS chromosomes to be identified. The presence of the 1RS arm from Kriszta, besides that of Petkus, was demonstrated in the F₁ hybrids using the rye SSR markers RMS13 and SCM9. In four of the 22 BC₁ progenies analyzed, only Kriszta-specific bands were observed with these markers, although the presence of the T1BL·1RS translocation was detected using GISH. We concluded that recombination occurred between the Petkus and Kriszta 1RS chromosome arms in the translocated chromosome in these plants.

GISH reveals different levels of meiotic pairing with wheat for individual *Ae. biuncialis* chromosomes. The *T. aestivum*-*Ae. biuncialis* (2n=4x=28; U^bU^bM^bM^b) disomic addition lines 2M^b, 3M^b, 7M^b, and 3U^b were crossed with the wheat cultivar Chinese Spring *ph1b* mutant genotype in order to induce homoeologous pairing, with the final goal of introgressing *Ae. biuncialis* chromatin into cultivated wheat. Wheat-*Aegilops* homoeologous chromosome pairing was studied in metaphase I of meiosis in the F₁ hybrid lines. Using U and M genomic probes, GISH demonstrated the occurrence of wheat-*Aegilops* homoeologous pairing for chromosomes 2M^b, 3M^b, and 3U^b, but not for 7M^b. The wheat-*Aegilops* pairing frequency decreased in the following order: 2M^b > 3M^b > 3U^b > 7M^b, which may reflect differences in

the wheat-*Aegilops* homoeologous relationships between the examined *Aegilops* chromosomes. The selection of wheat-*Aegilops* homoeologous recombinations could be successful in later generations.

Molecular cytogenetic evaluation of chromosome instability in *T. aestivum*–*S. cereale* disomic addition lines. The genetic stability of wheat-rye (Chinese Spring–Imperial) disomic addition lines was checked using the Feulgen method and FISH. Feulgen staining detected varying proportions of disomic, monosomic, and telosomic plants among the progenies of the disomic addition lines. The greatest stability was observed for the 7R addition line, whereas the most unstable lines were those with 2R and 4R additions. Chromosome rearrangements also were detected using FISH. Based on the specific hybridization patterns of repetitive DNA probes pSc119.2 and (AAC)5, as well as ribosomal DNA probes (5S and 45S), isochromosomes were identified in the progenies of 1R and 4R addition lines. These results draw attention to the importance of continuous cytological checks on basic genetic materials by using FISH, because this method reveals chromosome rearrangements that could not be detected either with the conventional Feulgen staining technique or with molecular markers.

Selection of U and M genome-specific wheat SSR markers using wheat-*Ae. biuncialis* and wheat-*Ae. geniculata* addition lines. Wheat SSR markers specific to the U and M genomes of *Aegilops* species were selected. A total of 108 wheat SSR markers were successfully tested on *Ae. biuncialis* ($2n = 4x = 28$, $U^bU^bM^bM^b$), on five wheat-*Ae. biuncialis* addition lines (2M^b, 3M^b, 7M^b, 3U^b, and 5U^b) and on a wheat-*Ae. geniculata* (1U^g, 2U^g, 3U^g, 4U^g, 5U^g, 7U^g, 1M^g, 2M^g, 4M^g, 5M^g, 6M^g, and 7M^g) addition series. Among the markers, 86 (79.6%) were amplified in the *Ae. biuncialis* genome. Compared with wheat, polymorphic bands of various lengths were detected in *Ae. biuncialis* for 35 (32.4%) of the wheat microsatellite markers. Three of these (8.6%) exhibited specific PCR products in wheat-*Ae. biuncialis* or wheat-*Ae. geniculata* addition lines. The primers GWM44 and GDM61 gave specific PCR products in the 2M^b and 3M^b wheat-*Ae. biuncialis* addition lines, but not on the 2M^g addition line of *Ae. geniculata*. A specific band was observed on the 7U^g wheat-*Ae. geniculata* addition line using the BARC184 primer. These three markers specific to the U and M genomes are helpful for the identification of 2M^b, 3M^b, and 7U^g chromosome introgressions into wheat.

Publications.

- Molnár-Láng M, Cseh A, Szakács É, and Molnár I. 2010. Development of a wheat genotype combining the recessive crossability alleles *kr1kr1kr2kr2* and the 1BL·1RS translocation, for the rapid enrichment of 1RS with new allelic variation. *Theor Appl Genet* 120:1535–1545.
- Molnár I and Molnár-Láng M. 2010. GISH reveals different levels of meiotic pairing with wheat for individual *Aegilops biuncialis* chromosomes. *Biol Plant* 54:259–264.
- Szakács É and Molnár-Láng M. 2010. Molecular cytogenetic evaluation of chromosome instability in *Triticum aestivum*–*Secale cereale* disomic addition lines. *J Appl Genet* 51:149–152.
- Schneider A, Molnár I, and Molnár-Láng M. 2010. Selection of U and M genome-specific wheat SSR markers using wheat-*Aegilops biuncialis* and wheat-*Ae. geniculata* addition lines. *Euphytica* 175:357–364.

ITEMS FROM INDIA

BHABHA ATOMIC RESEARCH CENTRE

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Application of Real-Time PCR in marker-assisted selection for stem rust resistance gene Sr24.

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Introduction. Real-Time PCR (RT–PCR) is a technique mainly used to amplify and simultaneously quantify a targeted DNA molecule (Gibson et al. 1996). Currently, four different chemistries, TaqMan® (Applied Biosystems, Foster City, CA, USA); Molecular Beacons (Newark, New Jersey, USA); Scorpions® (Sigma-Aldrich, St. Louis, MO, USA); and SYBR® Green (Life Technologies, Carlsbad, CA, USA), are available for RT-PCR. All of these chemistries allow