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Department of Plant Genetic Resources and Organic Breeding

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Association between pericentromeric SSR bands and intergenomic translocation breakpoints in natural populations of *Aegilops biuncialis* and *Ae. geniculata*. Repetitive DNA sequences are thought to be involved in the formation of intergenomic translocations, which is an important step in allopolyploid speciation. The allotetraploids *Ae. biuncialis* and *Ae. geniculata*, wild, genetic resources for wheat improvement, are excellent objects to study the role of microsatellite clusters in the formation of intergenomic translocations. The chromosomal localization of (ACG)_n and (GAA)_n microsatellite sequences in *Ae. biuncialis* and *Ae. geniculata* and their diploid progenitors *Ae. comosa* and *Ae. umbellulata* was investigated by sequential *in situ* hybridization with SSR probes and repeated DNA probes (pSc119.2 Afa family and pTa71) and by dual-color GISH. Thirty-two *Ae. biuncialis* and 19 *Ae. geniculata* accessions were screened by GISH for translocations, which were further identified by FISH and GISH. Single pericentromeric (ACG)_n signals were localized on most U- and on some M-genome chromosomes, whereas strong pericentromeric and several intercalary and telomeric (GAA)_n sites were observed on the *Aegilops* chromosomes. Three *Ae. biuncialis* accessions carried T7U^b–7M^b reciprocal translocations and one had a T7U^b–1M^b rearrangement, and two *Ae. geniculata* accessions carried T7U^s–1M^s or T5U^s–5M^s translocations. Conspicuous (ACG)_n and/or (GAA)_n clusters were located near the translocation breakpoints in eight out of the ten translocated chromosomes analyzed, SSR bands and breakpoints being statistically located at the same chromosomal site in six of them. SSR clusters seem to be involved in the formation of intergenomic translocations between the constituent genomes of allopolyploid *Aegilops* species. The (ACG)_n and (GAA)_n SSR motifs serve as additional chromosome markers for the karyotypic analysis of UM-genome *Aegilops* species.

Chromosome isolation by flow sorting in *Ae. umbellulata* and *Ae. comosa* and their allotetraploid hybrids *Ae. biuncialis* and *Ae. geniculata*. An international cooperation with Jaroslav Doležel (Centre of the Region Haná for Biotechnological and Agricultural Research, Institute of Experimental Botany, Olomouc, Czech Republic) was carried out to study the potential of flow cytometry for chromosome sorting in the wild wheats *Ae. umbellulata* (2n=2x=14, UU) and *Ae. comosa* (2n=2x=14, MM) and in their natural hybrids *Ae. biuncialis* (2n=4x=28, U^bU^bM^bM^b) and *Ae. geniculata* (2n=4x=28, U^sU^sM^sM^s). Histograms of fluorescence intensity (flow karyotypes) obtained after the analysis of DAPI-stained chromosomes were characterized and the chromosome content of the four peaks on flow karyotypes was determined for the first time for these *Aegilops* species. Chromosomes sorted onto microscope slides were identified after FISH with probes pSc119.2, Afa repeats, and pTa71. Only chromosome 1U could be discriminated by the peak I on flow karyotypes of *Ae. umbellulata* and *Ae. biuncialis* with the standard karyotype. Microscopic evaluation of the sorted fractions showed that chromosome 1U could be sorted at purity >95%. Remaining chromosomes formed composite peaks and could be sorted only as groups. Distribution of chromosomes among the peaks of flow karyotypes reflected different size modifications for the chromosomes 4U, 1M, 2M, 3M, 4M, and 6M in the *Ae. biuncialis* and *Ae. geniculata*. Twenty-four wheat SSR markers were used to map the U- and M-genome chromosomes by the use of DNA amplified from the sorted chromosome fractions and wheat–*Ae. geniculata* addition lines. Ten SSR markers located unambiguously on the *Aegilops* chromosomes supported the chromosome content of sorted fractions and confirmed the suitability of flow-sorted chromosomes for physical mapping. These SSR markers are suitable tools for the marker-assisted production of wheat–*Aegilops* introgression lines. These results open the way for the construction and sequencing of large-insert,

chromosome 1U-specific, DNA libraries in *Ae. umbellulata* and *Ae. biuncialis*, which would greatly promote the targeted isolation of molecular markers and the discovery of novel genes for the wheat improvement.

Characterization of a new T4BS-7HL wheat/barley translocation line using GISH, FISH, and SSR markers and its effect on the β -glucan content of wheat. A spontaneous interspecific Robertsonian translocation was revealed by GISH in the progenies of a monosomic 7H addition line originating from a new wheat/barley, 'Asakaze komugi/Manas' hybrid. FISH with repetitive DNA sequences Afa family, pSc119.2, and pTa71 allowed identification of all wheat chromosomes, including wheat chromosome arm 4BS involved in the translocation. FISH using barley telomere- and centromere-specific repetitive DNA probes (HvT01 and AGGGAG) confirmed that one of the arms of barley chromosome 7H was involved in the translocation. SSR markers specific to the long and short arms of barley chromosome 7H identified the translocated chromosome segment as 7HL. Further analysis of the translocation chromosome clarified the physical position of genetically mapped SSRs within 7H, with a special focus on its centromeric region. The presence of the HvCslF6 gene, responsible for (1,3;1,4)- β -D-glucan production, was revealed in the centromeric region of 7HL. An increased (1,3;1,4)- β -D-glucan level also was detected in the translocation line, demonstrating that the HvCslF6 gene is of potential relevance for the manipulation of wheat (1,3;1,4)- β -D-glucan levels.

Constructing a detailed mcFISH karyotype and studying chromosome polymorphisms of *Elytrigia elongata* E genome using highly repetitive and rDNA sequences. *Elytrigia elongata* (= *Agropyron elongatum*, *Thinopyrum elongatum*, $2n=2x=14$, EE) has long been used as a source of various resistance for wheat improvement, and numerous transfers have been made. However, despite heavy use, the species has never enjoyed cytological attention it deserves and no high resolution karyotype exists, perhaps because its chromosomes do not C-band well and the genome is rather symmetrical. As the interest in *E. elongata* for wheat improvement does not appear to abate, and alien transfers are now routinely detected and followed using the techniques of *in situ* probing with labelled DNA, a detailed FISH karyotype of the E genome was generated and verified in several accessions. The karyotype itself was generated using highly repetitive DNA sequences and sequential GISH-mcFISH; chromosome identification was using the complete *E. elongata* disomic chromosome addition series and 11 ditelosomic addition lines in Chinese Spring wheat. The E-genome chromosomes in a wheat background were detected by probing with the total genomic DNA of *E. elongata*, followed by mcFISH with five repetitive DNA probes. Of these, two clones failed to hybridize to the E-genome chromosomes. Based on the successful mcFISH, each complete chromosome and each telocentric studied was unambiguously identified. Validation of the karyotype in four *E. elongata* accessions with different geographical origins showed extensive variation of the probe hybridization patterns but this did not prevent positive chromosome identification. We believe that this karyotype will be useful in quick identification of potential donor chromosomes in wheat improvement programs so that proper alien transfer approaches can be selected and implemented.

Development of synthetic amphiploids based on different *Triticum turgidum* \times *T. monococcum* crosses to improve the adaptability of cereals. Cultivated einkorn (*T. monococcum* subsp. *monococcum*) is an excellent source of resistance against several wheat diseases and quality parameters. Semidwarf, einkorn lines with good crossability were identified in order to produce '*T. turgidum* \times *T. monococcum*' synthetic amphiploids. Two combinations were used to develop the amphiploids: 'durum \times einkorn' and 'emmer \times einkorn'.

After the genome duplication of the F_1 seed, highly fertile amphiploids were developed. The A^uBA^m genome structure of the progenies was confirmed by GISH. Lines derived from 'durum \times einkorn' and 'emmer \times einkorn' crosses were studied for agronomic performance, disease resistance, and genetic variability. Both amphiploid combinations showed excellent resistance against certain wheat diseases (leaf rust and powdery mildew), but not against Fusarium. The durum-based synthetic amphiploid lines showed a higher level of phenotypic diversity. The newly produced '*T. turgidum* \times *T. monococcum*' synthetic hexaploids are promising genetic resources for wheat breeding. Selected 'durum \times einkorn' lines are currently used in bread wheat improvement to transfer the useful properties of einkorn into cultivated hexaploid wheat via bridge crosses.

Martonvásár Cereal Gene Bank and Organic Plant Breeding. The Cereal Gene bank activity mainly concentrated on the characterization of the different resistance sources such as *T. monococcum* and *T. timopheevii* accessions, and their possible use in the development of new synthetic amphiploids. New, highly crossable, semidwarf, *T. monococcum* lines have been developed, and crossed with selected *T. turgidum* subsp. *durum* and *T. timopheevii* accessions or selected breeding lines. Triploids were identified in the F_1 generation, and treated with colchicine to double the chromosome number of the interspecific hybrids. Fertile hexaploids were selected in the C_2 generation and multiplied under greenhouse conditions. The new synthetics will be used in wheat-improvement programs and are target of independent breeding line development.

Our organic breeding program is highly focused on the development of new, organic cultivars of alternative or underutilized cereals such as einkorn and emmer. A new, organically bred, einkorn cultivar, **Mv Menket**, was released last year. This is the first semidwarf einkorn cultivar in the market, with elevated yield potential, and excellent resistance against most of the wheat diseases, except Fusarium. Mv Menket is an organic cultivar, because it is highly sensitive against all herbicides used in the Hungarian farming practice, and there is no possibility to use it in traditional agricultural practices.

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ITEMS FROM INDIA

DIRECTORATE OF WHEAT RESEARCH

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A study of floral biology traits in bread wheat.

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Summary. Three floral biology traits, anther length, stigma length, and anther extrusion, were investigated in 92 elite wheat germ plasm lines. A wide range of variability was observed for these floral traits and promising genotypes were identified for their further utilization as parents for hybrid wheat development.

Wheat productivity levels in the present genotypes have reached a saturation level, which limits higher production to meet the targets for food security. Newer, innovative techniques can be promising approaches in order to break yield barriers and, in this regard, hybrid wheat development through exploitation of heterosis may be a potential tool. Wheat production in the Northwest Plains Zone has reached peak yields. Knowledge about the variability and character association between floral characteristics is crucial to identify suitable male or female parental lines for further utilization in hybrid development programs. Our objective was to study the extent of variability present in some wheat genotypes for various floral characters and establish correlations between them.

Materials and methods. A total of 92 elite genotypes maintained by the Hybrid Wheat Programme were used in the study. These entries included elite selections from international nurseries and trials from CIMMYT; genetic stocks;