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ARTICLE

Incorporation of *Aegilops biuncialis* chromosomes into wheat and their identification using fluorescent *in situ* hybridization

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ABSTRACT The aim of the study was to select *Aegilops biuncialis* chromosomes from the progeny of the BC_2 and BC_3 generations of the wheat × *Ae. biuncialis* hybrids, which differ from the chromosomes 2M, 3M, 7M, 3U and 5U found in the wheat-*Ae. biuncails* addition lines produced earlier in Martonvásár. Besides the above mentioned addition lines exists a 5U, 6U double disomic addition line. Chromosomes of the progeny of the BC_2 and BC_3 generations and the 5U, 6U double disomic addition line were counted with Feulgen method, while alien (*Ae. biuncialis*) chromosomes were identified with fluorescent *in situ* hybridization (FISH) using pSc119.2 and AFA family DNA probes. During the present experiment it was found that the transmission percentage of the chromosome 5U is 67,55% in the progeny of the BC_2 and BC_3 generations, while in the 5U, 6U disomic addition line chromosome 5U showed 100% transmission. Besides the chromosomes incorporated in the wheat- *Ae. biuncialis* addition lines produced in Martonvásár, some plants containing the chromosomes 1U, 2U, 4U, 6U, 7U, 5M and 6M without the presence of the chromosome 5U exist. These plants are potential sources of the production of new wheat-*Ae. biuncialis* addition lines.
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KEY WORDS

Aegilops biuncialis
addition and translocation lines
preferential transmission
fluorescent *in situ* hybridization (FISH)

Aegilops species played an important role in the evolution of cultivated wheat. The D genome ancestor of wheat is the species *Aegilops tauschii* (2n=2x=14, DD), while the S genome of *Ae. speltoides* (2n=2x=14, SS) bears the greatest resemblance to the B genome of cultivated wheat. *Ae. tauschii,* carrying D genome, belongs to the primary gene pool of common wheat, the secondary gene pool of cultivated wheat includes *Aegilops* species which contain S genome. Species belonging to the tertiary gene pool carrying other genomes are more distantly related. *Aegilops* species are valuable sources of several resistance genes, which can be incorporated into the wheat genome via intergeneric crossing, where necessary, followed by the development of chromosome addition lines from the resulting hybrids. The transfer of a single segment from an alien chromosome can be achieved by developing translocations. Up to the present several wheat–*Aegilops* interspecific hybrids and addition lines have been developed (Friebe et al. 1996 b, 1999, 2000), and numerous genes of agronomic interest were incorporated into common wheat (for review see Friebe et al. 1996a; Schneider et al. 2008), however, there have been no reports on the production of a complete set of wheat-*Ae. biuncialis* addition lines, moreover, *Ae. biuncialis* resistance genes were not exploited, not transferred into cultivated wheat.

The aim of the present study was to utilize useful gene sources of *Ae. biuncialis*, to transfer drought tolerance (Molnár et al. 2004), salt tolerance (Colmer et al. 2006) and other resistance genes into wheat, moreover, to visualize the *Ae. biuncialis* chromosomes and chromosome segments in wheat background and to identify the alien chromosomes with fluorescent *in situ* hybridization (FISH). Further aim of the experiment was to identify *Ae. biuncialis* chromosomes incorporated into progenies of the backcross generations of the wheat × *Ae. biuncialis* hybrids. It is hoped that new *Ae. biucialis* chromosomes will be selected in the backcross progenies of the wheat - *Ae. biuncialis* hybrids, which differ from the chromosomes 2M, 3M, 7M, 3U and 5U found in the wheat - *Ae. biuncalis* addition lines produced earlier in Martonvásár (Schneider et al. 2005).

Materials and Methods

Plant materials

- Progenies of the BC_2 and BC_3 generations of the Mv9 kr1 × *Ae. biuncialis* hybrids produced in Martonvásár (Logojan and Molnár-Láng 2000)
- Mv9 kr1 - *Ae. biuncialis* 5U, 6U double disomic addition line produced in Martonvásár.

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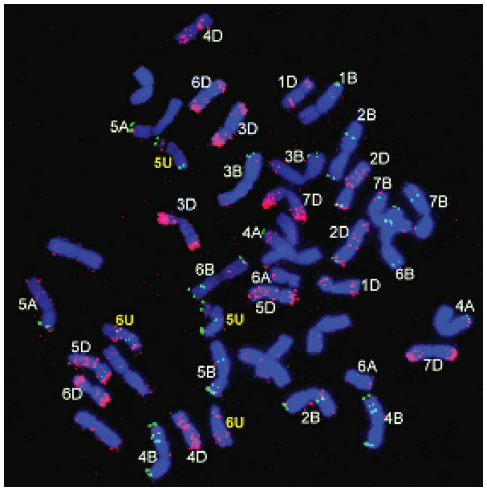

Figure 1. Fluorescent *in situ* hybridization (FISH) patterns on the metaphase chromosome spread of the 5U, 6U double disomic addition line using pSc119.2 (green) and AFA family (red) repetitive DNA probes. *Ae. biuncialis* chromosomes are labelled with yellow characters.

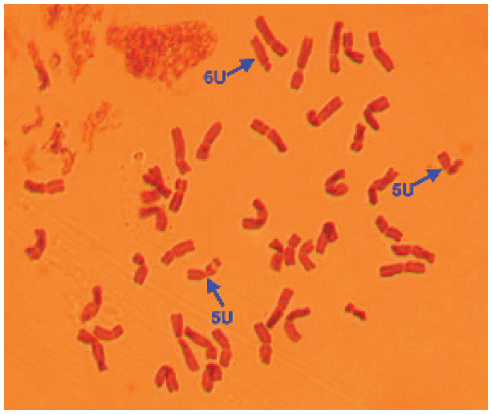

Figure 2. Feulgen staining of one plant of the 5U, 6U double disomic addition line. On the preparation a pair of 5U chromosome and one 6U chromosome are visible (labelled with arrows).

Figure 3. Spikes of 5U, 6U double disomic wheat-*Ae. biuncialis* addition line (two spikes are displayed on the figure, pictures were taken front-viewed and side-viewed).

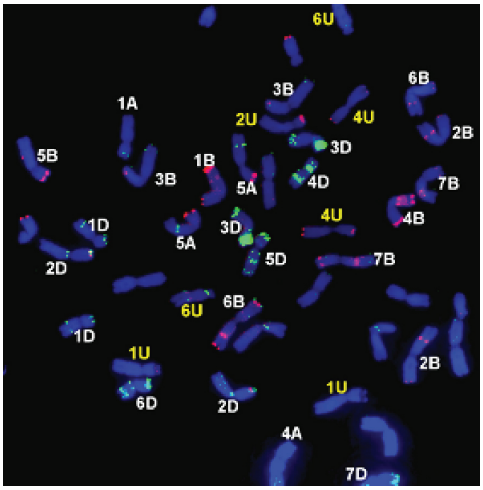

Figure 4. Fluorescent *in situ* hybridization (FISH) pattern on mititic chromosome spread preparation of the plant with identification No. 07318 using pSc119.2 (red) and AFA family (green) DNA probes. *Ae. biuncialis* chromosomes are labelled with yellow characters. Chromosomes 1U, 2U, 4U and 6U were identified in wheat background.

Figure 5. Morphology of the plant with cytological No. 07318. When this picture was taken, the other plants in the phytotron chamber were earing (3.5 months after planting).

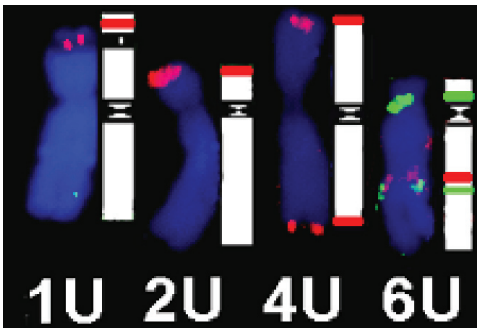

Figure 6. FISH pattern of the chromosomes detected in plant with identification No. 07318 using pSc119.2 (red) and Afa family (green) DNA probes.

Methods

Feulgen staining

Fluorescent *in situ* hybridization (FISH), using pSc119.2 (Bedbrook et al. 1980) and AFA family (Nagaki et al. 1995) repetitive DNA probes, was carried out according to Szakács et al. (2005) and Sepsi et al. (2008) with minor modifications. The DNA probes were labelled by nick translation with Fluorogreen and Fluorored (Roche).

Results

Five different wheat - *Ae. biuncialis* addition lines were produced earlier (2M, 3M, 7M, 3U and 5U, see Schneider et al. 2005), moreover, a 5U, 6U double disomic addition line was also developed. The karyotype described in the diploid *Ae. umbellulata* and *Ae. comosa* by Badaeva et al. (1996) was taken as a basis to produce FISH karyotype of the *Ae. biuncialis* accession which was used to develop wheat-*Ae. biuncialis* addition lines (Schneider et al. 2005), in order to facilitate the identification of *Ae. biuncialis* chromosomes in the wheat-*Ae. biuncialis* BC_2 and BC_3 generations and in the 5U, 6U double disomic addition line using FISH. The 5U, 6U double disomic addition line contains 42 wheat and two pairs of *Ae. biuncialis* chromosomes, thus, it carries 46 chromosomes. It was observed that the 5U chromosome did not eliminate from the 5U, 6U double disomic addition line, the genome constitution with 46 chromosomes is stable. Thirty seeds of the 5U, 6U double disomic addition line were germinated to carry out FISH experiments. Among the 13 plants analysed all contained chromosome 5U (Figs. 1 and 2 and Table 1). After the analysis of FISH patterns it was established that genome constitution with 46 chromosomes is stable, therefore, plants were analysed with Feulgen method (Fig. 2.). Chromosomes 5U and 6U have specific morphological characters, hence, they can be distinguished from wheat chromosomes using Feulgen method (Fig. 2). Chromosome 5U is a satellited, but smaller sized chromosome, than satellited chromosomes of wheat. Chromosome 6U is an acrocentric chromosome, therefore, it can be differentiated from metacentric and submetacentric wheat chromosomes. Among the 75 plants examined with Feulgen staining, 60 plants were suitable for analysis, 59 plants contained a pair of 5U and a pair of 6U chromosomes. One plant carried only a pair of 5U and one 6U chromosome (Fig. 2 and Table 1). However, analysis of progenies of this plant showed that it contains both (5U and 6U) chromosome pairs. Plants were grown in the phytotron. Some of the morphological features were different from the addition lines produced earlier. Plants have characteristic thick, dark greenish leaves, the spikes appeared to resemble to those of Mv9 kr1 (Fig. 3).

The high transmission rate of chromosome 5U raises difficulties in the development of new wheat-*Ae. biuncialis* addition lines. Progenies of the BC_2 and BC_3 generations of the Mv9 kr1 × *Ae. biuncialis* hybrids were analysed using FISH to produce new addition lines. The aim of the experiments was to select the plants from the progenies of the BC_2 and BC_3 generations that do not carry chromosome 5U, but contain other *Ae. biuncialis* chromosomes that cannot be found in the addition lines produced earlier. FISH data are available of 188 BC_2 and BC_3 plant progenies altogether. 127 plants contained chromosome 5U (these plants generally carried other *Ae. biuncialis* chromosomes), 29 plants had no chromosome 5U (but carried other *Ae. biuncialis* chromosome), while *Ae. biuncialis* chromosome eliminated in 32 plants. Experiments showed that 5U chromosome is present in most plant progenies containing other *Ae. biuncialis* chromosomes. Previous observations confirm that the transmission rate of this chromosome is very high. FISH analysis of most important plant progenies of the BC_2 and BC_3 plants, which contain different chromosomes from those of present in the addition lines produced earlier, are represented in Table 2. The development of the plant with the identification number 07318 not containing chromosome 5U stopped at the tillering phase, it did not ear, and remained dwarf (Figs. 4, 5 and 6). It is widely known that certain hybrid combinations lead to the development of dwarf plants incapable of living. Plants demonstrated in Table 2 are being grown in the phytotron, except for the plant with identification number 07318. Besides the chromosomes 2M, 3M, 7M, 3U, 5U and chromosome combination 5U, 6U found in the addition lines developed so far, some plants are now available that carry the chromosomes 1U, 2U, 4U, 6U, 7U, 5M and 6M without the presence of chromosome 5U.

Table 1. Summarized data of cytological analysis on 5U, 6U wheat- *Ae. biuncalis* double disomic addition line.

No. of seeds germinated (pieces)	No. of plants grown (pieces)	Method of analysis	No. of plants analysed (pieces)	Results
30	20	fluorescent *in situ* hybridization	13	contains chromosomes 5U and 6U
55	55	Chromosome counting on mititic chromosome spread preparations with Feulgen staining	53	contains chromosomes 5U and 6U
20	12	Chromosome counting on meiotic chromosome spread preparations with Feulgen staining	7	Good metaphase was observed in three plants, 46^{II}, so it contains chromosomes 5U and 6U

Table 2. FISH analysis data of some plants of the progeny of the BC_2 and BC_3 generations selfed (iso=isolated).

Identification Nos. of plants	Combination	FISH DATA
07285	(Mv9kr1 × Ae. biuncialis) wheat2)iso3	3U 2 pcs, 1U 2 pcs, 5U 1 pc
07286	(Mv9kr1 × Ae. biuncialis) wheat 2)iso3	5U 2 pcs, 2U 2 pcs, 3U 2 pcs, 4U 1 pc, 7M 2 pcs
07297	(Mv9kr1 × Ae. biuncialis) wheat 2)iso4	5U 2 pcs, + small metacentric chromosome with pSc119.2 sites on both telomeres
07304	(Mv9kr1 × Ae. biuncialis) wheat 2)iso4	5U 2 pcs, 1U 1 pc
07305	(Mv9kr1 × Ae. biuncialis) wheat 2)iso4	5U 2 pcs, + big metacentric chromosome with pSc119.2 sites on both telomeres
07308	(Mv9kr1 × Ae. biuncialis) wheat 2)iso4	5U 2 pcs 3U 2 pcs, + big metacentric Ae. biuncialis chromosome
07309	(Mv9kr1 × Ae. biuncialis) wheat 2)iso4	5U 1 pc, + small metacentric chromosome with pSc119.2 sites on both telomeres
07311	(Mv9kr1 × Ae. biuncialis) wheat 2)iso4	5U 1 pc, 1U 2 pcs, 2U 2 pcs, 4U 1 pc, 6U 2 pcs
07312	(Mv9kr1 × Ae. biuncialis) wheat 2)iso4	5U 2 pcs, 1U 1 pc, 2U 2 pcs, 6U 2 pcs
07313	(Mv9kr1 × Ae. biuncialis) wheat 2)iso4	5U 2 pcs, 2U 2 pcs, 4U 1 piece, 6U 2 pcs
07318	(Mv9kr1 × Ae. biuncialis) wheat 2)iso6	1U 2 pcs, 2U 2 pcs, 4U 2 pcs, 6U 2 pcs (no 5U)
07320	(Mv9kr1 × Ae. biuncialis) wheat 2)iso6	5U 2 pcs, 2U 2 pcs, 4U 2 pcs, 6U 2 pcs
07321	(Mv9kr1 × Ae. biuncialis) wheat 2)iso6	5U 2 pcs, 6U 1 pc
07324	(Mv9kr1 × Ae. biuncialis) wheat 2)iso5	5U 2 pcs, 1U 1 pc, 2U 2 pcs, 6U 2 pcs, 7M 2 pcs
0865	(Mv9kr1 × Ae. biuncialis) wheat 2)iso2	3U 2 pcs, 6M 2 pcs (no 5U)
0868	(Mv9kr1 × Ae. biuncialis) wheat 2)iso2	7U 2 pcs, 6U 2 pcs, 5U 1 pc, 3U 1 pc, 2U 2 pcs, 6M 1 pc
0869	(Mv9kr1 × Ae. biuncialis) wheat 2)iso2	6U 1 pc (no 5U)
0877	(Mv9kr1 × Ae. biuncialis) wheat 2)iso	2U 1 pc (no 5U)
0879	(Mv9kr1 × Ae. biuncialis) wheat 2)iso	big metacentric chromosome with pSc119.2 sites on both telomeres (no 5U)
0884	(Mv9kr1 × Ae. biuncialis) wheat 2)iso3	7M 1 pc, 5M 1 pc (no 5U)
0886	(Mv9kr1 × Ae. biuncialis) wheat 2)iso	1U 1 pc, 4U 1 pc (no 5U)
0887	(Mv9kr1 × Ae. biuncialis) wheat 2)iso	1U 1 pc
0892	(Mv9kr1 × Ae. biuncialis) wheat 2)iso2	2U 2 pcs, 3U 1 pc, 6U 1 pc (no 5U)
08145	(Mv9kr1 × Ae. biuncialis) wheat 2)iso2	1U 1 pc, 5U 2 pcs, 6U 1 pc, 7U 1 pc, 2U 1 pc, 3U 1 pc, 5M 1 pc, 7M 1 pc

Discussion

It was observed, similarly to the chromosome 5U of *Ae. biuncialis*, that individual chromosomes are transmitted with different frequency to the progenies in other *Aegilops* species as well. Miller et al. (1982) intended to produce a complete set of wheat- *Ae. sharonensis* (2n=2x=14, SS) addition lines, but it was experienced that the transmission rate of chromosome 4S is 100%, therefore, chromosome 4S was denominated as 'Cuckoo' chromosome. Similar 'Cuckoo' chromosomes were found in several *Aegilops* species (for review see Schneider et al. 2008) and in other cereal species (for example barley) (Koba et al. 1991; Molnár-Láng et al. 2005). The reason of the preferential transmission of some *Aegilops* chromosomes has not been clarified yet.

The successful transfer of agronomically useful traits (salt and drought tolerance, disease resistance) into the wheat genome requires to develop the complete set of wheat-*Ae. biuncialis* addition lines in the future. Production of a complete set of addition lines is time consuming, takes several years, as some of the individual *Ae. biuncialis* chromosomes are transmitted with higher frequency to the progenies (for example chromosome 5U), other chromosomes eliminate easily, which raises difficulties in the production of the whole set of addition lines. The production of addition lines requires numerous crossings and backcrossings, it is necessary to grow up several plant generations and to analyse them using molecular cytogenetical methods. The production of a complete set of wheat- *Ae. biuncialis* chromosome addition lines allow the studying of the genetic effects of individual *Ae. biuncialis* chromosomes in cultivated wheat, enabling the selection of chromosomes that influence especially drought tolerance and other agronomically beneficial traits. After the effect of each *Ae. biuncialis* chromosome is analysed, it is possible to produce introgression lines that carry genes responsible for agronomically useful traits.

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