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ARTICLE

Molecular cytogenetic analysis of the wheat-*Agropyron elongatum* partial amphiploid BE-1

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ABSTRACT Multicolor genomic in situ hybridization (mcGISH) and fluorescence *in situ* hybridization (FISH) using repetitive DNA probes were used to characterize the genomic composition of the wheat-*Thinopyrum ponticum* partial amphiploid BE-1. The amphiploid is a high-protein line having resistance to leaf rust (*Puccinia recondita* f. sp. tritici) and powdery mildew (*Blumeria graminis* f. sp. tritici) and has in total 56 chromosomes per cell. Multicolor GISH revealed 16 chromosomes originating from *Thinopyrum ponticum* and 14 A genome, 14 B genome and 12 D genome chromosomes. Rearrangements involving *Thinopyrum* chromosomes and the A, B and D genomes of wheat were visualised. FISH using repetitive DNA probes allowed the identification of all wheat chromosomes present and the determination of the chromosomes involved in translocations. **Acta Biol Szeged 52(1):139-141 (2008)**

KEY WORDS

Triticum aestivum
Thinopyrum ponticum
amphiploid
multicolor GISH
FISH

The production of stable wheat-alien amphiploids is an important intermediate step for transfering agronomically useful genes into bread wheat (*Triticum aestivum* L.), because they allow the reliable analysis of the effects of alien genes in the genetic background of wheat and their fertility allows gene transfer even when the F1 hybrid is almost completely sterile.

BE-1 produced by Szalay (1979), is a wheat–*Thinopyrum ponticum* (Popd.) [syn *Agropyron elongatum* (Host)] partial amphiploid with 56 chromosomes having high protein content and resistance to leaf rust and powdery mildew (Szalay 1979). Being highly fertile, this genetic material could be a potential source for wheat improvement.

It is important to describe the genomic composition of plants carrying the desired traits. Multicolor GISH using several different genomic probes is a useful technique for simultaneously discriminating three or more genomes in wheat-alien species amphiploids (Mukai et al. 1993).

Fluorescence *in situ* hybridization (FISH) using repetitive DNA clones is a powerful tool for identifying chromosomes within a species (Bedbrook et al. 1980).

The aim of this study was to characterize the chromosome composition of the wheat-*Thinopyrum ponticum* partial amphiploid BE-1 by means of multicolor GISH and FISH.

Materials and Methods

Plant material

The wheat-*Thinopyrum ponticum* partial amphiploid was derived from a cross between hexaploid wheat (*Triticum aestivum* cv Bánkúti) and *Thinopyrum ponticum* (Szalay 1979). The line BE-1 was selected from the F3 generation of the cross.

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In situ hybridization

Chromosome preparation was carried out as described by Lukaszewski et al. (2004) and the slides were stored at -20°C for several weeks.

Pre-treatments and post-hybridization washing were carried out as described by Molnár-Láng et al. (2000) The procedures employed for probe labeling and for the hybridization (GISH and FISH) were identical to those described previousely by Sepsi et al (2008).

Results

Using biotinylated J genomic DNA and digoxigenated A genomic DNA, 16 *Th. ponticum* chromosomes, 14 A genome chromosomes and 26 unlabeled chromosomes were detected, indicating that BE-1 carries a complete set of A genome chromosomes, while one pair of wheat chromosomes was substituted by a pair of alien chromosomes (Fig. 1). Four of the 16 Th. ponticum chromosomes showed no green fluorescent signal near their centromeric regions (Fig. 1, Fig. 3). This suggested that they were involved in intergenomic translocations. Among the 14 red-fluorescing chromosomes, 2 pairs carried a terminal unlabelled region, with fraction lengths 0.7 and 0.8, respectively, on the relevant arm, suggesting that intergenomic rearrangement had taken place in the wheat genome (Fig.1). These chromosomes were later identified using FISH as 4A and 7A, respectively (Fig. 2).

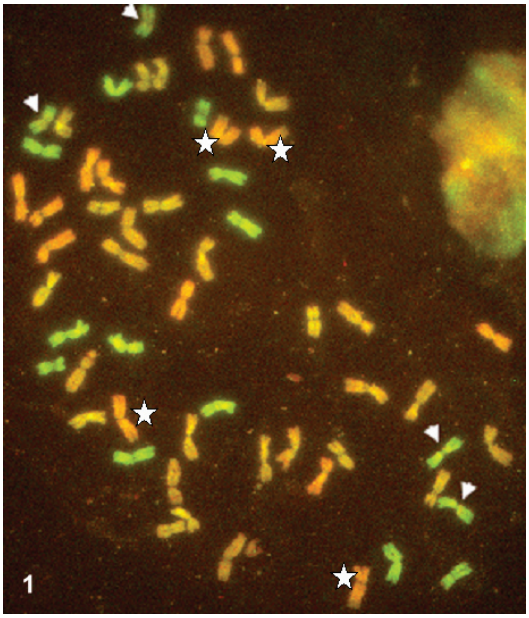

Figure 1. Multicolor genomic *in situ* hybridization on mitotic chromosomes of BE-1 using J and A genomic probes. J genome visualised in green, A genome chromosomes visualised in red, B, D genomic chromosomes are brown. The four *Th. ponticum* translocation chromosomes are marked with arrows while the 4A/7B translocation chromosomes and the 7A translocation chromosomes are indicated with asterisk

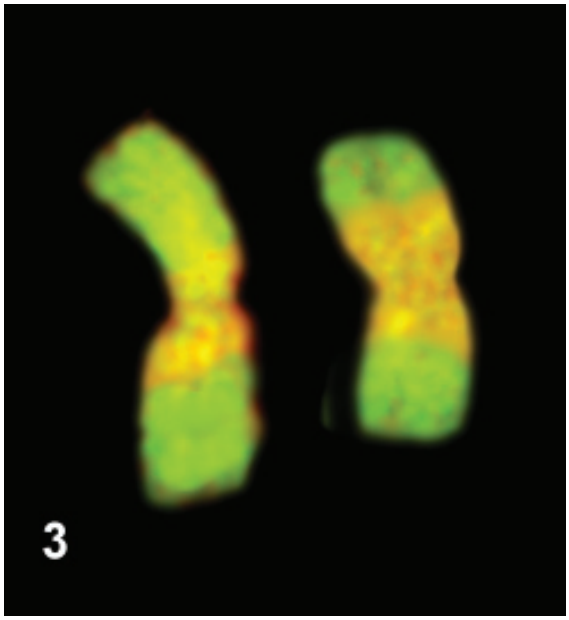

Figure 3. GISH pattern of the two types of *Th. ponticum* translocation chromosomes in the wheat-*Th. ponticum* partial amphiploid BE-1. J genomic probe vizualized in green. The chromosomes are only labeled by biotinylated J genome probe in the terminal regions, the centromeric region remained unlabeled.

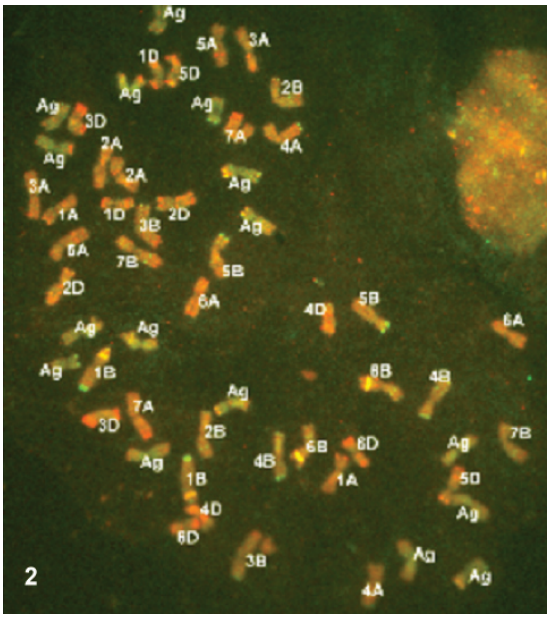

Figure 2. Chromosome identification of BE-1 using Afa family (red), pSc119.2 (green) and pTa71 (yellow) repetitive DNA probes.

In further probing experiments biotinylated J genomic DNA and digoxigenated D genomic DNA were used as probes. This study revealed only 12 D genome chromosomes, indicating that the two wheat chromosomes missing from BE-1 belonged to the D genome.

One pair of the A chromosomes which showed unlabeled regions when using the A genome probe exhibited red fluorescence in the same regions in this experiment. This suggested that D-A genomic translocations had taken place.

The repetitive DNA probes pSc 119.2, Afa family and pTa71, has been simultaneously hybridized on mitotic metaphase cells of BE-1. All the wheat chromosomes present were unequivocally identified (Fig. 2). This experiment showed the complete absence of the 7D chromosome pair (7D nullisomy). The sixteen added *Th. ponticum* chromosomes can be arranged in eight pairs and differentiated from each other by their GISH and FISH patterns.

Discussion

The BE-1 wheat–*Thinopyrum ponticum* partial amphiploid was produced in the 1950s by Szalay (1979) and was used for years as a multiresistant line with high protein content though its chromosome composition remained unknown. The aim of this work was to describe the chromosome composition of BE-1 by means of multicolor GISH and FISH in order to compare it with other wheat–Th. ponticum amphiploids.

Fedak et al. (2000) reported the genomic composition of six wheat-*Th. ponticum* amphiploids revealed by GISH using

S genomic DNA as a probe. GISH analysis confirmed that partial amphiploids originating from the same alien parent do not carry the same combination of alien chromosomes in all cases, but the alien chromosomes were not identified.

Four other wheat-*Th. ponticum* amphiploids were characterized by Oliver et al. (2006) using DNA from *Th. ponticum* as a probe. GISH detected 56 chromosomes per cell, but the number of chromosomes belonging to different genomes varied among the genetic materials.

In the present study mcGISH revealed intergenomic rearrangements occurring in the wheat and in the *Thinopyrum* genomes and showed that the substituted wheat chromosome pair belonged to the D genome. Later FISH analysis identified it as 7D. Natural substitutions usually involve homoeologous chromosomes, and the alien homoeologous chromosome pair compensates for the loss of wheat chromosomes (Knott 1968).

McGISH revealed two chromosome pairs carrying telomeric translocations, both belonging to the A genome and identified as 4A and 7A by FISH. The alien fragment translocated on chromosome 7A was identified as a D genome segment by mcGISH. The translocation detected in this study on the terminal region of wheat chromosome arm 4AL has already been reported in the literature (Naranjo et al. 1987).

The detailed description of the various alien chromosomes in the partial amphiploid BE-1 reported in the present study makes it possible to trace the transfer of *Th. ponticum* chromosomes from this amphiploid into wheat. As a good source for improving disease resistance and quality, BE-1 could be a promising crossing partner in wheat breeding programmes.

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References

Bedbrook J, Jones J, O'Dell M, Thompson RD, Flavell RB (1980) A molecular description of telomeric heterochromatin in *Secale* species. Cell 19:545-560.

Fedak G, Chen Q, Conner RL, Laroche A, Petroski R, Armstrong KW (2000) Characterisation of wheat-*Thinopyrum* partial amphiploids by meiotic analysis and genomic *in situ* hybridization. Genome 43:712-719.

Knott DR (1968) Translocations involving *Triticum* chromosomes carrying rust resistance. Can J Genet Cytol 10:695-696.

Lukaszewski AJ, Rybka K, Korzun V, Malyshev SV, Lapinski B, Whitkus R (2004) Genetic and physical mapping of homoeologous recombination points involving wheat chromosome 2B and rye chromosome 2R. Genome 47:36-45.

Molnár-Láng M, Linc G, Friebe R B, Sutka J (2000) Detection of wheat-barley translocations by genomic in situ hybridization in derivatives of hybrids multiplied *in vitro*. Euphytica 112:117-123.

Mukai Y, Nakahara Y, Yamamoto M (1993) Simultaneous discrimination of three genomes in hexaploid wheat by multicolor fluorescence in situ hybridization using total genomic and highly repeated DNA probes. Genome 36:489-494.

Naranjo T, Roca P, Goicoechea PG, Giraldez R (1987) Arm homoeology of wheat and rye chromosomes. Genome 29:873-882.

Oliver RE, Xu SS, Stack RW, Friesen TL, Jin Y, Cai X (2006) Molecular cytogenetic characterization of four partial wheat-*Thinopyrum ponticum* amphiploids and their reaction to *Fusarium* head blight, tan spot, and *Stagonospora nodorum* blotch. Theor Appl Genet 112:.1473-1479.

Sepsi A, Molnár I, Szalay D, Molnár-Láng M (2008) Characterization of a leaf rust resistant wheat–*Thinopyrum ponticum* partial amphiploid BE-1, using sequential multicolor GISH and FISH. TAG 116:.825-834.

Szalay D (1979) Faj- és nemzetséghibridek felhasználása a búzanemesítésben (Use of interspecific and intergenomic hybrids in wheat breeding). In A búza jelene és jövője (The present and future of wheat), ed., Bálint Andor, Mezőgazdasági kiadó.