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ORIGINAL PAPER

Characterization of a leaf rust-resistant wheat–*Thinopyrum ponticum* partial amphiploid BE-1, using sequential multicolor GISH and FISH

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Abstract In situ hybridization (multicolor GISH and FISH) was 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 using J, A and D genomic probes showed 16 chromosomes originating from *Thinopyrum ponticum* and 14 A genome, 14 B genome and 12 D genome chromosomes. Six of the *Th. ponticum* chromosomes carried segments different from the J genome in their centromeric regions. It was demonstrated that these alien chromosome segments did not originate from the A, B or D genomes of wheat, so the translocation chromosomes were considered to be J^s type chromosomes carrying segments similar to the S genome near the centromeres. Rearrangements between the A and D genomes of wheat were detected. FISH using Afa family, pSc119.2 and pTa71 probes allowed the identification of all the wheat chromosomes present and the determination of the chromosomes involved in the translocations. The 4A and 7A chromosomes were identified as being involved in intergenomic translocations. The replaced wheat chromosome was identified as 7D. The localization of these repetitive DNA clones on the *Th. ponticum* chromosomes of the amphiploid was described in the present study. On the basis of their multicolor FISH patterns, the alien chromosomes could be arranged in eight pairs and could also be differentiated unequivocally from each other.

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Introduction

Alien gene transfer into bread wheat (*Triticum aestivum* L.) via wide hybridization makes it possible to increase resistance to biotic and abiotic stresses and to improve quality. Wild relatives of common wheat have been used extensively to transfer agronomically useful traits into wheat (Friebe et al. 1996; Bommineni and Jauhar 1997).

Thinopyrum ponticum (Popd.) Barkworth & D. R. Dewey [syn *Agropyron elongatum* (Host) Beauvoir ssp. *ruthenicum* Beldie] ($2n = 10x = 70$) has frequently been used in wheat improvement as a donor of various disease-resistance genes, in particular for leaf rust (*Puccinia recondita* f. sp. *Tritici*; Sharma and Knott 1966; Knott 1968; Sears 1973; Friebe et al. 1996) and wheat streak mosaic virus (Martin et al. 1976; Jiang et al. 1993).

Although several studies were carried out on the frequency of multivalent formation among *Th. ponticum* chromosomes during meiosis (Cauderon 1966; Muramatsu 1990) and on the pairing frequency between the chromosomes of hexaploid wheat and *Th. ponticum* in wheat–*Th. ponticum* hybrids (Dvorak 1981; Jauhar 1995; Cai and Jones 1997), the genomic composition of *Th. ponticum* was not completely clarified. More recent studies using GISH, with S genomic DNA isolated from the diploid *Pseudoroegneria strigosa* (M. Bieb), revealed that the genomic composition of *Th. ponticum* was J^sJ^sJJJ. The J genome of *Th. ponticum* is homologous to the J genome of the diploid *Thinopyrum bessarabicum*, while the J^s genome is a modified J genome of unknown origin characterized by the presence of an S genome-specific hybridization signal near the centromere (Chen et al. 1998c).

In wheat breeding programs, the production of stable amphiploids is an important intermediate step for successful gene transfer, 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 F_1 hybrid is almost completely sterile (Gale and Miller 1987; Jiang et al. 1994; Ellneskog-Staam and Merker 2002). Several *Th. ponticum*–wheat amphiploids have been obtained, such as Agrotana, OK7211542, PWM706, PWMIII and PWM 209, which were proved to carry many agronomically useful traits (resistance to wheat streak mosaic virus, barley yellow dwarf virus, common root rot, *Fusarium* head blight, tan spot and *Stagonospora nodorum*) from the *Th. ponticum* progenitor. These have been analyzed and exploited as alien sources of disease resistance in wheat improvement (Chen et al. 1998a, 1998b; Thomas et al. 1998; Fedak et al. 2000; Li et al. 2004; Oliver et al. 2006). These amphiploids, which contain a complete set of ABD genomes from wheat and two monoploid sets of the alien genome show ploidy stabilization at the 8*x* level ($2n = 56$). However, in a few cases the substitution of wheat chromosomes was observed (Fedak and Han 2005).

BE-1, produced by Szalay (1979), is a wheat–*Thinopyrum ponticum* 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.

In order to transfer agronomically important genes from related species into wheat by means of wide hybridization, it is important to determine the genomic composition of plants carrying the desired traits. Genomic in situ hybridization (GISH) using the total genomic DNA of the introgressed species as a probe is a useful technique for detecting alien chromatin in wheat-alien species amphiploids (Le et al. 1989; Schwarzacher et al. 1989; Molnár-Láng et al. 2000; Cai et al. 2001). Few techniques are known for the simultaneous discrimination of the three genomes in hexaploid wheat, thus providing the opportunity for more precise genome analysis. Zhang et al. (2004) successfully used BAC-FISH to differentiate the A, B and D genomes of wheat and detected intergenomic translocations involving the A genome and the A and D genomes of hexaploid wheat. Multicolor GISH using several different genomic probes is another possibility to visualize simultaneously two or more genomes in a polyploid species. Mukai et al. (1993) simultaneously visualized the A, B and D genomes of hexaploid wheat using A and D genomic probes. Han et al. (2003) used multicolor GISH to analyze wheat–*Thinopyrum intermedium* derivatives and detected intergenomic rearrangements involving *Th. intermedium* chromosomes and the A and B genomes of wheat.

Fluorescence in situ hybridization (FISH) using repetitive DNA clones is a powerful tool for identifying chromosomes within a species (Bedbrook et al. 1980; Rayburn and Gill 1987; Mukai et al. 1993; Pedersen and Langridge 1997) or tracing intergenomic chromosome rearrangements in a polyploid species (Linc et al. 1999). The combination of the mcGISH technique with sequential FISH on wheat-alien hybrids enables chromosomes belonging to different genomes to be detected and identified and intergenomic rearrangements within a polyploid species to be visualized (Sánchez-Morán et al.1998; Nagy et al. 2002; Wang et al.2005).

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

A wheat–*Thinopyrum ponticum* partial amphiploid was derived from a cross between hexaploid wheat (*Triticum aestivum* cv. Bánkúti) and *Thinopyrum ponticum* (Popd.) Barkworth & D. R. Dewey (Szalay 1979). The line BE-1 was selected from the F_3 generation of the cross.

Chromosome preparation

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

Probe labeling

Total genomic DNA was extracted from fresh young leaves of *T. urartu* ($2n = 2x = 14$, AA), *Aegilops bicornis* ($2n = 2x$, SS = BB), *Ae. tauschii* ($2n = 2x = 14$, DD), *T. durum* ($2n = 2x = 28$, AABB), *T. aestivum* cv. Bánkúti ($2n = 6x = 42$, AABBDD), *Elytrigia elongata* ($2n = 2x = 14$, EE) and *Thinopyrum bessarabicum* ($2n = 2x = 14$, JJ), following the phenol-chloroform method described by Sharp et al. (1988).

The total genomic DNA of *T. urartu and Ae. tauschii* was labeled with digoxigenin-16-dUTP by nick translation (Dig-Nick Translation Mix, Roche), while DNA isolated from *Elytrigia elongata* and *Thinopyrum bessarabicum* was labeled with biotin-11-dUTP by nick translation (Biotin-Nick Translation Mix, Roche).

FISH was carried out using the following repetitive sequences: Afa family (Nagaki et al. 1995), a subfamily clone of the pAs1 repetitive sequence originally cloned by Rayburn and Gill (1986) from *Aegilops squarrosa*, the rye subtelomeric heterochromatic sequence pSc119.2 (Bedbrook et al. 1980), the GAA satellite sequence and the 18S-5.8S-26S rDNA clone, pTa71 (Gerlach and Bedbrook 1979).

For three-color FISH, the pSc119.2 and Afa-family DNA sequences were amplified and labeled by PCR with biotin-11-dUTP (Roche) and digoxigenin-16-dUTP (Roche), respectively (Contento et al. 2005; Nagaki et al. 1995). The clone pTa71 was labeled combinatorially with 50% Biotin-11-dUTP and 50% Dig-11-dUTP. GAA satellite sequences were amplified from the genomic DNA of *Hordeum vulgare* and labeled using PCR with biotin-11-dUTP according to Vrana et al. (2000).

Digoxigenin and biotin were detected using anti-digoxigenin-rhodamine Fab fragments (Roche) and streptavidin-FITC (Roche), respectively.

GISH

Pre-treatments and post-hybridization washing were carried out as described by Molnár-Láng et al. (2000). The hybridization solution (25 μL/slides) contained 80 ng of biotinylated J genome specific probe, and 70 ng of digoxigenated D genomic probe or 70 ng of digoxigenated A genomic probe. For blocking, 3.2 μg AB-genomic DNA, or 1.6 μg B genomic DNA and 1.6 μg D genomic DNA were applied. The probes were mixed with 50% formamide and 10% dextrane sulfate in 2× SSC.

The probes were denatured at 80°C for 10 min and chilled on ice for 5 min. The chromosomes were denatured in the presence of the hybridization solution at 80°C for 2 min, then incubated at 42°C overnight.

In order to detect biotinylated and digoxiginated sequences, the slides were incubated with 10 μg/mL streptavidin-FITC (fluorescein isothiocyanate) and 10 μg/mL anti-digoxigenin-rhodamine in TNB detection buffer for 45 min at 37°C. The slides were mounted in Vectashield antifade solution (Vector Laboratories) containing 2 μg/mL 4′-6-diamino-2-phenylindole (DAPI). Fluorescent signals were visualized with a Zeiss Axioscope 2 epifluorescence microscope equipped with a filter for detecting DAPI (Zeiss, Filterset 01) and a dual band filter set (Zeiss, Filterset 24) for the observation of FITC and rhodamine signals simultaneously. Photographs were taken with a Spot CCD camera (Diagnostic Instruments, Inc., USA). The image processing was carried out using Image-Pro Plus 5.1 (Media Cybernetics, USA) software.

FISH

After washing the GISH hybridization signals off the slides in 4× SSC Tween at 25°C overnight, multicolor FISH was performed on them according to a protocol similar to GISH, except that the hybridization temperature was 37°C in this case. FISH was carried out by hybridizing three labeled repetitive DNA clones simultaneously (pSc119.2, the Afa-family and pTa71). Salmon sperm DNA was added for blocking. The procedure used for detection and counterstaining was the same as that described for GISH.

SSR marker analysis

Total genomic DNA from the wheat cultivar Bánkúti 1201, the amphiploid BE-1 and from the diploid *Thinopyrum bessarabicum* and *Pseudoroegneria stipifolia* was extracted according to Anderson et al. (1992).

Ten SSR markers distributed over the 7D chromosome were selected from different sources (Xgwm: Röder et al. 1998a, 1998b; Xbarc, Xwmc: Somers et al. 2004; Xgdm: Pestsova et al. 2000).

PCR reactions were performed in an Eppendorf Mastercycler (Eppendorf-Netheler–Hinc Inc., Hamburg, Germany) using the following conditions for amplification of DNA from the Xgdm 46, Xgdm67, Xgdm130, Xgdm84, Xgwm 295, Xwmc506, Xgdm150 loci: 1 min at 94°C, 30 cycles of 25 s at 94°C, 25 s at 60°C, 25 s at 72°C and a final extension step of 5 min at 72°C. The same conditions were used for the Xgwm437, Xwmc94 and Xbarc172 loci except that the annealing temperature was 50°C.

The total volume of 16 μL reaction mixture contained 30 ng genomic DNA, 5× PCR buffer, 0.45 U Taq DNA polymerase (both Promega, Madison, WI), 0.3 μM of forward and reverse primers, 200 μM dNTPs and 1.5 mM $MgCl_2$. The PCR products were separated on 2% agarose gels.

Results

The genomic constitution of the wheat–*Thinopyrum ponticum* partial amphiploid BE-1 was analyzed in somatic metaphase cells by multicolor GISH and FISH. Chromosome counting was carried out on metaphase spreads after mcGISH and the chromosome number per cell was 56.

Genome differentiation of the wheat–*Th. ponticum* amphiploid by multicolor GISH

By probing with biotinylated J genomic DNA and digoxigenated A genomic DNA and blocking with B and D 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. 1a, c). Four of the 16 *Th. ponticum* chromosomes (later identified by FISH as two pairs) showed no green fluorescent signal near their centromeric regions (chromosomes 3 and 7 on Fig. 2b, c); however, they exhibited a faint red signal in the centromeric region (chromosomes No. 3 and 7 on

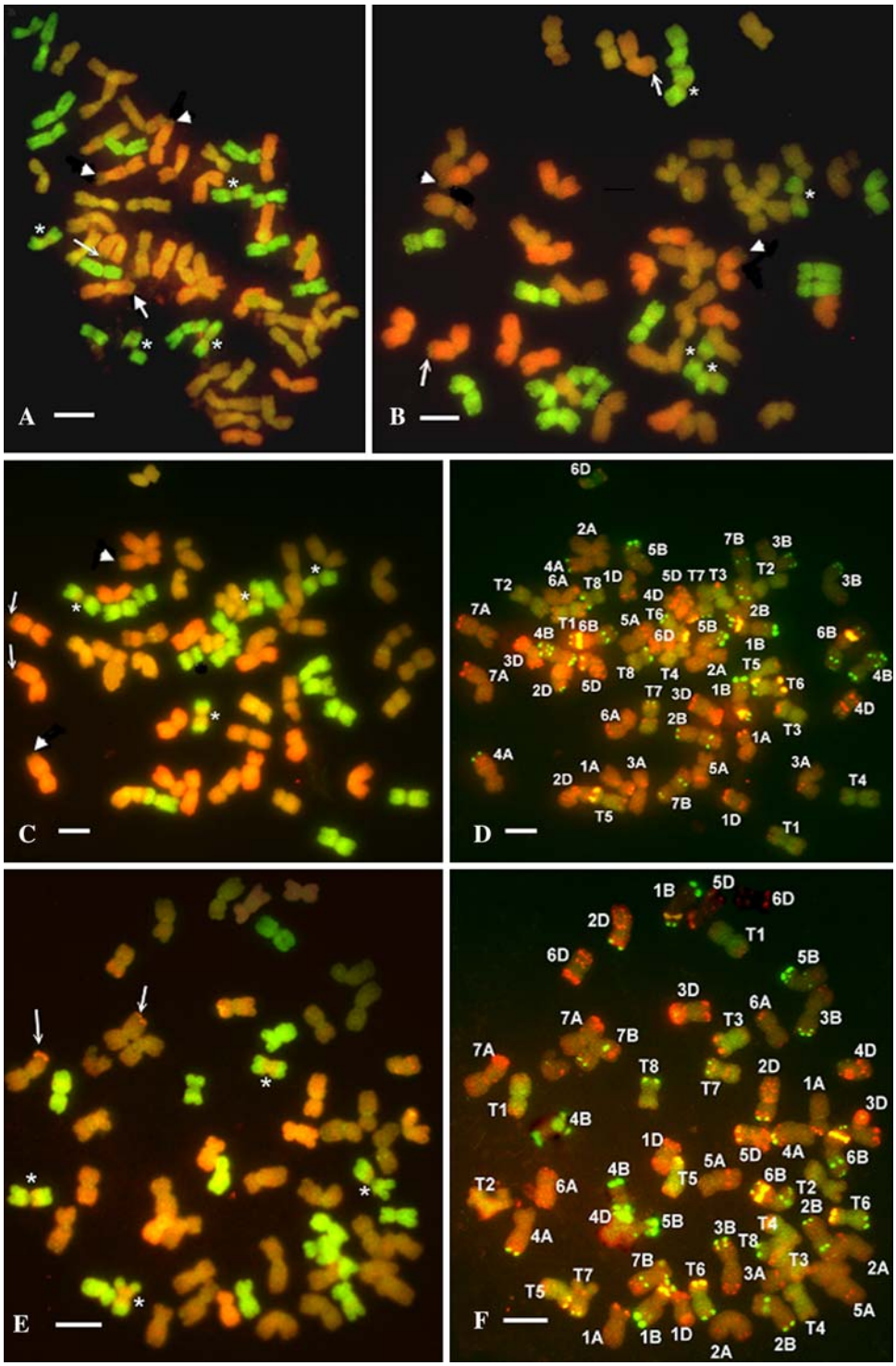

Fig. 2b, c), though the signal was weaker than that given by the A genome chromosomes. This suggested that they were involved in intergenomic translocations (Fig. 1a, c, Fig. 2b, c). One pair of *Thinopyrum* chromosomes (chromosome No 8) showed a weaker fluorescent signal in the centromeric region when J genomic probe was used, but this hybridization pattern was clearly different from that of chromosomes 3 and 7. Among the 14 red-fluorescing chromosomes, 2 pairs carried a terminal unlabeled region, with fraction lengths of ±0.7 and ±0.8, respectively, on the relevant arm, suggesting that intergenomic rearrangement had taken place in the wheat genome (Fig. 1a–c). These chromosomes were later identified using FISH as 4A and 7A, respectively. Translocation involving the terminal region of wheat

◀ **Fig. 1** **a** Multicolor genomic in situ hybridization on mitotic chromosomes of BE-1 using J and A genomic probes. J genome visualized in *green*, A genome chromosomes visualized in *red*, B and D genomic chromosomes are *brown*. The four *Th. ponticum* translocation chromosomes are marked with an *asterisk*; the 4A/7B translocation chromosomes are indicated with *arrowheads*, while the 7A translocation chromosomes are indicated with *arrows*. **b** Multicolor genomic in situ hybridization on mitotic chromosomes of BE-1 using E and A genomic probes. E genomic probe visualized in *green*, A genome chromosomes visualized in *red*, B and D genomic chromosomes *are* brown. The four *Th. ponticum* translocation chromosomes are marked with an *asterisk*; the 4A/7B translocation chromosomes are indicated with *arrowheads*, while the 7A translocation chromosomes are indicated with *arrows* **c** Multicolor genomic in situ hybridization on mitotic chromosomes of BE-1 using J and A genomic probes. J genome visualized in *green*, A genome chromosomes visualized in *red*, B and D genomic chromosomes are *brown*. The four *Th. ponticum* translocation chromosomes are marked with an *asterisk*; the 4A/7B translocation chromosomes are indicated with *arrowheads*, while the 7A translocation chromosomes are indicated with *arrows*. **d** Fluorescent in situ hybridization on mitotic chromosomes of BE-1 using Afa family (*red*), pSc119.2 (*green*) and pTa71 (*yellow*) repetitive DNA probes. **e** Multicolor genomic in situ hybridization on mitotic chromosomes of BE-1 using J and D genomic probes. J genome visualized in *green*, D genome chromosomes visualized in *red*, A and B genomic chromosomes are *brown*. The four *Th. ponticum* translocation chromosomes are marked with an *asterisk*; the 4A/7B translocation chromosomes are indicated with *arrowheads*, while the 7A translocation chromosomes are indicated with *arrows*. **f** Fluorescent in situ hybridization on mitotic chromosomes of BE-1 using Afa family (*red*), pSc119.2 (*green*) and pTa71 (*yellow*) repetitive DNA probes

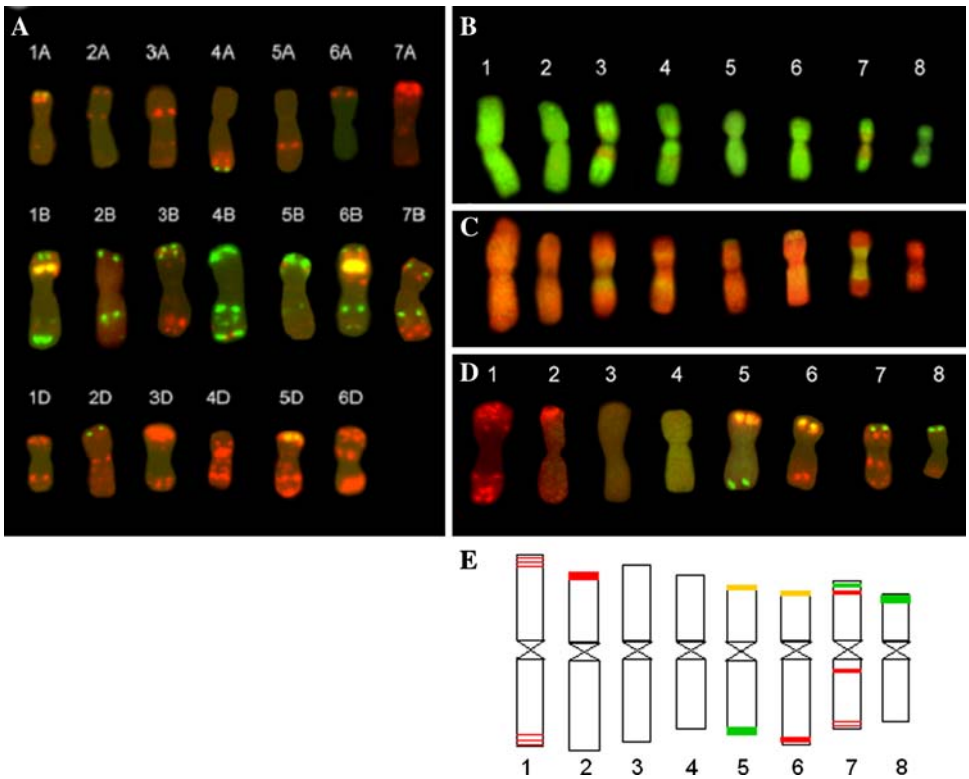

Fig. 2 **a** FISH pattern of wheat chromosomes of BE-1 using Afa family (*red*), pSc119.2 (*green*) and pTa71 (*yellow*) repetitive DNA probes. **b** GISH pattern of the eight types of *Th. ponticum* chromosomes in the wheat–*Th. ponticum* partial amphiploid BE-1. J genomic probe vizualized in *green*. Chromosomes No 3 and 7 were only labeled by biotinylated J genome probe in the terminal regions, the centromeric region remained unlabeled. **c** GISH pattern of the eight types of *Th. ponticum* chromosomes in the wheat–*Th. ponticum* partial amphiploid BE-1. J genomic probe vizualized in *red*. Chromosomes No 3 and 7 are only labeled by biotinylated J genome probe in the terminal regions; the centromeric region remained unlabeled. **d** FISH pattern of the eight types of *Th. ponticum* chromosomes in the wheat–*Th. ponticum* partial amphiploid BE-1 using Afa family (*red*), pSc119.2 (*green*) and pTa71 (*yellow*) repetitive DNA probes. **e** Idiogram of the *Th. ponticum* chromosomes in the wheat–*Th. ponticum* partial amphiploid BE-1 showing the distribution of Afa family (*red*), pSc119.2 (*green*) and pTa71 (*yellow*) repetitive DNA probes

chromosome arm 4AL has already been reported in the literature (Naranjo et al. 1987, 1990; Chao et al. 1989; Liu et al. 1992; Devos et al. 1995; Fig. 3).

When J genomic DNA was replaced by E genomic DNA from *Elytrygia elongata*, the same hybridization pattern was observed as for J genomic DNA (Fig. 1a, b).

In further probing experiments, biotinylated J genomic DNA and digoxigenated D genomic DNA were used as probes and AB genomic DNA isolated from *Triticum durum* was used for blocking. This study revealed only 12 D genome chromosomes, indicating that the two wheat chromosomes missing from BE-1 belonged to the D genome (Fig. 1e).

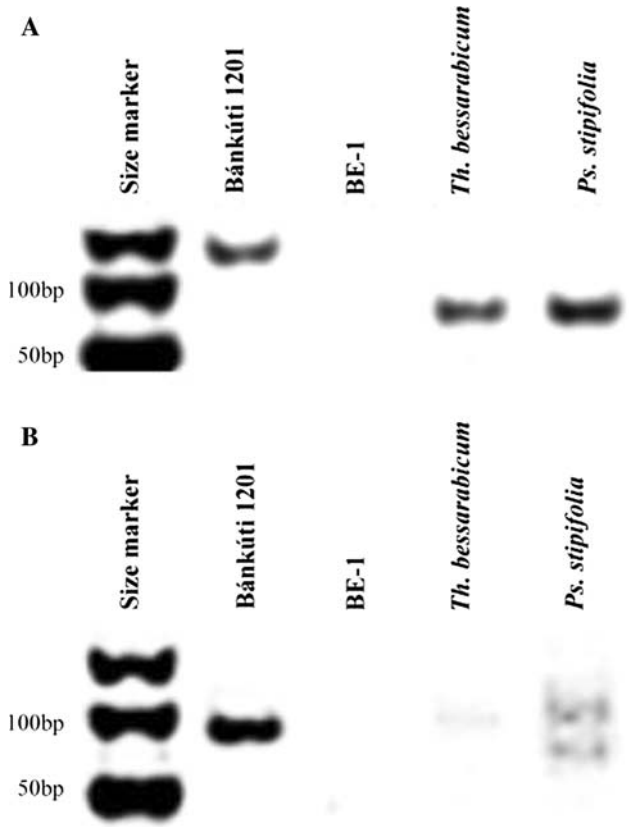

Fig. 3 Agarose gel electrophoresis pattern of PCR products amplified by the Xgdm46 (**a**) and the Xgdm130 (**b**) SSR markers on the wheat cultivar Bánkúti 1201, the partial amphiploid BE-1, *Thinopyrum bessarabicum* (J genome) and *Pseudoroegneria stipifolia* (S genome). The partial amphiploid BE-1 lacked the 7D-specific bands given by Bánkúti 1201, confirming the absence of the 7D chromosome from BE-1

One pair of A chromosomes, which showed unlabeled regions when using the A genome probe exhibited red fluorescence in the same region in this experiment. This suggested that D–A genomic translocations had taken place (Fig. 1e). Two pairs of *Th. ponticum* translocation chromosomes, which were involved in centromeric translocations and showed a faint fluorescent signal on the centromeric region when A genomic DNA was used as a probe, also gave a faint fluorescent signal when probed with D genome DNA. The other pair of *Th. ponticum* translocation chromosomes exhibited no fluorescent signals on the centromeric regions when A or D genome probe was added (Fig. 1e).

Identification of wheat and *Th. ponticum* chromosomes with repetitive DNA probes by FISH

Three-color FISH, with the simultaneous hybridization of the repetitive DNA probes pSc 119.2 and Afa family and a 18S-5.8S-25S rDNA probe (pTa71), has been usefully employed on mitotic metaphase cells of BE-1. All the wheat chromosomes present were unequivocally identified and the FISH pattern of the *Th. ponticum* chromosomes carried by BE-1 was determined with these probes (Fig. 1d, f, 2a, d). The simultaneous in situ hybridization of the repetitive DNA clones showed the complete absence of the 7D chromosome pair (7D nullisomy). The genomic characterization of the *Th. ponticum* chromosomes detected in the amphiploid by GISH and FISH was also carried out in this study. The 16 added *Th. ponticum* chromosomes could be arranged in eight pairs and were differentiated from each other by their GISH and FISH patterns (Fig. 1d, f, 2a, d).

*Chromosome No. 1 (*Th. ponticum*)*

The longest added chromosome pair was metacentric and showed a uniform green signal along its entire length when biotinylated total J genomic DNA was added as probe, indicating that the chromosome belonged to the J genome. It exhibited a faint dispersed Afa-family signal on the telomeric region of each arm (No. 1 on Fig. 2b–d).

Chromosome No. 2

The second chromosome pair, a submetacentric chromosome, belonged to the J genome and carried a strong Afa family signal on the terminal region of its short arm (No. 2 on Fig. 2b–d).

Chromosome No. 3

The third chromosome pair was only labeled by the biotinylated J genome probe in the terminal regions, while the centromeric region remained unlabeled. The fraction lengths were ±0.35 and ±0.26. When digoxigenated A or D genomic DNA was added, the centromeric region showed a faint fluorescent signal. The intensity of the signal was weaker than that on the A or D genome chromosomes, but stronger than that observed for the telomeric region of the same chromosome. This chromosome exhibited no FISH signal with the four repetitive DNA clones used (No. 3 on Fig. 2b–d).

Chromosome No. 4

The fourth chromosome pair, also a submetacentric chromosome, had a narrow unlabeled band in the intercalary region of each arm and no FISH signal (No. 4 on Fig. 2b–d).

Chromosome No. 5

The fifth was an acrocentric chromosome with a pTa71 signal on the short arm and an Afa signal on the subtelomere of the long arm (No. 5 on Fig. 2b–d).

Chromosome No. 6

The sixth was a chromosome of the J genome type with a pTa71 signal on the distal region of the short arm and a pSc119.2 signal on the terminal region of the long arm (No. 6 on Fig. 2b–d).

Chromosome No. 7

The seventh chromosome was submetacentric and carried a translocation in the centromeric region with fraction lengths of ±0.48 and ±0.41. The A and D genome probes hybridized weakly to the centromeric region, as seen for the second largest *Th. ponticum* chromosome. It carried a pSc119.2 signal on the end of the short arm and an Afa signal on the subtelomeric region. On the long arm, two other Afa family signals were observed, one close to the centromere (near to the translocation breakpoint) and the other close to the telomere. It was the only added chromosome that had a strong GAA signal near the centromeric region (No. 7 on Fig. 2b–d).

Chromosome No. 8

The eighth and smallest chromosome was strongly labeled by the J genome probe at the telomeres, but was only partly labeled close to the centromere. The centromeric region was unlabeled when using the A or D genome probes. A pSc119.2 signal was observed in the terminal region of the short arm (No. 8 on Fig. 2b–d).

SSR marker analysis

Ten SSR markers were used in order to confirm the substitution of the 7D chromosome pair in the partial amphiploid BE-1. All the markers selected were previously tested and reported to be specific for chromosome 7D (Röder et al. 1998; Pestsova et al. 2000; Somers et al. 2004). Six of them were mapped to the long arm of chromosome 7D and four to the short arm.

Four of the markers (Xgwm295, Xgdm84, Xbarc172, Wgdm150) gave the same PCR product on control DNAs as on the BE-1 and on the Bánkúti 1201, so they could not be used to prove the presence or absence of chromosome 7D.

Six markers (Xgwm437, Xgdm46, Xgdm67, Xgdm130, Xwmc94, Xwmc506) showed clear polymorphism between the wheat and control DNAs. The 7D-specific PCR products given by these markers on wheat were not observed on BE-1, indicating that the 7D chromosome pair was missing from the partial amphiploid.

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 (leaf rust, powdery mildew) line with high protein content (20.8% protein and 49.9% gluten; personal communication, M. Rakszegi), 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.

Chromosome counting on the metaphase spreads after mcGISH revealed 56 chromosomes in the partial amphiploid BE-1. A total of 16 chromosomes derived from *Th. ponticum* and 40 wheat chromosomes were detected. The substituted wheat chromosome pair was identified by FISH as 7D. Similar results were obtained by Chen et al. (1998b) who found 40 wheat and 16 alien chromosomes when applying GISH on three wheat–*Th. ponticum* amphiploids (Agrotana, OK7211542, ORRPX) using E, J and S genomic probes. Using the S genome as a probe, eight J^s type chromosomes and eight J genome chromosomes were detected in each of the amphiploids, but the substituted wheat chromosomes were not identified.

Fedak et al. (2000) reported the genomic composition of six wheat–*Th. ponticum* amphiploids (PMW706, PMW206, PMW209, PMWIII, OK7211542 and an *Agropyron*-wheat hybrid) revealed by GISH using S genomic DNA as a probe. The number of alien chromosomes varied from 12 to 18 among the amphiploids, but the chromosome number per cell was consistently 56. This suggested that chromosome substitutions occurred from the wheat genome, but the identity of the substituted chromosomes is unknown. 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 individual alien chromosomes were not identified. Li et al (2003) analyzed the chromosome composition of the leaf-rust-resistant wheat–*Th. ponticum* amphiploid line 693 using labeled S genomic DNA isolated from *Pseudoroegneria strigosa* and detected 40 wheat chromosomes and 16 *Th. ponticum* chromosomes belonging to the J and J^s genome, reaffirming that the alien genomes of *Th. ponticum* are present in a synthetic form and that both J and J^s genomes are represented in the partial amphiploids.

Four other wheat–*Th. ponticum* amphiploids (SS5, SS156, SS363, SS660) 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. SS5 and SS156 carried 42 wheat and 14 alien chromosomes, while SS363 carried 40 wheat, 14 alien

and 2 translocation chromosomes. SS660 carried 16 *Th. ponticum* and 40 wheat chromosomes. Based on meiotic pairing analysis in the F_1 hybrids of these four amphiploids they were found to carry a similar set of *Th. ponticum* chromosomes; the missing chromosomes and the chromosomes involved in the translocations were not identified.

Using GISH and multicolor GISH, Han et al. (2004) characterized the genomic composition of five wheat–*Th. intermedium* amphiploids classified into two types (type I: Zhong 1 and Zhong 2, type II: Zhong 3, Zhong 5). Although the amphiploids belonged to the same type and were derived from the same backcrossing pedigrees, different types of genomic rearrangements were detected. Zhong 1 carried a translocation between a pair of A-genome chromosomes and *Th. intermedium* chromosomes, which was not detected in Zhong 2. In Zhong 4, rearrangements were detected between the A and D genomes of wheat, while Zhong 3 and Zhong 5, which were derived from the same pedigree, did not carry the same genomic changes. The gliadin and HMW glutenin pattern of this plant material showed novel expression patterns compared to the parental progeny, showing that allopolyploidization accelerates genomic changes in wheat.

Fedak and Han (2005) described the mcGISH pattern of addition lines ($2n = 44$) derived from the wheat–*Th. intermedium* amphiploids TAF 46 and Zhong 5 and detected rearrangements involving the A, B and D genomes of wheat and the genomes of *Thinopyrum intermedium*. The addition line Z5, derived from the amphiploid Zhong 5, contained a pair of translocated chromosomes, which carried segments of A and D genome chromosomes and a segment of a *Thinopyrum* chromosome. Z4 contained two pairs of translocation chromosomes, one carrying an A- and a *Thinopyrum* chromosome segment, while the other pair was a multiple translocated chromosome similar to Z5. Line Z6 contained a translocation between a B genome chromosome and a *Th. intermedium* chromosome.

Chen et al. (1998c) reported that *Th. ponticum* contains three sets of J genome chromosomes and two sets of J^s genome chromosomes. The J genome chromosomes are completely labeled when J genomic probe is added, while the J^s type chromosomes can only be detected using the S genome probe of *Pseudoroegneria strigosa*, which hibridizes to the centromeric region of these chromosomes and only the telomeres give J genome-specific signals.

In the present study, an indirect method was used to detect chromosomes of the J^s type. After detecting *Th. ponticum* chromosomes unlabeled in their centromeric regions with J genomic probe, A and D genomic probes and GAA repetitive sequences (data not shown) were used to demonstrate whether these unlabeled regions originated from any of the three homoeologous genomes of hexaploid wheat. McGISH using these probes confirmed that the alien chromosome segments in the centromeric region of the translocated chromosomes did not originate from the A, B or D genome of wheat, though they may have had some similarity with the A and D genomes, as a faint fluorescent signal was observed when using these probes.

McGISH also gave information about intergenomic rearrangements occurring in the wheat genomes and revealed that the substituted wheat chromosome pair belonged to the D genome. Later, FISH analysis identified it as 7D. The absence of the 7D chromosome pair was further confirmed using 7D-specific SSR markers, as PCR products given by the polymorphic markers were not found on BE-1. Natural substitutions usually involve homoeologous chromosomes, and the alien homoeologous chromosome pair compensates for the loss of wheat chromosomes (Knott 1968, 1987; Sharma 1997; Li et al. 2004).

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 literature (Naranjo et al. 1987, 1990; Chao et al. 1989; Liu et al. 1992; Devos et al. 1995).

Using the in situ hybridization pattern of repetitive sequences, pSc119.2 and Afa family, Kubalakova et al. (2005) were able to identify all the chromosomes of durum wheat. In the present work, when the same repetitive DNA clones were combined with the pTa71 rDNA sequence, FISH resulted in the identification of all the chromosomes of hexaploid wheat. The localization of the repetitive DNA clones mentioned above on the *Th. ponticum* chromosomes of the amphiploid has been described in the present study. On the basis of their FISH patterns, the *Th. ponticum* chromosomes could be arranged in eight pairs and could also be differentiated unequivocally from each other.

Li and Zhang (2002) analyzed the number and localization of the 18S-5.8S-25S rDNA loci in three accessions of *Th. ponticum* and detected 20 major sites in the telomeric position on the short arms of the chromosomes. The hybridization pattern was the same for each accession. By contrast, in another accession of *Th. ponticum*, Brasileiro-Vidal et al. (2003) detected only 17 loci of 18S-5.8S-25S rDNA using the pTa71 probe and 17 chromosomes carrying the pSc119.2 signal. These results suggest heterogeneity in the distribution of repetitive sequences among accessions of *Th. ponticum*, but more data will be required before a definite conclusion can be drawn.

Resistant *Th. ponticum* amphiploids were produced by several authors and studied by means of GISH using total genomic DNA probes isolated from *Pseudoroegneria strigosa* to determine the number of J and J^s genome chromosomes.

However, the simultaneous hybridization of different genomic and repetitive DNA probes was not used to describe their precise genomic composition. The absence of a detailed description of the FISH patterns of the individual chromosomes of *Th. ponticum* makes comparison with the *Th. ponticum* chromosomes of BE-1 impossible.

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 programs. The BE-1 partial amphiploid was crossed with winter wheat cultivars and resistant progenies were selected through ten generations of selfing at the Cereal Resistance Breeding Department (O. Veisz, personal communication) at the Agricultural Research Institute of the Hungarian Academy of Sciences in Martonvásár. Some selected wheat lines have very good leaf rust resistance and are genetically stable, having 42 chromosomes. It is planned to analyze the chromosome constitution of these lines using GISH in the near future to determine whether they have a translocated segment from *T. ponticum* in a wheat background. Resistant progenies of this partial amphiploid are used as leaf rust resistance sources in the wheat breeding crossing program in Martonvásár.

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