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Research Article

Molecular cytogenetic characterization of parental genomes in the partial amphidiploid *Triticum aestivum* × *Thinopyrum ponticum*

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Abstract

The wheat line PF 839197 and six hybrid derivatives from a cross between PF 839197 and *Thinopyrum ponticum* were cytologically characterized by fluorescent *in situ* hybridization (FISH). Probes for the 5S and 45S rDNA genes (pTa794 and pTa71, respectively), a highly repetitive rye sequence (pSc119.2), the synthetic oligonucleotide $(AAG)_5$, and total genomic DNA from *Th. ponticum* and rye were used. In the wheat line, a 1RS.1BL translocation was revealed by the labeling patterns produced with pSc119.2 and $(AAG)_5$, and confirmed by genomic *in situ* hybridization (GISH) using rye genomic DNA as a probe. Analyses of partial amphiploids confirmed previous results indicating mitotic instability, with a tendency to stabilize at 2n = 42 or 56. GISH with *Th. ponticum* genomic DNA showed that in one hybrid derivative, with lower chromosome numbers (2n = 42-45), chromosomes were not labeled, whereas in the hybrids with 2n = 48-56 up to 14 chromosomes were labeled. These data suggest that the original chromosome set of these hybrids was 2n = 56, and that chromosomes from both genomes were lost by mitotic instability. FISH using the rDNA probes and GISH with *Thinopyrum* genomic DNA suggested that cells with 2n = 56 contained an entire wheat genome plus two monoploid chromosome sets of *Th. ponticum*.

Key words: wheat, *Thinopyrum ponticum*, rye, hybrid derivatives, GISH.

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Introduction

Thinopyrum ponticum (Podp.) Barkworth & D. R. Dewey has been extensively used in wheat breeding programs as a source for new genes, such as the resistance genes against leaf and stem rust (McIntosh *et al.*, 1998). This species is decaploid (2n = 10x = 70) including the genomes JJJ^sJ^s, with J and J^s proximally related (Chen *et al.*, 1998a). As other Triticeae species, *Th. ponticum* has a symmetric karyotype, hindering the identification of individual chromosomes. Furthermore, no banding pattern allowing the identification of each homoeology group was reported for this species.

In a recent attempt to better characterize the karyotype of *Th. ponticum*, Brasileiro-Vidal *et al.* (2003) reported the presence of 20 5S rDNA and 17 45S rDNA sites, in addition to other sites of satellite DNA sequences. The 45S rDNA sites were terminally located on the short arms of the 5S rDNA bearing chromosomes. Considering the basic number x = 7, the decaploid nature and the chromosome morphology of this species, the 17 linked 5S-45S rDNA loci and the three single 5S rDNA loci seem to be restricted to two homeologous groups.

Many partial wheat × *Thinopyrum* amphiploids with 2n = 8x = 56 have been produced by backcrossing the wheat × *Thinopyrum* hybrid with wheat. These amphiploids usually contain a complete wheat complement (42 chromosomes) and two monoploid *Thinopyrum* complements (14 chromosomes), but sometimes there are some substitutions of wheat chromosomes by *Thinopyrum* chromosomes or the opposite (Fedak *et al.*, 2000). The genomic composition of these amphiploids has been analyzed by genomic *in situ* hybridization (GISH) using genomic DNA of several species as probes (Chen *et al.*, 1998b; Cai *et al.*, 2001). Thus, the J and J^s genomes have been distinguished by the presence of sequences from the St genome (*Pseudoroegneria strigosa*, StSt), mainly in the centromeric regions of J^s chromosomes (Chen *et al.*, 1998a).

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Other probes, such as synthetic oligonucleotides and the repetitive sequence pSc119.2 isolated from rye, have also been very useful for genomic characterization of interspecific hybrids (Cuadrado and Schwarzacher, 1998). In wheat, fluorescent *in situ* hybridization (FISH) with $(AAG)_5$ or with a probe rich in GAA repeats results in a banding pattern similar to the N banding (Pedersen and Langridge, 1997; Cuadrado *et al*., 2000). As a result, the seven pairs of the B genome are easily recognizable, as well as some A and D chromosomes in intergeneric hybrids (Cuadrado and Schwarzacher, 1998).

The research unit "Embrapa Trigo", located in Passo Fundo (state of Rio Grande do Sul, Brazil), has developed a program to generate introgressions of *Th. ponticum* genes, mainly for leaf and stem rust resistance, in local wheat cultivars. Six accessions obtained from the wheat × *Th. ponticum* hybrid backcrossed twice and self-fertilized for five to seven generations were analyzed for chromosome number and DNA content. The results revealed a chromosome number variation generated at least partially by mitotic instability (Brasileiro-Vidal, 2003). Five of these accessions revealed wide intra-individual variation, with higher chromosome numbers (around 2n = *ca.* 56), while a single accession showed a more or less stable chromosome number, 2n = 42 being the most frequent one.

In the present work, a combination of cytological methods, GISH and FISH, was used to characterize the genomic composition of the six above mentioned accessions and of the wheat line PF 839197 used as a parent in the first cross. The cultivar 'Alondra' is one of the ancestral lines of this wheat line and putatively carries a wheat-rye translocation on the short arm of chromosome 1, designated as 1BL.1RS (Schlegel, 2003). However, it is not known if the rye short arm was transmitted to the progeny and if it is still present in PF839197.

Materials and Methods

Plant material and chromosome preparation

The genotypes analyzed included the wheat line PF 839197 and six wheat-*Th. ponticum* partial amphiploids. The latter were produced by crossing line PF 839197 with the accession PF Ag. el. 84001 of *Th. ponticum*. The hybrid was backcrossed twice with the wheat cultivar 'CEP 19' (PF 839197/PF Ag. el. 84001//2*'CEP 19'). Three accessions (PF 984501, PF 984504, PF 984506) were obtained by selfing for seven generations (BC_2F_7), and other three accessions (PF 984901A, PF 984902, PF 984903A) were derived from selfing for five generations (BC_2F_5) followed by haplodiploidization, as described by Laurie and Bennett (1986; 1989), modified by Suenaga and Nakajima (1989) and Inagaki and Tahir (1990). Haplodiploidization was achieved by crossing individuals of the BC_2F_5 generation to maize plants. The resulting hybrid lost all maize chromosomes by somatic elimination, whereas the entire haploid chromosome set of the original hybrid derivative was maintained. Chromosome doubling was performed when the haploid plants reached the 4-5 tiller stage, using a solution containing 0.25% colchicine and 10% dimethyl sulfoxide for 3-4 hours (Brammer, 2000). The described material is part of the wheat breeding program of the Brazilian Agricultural Research Corporation (Embrapa Trigo, Passo Fundo, RS, Brazil).

Root tips were pretreated in ice-cold water (*ca.* 0 °C) for 24 h, fixed in ethanol: acetic acid (3:1, v/v), and stored at -20 °C. The material was digested in an enzyme mixture containing 2% (w/v) cellulase (Onozuka R10) and 20% (v/v) pectinase (Sigma) for 1.5 h at 37 °C. Root tips were squashed in a drop of 45% acetic acid and frozen in liquid nitrogen.

DNA probes and labeling

Six DNA probes were used for *in situ* hybridization: (1) total genomic DNA of *Th. ponticum*; (2) total genomic DNA of *Secale cereale*; (3) clone pTa71, containing the 18S-5.8S-26S rDNA repeat unit from *T. aestivum* (Gerlach and Bedbrook, 1979); (4) clone pTa794, that includes the complete 5S rRNA gene unit from *T. aestivum* (Gerlach and Dyer, 1980); (5) clone pSc119.2, containing the 120-pb repeat unit of a tandemly arranged DNA family derived from *S. cereale* (McIntyre *et al*., 1990), and (6) the synthetic oligonucleotide $(AAG)_5$.

Probes were labeled with digoxigenin-11-dUTP (Roche), biotin-11-dUTP (Sigma) or rhodamine-5-dUTP (Sigma) using nick translation (genomic DNA and pTa71), random primer (synthetic oligonucleotide) or the polymerase chain reaction (pTa794 and pSc119.2) with universal forward and reverse primers, at an annealing temperature of 55 °C. In the GISH analyses, the genomic DNA probes of *Th. ponticum* and *S. cereale* were added to the hybridization mixture at the ratios of 1:40 and 1:10, respectively, with non-labeled blocking genomic wheat DNA.

In situ hybridization

In situ hybridization was performed essentially as described by Heslop-Harrison *et al*. (1991), at 85% stringency. Digoxigenin-labeled probes were detected using anti-digoxigenin-fluorescein isotiocyanate (FITC) conjugate (Boehringer), while biotin-labeled probes were detected using avidin-rhodamine or avidin-FITC conjugate (Vector). All preparations were counterstained with 2 μg/mL 4', 6-diamidino-2-phenylindole (DAPI) and mounted in Vectashield H-1000 (Vector). The best cells were captured with a Leica DMLB microscope equipped with a Cohu CCD camera and the Leica QWin software or photographed on Fuji Super G or Kodak Ultra ASA 400 color films, and scanned at 300 dpi. The images were optimized for best contrast and brightness with Adobe Photoshop 6.0.

Results

The hybridization patterns obtained with the probes pTa794, pTa71, pSc119.2 and $(AAG)_5$ on the cultivar PF 839197 were similar to that previously described for 'Chinese Spring' (Mukai *et al.*, 1990; 1991; Cuadrado and Jouve, 1994; Cuadrado *et al.*, 2000), except for the short arm of chromosome 1B, which exhibited a different pattern when using the probes pSc119.2 and $(AAG)_5$ (Figures 1a, b). With these two probes, the chromosome arm 1BS showed the typical pattern of rye 1RS: probe pSc119.2 hybridized to both terminal and intercalary positions along this arm, with no hybridization signal with the $(AAG)_5$ oligonucleotide probe. Using GISH with rye genomic DNA as probe, two chromosome arms of rye were observed, confirming the translocation 1BL.1RS in this wheat line (Figure 1c).

A second GISH experiment using genomic DNA of *Th. ponticum* as probe in one of the BC_2F_5 lines (PF 984902), with 2n = 42 to 2n = 45, indicated the absence of fragments or whole chromosomes of *Thinopyrum*. On the other hand, in five other accessions analyzed (two BC_2F_5 and three BC_2F_7), the same probe revealed a maximum of 14 out of a total of 56 chromosomes, with uniform labeling along the whole chromosomes (Figure 1d). Due to the mi-

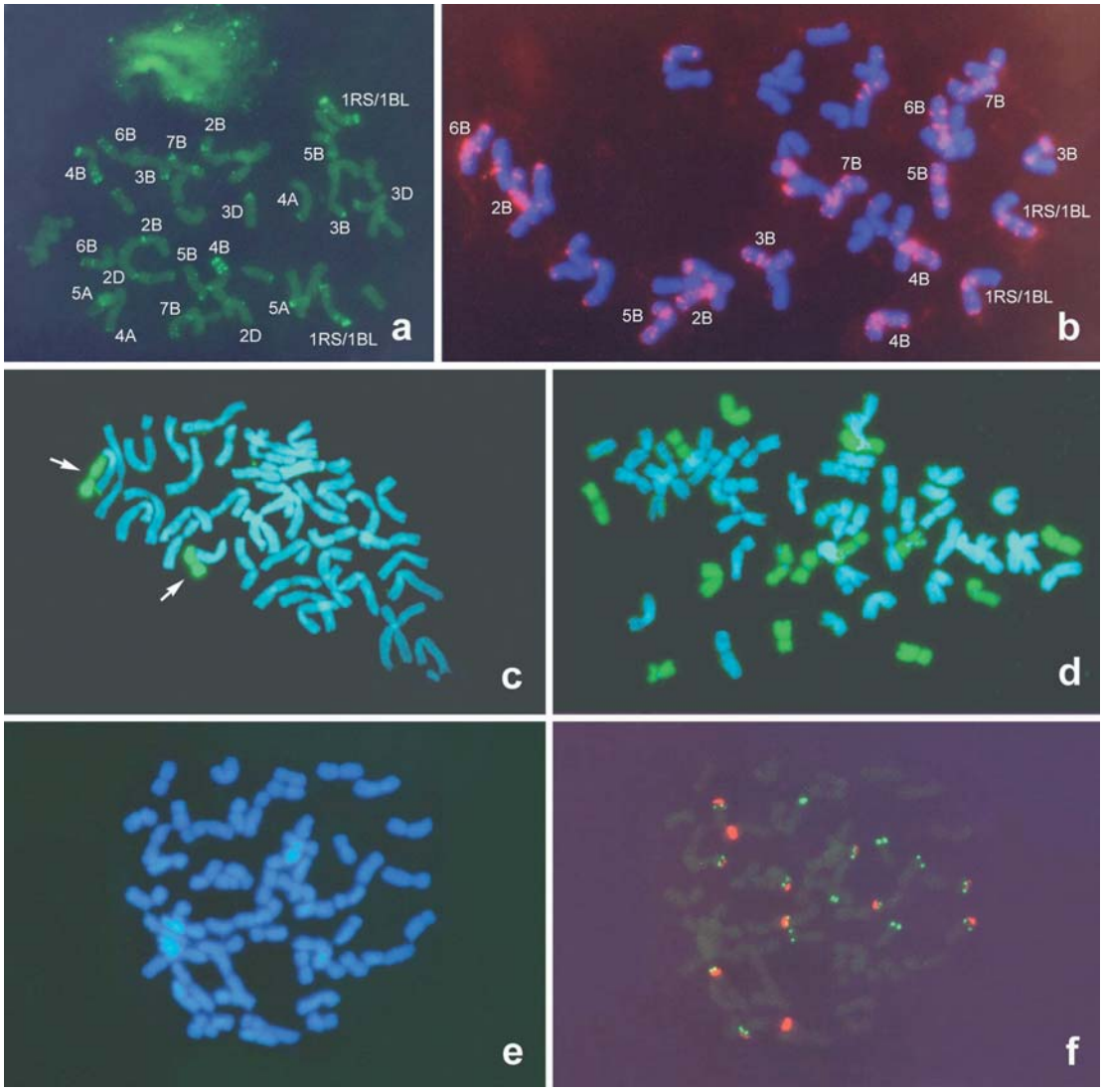

Figure 1 - *In situ* hybridization of mitotic metaphases of the wheat line PF 839197 and hybrid accessions derived from PF 839197 *Thinopyrum ponticum*. (a) Complete metaphase of the wheat line PF 839197 with 2n = 42, revealing the distribution of pSc119.2 (green). Chromosomes were identified according to the distribution pattern of pSC119.2, as described by Cuadrado and Jouve (1994). (b) A metaphase of the same line after hybridization with $(AAG)_5$ oligonucleotide (pink). Chromosomes are counterstained in blue (DAPI). The B genome chromosomes of wheat were identified according to Cuadrado *et al*. (2000). (c) GISH in PF 839197, confirming the 1RS translocation (green) to a wheat 1BL arm. Arrows indicate the pair of translocated 1RS.1BL chromosomes. (d) GISH of the amphidiploid PF 984506 with 2n = 56 and 14 *Th. ponticum* chromosomes (green). (e, f) Metaphase of the same accession with 2n = 56, stained with DAPI (e) and showing sixteen 5S rDNA sites (green) and twelve 45S rDNA sites (red) (f).

totic instability, 59% of the 44 analyzed cells showed lower chromosome numbers (2n = 48 to 2n = 55), with 11 to 14 labeled chromosomes. No wheat-*Thinopyrum* translocation could be observed.

In situ hybridization with rDNA probes in cells with 2n = 56 revealed six chromosomes bearing only 5S rDNA sites (probably 5A, 5B and 1D), two with only 45S rDNA sites (probably 6B), and six with both 5S and 45S rDNA sites (probably 1A, 1B and 5D), as previously observed in wheat (Mukai *et al.*, 1990; 1991), plus four other chromosomes bearing 5S and 45S rDNA (Figures 1e, f).

Discussion

The presence of the rye 1RS chromosome arm in wheat cultivars affects their performance positively and also confers resistance against insect and pathogen attacks (Caver and Rayburn, 1994). Many wheat cultivars carry the 1RS arm, but in most cases one group 1 chromosome has its original arm substituted by 1RS (Berzonsky and Francki, 1999). The present *in situ* evaluation using rye genomic DNA and the probes pSc119.2 and $(AAG)_5$ revealed the presence of the 1BL.1RS translocation in the line PF 839197, probably derived from the cultivar 'Alondra'.

The mitotic instability, reported by Brasileiro-Vidal (2003) in the same accessions, led to chromosome numbers around 2n = 42 in the double-haploid PF 984902 (BC_2F_5 line) and around 2n = 56 in the remaining partial amphidiploids. In the first case (2n = 42), no *Th. ponticum* chromosomes were found in the accession mentioned. Likewise, Cai *et al.* (1998b) also observed that the *Th. ponticum* genome had been completely eliminated from some BC_1F_2 individuals of the cross wheat × *Thinopyrum*. In the remaining accessions, the cells with 2n = 56 had always 42 wheat and 14 *Thinopyrum* chromosomes, whereas cells with 2n = 48 to 2n = 55 had a variable number of both genomes. These data suggest that the zygotic chromosome number of these individuals is likely to be 2n = 56, and that the mitotic instability has produced chimerical tissues with different chromosome numbers and an uneven proportion of wheat and *Thinopyrum* genomes. Brasileiro-Vidal (2003) observed that this mitotic instability was already present in the cultivar used as recurrent wheat parent ('CEP 19'). The same phenomenon was previously reported for other Brazilian wheat cultivars (Guerra and Moraes-Fernandes, 1977), and it was probably enhanced by the hybrid condition. Influence of the parental genotypes on chromosome elimination has also been observed in wide-cross hybrids, and differential elimination seems to be dependent on the ordered spatial arrangement of chromosomes throughout the cell cycle (see Heslop-Harrison and Schwarzacher, 1993).

The biparental chromosome losses found in the present work are in contrast to previous reports for wide-cross hybrids, such as barley × *Hordeum bulbosum*, wheat × maize, wheat × sorghum, and barley × maize, in which the mitotic chromosome elimination was always uniparental (Laurie and Bennett, 1988; Finch, 1983). However, hybrids of wheat with closely related genera, like *Agropyron* and *Thinopyrum*, tend to be rather stable (Chen *et al.*, 1992; Cai *et al.*, 1998b). Therefore, if mitotic instability is present in such hybrids, it should affect both parental genomes.

Partial amphidiploids of wheat × *Th. ponticum* with 2n = 56 chromosomes were reported before, but their genomic composition was variable. In line AT 3425, for example, seven *Th. ponticum* chromosome pairs could be identified, as well as three additional pairs of translocated wheat-*Thinopyrum* and 18 pairs of wheat chromosomes (Cai *et al*., 1998a). In the line Agrotana, a total of 40 wheat and 16 *Th. ponticum* chromosomes were observed (Chen *et al*., 1995). Line 784 had a similar composition as observed in the present work, with 42 wheat and 14 *Th. ponticum* chromosomes (Zhang *et al*., 1996).

Chen *et al*. (1995) suggested that Agrotana could be derived from the fusion of reduced or non-reduced gametes, followed by the loss of chromosomes at the beginning of the subsequent backcrosses and selfing procedures. Chen *et al*. (1999b) suggested that the genome of the donating species in partial amphidiploids of wheat × *Thinopyrum* may not necessarily be an intact genome of the donor species, but a synthetic genome combining chromosomes from both species. The alien genomic composition of the partial amphidiploid TAF46, for example, is a synthetic genome containing seven pairs of homoeologous chromosomes, that works as a single unit in terms of stability and transmission of its chromosomes through the gametes. The alien genome, once formed, is quite stable. The data presented here seem to confirm this observation, since five of the six accessions maintained ca. 14 chromosomes of *Th. ponticum,* and only one accession was entirely depleted of *Thinopyrum* chromosomes.

Partial amphidiploids of this type, with 2n = 56, have normally a regular meiosis with high frequencies of bivalent and low multivalent formation (Fedak *et al*., 2000). In Agrotana, for example, eight *Thinopyrum*-*Thinopyrum* bivalents, 16-20 wheat-wheat bivalents and no allosyndetic pairing were observed. This meiotic behavior could guarantee high fertility and chromosome stability (Chen *et al*., 1995). In the cells with 2n = 56 analyzed here, the detection of four chromosomes with 5S and 45S rDNA sites, in similar positions to those observed in *Th. ponticum* (Brasileiro-Vidal *et al.*, 2003), suggests that the 14 *Th. ponticum* chromosomes represent two monoploid complements, since each monoploid chromosome set of *Th. ponticum* has two 5S and two 45S rDNA sites (Brasileiro-Vidal *et al*., 2003). It was not possible to recognize to which genome (J or J^s) these chromosomes belonged. Partial amphidiploids do not have necessarily the same chromosome set as the introgressed species; there-

fore, they can be composed of different rates of J and J^s chromosomes. Zhang *et al*. (1996) suggested that, of the 14 *Th. ponticum* chromosomes of line 784, six pairs came from the J^s and one from the J genome. On the other hand, Chen *et al*. (1998b; 1999a) identified eight J and eight J^s chromosomes in Agrotana.

If the 14 *Th. ponticum* chromosomes reported here in cells with 2n = 56 form seven bivalent pairs in meiosis, they could be maintained by selfing with a stable meiotic transmission. The mitotic instability of these accessions generates a mosaic of cells with chromosome numbers different from 2n = 56, but it does not seem to affect the production of gametes to the next generation. A GISH evaluation of meiotic cells may reveal the maintenance mechanism of such amphidiploids even in the presence of the observed mitotic instability.

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