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), 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), 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 JJJJJsJ, with J and Js 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 homeologous 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.

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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 Js genomes have been distinguished by the presence of sequences from the St genome (Pseudoroegneria strigosa, SSt), mainly in the centromeric regions of J′ chromosomes (Chen et al., 1998a).
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), 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 (BC2F7), and other three accessions (PF 984901, PF 984902, PF 984903A) were derived from selfing for five generations (BC2F5) 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 BC2F5 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). 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 isothiocyanate (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 BC2F5 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 BC2F5 and three BC2F7), 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.](image)
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)6 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 (BC2F5 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 BC2F2 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 unparental (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 multivalence 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′) 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' chromosomes. Zhang et al. (1996) suggested that, of the 14 \textit{Th. ponticum} chromosomes of line 784, six pairs came from the J' and one from the J genome. On the other hand, Chen et al. (1998b; 1999a) identified eight J and eight J' chromosomes in Agrotana.

If the 14 \textit{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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