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Research Article

Chromosome characterization in *Thinopyrum ponticum* (Triticeae, Poaceae) using *in situ* hybridization with different DNA sequences

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Abstract

Thinopyrum ponticum (2n = 10x = 70, JJJ^sJ^s) belongs to the Triticeae tribe, and is currently used as a source of pathogen resistance genes in wheat breeding. In order to characterize its chromosomes, the number and position of 45S and 5S rDNA sites, as well as the distribution of the repetitive DNA sequences pAs1 and pSc119.2, were identified by fluorescent *in situ* hybridization. The number of nucleoli and NORs was also recorded after silver nitrate staining. Seventeen 45S and twenty 5S rDNA sites were observed on the short arms of 17 chromosomes, the 45S rDNA was always located terminally. On three other chromosomes, only the 5S rDNA site was observed. Silver staining revealed a high number of Ag-NORs (14 to 17) on metaphase chromosomes, whereas on interphase nuclei there was a large variation in number of nucleoli (one to 15), most of them (82.8%) ranging between four and nine. The pAs1 probe hybridized to the terminal region of both arms of all 70 chromosomes. In addition, a disperse labeling was observed throughout the chromosomes, except in centromeric and most pericentromeric regions. When the pSc119.2 sequence was used as a probe, terminal labeling was observed on the short arms of 17 chromosomes and on the long arms of five others. The relative position of 45S and 5S rDNA sites, together with the hybridization pattern of pAs1 and pSc119.2 probes, should allow whole chromosomes or chromosome segments of *Th. ponticum* to be identified in inbred lines of wheat x *Th. ponticum*.

Key words: *Thinopyrum*, silver staining, 45S and 5S rDNA, pSc119.2, pAs1.

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Introduction

Thinopyrum ponticum (Podp.) Barkworth & D. R. Dewey, previously named *Agropyron elongatum* auct. non (Host) P. Beauv., *Elytrigia pontica* (Podp.) Holub. or *Lophopyrum ponticum* (Podp.) Á. Löve, is an important source of genes for wheat, especially for leaf and stem rust resistance (McIntosh *et al*., 1998). Because of its decaploid nature (2n = 10x = 70) and chromosome pairing complexity, various genome formulas have been reported for this species (Muramatsu, 1990; Wang *et al.*, 1991; Zhang *et al*., 1996). More recently, Chen *et al*. (1998) suggested, by using genome *in situ* hybridization, that *Th. ponticum* consists of five closely related genomes represented by the formula JJJ^sJ^s, and that J, J^s, E and St genomes from the *Thinopyrum* species are closely related.

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Due to the importance of *Th. ponticum* genes in wheat breeding, cytological markers associated with chromosomes or chromosome segments have been used in gene introgression programs. The use of fluorescent *in situ* hybridization (FISH) has made it possible to locate several specific DNA sequences throughout the chromosomes of various species of Triticeae, allowing alien chromatin to be identified in interspecific hybrids. Probes corresponding to multigenes, such as 45S and 5S rDNA, and highly repetitive DNA sequences, are the most commonly used markers of specific chromosomes or genome regions. For example, in *Triticum aestivum* L., Mukai *et al*. (1990; 1991) labeled five chromosome pairs with 45S rDNA sites (1AS, 1BS, 6BS, 5DS, 7DL) and six pairs with 5S rDNA sites (1AS, 1BS, 1DS, 5AS, 5BS e 5DS). The pAs1 probe, originally obtained from *Aegilops tauschii* Coss (D genome), allowed the D genome chromosomes (Rayburn and Gill, 1986) to be identified, along with small sites on chromosomes 1A, 4A, 1B, 3B, 6B and 7B in wheat (Mukai *et al.*, 1993). Similarly,

the pSc119.2 probe from *Secale cereale* L. (R genome) distinguished all B genome chromosomes and 4A, 5A, 2D, 3D, 4D e 5D chromosomes. The simultaneous use of these two probes allowed 17 out of 21 chromosome pairs of *T. aestivum* to be identified (Mukai *et al.*, 1993). More recently, Cuadrado *et al.* (2000) characterized the seven pairs of the B genome and some chromosomes of the A and D genomes of *T. aestivum* using two simple sequence repeats (SSR), $(AAC)_5$ and $(AAG)_5$.

Besides the FISH, another procedure used to characterize chromosomes has been silver staining, which was developed to detect nucleoli in interphase cells and nucleolus organizer regions (NORs) in metaphase chromosomes (Fernandez-Gomez *et al*., 1969; Hizume *et al*., 1980). The activity of 45S rDNA genes is usually associated with NORs and secondary constrictions, and has been studied at cytological level by recording the number and volume of nucleoli in interphase cells and NORs in metaphase chromosomes by silver staining (Martini and Flavell, 1985; Vieira *et al*., 1990; Lima-Brito *et al*., 1998).

In the genus *Thinopyrum*, the localization of rDNA sites has been reported in some species. In *Th. distichum* (Thunb.) Á. Löve (2n = 4x = 28, JJ)[1], two 45S rDNA sites have been located in homeologous groups 5 and 6, and two 5S rDNA sites have been found in homoeologous group 5 and in a non-defined group (Fominaya *et al*., 1997). In *Th. elongatum* (Host) D. R. Dewey [= *Agropyron elongatum* (Host) P. Beauv., *Elytrigia elongata* (Host) Nevski, *Lophopyrum elongatum* (Host) Á. Löve] (2n = 2x = 14, E), the 5S rDNA sites were associated with chromosomes 1E and, possibly, 5E (Dvorák *et al*., 1989). Similarly to related diploid species, the decaploid *Th. ponticum* has two 45S rDNA loci per monoploid genome, in all a total of 20 sites (Li and Zhang, 2002). Highly repetitive DNA sequences, such as pSc119.2 cloned from rye (Lapitan *et al*., 1987) and pLeUCD2 of *Th. elongatum* (Zhang and Dvorák, 1990; Kim *et al.*, 1993), have been isolated and used in the characterization of *Thinopyrum* chromosomes and for the identification of introgressed alien chromatin in wheat x *Thinopyrum* hybrids. In the present study, FISH was used to identify the number and position of 45S and 5S rDNA sequences, as well as the distribution of the highly repetitive DNA sequences pAs1 and pSc119.2 in the *Th. ponticum* genome. The number of nucleoli and NORs was also recorded to elucidate whether all 45S rDNA loci were active in this allopolyploid.

Material and Methods

Plant material and chromosome preparation

The clone of *Th. ponticum* used in this study was the PF Ag. el. 84001 accession, maintained under cultivation at Embrapa Trigo, Passo Fundo (Rio Grande do Sul, Brazil).

1. Genome designation of *Thinopyrum* species was based on Chen *et al*. (1998) and Chen *et al*. (2001).

Root tips were pretreated in ice-cold water for 24 h, fixed in ethanol: acetic acid (3:1, v/v) and then 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 2 to 3 h at 37 °C. Afterward, each root tip was squashed in a drop of 45% acetic acid and frozen in liquid nitrogen.

DNA probes and labeling

Four DNA probes were used for FISH analysis: (1) clone pTa71, containing the repeat unit of 18S-5.8S-26S rDNA from *T. aestivum* (Gerlach and Bedbrook, 1979); (2) clone pTa794, which corresponds to the complete 5S gene unit from *T. aestivum* (Gerlach and Dyer, 1980); (3) clone pSc119.2, containing the 120-pb repeat unit of a tandemly arranged DNA family derived from *S. cereale* (McIntyre *et al*., 1990); and (4) clone pAs1, including a 1 kb repetitive DNA sequence from *Ae. tauschii* (Rayburn and Gill, 1986). Probes were labeled with digoxigenin-11-dUTP (Roche), biotin-11-dUTP (Sigma) or rhodamine-5-dUTP (Amersham) using nick translation for pTa71 or the polymerase chain reaction for pTa794, pSc119.2 and pAs1.

Fluorescent *in situ* hybridization (FISH)

FISH was performed essentially as described by Heslop-Harrison *et al*. (1991) at 85% stringency. No immunocytochemistry was necessary to detect the direct rhodamine labeling. Biotin-labeled probes were detected using avidin-rhodamine conjugate (Vector), while digoxigenin-labeled probes were detected using anti-digoxigenin-fluorescein isotiocyanate (FITC) conjugate (Roche). All preparations were counterstained with 2 µg/mL 4', 6-diamidino-2-phenylindole (DAPI) and mounted in Vectashield H-1000 (Vector). Photographs were taken on Fujicolor Super HG or Kodak Ultra 400 print film, and scanned at 300 dpi. The images were optimized for best contrast and brightness with Adobe Photoshop 6.0.

Silver staining

The silver staining technique employed was that described by Vieira *et al.* (1990). After cold treatment, roots were fixed in 50% ethanol, glacial acetic acid and 37% formaldehyde (18:1:1, v/v/v) for a minimum of 4 h at room temperature and a maximum of three days at 0 °C to 2 °C. Fixed roots were washed several times in distilled water to eliminate any residues of fixative. Once clean, they were immersed overnight in an aqueous solution of 20% $AgNO_3$ at 60 °C. Residual silver was eliminated by washing with distilled water and the staining developed with 1% hydroquinone and 10% formaldehyde (1:1, w/v) for one to 10 min, depending on the intensity required. After a further wash with distilled water, the root tips were squashed in 45% acetic acid containing a drop of 1% carmine, which aids viewing in normal light microscopy.

The number of nucleoli was recorded in 1,064 interphase nuclei and the number of active NORs was ob-

served in 42 metaphases. Best cells were photographed on Kodak Imagelink ASA 25 print film, and scanned at 300 dpi. The images were optimized for best contrast and brightness with Adobe Photoshop 6.0.

Results

In situ hybridization

Both simultaneous and sequential FISH with a two-by-two combination of probes were used to study the metaphase chromosomes of *Th. ponticum*. The 5S and the 45S rDNA genes were located simultaneously using pTa794 and pTa71 probes, respectively. For both sequences, minimal differences of intensities were detected between loci. Twenty 5S rDNA sites were observed at interstitial position in the short arms of 20 chromosomes. Seventeen 45S rDNA were located at a terminal position in the short arms of 17 out of the 20 chromosomes carrying the 5S rDNA, on a double labeling pattern (Figures 1a, b). Considering the number of rDNA sites, as well as the ploidy level (2n = 10x = 70) and the chromosome morphology of *Th. ponticum*, double labeling seemed to be restricted to chromosomes of two homoeologous groups, both submetacentrics.

The pAs1 probe produced terminal labels on both arms of all 70 chromosomes. In addition, dispersed labeling was observed throughout the chromosomes, except for centromeric and most pericentromeric regions (Figure 1c). The pSc119.2 probe produced labeling on terminal positions of the short arms of 17 chromosomes. Two of them, with different morphologies, also showed telomeric labels on the long arm. In addition, pSc119.2 labels were observed on the long arm of three chromosomes with 45S rDNA sites (Figure 1d).

Silver staining

An attempt was made to analyze the activity of different NORs in cells undergoing interphase to metaphase. Silver staining revealed a high number of Ag-NOR sites (14 to 17). Seventeen were observed in 76% of the 46 metaphases studied (Figure 2a). However, in the 1,064 interphase nuclei studied, the number of nucleoli varied from one to 15, most (82.8%) showing four to nine nucleoli (Figure 2b).

Discussion

Ribosomal genes

The species of the Triticeae tribe vary greatly in number of 45S ribosomal DNA sites. For example, *Ae. comosa*

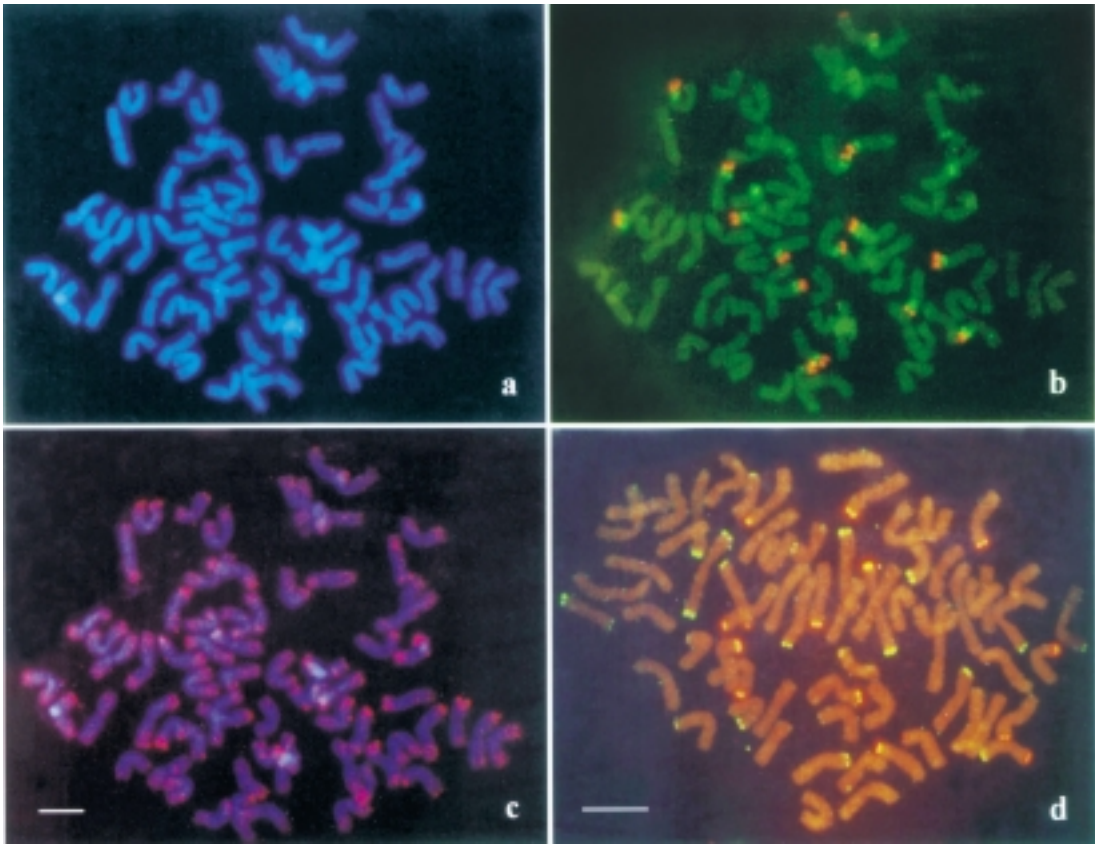

Figure 1 - Location of specific sites in the chromosomes of *Thinopyrum ponticum*. a. Complete metaphase with 2n = 70 DAPI stained chromosomes. b. The same cell showing seventeen 45S rDNA sites (red) and 20 sites of 5S rDNA (green). c. Reprobing of the same cell revealing the distribution of the highly repetitive DNA sequence pAs1. d. Partial prometaphase displaying 15 sites of 45S rDNA (red) and the terminal locating of the pSc119.2 probe (green). Bars = 10 μm.

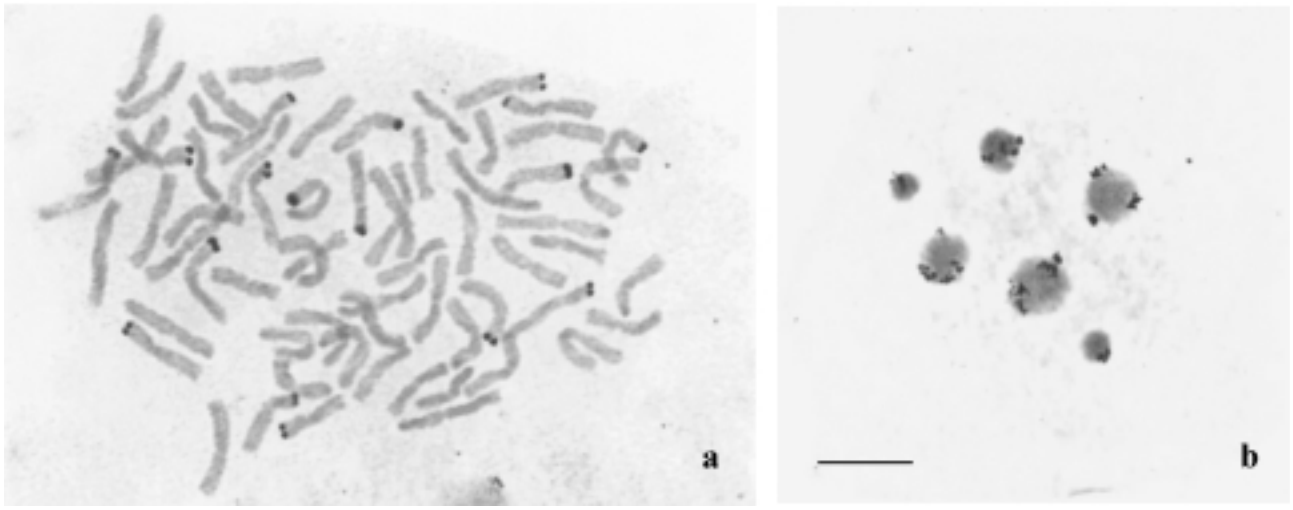

Figure 2 - Analysis of NOR activity, using the silver-staining procedure. a. Partial metaphase showing the maximum number of Ag-NORs found in a single cell (17 sites). b. Interphase nuclei with six nucleoli. Bar = 10 μm.

Sibth. & Sm. ssp. *comosa* shows nine pairs of rDNA loci distributed among their seven chromosome pairs, whereas *S. cereale* has only one pair of these loci (Appels *et al*., 1980). Nevertheless, the main 45S rDNA loci occur preferentially in the homoeologous groups 1, 5 and 6 (Lacadena *et al.*, 1988; Badaeva *et al.*, 1996). In *Th. elongatum* (2n = 2x = 14, E) and in *Th. distichum* (2n = 4x = 28, JJ), the 45S rDNA loci are located on the short arms of two chromosome pairs named as 5 and 6 (Dvorák *et al.*, 1984; Fominaya *et al.*, 1997). In the *Th. ponticum* analyzed, the 45S rDNA sites were located in a similar position in 17 chromosomes, suggesting that, except in three chromosomes, the ribosomal genes are present in two chromosome pairs of each one of the five genomes ($JJJJ^sJ^s$). The occurrence of these sites in 17 instead of 20 chromosomes, as observed by Li and Zhang (2002), most likely indicates a reduction in the number of repeats or a deletion of sites from this accession. A similar reduction has already been described in other polyploids (Linde-Laursen *et al*., 1992) and it may be more frequent in higher polyploids. In common wheat, the elimination of DNA sequences is one of the main responses to allopolyploidization, occurring after the formation of the polyploid and involving a large fraction of the genome, including 45S rDNA repetitive units (Shaked *et al.*, 2001). For example, in the chromosome 1AS of *T. monococcum* L., the number of repetitive units of 45S rDNA was largely reduced after its incorporation into the polyploid wheats (Jiang and Gill, 1994). In addition, the terminal position of this locus in *Th. ponticum* might facilitate its partial or total elimination in some sites (Pestsova *et al.*, 1998).

The number of Ag-NOR sites of *Th. ponticum* revealed after silver staining was generally similar to that of 45S rDNA labeled after FISH (17). However, the mean number of nucleoli per nucleus was much lower. This indicates that all or nearly all rDNA sites are necessary for normal cell function. At interphase, the Ag-NOR sites tend to coalesce, as suggested by Lacadena *et al.* (1988).

In Triticeae, the 5S rDNA sites consist of a 120 bp coding sequence alternating with non-transcribed spacers, which are classified into two types according to their length and sequence (Gerlach and Dyer, 1980). In most genomes the sites with short spacers are located in homeologous group 1, whereas those with long spacers are located in group 5 (Reddy and Appels, 1989; Dvorák *et al.*, 1989). In *Th. elongatum*, the presence of 5S rDNA in the short arms of chromosomes of the homeologous group 1 and probably 5 has been suggested (Scoles *et al.*, 1988; Dvorák *et al.*, 1989). In *Th. ponticum*, we found two 5S rDNA sites per haploid genome. Considering the distribution of these sites in other species, and the proximity of these sites to the 45S rDNA sites, it is tempting to conclude that these chromosomes belong to homoeologous groups 1 and 5.

In *Th. ponticum*, the 45S rDNA locus was always distally located in relation to the 5S rDNA locus. This disposition is similar to that found in homoeologous group 5 of the D genome of *T. aestivum* (Mukai *et al*., 1990; Mukai *et al*., 1991), of *Ae. umbellulata* Zhuk. (Castilho and Heslop-Harrison, 1995) and of *Th. distichum* (Fominaya *et al*., 1997). However, in homoeologous group 1 of the B genome of *T. aestivum* (Mukai *et al*., 1990; Mukai *et al*., 1991) and *Ae. umbellulata* (Castilho and Heslop-Harrison, 1995), these sites are located in an inverted position. In *Th. elongatum*, one pair of 45S and 5S rDNA loci are separately located in chromosomes 6E and 1E, respectively (Dvorák *et al.*, 1984; Dvorák *et al.*, 1989).

pSc119.2 and pAs1 repetitive sequences

The sequence pSc119.2 from *S. cereale* has been widely used in FISH experiments due to its high copy number and polymorphic location in many species of the Triticeae. Lapitan *et al.* (1987), studying the distribution of

this sequence in three species of *Thinopyrum*, found 10 terminal and only one subterminal site in *Th. bessarabicum* (Savul & Rayss) Á. Löve (2n = 2x = 14, J genome). In *Th. elongatum* (2n = 2x = 14, E genome), these authors found 10 terminal sites plus other sites distributed throughout the entire length of all chromosomes, except in the telomeres of one chromosome. On the other hand, in the hexaploid *Th. intermedium* (Host) Barkworth & D.R. Dewey (JJ^sSt), the pSc119.2 sequence hybridized to a few chromosomes. Similarly, in the decaploid *Th. ponticum* studied in the present work, a reduction in the number of pSc119.2 sites was found, when compared to those of the two diploid species referred to above.

The pAs1 probe has also been widely used in the Triticeae. In *T. aestivum*, the identification of all chromosomes was possible when this probe was associated with a GAA-rich satellite sequence. The authors observed 48 pAs1 bands (Pedersen and Langridge, 1997) and most large sites were located on the terminal region of D genome chromosomes, as described by Rayburn and Gill (1986). In fact, most large blocks of tandem repetitive sequences are usually located in a similar position in all the chromosomes of a given species. It is possible that these chromosome regions favor or show greater tolerance to the presence of such tandem repetitions (Flavell, 1982). In the present study, *Th. ponticum* showed terminal bands on both arms of the 70 chromosomes. Another characteristic of this probe in *Th. ponticum* was the disperse labeling throughout all the chromosomes, except in the centromeric and most of the pericentromeric regions. This diffuse labeling in *Th. ponticum*, unlike that found in *T aestivum*, makes this sequence an important tool for chromosome differentiation in *T. aestivum* x *Th. ponticum* hybrids.

These data show that the number and position of the sites revealed by the probes pTa 71, pTa794, pAs1 and pSc119.2 are valuable markers to characterize and identify the chromosomes of *Th. ponticum* in inbred lines of wheat x *Th. ponticum*.

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References

Appels R, Gerlach WL, Dennis ES, Swift H and Peacock WJ (1980) Molecular and chromosomal organization of DNA sequences coding for the ribosomal RNAs in cereals. Chromosoma 78:293-311.

Badaeva ED, Friebe B and Gill BS (1996) Genome differentiation in *Aegilops*. 2. Physical mapping of 5S and 18S-26S ribosomal RNA gene families in diploid species. Genome 39:1150-1158.

Castilho A and Heslop-Harrison JS (1995) Physical mapping of 5S and 18S-25S rDNA and repetitive DNA sequences in *Aegilops umbellulata*. Genome 38:91-96.

Chen Q, Conner RL, Laroche A and Thomas JB (1998) Genome analysis of *Thinopyrum intermedium* and *Thinopyrum ponticum* using genomic *in situ* hybridization. Genome 41:580-586.

Chen Q, Eudes F, Conner RL, Graf R, Comeau A, Collin J, Ahmad F, Zhou R, Li H, Zhao Y and Laroche A (2001) Molecular cytogenetic analysis of a durum wheat x *Thinopyrum distichum* hybrid used as a new source of resistance to Fusarium head blight in the greenhouse. Plant Breed 120:375-380.

Cuadrado A, Schwarzacher T and Jouve N (2000) Identification of different chromatin classes in wheat using *in situ* hybridization with simple sequence repeat oligonucleotides. Theor Appl Genet 101:711-717.

Dvorák J, Lassner MW, Kota RS and Chen KC (1984) The distribution of the ribosomal RNA genes in the *Triticum speltoides* and *Elytrigia elongata* genomes. Can J Genet Cytol 26:628-632.

Dvorák J, Zhang H-B, Kota RS and Lassner MW (1989) Organization and evolution of the 5S ribosomal RNA gene family in wheat and related species. Genome 32:1003-1016.

Fernández-Gomez M-E, Stockert JC, Lopez-Saez JP and Gimenez-Martin G (1969) Staining plant nucleoli with Ag-NO_3 after formalin-hydroxiquinone fixation. Stain Technol 44:48-49.

Flavell R (1982) Sequence amplification, deletion and rearrangement: major sources of variation during species divergence. In: Dover GA and Flavell RB (eds) Genome Evolution. Academic Press, London, pp 301-323.

Fominaya A, Molnar S, Kim N-S, Chen Q, Fedak G and Armstrong KC (1997) Characterization of *Thinopyrum distichum* chromosomes using double fluorescence *in situ* hybridization, RFLP analysis of 5S and 26S rRNA, and C-banding of parents and addition lines. Genome 40:689-696.

Gerlach WL and Bedbrook JR (1979) Cloning and characterization of ribosomal RNA genes from wheat and barley. Nucleic Acids Res 7:1869-1885.

Gerlach WL and Dyer TA (1980) Sequence organization of the repeated units in the nucleus of wheat, which contains 5S rDNA genes. Nucleic Acids Res 8:4851-4865.

Heslop-Harrison JS, Schwarzacher T, Anamtawat-Jonsson K, Leitch AR, Shi M and Leitch IJ (1991) *In situ* hybridization with automated chromosomes denaturation. Technique 3:109-116.

Hizume M, Sato S and Tanaka A (1980) A highly reproducible method of nucleolus organizing regions staining in plants. Stain Tech 55:87-90.

Jiang J and Gill BS (1994) New 18S-26S ribosomal RNA gene loci: chromosomal landmarks for the evolution of polyploid wheats. Chromosoma 103:179-185.

Kim N-S, Armstrong KC, Fedak G, Fominaya A and Whelan EWP (1993) Cytological and molecular characterization of a chromosome interchange and addition lines in Cadet involving chromosome 5B of wheat and 6Ag of *Lophopyrum ponticum*. Theor Appl Genet 86:827-832.

Lacadena JR, Cermeño MC, Orallana J and Santos JL (1988) Nucleolar competition *Triticeae*. In: Brandham PE (ed) Proc. Third Kew Chrom. Conf., 1987, HMSO, London, pp 151-165.

Lapitan NLV, Gill BS and Sears RG (1987) Genomic and phylogenetic relationships among rye and perennial species in the Triticeae. Crop Science 27:682-687.

Li D and Zhang X (2002) Physical localization of the 18S-5.8S-26S rDNA and sequence analysis of ITS regions in *Thinopyrum ponticum* (Poaceae: Triticeae): implications for concerted evolution. Genome 41:763-768.

Lima-Brito J, Guedes-Pinto H and Heslop-Harrison JS (1998) The activity of nucleolar organizing chromosomes in multigeneric F_1 hybrids involving wheat, triticale, and tritordeum. Genome 41:763-768.

Linde-Laursen I, Isben E, Von Bothmer R and Giese H (1992) Physical localization of active and inactive rRNA gene loci in *Hordeum marinum ssp. gussoneanum* (4x) by *in situ* hybridization. Genome 35:1032-1036.

Martini GM and Flavell RB (1985) The control of nucleolus volume in wheat, a genetic study at three development stages. Heredity 54:111-120.

McIntosh RA, Hart GE, Devos KM, Gale MD and Rogers WJ (1998) Patogenic disease/pest reaction. In: McIntosh RA, Hart GE, Devos KM, Gale MD and Rogers WJ (eds) Proc. 9th Int. Wheat Genetics Symp., 1998, University of Saskatchewan, Saskatoon, v 5, pp 129-139.

McIntyre CL, Pereira S, Moran LB and Appels R (1990) New *Secale cereale* (rye) DNA derivatives for the detection of rye chromosome segments in wheat. Genome 33:635-640.

Mukai Y, Endo TR and Gill BS (1991) Physical mapping of the 18S.26S rRNA multigene family in common wheat: Identification of a new locus. Chromosoma 100:71-78.

Mukai Y, Endo TR and Gill BS (1990) Physical mapping of the 5S rRNA multigene family in common wheat. J Hered 81:290-295.

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

Muramatsu M (1990) Cytogenetics of decaploid *Agropyron elongatum* (*Elytrigia elongata*) (2n = 70). I. Frequency of decavalent formation. Genome 33:811-817.

Pedersen C and Langridge P (1997) Identification of the entire chromosome complement of bread by two-colour FISH. Genome 40:589-593.

Pestsova EG, Goncharov NP and Salina EA (1998) Elimination of a tandem repeat of telomeric heterochromatin during the evolution of wheat. Theor Appl Genet 97:1380-1386.

Rayburn AL and Gill BS (1986) Molecular identification of the D-genome chromosomes of wheat. J Hered 77:253-255.

Reddy P and Appels R (1989) A second locus for the 5S multigene family in *Secale* L.: sequence divergence in two lineages of the family. Genome 32:457-467.

Scoles GJ, Gill BS, Xin Z-Y, Clarke BC, McIntyre CL, Chapman C and Appels R (1988) Frequent duplication and deletion events in the 5S RNA genes and the associated spacer regions of the *Triticeae*. Plant Syst Evol 160:105-122.

Shaked H, Kashkush K, Ozkan H, Feldman M and Levy AA (2001) Sequence elimination and cytosine methylation are rapid and reproducible responses of the genome to wide hybridization and allopolyploidy in wheat. Plant Cell 13:1749-1759.

Vieira R, Queiroz A, Morais L, Barao A, Mello-Sampayo T and Viegas W (1990) Genetic control of 1R nucleolus organizer region expression in the presence of wheat genomes. Genome 33:713-718.

Wang RR-C, Marburger JE and Hu C-J (1991) Tissue-culture-facilitated production of aneupolyhaploid *Thinopyrum ponticum* and amphidiploid *Hordeum violaceum* x *H. bogdanii* and their uses in phylogenetics studies. Theor Appl Genet 81:151-156.

Zhang H-B and Dvorák J (1990) Characterization and distribution of an interspersed repeated nucleotide sequence from *Lophopyrum elongatum* and mapping of a segregation-distortion factor with it. Genome 33:927-936.

Zhang X, Dong Y and Wang RR-C (1996) Characterization of genomes and chromosomes in partial amphiploids of the hybrid *Triticum aestivum* x *Thinopyrum ponticum* by *in situ* hybridization, isozyme analysis, and RAPD. Genome 39:1062-1071.W

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