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Detection of 5S rDNA and other repeated DNA on supernumerary B chromosomes of *Triticum* species (*Poaceae*)

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Abstract: The supernumerary B chromosomes of *Triticum speltoides* and *T. tripsacoides* were analyzed in mitotic metaphases of spike primordium cells by C-banding and in situ hybridization (ISH) analyzes. B chromosomes of *T. speltoides* have larger telomeric and interstitial C-bands, whereas those of *T. tripsacoides* are almost completely devoid of C-bands. A prominent ISH site of rye related DNA sequences (using probe pSc119) was detected on B chromosomes of *T. tripsacoides* and only a minor ISH site was observed on the *T. speltoides* B chromosomes. However, two ISH sites of 5S rRNA loci were detected at a terminal and an interstitial location of the *T. speltoides* B chromosomes. These sites were absent on B chromosomes of *T. tripsacoides*. The results are discussed with respect to the phylogenetic origin of these B chromosomes.

B chromosomes are supernumerary chromosomes that may or may not be present in some individuals or populations in addition to the normal A chromosome complement of a species. B chromosomes are found in animals as well as in plants (for review, see Jones 1975, Jones & Rees 1982). They are usually smaller and do not pair with A chromosomes, often are heterochromatic, and in general, do not possess major genes. Plant B chromosomes sometimes have nucleolar organizer regions (NORs) as revealed by their ability to organize nucleoli, Ag-NOR-banding, and in situ hybridization (ISH) analysis (Bosemark 1957; Powell & Burton 1966, Stack 1974, Brandham & Bhattarai 1977, Carr & Carr 1982, Loidl 1982, Guillén & Rejón 1984, Friebe 1989, Maluszynska & Schweizer 1989).

In *Triticum* (syn. *Aegilops*) species, B chromosomes are known to be present only in two diploid species, *T. speltoides* (Tausch.) Gren. ex Richter, 2n = 2x = 14, genome formula SS (syn. *Ae. speltoides* Boiss.) (Simchen & al. 1971; Mendelson & Zohary 1972; Zarchi & al. 1974; Sano & Tanaka 1980, 1982) and in *T. tripsacoides* (Jaub. & Sp.) Bowen, 2n = 2x = 14, genome formula TT (syn. *Ae. mutica* Boiss.) (Mochizuki 1957, 1960; Dover & Riley 1972; Vardi & Dover 1972; Ohta & Tanaka 1982; Ohta 1991). Both species are the only cross-pollinating *Triticum* species and grow sympatrically in some habitats in the Anatolian Plateau in Turkey (Ohta

1991). In both species, the B chromosomes are only present in aerial tissues such as shoots and meiocytes, making it more difficult to analyze their structure. In the present study we report results on the chromosomal structure of *Triticum* B chromosomes analyzed by C-banding and in situ hybridization (ISH) analyses.

Material and methods

Plant material. Seed samples of *T. speltoides* acession no. KU7717C (collected 13.2 km S from Sulaymaniyah to Qara Dagh, Iraq, at 950 m s.m., collection no. 1970-5-27-1-18) and *T. tripsacoides* accession no. KU12008 (collected 27.6 km E from Kirikkale to Yozgat, Turkey, at 900 m s.m., collection no. 1982-8-11-2-3) were kindly provided by Dr S. OHTA, Plant Germ-plasm Institute, Kyoto University, Japan. Seeds of the *T. speltoides* accession were obtained from a plant that had 2n = 14 + 3 B chromosomes after self-pollination. Seeds of the *T. tripsacoides* accession were set on a plant that had 2n = 14 + 2 B chromosomes after open-pollination with plants having 0–2 B chromosomes and originally collected from the same population.

Cytogenetic analysis. Root tips and small spikes of about 2–3 cm length were treated for 3 h with 0.05% colchicine and fixed in alcohol glacial acetic acid (3 : 1). Chromosome numbers were determined in root tip meristems, spike primordium cells, and in pollen mother cells (PMCs) using conventional aceto-carmine staining. For chromosome identification, the C-banding protocol described by GILL & al. (1991) was used. For ISH three probes were used. The 18S · 26S rRNA gene probe consisted of the plasmid pUC8, having a single rRNA gene repeat unit that originated from the plasmid pTa71 (GERLACH & BEDBROOK 1979). The 5S rRNA gene probe, pTa794, consisted of the plasmid pBR322 and had 5S DNA units derived from *Triticum aestivum* L. em. THELL. (GERLACH & DYER 1980). Clone pSc119 contains a highly repetitive DNA sequence derived from *Secale cereale* L. inserted in the plasmid pBR322 (BEDBROOK & al. 1980). All probes were labelled by nick translation with biotin-11-dUTP (uridine 5′-triphosphate) according to the manufacturer's instruction (Enzo Diagnostics, Farmingdale, New York). Signal detection with streptavidin horseradish peroxidase and DAB (diaminobenzidine tetrahydrochloride) was according to RAYBURN & al. (1985). For fluorescence ISH (FISH), signals were detected with avidin-FITC (fluorescein isothiocyanate, Boehringer, Mannheim) after counterstaining with propidium iodide as described by JIANG & GILL (1994 a, b). Microphotographs were taken with a Zeiss photomicroscope III using Kodak Imagelink HQ microfilm 1461 and after ISH and FISH and with an Olympus BH-2 photomicroscope using either Kodak technical Pan film 2415 or Kodak EKTAR 1000 film.

Results

Chromosome numbers of all *T. speltoides* and *T. tripsacoides* plants determined in root tip meristems were 2n = 14. Two plants were obtained from the *T. speltoides* accession KU7717C, one had 2n = 14 and the other had 2n = 14 + 6 B chromosomes in spike primordium cells and in PMCs. Nine plants germinated from the *T. tripsacoides* accession KU12008, three of them had 2n = 14 and six had 2n = 14 + 1 B chromosomes in spike primordium cells and in PMCs. The B-chromosomes of *T. speltoides* and *T. tripsacoides* were smaller than the A chromosomes. The B chromosomes of *T. speltoides* were submetacentric, whereas in *T. tripsacoides* they were nearly metacentric.

C-banded mitotic metaphases of *T. speltoides* and *T. tripsacoides* are shown in Fig. 1 and karyotypes of these species are shown in Fig. 2. Chromosome designations reflect their relationship with the seven homoeologous groups of wheat and

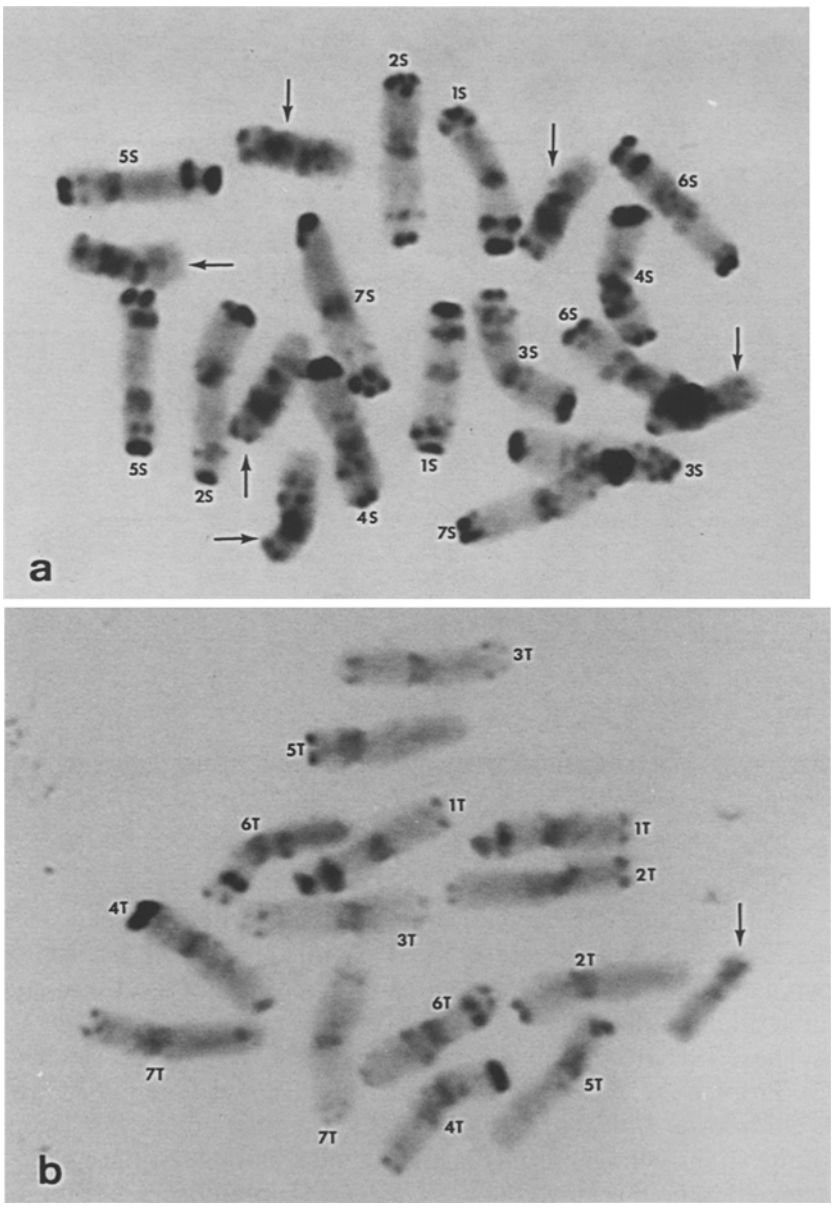

Fig. 1. C-banded mitotic metaphases from spike primordium cells. *a Triticum speltoides* (2n = 14 + 6B), *b T. tripsacoides* (2n = 14 + 1B). B chromosomes are marked by arrows

were determined by comparison with the generalized idiograms of *T. speltoides* and other S genome species (Friebe & al. 1993, Friebe & Gill 1995). In general, the S genome chromosomes of *T. speltoides* have more and larger C-bands than the T genome chromosomes of *T. tripsacoides*.

In situ hybridization using the 18S · 26S ribosomal rRNA gene probe pTa71 detected two pairs of hybridization sites in *T. speltoides* and *T. tripsacoides*, corresponding to the NORs on chromosomes 1S/1T and 6S/6T, respectively. The 5S

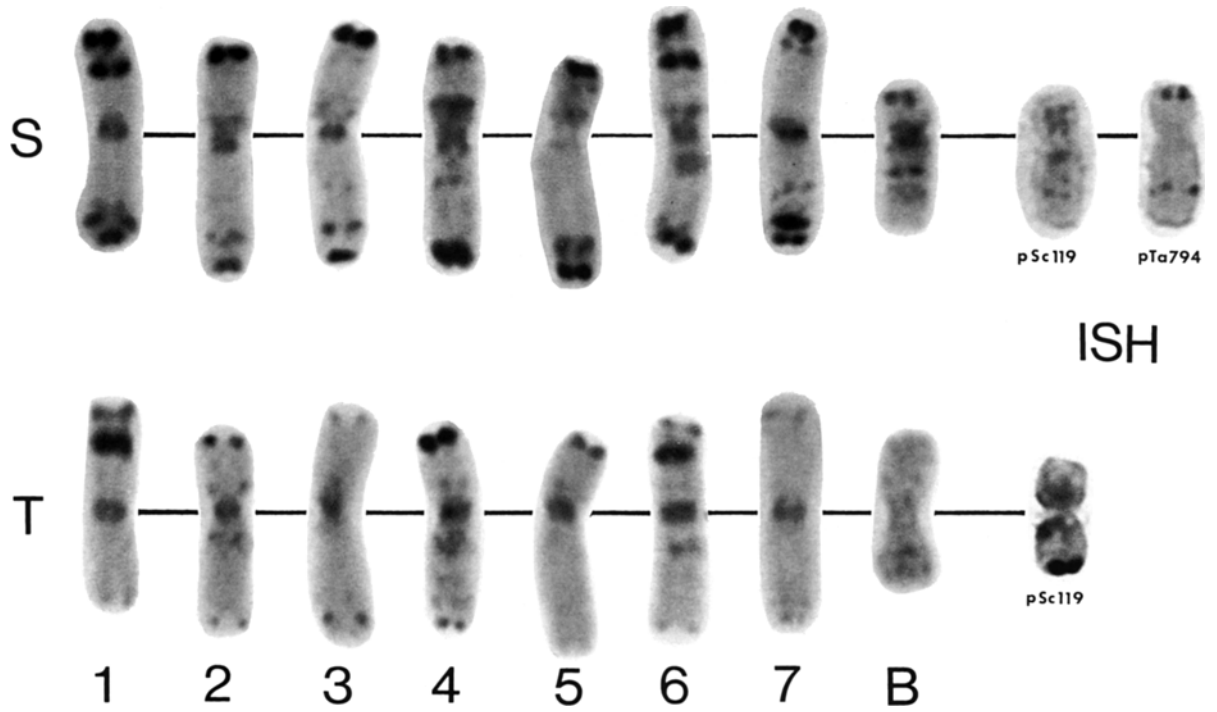

Fig. 2. C-banded karyotypes and in situ hybridization (ISH) patterns, using probes pSc119 and pTa794, of *Triticum speltoides* (upper row) and *T. tripsacoides* (lower row) B chromosomes

rRNA probe pTa794 identified only one pair of hybridization sites on a pair of A chromosomes of *T. tripsacoides*. One pair of 5S rRNA ISH sites on an A chromosome pair and two additional ISH sites, one at the telomere of the short arm and the other at an interstitial region of the long arm, were present on all *T. speltoides* B chromosomes. These ISH sites correspond to C-banded regions in those chromosomes (Fig. 2). Probe pSc119 detected ISH sites on all S and T genome chromosomes. The B chromosome of *T. tripsacoides* has a prominent pSc119 ISH site at the telomere of the slightly longer arm (Figs. 2, 3) and small interstitial pSc119 ISH sites were detected in both arms of the *T. speltoides* B chromosomes (Fig. 2).

Discussion

The present study confirmed earlier analyses in showing that B chromosomes in *T. speltoides* and *T. tripsacoides* are only present in aerial plant tissues and absent from root tip meristems (Mochizuki 1957, Mendelson & Zohary 1972). The *T. speltoides* and *T. tripsacoides* B chromosomes are similar in behaviour to those in other species, in that they have tendency to accumulate because non-disjunction at anaphase of the first division pollen grain mitosis is followed by preferential inclusion of both sister chromatids in the generative nucleus.

The C-banding patterns of the S and T genome chromosomes of *T. speltoides* and *T. tripsacoides* are quite distinct from each other, although both species are closely related. Their interspecific hybrids are partially fertile and the chromo-

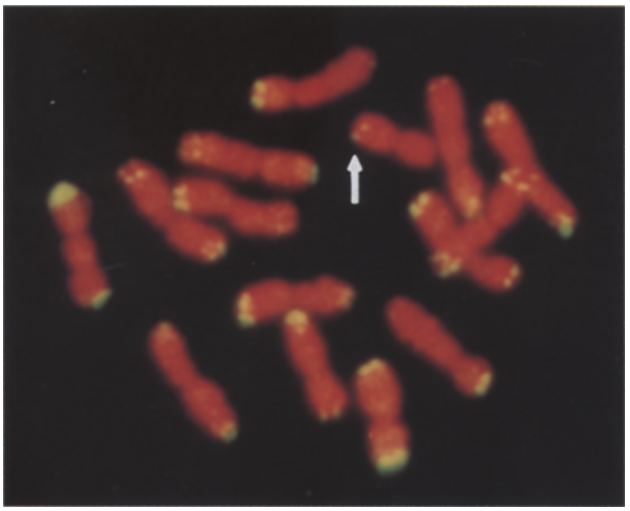

Fig. 3. Fluorescence in situ hybridization pattern of a spike primordium mitotic metaphase of *Triticum tripsacoides* (2n = 14 + 1B) using pSc119 as a probe. B chromosome marked by an arrow

somes pair as seven ring bivalents in about 12% of the PMCs (Ohta 1988, 1991). The S genome chromosomes of *T. speltoides* have larger telomeric and interstitial C-bands, whereas the T genome chromosomes of *T. tripsacoides* have smaller and mainly telomeric C-bands. This trend was also observed in the C-banding patterns of their B chromosomes. The *T. speltoides* B chromosome had prominent telomeric and interstitial C-bands, whreas the *T. tripsacoides* B chromosome was almost completely euchromatic. Since the C-banding technique differentially stains regions that contain highly repetitive DNA, our results indicate that *T. speltoides* B chromosomes have a larger or more localized amount of these sequences compared to those of *T. tripsacoides*. No similarities of the *T. speltoides* and *T. tripsacoides* B chromosomes were observed with any of the normal S and T genome chromosomes.

In situ hybridization using the rye derived probe pSc119 detected ISH sites on all S and T genome chromosomes. Most of the sites correspond to C-band positive regions. Only minor pSc119 ISH sites were observed in the *T. speltoides* B chromosomes, whereas those of *T. tripsacoides* had a prominent pSc119 ISH site at the telomere of one arm that did not correspond to a C-band positive region. The B chromosomes of cultivated rye were shown to be composed of DNA sequences, some of them common to both the A and B chromosomes, whereas others are B chromosome-specific (Sandery & al. 1990, 1991; Tsujimoto & Niwa 1992; Blunden & al. 1993). Similarly, B chromosomes of the greater glider, *Petauroides volans*, were shown to be composed of a heterogeneous mixture of sequences, some present on A and B chromosomes, and others unique to B chromosomes (McQuade & al. 1994). These results indicate that B chromosomes have a large extent diverged from A chromosomes.

The present study revealed the presence of two 18S · 26S rRNA loci in *T. speltoides* and in *T. tripsacoides* corresponding to the NORs on chromosomes 1S/1T and 6S/6T. These results are in agreement with earlier reports (Teoh & al. 1983, Yamamoto & Mukai 1995). However, an additional minor locus was detected recently in the long arm of the *T. speltoides* chromosome 1S, also present in 1B of *T. aestivum* and 1G of *T. timopheevii* (Jiang & Gil 1994 a). In

Tricum species major 18S · 26S rNA loci were mapped on homoeologous groups 1, 5, and 6 and two minor loci were detected on *T. aestivum* chromosomes 3D and 7D (MUKAI & al. 1991, JIANG & GILL 1994 a). In the present analysis, no 18S · 26S rRNA loci were detected in either the *T. speltoides* or the *T. tripsacoides* B chromosomes.

Our analysis further shows the presence of a 5S rRNA locus on a pair of A chromosomes in *T. speltoides* and *T. tripsacoides* and two additional loci on the B chromosomes of *T. speltoides*. In diploid *Triticum* species, 5S rRNA loci are on group 1 and 5 chromosomes, with only one locus on *T. speltoides* chromosome 5S (DVÓRÁK & al. 1989). Similarly, in hexaploid wheat, the 5S rRNA loci were physically mapped by ISH to the short arms of all group 1 and 5 chromosomes (MUKAI & al. 1990). This is the first study on the distribution pattern of constitutive heterochromatin in B chromosomes of wild wheats and, to our knowledge, is also the first report of 5S rRNA loci on plant B chromosomes.

Another interesting effect of the *T. speltoides* and *T. tripsacoides* B chromosomes is that they have no effect on homologous chromosome pairing, but that they drastically reduce the amount of homoeologous chromosome pairing in interspecific hybrids and can compensate for the loss of the *Ph1* gene (MOCHIZUKI 1964; DOVER & RILEY 1972; RILEY & al. 1961, 1973; SANO & TANAKA 1980, 1982; OHTA & TANAKA 1982; OHTA 1991). The *Ph1* gene is on the long arm of wheat chromosome 5B and suppresses the pairing of genetically related or homoeologous A, B, and D genome chromosomes, and therefore accounts for the diploid-like pairing behaviour of polyploid wheats (RILEY & CHAPMAN 1958; SEARS & OKAMOTO 1958).

The origin of B chromosomes is still under discussion. However, it is likely that they were derived from A chromosomes, either from the same complement or from a different genome, after interspecific hybridization. The close evolutionary relationship of *T. speltoides* and *T. tripsacoides* together with similarities in the tissue-specific distribution of their B chromosomes and their similar effects on homoeologous chromosome pairing suggest that both might have a common origin. Since the B chromosomes of *T. speltoides* have 5S rRNA loci and these are known to be located on group 1 and 5 chromosomes of *Triticum* species, one might speculate that the B chromosomes may have originated from a group 1 or 5 chromosome. In this respect it is interesting to note that the major gene controlling meiotic chromosome pairing in polyploid wheats is also located on a group 5 chromosome. It is also possible that the Bs were derived from a non-5S rDNA carrying chromosome and that the 5S rDNA sequences were transferred to this chromosome at a later stage. Due to the buffered allopolyploid nature, common wheat is unique in that it tolerates chromosome manipulations that are difficult to achieve in other species. To further analyze the chromosomal origin of the B chromosomes, we are now developing addition lines of all S and T genome and B chromosomes of *T. speltoides* and *T. tripsacoides* in *T. aestivum* cv. Chinese Spring wheat. Once these lines have been established, genetic maps of the *T. speltoides* and *T. tripsacoides* B chromosomes can be produced using molecular markers and compared to maps of the standard chromosomes of these species. This will eventually permit the determination of the phylogenetic relationship of the *T. speltoides* and *T. tripsacoides* B chromosomes and might also reveal further insights on their chromosomal origin.

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