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Different species-specific chromosome translocations in *Triticum timopheevii* and *T. turgidum* support the diphyletic origin of polyploid wheats

Jiming Jiang & Bikram S. Gill

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***Triticum timopheevii* ssp. *timopheevii* and *T. timopheevii* ssp. *araraticum* were analysed by sequential N-banding and genomic *in situ* hybridization. Three chromosomes, 6A^t, 1G and 4G, were involved in A^t-G intergenomic translocations in all six lines analysed. These chromosomes may be derived from a cyclic translocation that is species-specific to *T. timopheevii*. In contrast, *Triticum turgidum* has a species-specific cyclic translocation involving chromosomes 4A, 5A and 7B. The discovery of different species-specific chromosome translocations supports the diphyletic hypothesis of the evolution of tetraploid wheats. The results from genomic blocking analysis also revealed that the chromosomes of *Aegilops speltoides* are closer to the G genome than the B genome chromosomes. The possible role of species-specific translocations in the evolution of wheat is discussed.**

Key words: evolution, polyploid wheat, species-specific translocation, *Triticum timopheevii*, *Triticum turgidum*

Introduction

The tetraploid wheats are divided into two groups: the emmer wheat group (*Triticum turgidum* L.) with the genome formula AABB and the timopheevi group (*Triticum timopheevii* Zhuk.) with the genome formula A^{t}A^{t}GG. It is generally accepted that both A and A^t genomes were derived from *Triticum monococcum* L. ssp. *urartu* (Dovřák *et al.* 1993, Takumi *et al.* 1993). It has also been suggested that both B and G genomes originated from either *Aegilops speltoides* Tausch. (syn. *Triticum speltoides* (Tausch) Gren. ex Richter) or an ancestral form closely related to it (Sarkar & Stebbins 1956; Jaaska 1978, 1980, Tsunewaki & Ogihara 1983, Chen & Gill 1983, Ogihara & Tsunewaki 1988).

There are two different hypotheses on the origin of emmer and timopheevi wheats. According to the first hypothesis, emmer and timopheevi wheats had a diphyletic origin involving two independent hybridization events. Restriction pattern analysis of chloroplast DNA from tetraploid wheats and the possible B and G genome donor species supported the diphyletic hypothesis (Tsunewaki & Ogihara 1983). According to the second hypothesis, the AABB and A^{t}A^{t}GG wheats had a monophyletic origin which could be traced to a single hybridization event (Wagenaar 1961, Feldman 1966, Tanaka *et al.* 1978).

The B and G genome chromosomes are well differentiated, especially with respect to heterochromatic structure and chromosome translocations (Feldman 1966, Hutchinson & Miller 1982, Gill & Chen 1987). There are two types of chromosome translocations in wheat: (i) random, involving different chromosomes in different populations, and (ii) species-specific, involving particular chromosomes in every population. A species-specific 4A-5A-7B cyclic translocation was found in emmer and common wheat (*Triticum aestivum* L.) (Naranjo *et al.* 1987, Naranjo 1990, Liu *et al.* 1992). However, Gill & Chen (1987) reported that *T. timopheevii* lacked this cyclic translocation but instead carried a 6A^t-1G translocation. In this paper, we report the identification of a different cyclic translocation in timopheevi wheat. The implication of the finding on the phylogeny and evolution of tetraploid wheats is discussed.

Jiming Jiang and Bikram S. Gill (corresponding author) are at the Wheat Genetics Resource Center and Department of Plant Pathology, Kansas State University, Manhattan, KS 66506, USA. Fax: (+1) 913 532 5692.

Materials and methods

Three accessions of *Triticum timopheevii* ssp. *timopheevii*, TA103, TA140 (CI14133), TA141 (PI221421), and three accessions of *Triticum timopheevii* ssp. *araraticum*, TA2 (from Armenia), TA6 (from Turkey), TA12 (from Iraq), were used. *Triticum turgidum* var. *durum* cv. Langdon was used as an example for emmer wheat. All materials are maintained at the Wheat Genetics Resource Center at Kansas State University.

The above *Triticum* accessions were analysed by sequential N-banding and *in situ* hybridization (ISH) or genomic *in situ* hybridization (GISH) (Jiang & Gill 1993). ISH patterns were analysed using clone pSc119. This clone contains a 120 bp highly repeated sequence isolated from rye and also present in *Triticum* (Bedbrook *et al.* 1980). For GISH analysis, genomic DNA of *T. monococcum* was isolated and labelled with biotin-11-dUTP, and sheared genomic DNA of *Ae. speltoides* was added to the hybridization mixture to block any cross hybridization of genomic probe to the G and B genome chromosomes. For signal detection using a fluorescence technique, 100 µl rabbit anti-biotin antibody (Enzo Diagnostics USA) was applied to each preparation after the post-hybridization wash, and the slides were incubated at 37°C for 30 min. The slides were washed three times in 1 × PBS (5 min each) at room temperature, and incubated in 100 µl FITC-conjugated goat anti-rabbit antibody (Enzo Diagnostics USA) at 37°C for 30 min. After three washes in 1 × PBS at room temperature, a thin layer of anti-fade solution (Johnson *et al.* 1981) containing 1 µg/ml of propidium iodide was added for counterstaining.

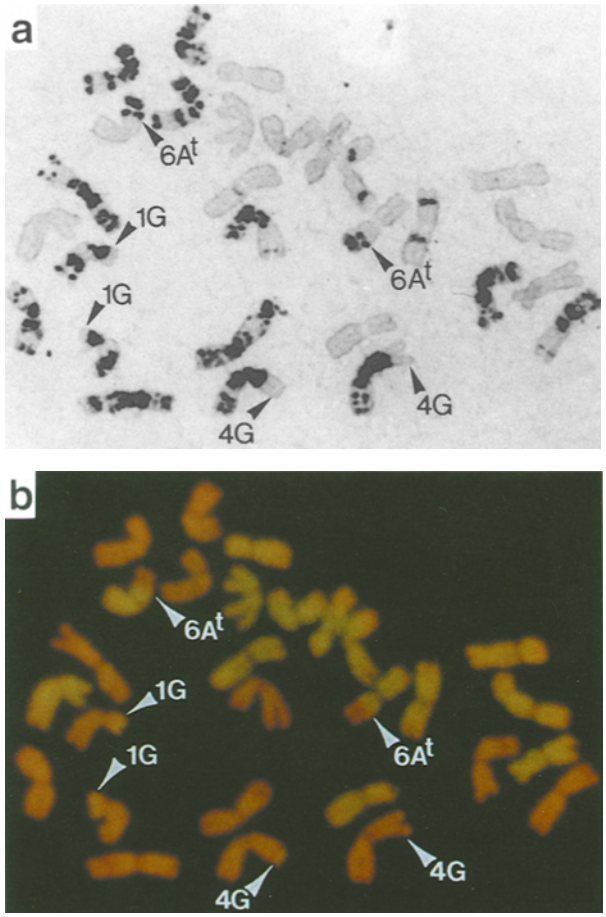

Figure 1. Sequential N-banding and genomic *in situ* hybridization analysis of *Triticum timopheevii* ssp. *timopheevii* line TA141. **a** N-banding of metaphase chromosomes of TA141. **b** Subsequent GISH on the same metaphase cell. *Triticum monococcum* DNA was used as a probe and *Aegilops speltoides* DNA as a blocker. Arrowheads point to the three A^t-G intergenomic translocation chromosomes 6A^t, 1G and 4G.

Results

All G genome chromosomes in both *T. timopheevii* ssp. *timopheevii* and *T. timopheevii* ssp. *araraticum* can be identified using the modified N-banding technique. For the A^t genome, chromosomes 6A^t and 7A^t consistently showed bands in different lines. In some lines, all A^t genome chromosomes can be identified (see Figure 5a): 2A^t, 3A^t, 5A^t, 6A^t, 7A^t have characteristic bands, and unbanded chromosomes 1A^t and 4A^t are identified based on their arm ratios, i.e. submetacentric and metacentric, respectively. The homoeology of *T. timopheevii* chromosomes is assigned according to their pairing with *T. turgidum* chromosomes and their spontaneous substituting ability for chromosomes of common wheat (Gill & Chen 1987, Badaeva *et al.* 1991).

Six different *T. timopheevii* lines were analysed by sequential N-banding and GISH. Figure 1a shows the N-banding of somatic metaphase chromosomes of *T. timopheevii* ssp. *timopheevii* line TA 141. Figure 1b shows the subsequent GISH pattern using *T. monococcum* DNA as a probe and *Ae. speltoides* DNA as a blocker. The A^t and G genome chromosomes show yellow and orange colours, respectively. By comparing Figure 1a and Figure 1b, each chromosome with a different banding pattern can be assigned to either A^t or G genomes. Three chromosomes, 6A^t, 1G and 4G, involving A^t-G intergenomic translocation, were found in all six *T. timopheevii* lines analysed. About 17% of the distal part of the short arm of 4G was derived from an A^t genome chromosome (Figures 1b and 2). The satellite, nucleolus-organizer region, and 25% of the distal part of the short arm of 6A^t belong to the G genome. Chromosome 1G has an intercalary A^t genome chromosome segment in the short arm. This segment represents 30% of the short arm.

The present results confirm the hypothesis of the structural modification of chromosome 4G (Rayburn & Gill 1985). Chromosome 4B in emmer and common wheats has a terminal hybridization site on the short arm when probed with a repeated sequence pSc119 (Figure 3). A similar hybridization site on the short arm of 4G is subterminal (Figure 3). The distal segment

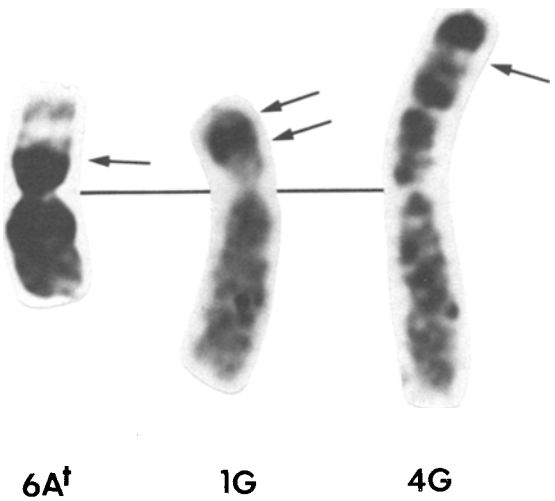

Figure 2. Genomic *in situ* hybridization patterns of chromosomes 6A^t, 1G and 4G. Arrows point to the break points.

beyond the pSc119 hybridization site is probably the A^t genome segment detected by GISH.

The genetic identity of the 6A^t/1G translocation was first reported by Gill & Chen (1987). They observed that the short arm of 1B paired with 6A^t, or chromosomes 1B, 6A^t and 6A paired as a trivalent at metaphase I in meiosis. Our results further confirm their observation and, in addition, reveal that the short arm of 1G contains an A^t genome segment at an intercalary location.

We postulate that 6A^t, 1G and 4G were derived from a cyclic translocation. Figure 4 depicts a possible mode of origin of the cyclic translocation. In the model, the initial translocation was reciprocal and involved chromosomes 1G and 6A^t. The result was a shift in part of the short arm of 1G, the nucleolus-organizer region, and the satellite to chromosome 6A^t. The distal end of

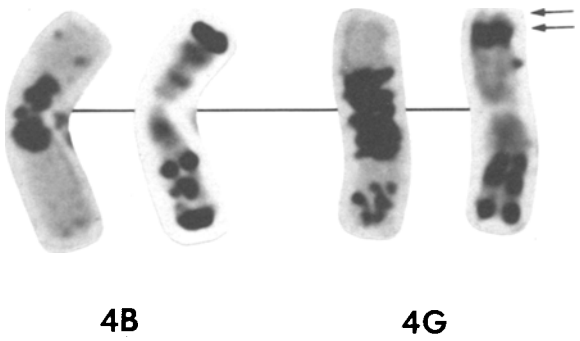

Figure 3. Sequential N-banding (left) and *in situ* hybridization using pSc119 as a probe (right) on chromosome 4B and 4G. Arrows point to the distal segment beyond the pSc119 hybridization site. This segment corresponds to the A^t genome segment detected by GISH.

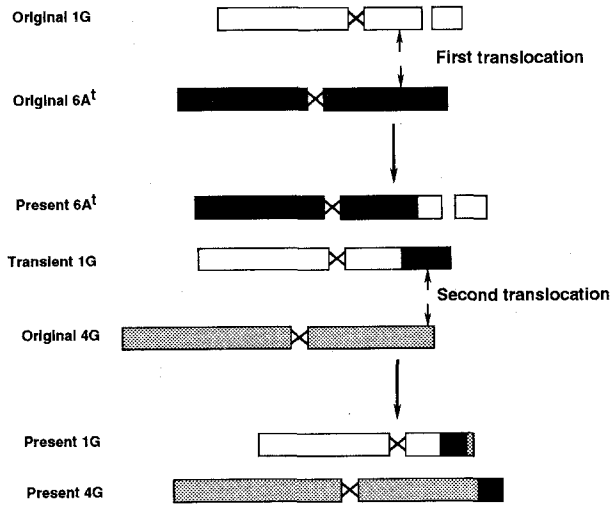

Figure 4. A possible mode of origin of the cyclic translocation involving 6A^t, 1G and 4G.

6A^t short arm was translocated to 1GS. As a result, chromosome 1G in all *T. timopheevii* accessions does not have a satellite. However, 6A^t always has a satellite (Badaeva *et al.* 1993). The translocated (transient) 1G and 4G were involved in a second translocation. A small distal part of the transient 1GS, originally from 6A^{t}S, was translocated to the distal end of 4GS. A tiny distal part of 4GS was translocated to the transient 1GS. As a result, the remaining 6A^{t}S segment in 1GS now occupies an intercalary position. The terminal area of 1GS has an ISH site of pSc119 (Figure 5). This ISH site probably originated from 4GS according to the cyclic translocation hypothesis.

The postulated presence of 6A^t segments on 1G and 4G needs to be verified by RFLP (restriction fragment length polymorphism) analysis. At present, we do not know whether more species-specific chromosome translocations exist in *T. timopheevii*, because intragenomic and small intergenomic translocations cannot be detected by GISH analysis.

Sequential chromosome N-banding and GISH analysis were also performed on *T. turgidum* var. *durum* cv. Langdon. The A and B genome chromosomes of Langdon were discriminated by GISH using *T. monococcum* DNA as a probe and *Ae. speltoides* DNA as a blocker, even though the discrimination was not as clear as that between A^t and G genomes in *T. timopheevii*. About 30% of the long arm of chromosome 4A in Langdon was derived from a B genome chromosome (7B). This was also reported in common wheat (Mukai *et al.* 1993). The ISH site of pSc119 on the terminal area of 4AL (Figure 5d) is probably derived from 7B. This is also confirmed by the presence of an ISH site on the terminal area of 7GS but not on 7BS (Figure 5b & d). The A genome segment on the terminal area of the short arm of 7B, detected by chromosome pairing and RFLP analysis (Naranjo *et al.* 1987, Liu *et al.* 1992), was not identified

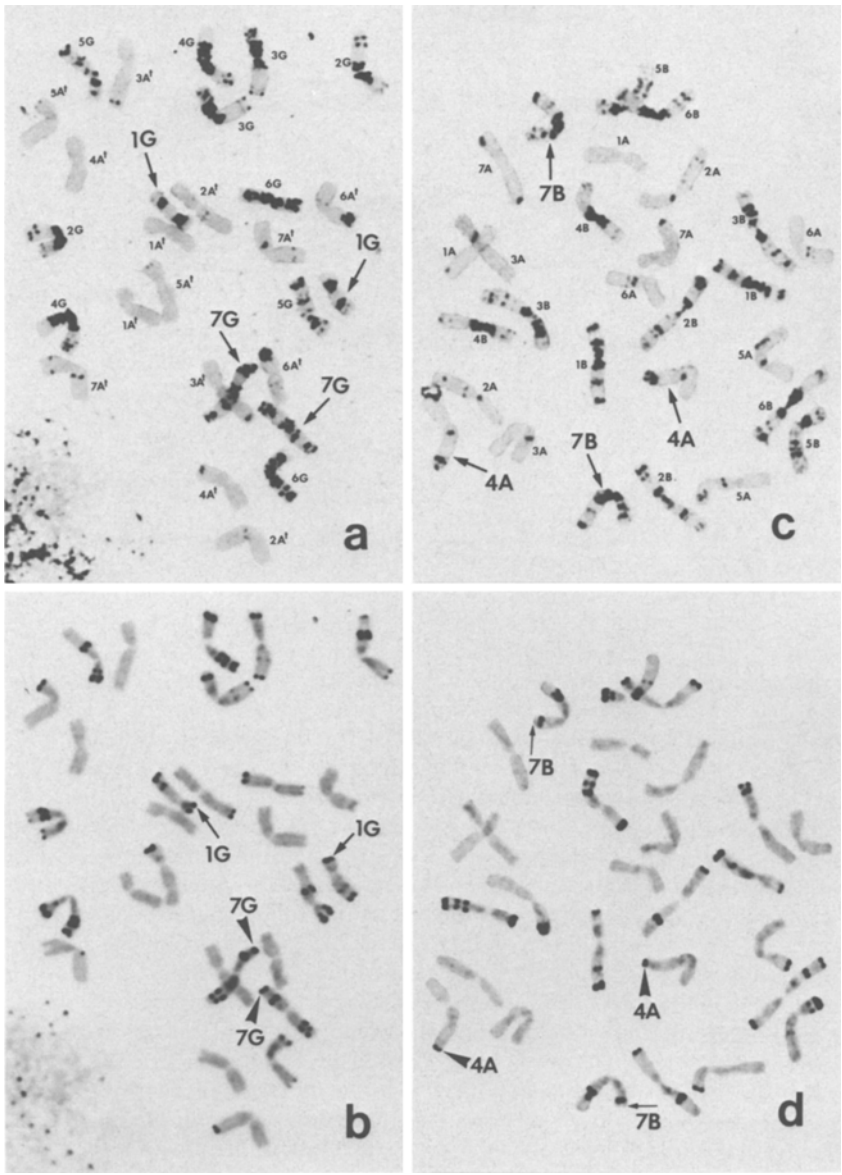

Figure 5. Sequential N-banding and *in situ* hybridization using pSc119 as a probe. **a** N-banding of *Triticum timopheevii* ssp. *araraticum* line TA2. **b** Subsequent ISH on the same metaphase cell. Arrows point to the ISH sites on the terminal area of 1GS. Arrowheads point to the ISH sites on the terminal area of 7GS. **c** N-banding of *Triticum turgidum* var. *durum* cv. Langdon. **d** Subsequent ISH on the same metaphase cell. Arrowheads point to the ISH sites on 4A. Note: the terminal area of 7BS (arrowed) has no ISH site.

by GISH. One possible reason is that the A genome segment on 7B is too small and perhaps undetectable by GISH.

Discussion

There are two unresolved issues relating to the evolution of emmer and timopheevi wheats. First, is their origin monophyletic or diphyletic? Second, what is the relationship between their genomes especially between the B and G genomes? The present results shed further light on both questions.

A 4A-5A-7B cyclic translocation was found in emmer and common wheat (Naranjo *et al.* 1987, Naranjo 1990, Liu *et al.* 1992). This translocation is specific to AABB and AABBDD species. The GISH analysis confirmed previous results that this cyclic translocation is not present in *T. timopheevii*. Instead, *T. timopheevii* has a cyclic translocation involving $6A^t$, 1G and 4G. Since the translocation patterns of these three chromosomes were found in all six *T. timopheevii* lines analysed, this cyclic translocation is species-specific to *T. timopheevii*. As a further support of this, more than one hundred accessions of *T. timopheevii* ssp. *araraticum* from different geographical regions have the $6A^t$ and 1G translocation chromosomes based on their characteristic C-banding patterns (Badaeva *et al.* 1993).

To explain the presence of species-specific chromosome translocations, Gill (1991) proposed a nucleocytoplasmic interaction (NCI) hypothesis of genome evolution and speciation in polyploid plants. According to this hypothesis, a new amphiploid must pass through a bottleneck of sterility resulting from the adverse interaction between the male nuclear genome

and both the nuclear and cytoplasmic genomes of the female parent. Certain bottleneck (=species-specific) chromosomal changes must occur in the nuclear genome to restore fertility and nucleo-cytoplasmic compatibility. The modifications of $6A^t$, 1G and 4G most likely belong to this type of bottleneck chromosomal change. First, they are species-specific to *T. timopheevii*. Second, they involve intergenomic chromosomes and cannot pre-exist in the progenitor species. The mechanism and gene action by which such chromosomal changes lead to fertility restoration remain to be explored.

The discovery of different species-specific chromosome translocations in emmer and timopheevi groups supports the diphyletic hypothesis on the evolution of tetraploid wheats. The generation and fixation of these modified chromosomes in natural populations are compatible with both the NCI hypothesis and with the evidence of cytoplasmic differentiation between A^tA^tGG and AABB species (Tsunewaki & Ogihara 1983). Emmer and timopheevi wheats were probably derived from two different original amphiploids. The females of these two amphiploids were two forms of *Ae. speltoides* with different cytoplasms, and the male was *T. monococcum*. Species-specific translocations occurred in both amphiploids. These translocations may play an important role in overcoming nucleo-cytoplasmic incompatibility and sterility of the raw amphiploids. Therefore, the generation of different species-specific chromosomal translocations in emmer and timopheevi are related to their cytoplasmic differences. Species-specific chromosome translocations may also be present in other species in both animal and plant kingdoms (Wienberg *et al.* 1990, Kenton *et al.* 1993).

Our results also reveal some information on genome relationships between emmer and timopheevi wheats. When *T. monococcum* DNA was used as a probe and *Ae. speltoides* DNA was used as a blocker in GISH, we found that the A^t and G genomes were easier to discriminate than the A and B genome chromosomes. This result suggested that G genome chromosomes share more similar repeated sequences with *Ae. speltoides* chromosomes because the repeated DNA sequences play the major role in genomic blocking (see also Anamthawat-Jónsson *et al.* 1990). Thus, in respect of repeated DNA sequences, *Ae. speltoides* is relatively more close to the G than the B genome. Therefore, the present results support *Ae. speltoides* as the G genome donor to *T. timopheevii*.

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References

Anamthawat-Jónsson K, Schwarzacher T, Leith AR, Bennett MD, Heslop-Harrison JS (1990) Discrimination between closely related Triticeae species using genomic DNA as a probe. *Theoret. Appl Genet* **79**: 721–728.

Badaeva ED, Budashkina EB, Badaev NS, Kalimina NP, Shkutina FM (1991) General features of chromosome substitutions in *Triticum aestivum* × *T. timipheevii* hybrids. *Theoret Appl Genet* **82**: 227–232.

Badaeva ED, Badaev NS, Gill BS, Filatenko AA (1993) Intraspecific karyotype divergence and speciation in *Triticum araraticum*. *Plant Syst Evol* (submitted).

Bedbrook JR, Jones J, O'Dell M, Thompson RJ, Flavell RB (1980) A molecular description of telomeric heterochromatin in *Secale* species. *Cell* **19**: 545–560.

Chen PD, Gill BS (1983) The origin of chromosome 4A, and genomes B and G of tetraploid wheat. *Proc 6th Internatl Wheat Genet Symp* pp. 39–48.

Dvořák J, di Terlizzi P, Zhang, H-B, Resta P (1993) The evolution of polyploid wheat: identification of the A genome donor species. *Genome* **36**: 21–31.

Feldman M (1966) Identification of unpaired chromosomes in F1 hybrids involving *Triticum aestivum* and *T. timopheevi*. *Can J Genet Cytol* **8**: 144–151.

Gill BS (1991) Nucleo-cytoplasmic interaction (NCI) hypothesis of genome evolution and speciation in polyploid plants. In: *Proc Dr. H. Kihara Memorial Intern Cytoplasmic Engin in Wheat* pp. 48–53.

Gill BS, Chen PD (1987) Role of cytoplasm-specific introgression in the evolution of the polyploid wheats. *Proc Natl Acad Sci USA* **84**: 6800–6804.

Hutchinson J, Miller TE (1982) Comparison of the chromosomes of *Triticum timopheevi* with related wheat using the techniques of C-banding and *in situ* hybridization. *Theoret Appl Genet* **64**: 31–40.

Jaaska V (1978) NADP-dependent aromatic alcohol dehydrogenase in polyploid wheats and their diploid relatives. On the origin and phylogeny of polyploid wheat. *Theoret Appl Genet* **53**: 209–217.

Jaaska V (1980) Electrophoretic survey of seedling esterases in wheats in relation to their phylogeny. *Theoret Appl Genet* **56**: 273–284.

Jiang J, Gill BS (1993) Sequential chromosome banding and *in situ* hybridization analysis. *Genome* **36**: 792–795.

Johnson GD, Nogueira C de, Aranjo JGM (1981) A simple method for reducing the fading of immunofluorescence during microscopy. *J Immunol Methods* **43**: 349–350.

Kenton A, Parokonny AS, Gleba YY, Bennett MD (1993) Characterization of the *Nicotiana tabacum* L. genome by molecular cytogenetics. *Mol Gen Genet* **240**: 159–169.

Liu CJ, Atkinson MD, Chinoy CN, Devos KM, Gale MD (1992) Nonhomoeologous translocations between group 4, 5 and 7 chromosomes within wheat and rye. *Theoret Appl Genet* **83**: 305–312.

Mukai Y, Nakahara Y, 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.

Naranjo T (1990) Chromosome structure of durum wheat. *Theoret Appl Genet* **79**: 397–400.

Naranjo T, Roca A, Goicoecha PG, Giraldz R (1987) Arm homoeology of wheat and rye chromosomes. *Genome* **29**: 873–882.

Ogihara Y, Tsunewaki K (1988) Diversity and evolution of chloroplast DNA in *Triticum* and *Aegilops* as revealed by restriction fragment analysis. *Theoret Appl Genet* **76**: 321–332.

Rayburn AL, Gill BS (1985) Molecular evidence for the origin and evolution of chromosome 4A in polyploid wheat. *Can J Genet Cytol* **27**: 246–250.

Sarkar P, Stebbins GL (1956) Morphological evidence concerning the origin of the B genome in wheat. *Am J Bot* **43**: 297–304.

Takumi S, Nasuda S, Liu YG, Tsunewaki K (1993) Wheat phylogeny determined by RFLP analysis of nuclear DNA. 1. Einkorn wheat. *Japan J Genet* **68**: 73–79.

Tanaka M, Kawahara T, Sano J (1978) The evolution of wild tetraploid wheat. *Proc 5th Internat Wheat Genet Symp* pp. 73–80.

Tsunewaki K, Ogihara Y (1983) The molecular basis of genetic diversity among cytoplasms of *Triticum* and *Aegilops* species. II. On the origin of polyploid wheat cytoplasms as suggested by chloroplast DNA restriction fragment patterns. *Genetics* **104**: 155–171.

Wagenaar EB (1961) Studies on the genome constitution of *Triticum timopheevi* Zhuk. I. Evidence for genetic control of meiotic irregularities in tetraploid hybrids. *Can J Genet Cytol* **3**: 47–60.

Wienberg J, Jauch A, Stanyon R, Cremer T (1990) Molecular cytotaxonomy of primates by chromosomal *in situ* suppression hybridization. *Genomics* **8**: 347–350.