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Chromosome substitutions of *Triticum timopheevii* in common wheat and some observations on the evolution of polyploid wheat species

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Abstract Whether the two tetraploid wheat species, the well known *Triticum turgidum* L. (macaroni wheat, AABB genomes) and the obscure *T. timopheevii* Zhuk. (A^tA^tGG), have monophyletic or diphyletic origin from the same or different diploid species presents an interesting evolutionary problem. Moreover, *T. timopheevii* and its wild form *T. araraticum* are an important genetic resource for macaroni and bread-wheat improvement. To study these objectives, the substitution and genetic compensation abilities of individual *T. timopheevii* chromosomes for missing chromosomes of *T. aestivum* 'Chinese Spring' (AABBDD) were analyzed. 'Chinese Spring' aneuploids (nullisomic-tetrasomics) were crossed with a *T. timopheevii* × *Aegilops tauschii* amphiploid to isolate *T. timopheevii* chromosomes in a monosomic condition. The F_1 hybrids were backcrossed one to four times to Chinese Spring aneuploids without selection for the *T. timopheevii* chromosome of interest. While spontaneous substitutions involving all A^t- and G-genome chromosomes were identified, the targeted *T. timopheevii* chromosome was not always recovered. Lines with spontaneous substitutions from *T. timopheevii* were chosen for further backcrossing. Six *T. timopheevii* chromosome substitutions were isolated: $6A^t$ (6A), 2G (2B), 3G (3B), 4G (4B), 5G (5B) and 6G (6B). The substitution lines had normal morphology and fertility. The $6A^t$ of *T. timopheevii* was involved in a translocation with chromosome 1G, resulting in the transfer of the group-1 gliadin locus to $6A^t$. Chromosome 2G substituted for 2B at a frequency higher than expected and may carry putative homoeoalleles of gametocidal genes present on group-2 chromosomes of several alien species. Our data indicate a common origin for tetraploid wheat species, but from separate hybridization events because of the presence of a different spectrum of intergenomic translocations.

Abstract *Triticum timopheevii* · *Triticum aestivum* · Chromosome substitution · C-banding

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Introduction

Triticum timopheevii Zhuk. is a tetraploid wheat having the A^t and G genomes, which are closely related to the A and B genomes of emmer and durum wheat (*T. turgidum* L.) and common wheat (*T. aestivum* L.). *T. timopheevii* was discovered in western Georgia by P.M. Zhukovskyi and shown to be a separate species from tetraploid emmer wheat. Although *T. timopheevii* is morphologically similar to *T. turgidum*, it crosses poorly with it and has a distinct karyotype (Badaeva et al. 1986; Gill and Chen 1987; Jiang and Gill 1994). *T. monococcum* L. ssp. *urartu* is generally accepted as the donor of the A^t and A genomes of *T. timopheevii* and *T. turgidum*, respectively (Dvorák et al. 1993). The B and G genomes are proposed to have originated from an S-genome species, either *Aegilops speltoides* or a closely related ancestral form (Sarkar and Stebbins 1956; Jaaska 1978; Chen and Gill 1983; Ogihara and Tsunewaki 1988).

The phylogeny of *T. timopheevii* has been a matter of some debate. Two hypotheses about the origin of the species in relation to wheat have been proposed. According to the monophyletic-origin hypothesis, *T. timopheevii* and *T. turgidum* originated from a single hybridization event, then diverged due to chromosomal rearrangements or introgressive hybridization with an unknown diploid species (Gill and Chen 1987). The

diphyletic-origin hypothesis suggests that *T. timopheevii* and *T. turgidum* resulted from separate hybridization events. The diphyletic hypothesis is supported by the presence of different species-specific chromosome translocations in the two species (Jiang and Gill 1994). In addition, a cytoplasm type has been identified in *A. speltoides* that corresponds to the cytoplasm of *T. timopheevii*. No cytoplasm identical to that of *T. turgidum* has been identified in *A. speltoides* (Ogihara and Tsunewaki 1988; Tsunewaki 1995).

The assignment of individual A^t- and G-genome chromosomes to homoeologous groups has been based on the similarity of chromosome banding patterns (Badaeva et al. 1986; Gill and Chen 1987), chromosome pairing in interspecific hybrids with wheat (Gill and Chen 1987), and compensating ability in spontaneous substitution lines (Badaeva et al. 1991; Gill et al. 1988).

Gyarfus (1968) produced a *Sr37* stem-rust-resistant hexaploid derivative from a cross of *T. aestivum*/*T. timopheevii* identified as a disomic 4G (4B) substitution by Dvorák (1983). Lines with chromosome 6G of *T. timopheevii* substituted for chromosomes 6A, 6B, and 6D of Chinese Spring wheat were produced by E. R. Sears (Shepherd and Islam 1988). Badaeva et al. (1991) described the C-banding karyotypes of hexaploid BC_1F_7 lines derived from crosses between three different *T. timopheevii* accessions and three hexaploid wheat cultivars. Chromosomes present as single disomic substitutions were $1A^t$, 5G, 6G, and 7G. Lines with a substitution of chromosomes of the wild form of timopheevi wheat, *T. timopheevii* var. *araraticum* (syn. *T. araraticum*), in a winter wheat background were produced by Gill et al. (1988) and Badaeva and Gill (1995). Chromosomes 2G and 6G were recovered as single disomic substitutions. The array and frequency of substitutions in these lines differed from that observed by Badaeva et al. (1991), indicating that parental genotypes may influence the pattern of chromosome substitutions.

All of the above-mentioned substitution lines were derived after one or two backcrosses to the wheat parent, with perhaps the exception of those lines produced by Sears. Development of a set of chromosome substitution lines in an isogenic background, and their molecular marker analysis, would be useful in the cytogenetic identification of individual chromosomes of *T. timopheevii*. Thus, the objectives of the present study were to isolate *T. timopheevii* chromosome substitution lines in a Chinese Spring background using two different approaches. The results of both approaches are pre-sented along with descriptions of the substitution lines obtained.

Material and methods

Plant material

An amphiploid of *T. timopheevii-A. tauschii* [genetically A^tA^tGGDD; Kansas State University Wheat Genetics Resource Center (WGRC) accession number TA 3432] was selected for crossing. The amphiploid was produced by E. R. Sears (University of Missouri) and seeds were supplied to the WGRC by S. S. Maan (North Dakota State University). Chinese Spring stocks used for crossing included nullisomic-tetrasomic (NT), monosomic (M), and monotelosomic (MT) lines (Sears 1954, 1966; Sears and Sears 1978).

Crossing scheme 1 used for the development of a $6A^t$(6A) substitution line is illustrated in Fig. 1a. It was designed to ensure that there was no recombination between the targeted *T. timopheevii* chromosome and its wheat homoeologue. It was used in an attempt to develop *T. timopheevii* substitutions for wheat chromosomes 1A, 3A, 5A, 6A, 1B, 3B, 4B, and 7B. TA 3432 was initially crossed as a male with the Chinese Spring nullisomic-tetrasomic lines: nulli1A-tetra1D (N1AT1D), N3AT3D, N5AT5D, N6AT6D, N1BT1D, N3BT3D, N4BT4D (derived from M4BT4D) and N7BT7D. Each cross combination was then backcrossed to the appropriate Chinese Spring NT from one to four times without selection. C-banding was done on F_2 or F_3 plants of random BC_1, BC_2, BC_3, or BC_4 families. N-banding was done on the progeny of lines selected for further crossing, and plants carrying *T. timopheevii*-chromosome substitutions were backcrossed to the appropriate monotelosomic or monosomic as a male. If plants were not male fertile, they were crossed as a female with a NT line. Lines were backcrossed to the appropriate Chinese Spring stock up to five additional times with selection for the targeted *T. timopheevii* chromosome done by N-banding or C-banding analysis after each backcross. Monosomic substitution lines were then selfed to obtain disomic substitution lines.

Crossing scheme 2 for the isolation of the 4G(4B) substitution line is shown in Fig. 1 b. This approach did not exclude the possibility of recombination between the targeted *T. timopheevii* chromosome and its wheat homoeologue. The BC_1 progeny of crosses between N1AT1D and N1BT1D with TA 3432 were highly fertile, particularly in the case of the cross combination N1AT1D *2/TA 3432. Lines derived from these crosses that had 2G (2B), 4G(4B), modified 5G (5B), and 6G (6B) substitutions were selected for backcrossing to the appropriate Chinese Spring monosomic or monotelosomic lines, as described above. A BC_1-derived line from the cross between N6AT6D and TA 3432 with 2G (2B) and 3G (3B) substitutions was backcrossed to MT3BL to produce the 3G(3B) substitution line.

Cytogenetic and protein analysis

The N- and C-banding techniques described by Endo and Gill (1984) and Gill et al. (1991) were used for chromosome identification. The identification of *T. timopheevii* chromosomes was according to the nomenclature of Badaeva et al. (1991). Gliadin electrophoresis patterns of substitution lines were analyzed after the extraction of gliadin proteins using the method described by Morris et al. (1990). Separation of proteins by electrophoresis was on vertical-slab polyacrylamide gels, according to Lookhart et al. (1986). Proteins were visualized by staining in a 6.25% trichloracetic acid – 0.3% Coomassie blue solution.

Extraction of protein for acid phosphatase (ACPH) isozyme analysis was done by grinding 0.1 g of leaf tissue in 0.5 ml of extraction buffer (Carlson 1972) in a chilled mortar. Crude extract was employed for electrophoresis. Polyacrylamide gels were prepared according to the method of Laemmli (1970) except that no SDS was used. Gels were run in a 4°C-cooler for 30 min at 50 V then for 1.5 h at 100 V. Staining for enzymatic activity was according to Hart and Langston (1977).

Results

The C-banded A^t- and G-genome chromosomes in TA 3432, the *T. timopheevii-A. tauschii* amphiploid, are similar to the standard karyotype of *T. timopheevii*, indicating that there have been no major modifications of these genomes in the amphiploid (Badaeva et al. 1994). The *T. timopheevii* and *A. tauschii* accessions used to synthesize the amphiploid are not known.

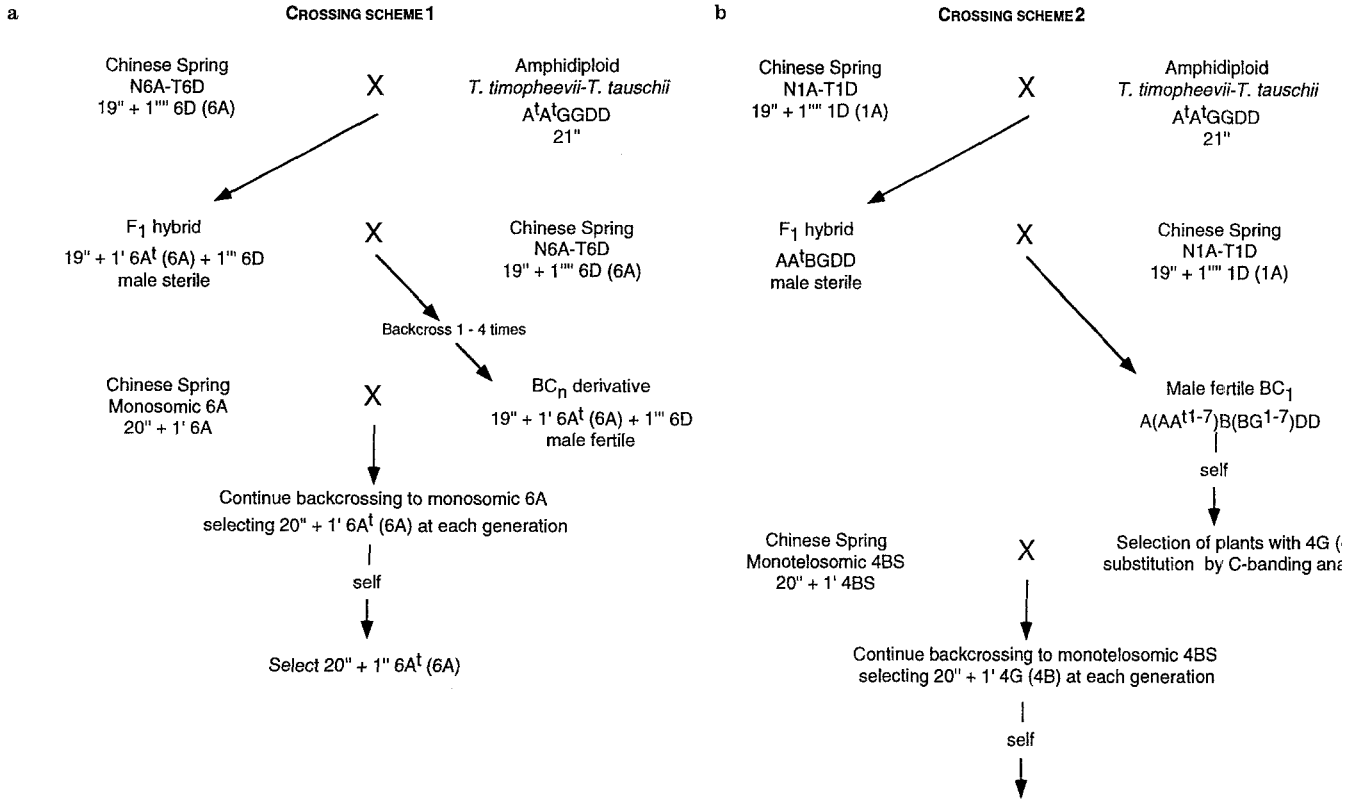
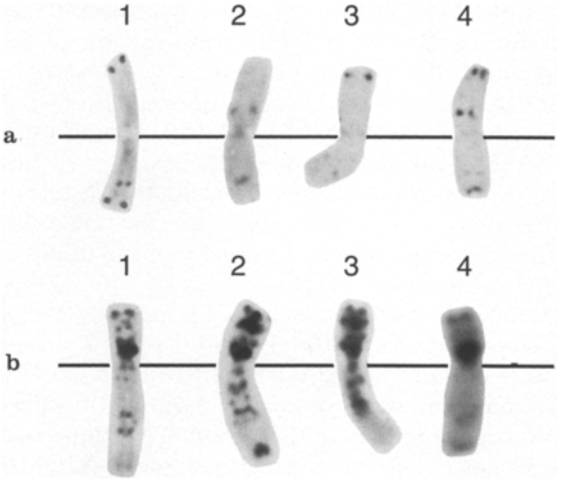


Fig. 1a, b Crossing schemes used for producing lines with *T. timopheevii* chromosome substitutions in 'Chinese Spring'. **a** Crossing scheme 1, with the method for the isolation of a disomic 6A^t (6A) substitution line shown as an example. **b** Crossing scheme 2, with the method used for the isolation of a disomic 4G(4B) substitution line shown as an example

C-banding analysis was done on 88 F_2 and F_3 progeny of BC_1-, BC_2-. BC_3-, and BC_4-derived lines from crosses between TA 3432 and Chinese Spring NT stocks. Substitutions involving all A^t-genome and G-genome chromosomes were identified. Most of these lines had multiple substitutions. Three of the *T. timopheevii* chromosomes identified had been modified. A modified chromosome 1G was found to substitute for chromosome 1B in crosses between N1BT1D rand TA 3432. A deletion of part of the terminal euchromatic region of the short arm of chromosome 1G was noted in six plants from this cross. One plant derived from the cross N6AT6D*2/TA 3432 had one normal chromosome 7A and a recombinant chromosome 7A^{t}S·7AL. Two rearranged 7A chromosomes were detected in the progeny of a plant heterozygous for chromosomes 7A and 7A^t. In a single plant, a recombinant chromosome 7A having a proximal N-band in the short arm, transferred from chromosome 7A^{t}S, as well as a recombinant 7AS·7A^{t}L were identified (Fig. 2). The 5G chromosome identified in plants from the cross N1AT1D*2/TA 3432 had lost the terminal C-band in the long arm (Fig. 2). One BC_2-derived line from the same lineage had a 5B chromosome with a telomeric C-band in the long arm (Fig. 2) indicating that the terminal C-band of 5GL was transferred to 5BL.

The number of different types of substitutions in each cross combination decreased as the number of backcrosses to wheat increased. In general, A^t-genome chromosomes substituted for their A-genome counterparts, while G-genome chromosomes substituted for their B-genome homoeologues. Exceptions to this were 3G (3A) and 1A^t (1D)

Fig. 2a, b C- and N-banding karyotypes of *T. timopheevii* chromosomes, wheat chromosomes, and recombinant *T. timopheevii*-wheat chromosomes. **a** N-banded chromosomes: *1* 7A; *2* 7A^t; *3* modified 7AL without a telomeric band; *4* recombinant 7AS with proximal N-band of 7A^{t}S. **b** C-banded chromosomes: *1* 5B; *2* 5G; *3* modified 5GL without telomeric C-band; *4* recombinant 5BL with the telomeric C-band of 5GL



substitutions. Substitutions of G-genome chromosomes for the respective B-genome homoeologue were detected more frequently than A^t (A) substitutions.

The frequency of substitution differed for different chromosomes. By far the most frequently detected substitution was 2G (2B), which was found in 60 of 88 plants derived from all cross combinations. Other chromosomes frequently substituted were 1A (23 plants), 3A (22 plants), 7B (20 plants) and 5B (17 plants). Chromosomes that were rarely substituted were 3B (5 plants) and 7A (2 plants).

Although substitutions involving all *T. timopheevii* chromosomes were identified in backcross-derived lines, the targeted *T. timopheevii* chromosomes were not maintained in several cross combinations. For example, in backcrosses of TA 3432 to N3BT3D, chromosome 3G was the targeted chromosome of *T. timopheevii*. However, chromosome 3G was not identified in lines that had been backcrossed three times to N3BT3D. Substitutions involving the targeted *T. timopheevii* chromosomes were $1A^t$(1A), $5A^t$(5A), $6A^t$(6A), and a modified 1G(1B). Chromosomes that were not maintained during backcrossing to the appropriate NT included $3A^t$, $7A^t$, 3G, 4G and 7G.

Lines with chromosomes $6A^t$, 2G, 3G, 4G, 5G and 6G were selected from crosses of TA 3432 with NT stocks for further backcrossing. A $6A^t$(6A) substitution line was developed via crossing scheme 1, whereas substitutions of G-genome chromosomes for their B-genome counterparts were developed using crossing scheme 2. Only those lines that have been backcrossed to Chinese Spring at least five times, and/or have only one or two *T. timopheevii* chromosomes, are described further.

$6A^t$(6A) substitution line

Chromosome $6A^t$ is involved in a species-specific cyclic translocation with chromosomes 1G and 4G (Jiang and Gill 1994). As a result of this translocation, part of the short arm of chromosome 1G, including the heterochromatic nucleolus-organizer region and the satellite, was transferred to chromosome $6A^t$ (Fig. 3). Though chromosome $6A^t$ has been significantly modified, it compensated for chromosome 6A in substitution lines. Monosomic $6A^t$(6A) substitution lines were fertile and used as males for backcrossing. Male transmission of $6A^t$ in monosomic substitution lines was 33%. After five backcrosses to wheat, $6A^t$(6A) substitutions were recovered only in conjunction with *T. timopheevii* chromosome 3G. The $6A^t$(6A) substitution lines were vigorous, though shorter than normal Chinese Spring. A $6A^t$S telosomic chromosome substituting for chromosome 6A was identified among the selfed progeny of the monosomic $6A^t$(6A) substitution line (Fig. 3).

Chromosome 6A of wheat has a locus on the short arm, *Gli-A2*, that encodes α gliadin protein sub-units that were absent in the $6A^t$(6A) substitution line (Fig 4). Additional gliadin bands were present in the substitution line that migrated in the region of gliadins encoded by the locus *Gli-B1* of wheat, which maps distal to the

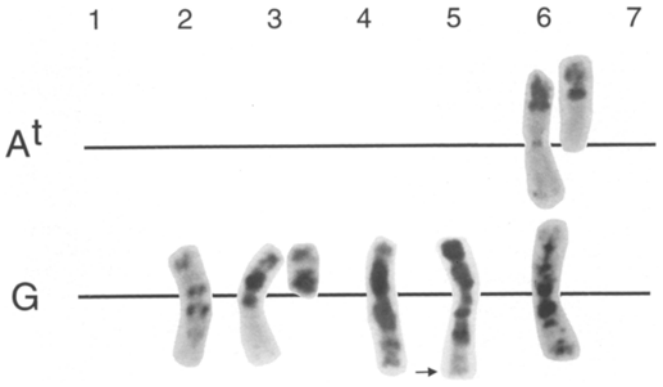


Fig. 3 C-banded karyotype of the A^t- and G-genome chromosomes and telosomes of *T. timopheevii* substitutions and additions. The arrow indicates the loss of a telomeric C-band in the long arm of chromosome 5G

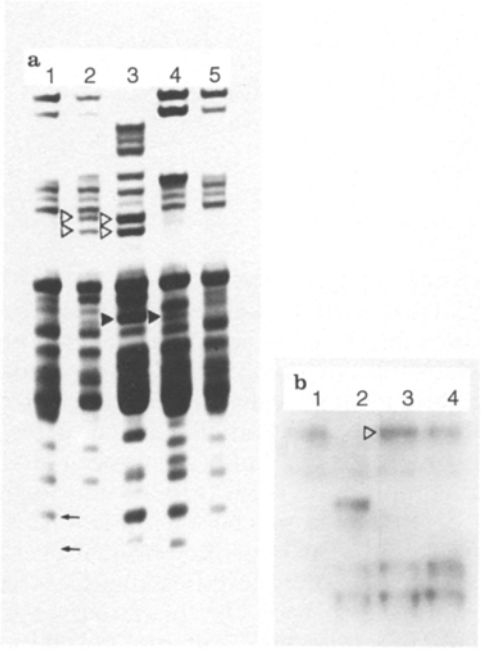


Fig. 4a, b Storage proteins and isozymes of *T. timopheevii* chromosome substitutions. **a** Gliadin electrophoresis patterns of Chinese Spring (*lanes 1 and 5*), disomic substitution $6A^t$ (6A) heterozygous 3G(3B) (*lane 2*), *T. timopheevii-A. tauschii* amphiploid (*lane 3*), disomic substitution 6G(6B) heterozygous 2G(2B) (*lane 4*). *Arrowheads* indicate proteins from the amphiploid: *open arrowheads*, chromosome $6A^t$; *arrowhead*, chromosome 6G. *Full arrows* indicate proteins in Chinese Spring that are absent in disomic substitution $6A^t$(6A). **b** Acid phosphatase zymogram phenotypes of *T. timopheevii* accession TA 103 (*lane 1*), Chinese Spring wheat (*lane 2*) disomic 4G (4B) substitution (*lane 3*), *T. timopheevii-A. tauschii* amphiploid (*lane 4*). The *open arrowhead* indicates the protein from *T. timopheevii*

NOR region of chromosome 1B. It is likely that a gliadin locus originally located on chromosome 1G of *T. timopheevii* is present on chromosome $6A^t$. Gill and Chen (1987) determined the genetic composition of the species-specific $6A^t$-1G translocation based on chromosome pairing. The presence of a locus encoding γ gliadins on chromosome $6A^t$ confirms their observations.

2G(2B) substitution line

The disomic 2G(2B) substitution line was derived from the cross of TA 3432 and N1BT1D. One BC_5F_2 plant was identified that was disomic for 2G(2B) and nullisomic for chromosome 1B, trisomic 1A and monosomic $1A^t$. This substitution line was backcrossed to wheat five times and is normal in morphology, vigor and fertility.

3G(3B) substitution line and 3GS ditelosomic addition line

The 3G (3B) substitution line was derived from a cross between the amphiploid and N6AT6D. This line was backcrossed to Chinese Spring MT3BL four times, with selection for chromosome 3G at each generation. After a total of five backcrosses to Chinese Spring, chromosome 2G was present in the 3G(3B) substitution line. The 3GS disomic telo-addition line had a normal complement of wheat chromosomes with the addition of two copies of chromosome 3GS.

4G(4B) substitution line

The disomic 4G(4B) substitution line was derived from a cross between N1AT1D and TA 3432 and has been backcrossed five times to wheat. It had normal morphology, fertility and vigor, indicating that chromosome 4G compensated for wheat chromosome 4B. Genes located on the distal portion of the short arm of homoeologous group-4 chromosomes are important for male fertility, resulting in the inability to maintain group-4 nullisomic stocks. When monosomic 4G(4B) substitutions were used as the pollen parent in backcrosses to MT4BS, 91% of the F_1 plants had chromosome 4G, indicating that pollen having chromosome 4G was more competitive than nullisomic-4G pollen.

An acid phosphatase zymogram of the 4G(4B) substitution line, along with Chinese Spring and TA 3432, is shown in Fig. 4. A band present in TA 3432 was also present in the substitution line but not in Chinese Spring. The corresponding Chinese Spring band was absent in the substitution line. The acid phosphatase genes are located on the long arms of wheat homoeologous group-4 chromosomes (Hart 1987). Gill et al. (1988) determined that a gene controlling acid phosphatase was present on chromosome 4G of *T. araraticum*. The presence of an acid phosphatase gene in the 4G(4B) substitution line further confirms that this chromosome belongs to wheat homoeologous group 4.

5G*(5B) substitution line

This line contains the normal complement of wheat chromosomes, except for a modified chromosome 5G (designated 5G*) that substitutes for wheat chromosome 5B. The terminal C-band in the long arm of normal chromosome 5G is absent in the substitution line (Fig. 3). The substitution line was derived by backcrossing a BC_1F_3 plant from the cross N1AT1D*2/TA 3432 five times to MT5BL. Chromosomes 5G and 5B were both present in the F_1 and BC_1 F_1 plants and had an opportunity to recombine (Fig. 2).

6G(6B) substitution line

A disomic substitution of chromosome 6G for chromosome 6B is present in this line. Though the line has been backcrossed to wheat six times, it is heterozygous for chromosomes 2G and 2B. The spikes of the substitution line have short awns, whereas Chinese Spring is awnless. The 6G(6B) substitution line differs from Chinese Spring in its gliadin protein profile. A β gliadin band present in the 6G(6B) substitution line was absent in Chinese Spring (Fig. 4).

Discussion

The production of "pure" substitution lines of A^t- and G- genome chromosomes for their wheat homoeologues in a hexaploid background was complicated by the fact that the A^t and G genomes are partially homologous with the A and B genomes. Thus, chromosomes of the A^t and G genomes pair with A- and B-genome chromosomes in the presence of the *Ph* gene which suppresses homoeologous pairing (Feldman 1966; Dvorák 1983; Gill and Chen 1987). To avoid recombination of substituted chromosomes, procedures used to isolate homologous substitution lines normally involve crosses to wheat monosomics using the donor line as the pollen parent. However, when trying to isolate *T. timopheevii* substitutions, this was not initially possible because of male sterility in the F_1 hybrid and the first few backcross generations. The *T. timopheevii* – *A. tauschii* amphiploid was used in this study to overcome this problem; but male sterility was still observed in early generations. Thus, nullisomic-tetrasomic stocks were utilized in crossing schemes because they could be used as a pollen parent. BC_1 plants from the cross between TA 3432 and N1AT1D, and BC_2 plants from the cross between TA 3432 and N1BT1D, were more fertile than early backcross generation plants from other cross combinations. Four of the substitution lines were derived from the progeny of these crosses.

Both crossing schemes used for substitution-line development had advantages and drawbacks. Crossing scheme 1 was designed to ensure that the targeted *T. timopheevii* chromosome had no oportunity pair with its wheat homoeologue. This scheme did not, however, exclude the possibility of translocated chromosomes of *T. timopheevii*, such as those involved in the $6A^t$-1G-4G cyclic translocation and a $3A^t$-$4A^t$ translocation (Gill

and Chen 1987), from pairing with wheat chromosomes having homoeologous regions.

An additional problem with crossing scheme 1 was that the nullisomic-tetrasomic stocks used already had a chromosome compensating for the absent A- or B-genome chromosome; therefore, the A^t- or G-genome homoeologue had to compete with this chromosome. Translocations present in *T. timopheevii* may reduce the compensating ability of the *T. timopheevii* chromosome. The targeted *T. timopheevii* chromosomes were not always recovered after backcrossing to the Chinese Spring NT lines in the absence of banding analysis and selection.

By utilizing crossing scheme 2, we were able to select fertile lines having a targeted chromosome substituted for its wheat homoeologue for further backcrossing. Crossing scheme 2 was used to develop substitutions involving G-genome chromosomes. This approach, which did not exclude recombination between the *T. timopheevii* chromosome of interest and its wheat homoeologue, was not used to obtain A^t(A) substitutions carried out because studies of pairing in *T. aestivum*/*T. timopheevii* and *T. turgidum*/*T. timopheevii* hybrids have shown that A- and A^t-genome homoeologues generally have a high pairing affinity (Feldman 1966; Gill and Chen 1987). Chromosomes of the B and G genomes are highly banded, making B-G recombinants easier to detect than A-A^t recombinants.

A gene for leaf rust resistance, *Lr18*, transferred from *T. timopheevii* to wheat, maps to chromosome 5B of wheat and is linked to a terminal C-band transferred to that chromosome (Yamamori 1994). Although TA 3432 is resistant to leaf rust, neither the 5G*(5B) substitution line nor the related line with the modified 5B chromosome were leaf rust-resistant (data not shown). Apparently TA 3432 has a leaf rust resistance gene different from *Lr18*. Whether the gene(s) was donated from the *T. timopheevii* or the *A. tauschii* parent of the amphiploid is not known. The other substitution lines were all susceptible when screened for reaction to leaf rust.

Although only one of the six lines described was produced using the crossing scheme that minimized the possibility of recombination between the substituted *T. timopheevii* chromosome and its wheat homoeologue, all of the lines are useful for studying the relationship of *T. timopheevii* and *T. aestivum*. Disomic substitutions involving chromosomes $6A^t$ and 3G have not been previously described. With the exception of the 5G*(5B) substitution line, the chromosomes in the substitution lines appear to be unmodified, although conserved molecular markers should be used to confirm this.

The 4G(4B) and 5G*(5B) substitution lines both had a single *T. timopheevii* chromosome substituting for its wheat homoeologue. Chromosome 2G was present in lines with 3G(3B) and 6G(6B) substitutions, though these lines have been backcrossed to wheat five and six times, respectively. Chromosome 2G was found to spontaneously substitute for 2B at a high frequency in crosses between TA 3432 and all Chinese Spring NT lines. After one to four backcrosses to wheat, chromosome 2G was detected in 68% of the lines analyzed. This may be due to some selective advantage of gametes having chromosome 2G, either by itself or in conjunction with other *T. timopheevii* chromosomes. Indirect evidence that chromosome 2G has 'meiotic drive' is available from a number of studies. Nyquist (1962) found that in some hexaploid backgrounds the transmission of an alien chromosome segment derived from *T. timopheevii*, which carried the stem-rust resistance gene *Sr36*, was favored. It was determined that a gene(s) causing differential fertilization was the same as, or else completely linked to, the *T. timopheevii*-derived gene, which maps to the long arm of chromosome 2B. Friebe et al. (1994) reported a 2G (2B) substitution in the wheat line TAM 104 that was derived from a male-sterile line of the wheat cultivar Sturdy in a *T. timopheevii* cytoplasm. The 2G (2B) substitution was maintained after additional backcrossing of the line to wheat (B. Friebe, personal communication). Brown-Guedira (1995) noted that chromosome 2G was recovered at a higher than expected frequency in the progeny of a hexaploid derivative of a cross between wheat and *T. araraticum* that was heterozygous for chromosomes 2G and 2B. Additionally, Badaeva et al. (1991) found 2G (2B) to be the most frequent disomic substitution in the progeny of *T. aestivum*/*T. timopheevii* hybrids.

Group-2 chromosomes with gametocidal genes have been isolated from several species that may show preferential transmission and cause breaks in wheat chromosomes (Tsujimoto 1995; Endo and Gill 1996). It is not known if these genes, including the one on 2G, constitute a homoeoalellic series.

Gliadin electrophoresis determined that no α gliadins corresponding to the gliadin proteins encoded by a gene on wheat chromosome 6A were present in the $6A^t$(6A) substitution line. Because the short arm of chromosome $6A^t$ is known to be involved in a cyclic translocation with chromosomes 1G and 4G (Jiang and Gill 1994), the gliadin locus (*Gli-A2*) present on the original chromosome $6A^t$ may have been transferred to either chromosomes 1G or 4G. Gliadin electrophoresis of the 4G(4B) substitution line did not detect additional α gliadins (data not shown). No substitution lines having chromosome 1G were available for testing. Another possibility is that either the gliadin locus was transferred to some other chromosome of *T. timopheevii* that was not tested or it was inactivated.

Most of the γ and ω gliadin genes in wheat are located on the short arms of chromosomes 1A, 1B, and 1 D (Beitz 1987). However, γ gliadin bands present in TA3432 were also present in the $6A^t$(6A) substitution line. The location of a gene orthologous to *Gli-B1* on chromosome $6A^t$ and the absence of a gene orthologous to *Gli-A2* confirms that a portion of the short arm of $6A^t$ was replaced by part of the short arm of chromosome 1G.

Substitution lines with two of the chromosomes involved in the cyclic translocation of chromosomes $6A^t$,

1G, and 4G, which is species-specific to *T. timopheevii*, were isolated. DNA markers that map to chromosomes 6A and 4B of wheat are currently being used to determine the translocation breakpoints of chromosomes $6A^t$ and 4G. The species-specific cyclic translocation 4A-5A-7B present in *T. turgidum* and *T. aestivum* also involves a group-4 chromosome (Naranjo et al. 1987; Naranjo 1990; Liu et al. 1992). A comparison of the translocation breakpoints of 4A and 4G should be useful in determining the significance of rearrangements involving homoeologous group-4 chromosomes in the genome evolution of polyploid wheats.

Full compensation for the fertility factors located on wheat chromosomes 4B and 6B (Sears 1966), together with compensation for *Ph1* of 5B (Gill et al. 1988) by G-genome homoeologues, demonstrates that the G-genome of *T. timopheevii* was derived from the same source as the B-genome of *T. turgidum* and *T. aestivum*. Given the high homology of the A and A^t genomes, the two tetraploid wheat species probably had the same diploid donors but arose from separate hybridization events, as indicated by the different spectrum of species-specific translocations.

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