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Studies on the origin and evolution of tetraploid wheats based on the internal transcribed spacer (ITS) sequences of nuclear ribosomal DNA

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Abstract In this study, the internal transcribed spacer (ITS) sequences of nuclear ribosomal DNA in the tetraploid wheats, *Triticum turgidum* (AABB) and *Triticum timopheevii* (AAGG), their possible diploid donors, i.e., *Triticum monococcum* (AA), *Triticum urartu* (AA), and five species in *Aegilops* sect. *Sitopsis* (SS genome), and a related species *Aegilops tauschii* were cloned and sequenced. ITS1 and ITS2 regions of 24 clones from the above species were compared. Phylogenetic analysis demonstrated that *Aegilops speltoides* was distinct from other species in *Aegilops* sect. *Sitopsis* and was the most-likely donor of the B and G genomes to tetraploid wheats. Two types of ITS repeats were cloned from *Triticum turgidum* ssp. *dicoccoides*, one markedly similar to that from *T. monococcum* ssp. *boeoticum* (AA), and the other to that from *Ae. speltoides* (SS). The former might have resulted from a recent integression event. The results also indicated that *T. turgidum* and *T. timopheevii* might have simultaneously originated from a common ancestral tetraploid species or be derived from two hybridization events but within a very short interval time. ITS paralogues in tetraploid wheats have not been uniformly homogenized by concerted evolution, and high heterogeneity has been found among repeats within individuals of tetraploid wheats. In some tetraploid wheats, the observed heterogeneity originated from the same genome (B or G). Three kinds of ITS repeats from the G genome of an individual of *T. timopheevii* ssp. *araraticum* were more divergent than that from inter-specific taxa. This study also demonstrated that hybridization and polyploidization might accelerate the evolution rate of ITS repeats in tetraploid wheats.

Keywords Tetraploid wheats · B genome · G genome · Concerted evolution

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Introduction

Tetraploid wheats include *Triticum turgidium* L. (AABB) and *Triticum timopheevii* Zhuk. (AAGG). *T. turgidum* consists of a wild subspecies *dicoccoides* (Korn.) Thell and some cultivated subspecies, such as ssp. *durum* Desf., ssp. *dicoccum* (Schrank) Thell, ssp. *turgidum* etc. *Triticum timopheevii* has one wild subspecies *araraticum* (Jukabz.) MK and one cultivated subspecies *timopheevii*. The origins of the A, B and G genomes of the tetraploid wheats have been the subject of considerable controversy. Based on previous studies, species in *Aegilops* sect. *Sitopsis* (SS), i.e. *Aegilops speltoides*, *Aegilops longissima*, *Aegilops searsii*, *Aegilops sharonensis* and *Aegilops bicornis*, were found to be closely related to the B genome, and all of them were proposed as the donor of the B genome to polyploid wheats (Kerby and Kuspira 1988; Dvorák and Zhang 1990; Friebe et al. 1993; Talbert et al. 1995; Daud and Gustafson 1996; Maestra and Naranjo 1998). Moreover, Zohary and Feldman (1962) proposed that the B genome be a recombined genome based on an intensive study on the cytology, morphology and natural hybridization of the *Aegilops-Triticum* group. The polyphyletic hypothesis was also supported by evidence based on karyotype (Giorgi and Bozzini 1969), isozyme (Nishikawa et al. 1992), chromosome pairing (Gill and Chen 1987) and RFLP (Ciaffi et al. 2000) analyses.

As for the G genome, some studies suggested that *T. turgidum* and *T. timopheevii* be derived from an ancient tetraploid species (Sachs 1953; Feldman 1966; Noda and Koulin 1989). But evidence for the diphyletic origin of *T. turgidum* and *T. timopheevii* was provided by Dvorák et al. (Dvorák et al. 1989; Dvorák and Zhang 1990). They

argued that both species originated from the hybridization of *Ae. speltoides* with *Triticum urartu* and that the origin of *T. turgidum* preceded that of *T. timopheevii*. More recent studies supported the diphyletic origin (Jiang and Gill 1994b; Miyashita et al. 1994; Mori et al. 1995; Maestra and Naranjo 1999; Rodriguez et al. 2000; Ciaffi et al. 2000).

Two diploid species, *Triticum monoccocum* (Gill and Kimber 1974; Jaaska 1980) and *Triticum urartu* (Caldwell and Kasarda 1978) were proposed as the possible A genome donors to tetraploid wheats based on the C-banding pattern of chromosomes, isozyme and seed storage protein profiles. Moreover, Konarev et al. (1979) proposed that the A genome of *T. turgidum* be derived from *T. urartu* and that of *T. timopheevii* from *T. monoccocum* based on the immunological character of seed storage proteins. Nishikawa et al. (1992) even suggested that the A genome of *T. turgidum* be possibly derived from both *T. monococcum* ssp. *boeoticum* and *T. urartu*. Studies by Dvorák et al. (1988, 1993) on repeated nucleotide sequences clearly showed that *T. urartu* was the ancestor of the A genome of *T. turgidum* and *T. timopheevii*, which has been supported by the recent studies of Allaby and Brown (2000) and Galili et al. (2000).

With the development of the techniques in molecular biology, DNA sequences have been used in phylogenetic and evolutionary studies. For example, the internal transcribed spacer (ITS) of 18S–26S nuclear ribosomal DNA (nrDNA) has proven useful for phylogenetic studies in plants, especially in lower-rank groups (Baldwin et al. 1995). It has also been used to study the evolution of wheat species in the early 1980s (Peacock et al. 1981; Dvorák and Appels 1982). Some other DNA molecules have also been examined, e.g. Dvorák and Zhang (1992) reconstructed the phylogeny of *Triticum* based on the variation in numerous repeated nucleotide sequence families, and Talbert et al. (1995) identified the donor of the B genome to hexaploid wheat using sequences of three low-copy DNA fragments.

The evolution of the ITS region is more complicated in hybrid and polyploid species (Baldwin et al. 1995; Wendel et al. 1995; Waters and Schaal 1996). Baldwin et al. (1995) proposed that ITS sequences would provide direct evidence of reticulate evolution if concerted evolution failed to homogenize the repeat units contributed by different parental species when the hybridization event was recent, or if nrDNA repeats were at different loci in the parental genomes and interlocus gene conversion was inoperative in their hybrid, or if the hybrid was asexual. Since the history of polyploid wheats is relatively short (Mori et al. 1995) and the ITS repeats in polyploid wheats are located at different loci (Dubkovsky and Dvorák 1995; Badaeva et al. 1996), it may be possible to identify ITS sequences of different parental origins in polyploid wheats and hence to identify their progenitors. In addition, the *Triticum* complex is a good model system for studying how hybridization and polyploidization could possibly affect the evolution of nrDNA.

In this study, the ITS sequences of tetraploid wheats, the possible diploid donors and *Aegilops tauschii* were amplified by PCR and cloned. The ITS1 and ITS2 regions were sequenced. A phylogenetic tree of diploid and tetraploid wheats was reconstructed, and the donors of the B and G genomes of tetraploid wheats were inferred from the phylogenetic tree. The concerted evolution of ITS repeats in tetraploid wheats is also discussed.

Materials and methods

Plant materials

Taxon, genome type, and the source of the plant materials used in this study are listed in Table 1. One individual was usually selected for each taxon except for two dipoid *Aegilops* and one tetraploid *Triticum* species. Seeds of all plant materials used were sterilized in 15% NaClO solution for 20 min and sown on MS culture medium to avoid fungal contamination. The leaves were taken for DNA extraction 7 days after germination and seedlings were then moved into pots in the greenhouse to grow to maturation for confirming their identification by morphological and/or cytological characters. *Taeniatherum caput-medusae* (L.) Nevski was used as an outgroup.

PCR amplification and cloning

Total DNA was isolated from leaves of a single plant, using the CTAB method (Murray and Thompson 1980).

ITS regions were amplified by PCR using primers "ITS-5" and "ITS-4" designed by White et al. (1990). PCR amplification was carried out in a 50-µl reaction mixture containing 5 µl of 10 × *Taq* polymerase buffer, 4 µl with 10 mM each of dNTPs, and 20–40 ng of DNA template. The reaction mixture was overlayed with 30 µl of light silicone oil and denatured at 94 °C for 5 min before adding 1 µl (3 units) of *Taq* polymerase. The following PCR regime was used: 35 cycles of denaturation for 1 min at 94 °C, annealing for 1 min at 52 °C and extension for 1 min at 72 °C. A 10-min extension at 72 °C followed the completion of the 35 cycles. The PCR products were electrophoresed on 1.2% agarose gels and then purified by electroelution using DEAE membrane. Purified DNA fragments were cloned into pBluescript SK.

Sequencing

Sequencing was carried out by the dideoxy chain-termination method using a Dye Primer Cycle Sequencing Kit (Applied Biosystems) with T7 and T3 primers from both 5′ and 3′ directions on an ABI 373A DNA automatic sequencer (Applied Biosystems). In order to detect polymorphism of the ITS sequences, PCR products of some taxa were sequenced directly using a T7 sequencing Kit (Phamacia). ITS sequences of *T. caput-medusae* and *T. monococcum* ssp *monococcum* were obtained from Hsiao et al. (1994, 1995a). At least two clones from one individual of diploid, and four clones from one individual of tetraploid, species were sequenced.

Phylogenetic analysis

ITS sequences of 15 taxa were aligned using the CLUSTAL V programs (Higgins et al. 1992) with some corrections manually at certain sites. The maximum-parsimonious (MP) trees for the sequences were constructed using PAUP 3.1.1 (Swofford 1993). The neighbor-joining (NJ) tree was calculated with MEGA (Kumar et al. 1993) based on the Kimura two-parameter genetic distance (Kimura 1980). A maximum-likelihood (ML) tree was constructed employing TREE-PUZZLE 5.0 (Schmidt et al. 2000) based on the Hasegawa-Kishino-Yano (HKY) substitution model (Hasegawa et al. 1985).

It has been demonstrated that *T. caput-medusae* was closely related to *Triticum* and *Aegilops* (Hsiao et al. 1995a), therefore

Table 1 The plant materials used in this study

Taxon	Genome	Accession No. and/or reference	EMBL accession number for DNA sequence		
			Clone number	ITS 1	ITS 2
T. caput-medusae (L.) Nevski	TT	Hsiao et al. 1995a	Tca	L36505	
T. monococcum ssp. *monococcum* L.	AA	Hsiao et al. 1994	Tmo	L11581	
T. monococcum ssp. *boeoticum* Boiss	AA	ICGR[a], Bo1, Zhang et al. 1998	Tbo-2	AJ242401	AJ238901
T. urartu Thum.	AA	ICGR, UR1, Zhang et al. 1998	Tur-2	AJ242402	AJ238902
T. turgidum ssp. *turgidum* (L.) Morris et Sears	AABB	ICGR, Zh1	Ttu-11	AJ242420	AJ238918
			Ttu-10	AJ242421	AJ238919
T. turgidum ssp. *dicoccoides* Korn.	AABB	ICGR, Ds5	Tdi-7	AJ242411	AJ238911
			Tdi-8	AJ242413	AJ238912
			Tdi-4	AJ242414	AJ238913
			Tdi-13	AJ242416	AJ238914
			Tdi-12	AJ242417	AJ238915
			Tdi-15	AJ242418	AJ238916
T. turgidum ssp. *dicoccum* Schrank.	AABB	ICGR, Dr8	Tdu-0	AJ242419	AJ238917
T. timopheevii ssp. *timopheevii* Zhuk.	AAGG	ICGR, Ti1	Tti-1	AJ242425	AJ238923
			Tti-2	AJ242426	AJ238924
T. timopheevii ssp. *araraticum* Jukabz.	AAGG	ICGR, Ar1	Tar-2	AJ242422	AJ238920
			Tar-3	AJ242423	AJ238921
			Tar-4	AJ242424	AJ238922
Ae. speltoides Tausch	SS	ICGR, Ae48, Zhang et al. 1998	AspI-4	AJ242403	AJ238903
	SS	ICGR, Ae 29	AspII-2	AJ242404	AJ238904
Ae. longissima Schweinf. & Mushl	SS^{l}	ICGR, Y 152	Alo-2	AJ242408	AJ238908
Ae. sharonensis Eig.	SS^{sh}	Liu Deng-Cai, no number	AshI-3	AJ242405	AJ238905
	SS^{sh}	Kawahara, KU5-1	AshII-2	AJ242406	AJ238906
Ae. bicornis (Forsk.) Jaub.	SS^{b}	ICGR, Ae 4	Abi-5	AJ242407	AJ238907
Ae. searsii Feld. and Kis.	SS^{S}	Kawahara, KU4-7	Ase-1	AJ242409	AJ238909
Ae. tauschii Coss.	DD	ICGR, Ae 42, Zhang et al. 1998	Ata-1	AJ242410	AJ238910

[a] ICGR abbreviates for the Institute of Crop and Germ-plasm Resource, China

T. caput-medusae was used as an outgroup. The 5.8S nrDNA sequence was not considered in this study since very little variation was found among the species of the tribe Triticeae and the subfamily Pooideae (Hsiao et al. 1995a, b).

Results

Length and variation of ITS1 and ITS2 sequences

From more than 80 clones sequenced, 24 clones of ITS1 and ITS2 with different nucleotide sequences were found from 13 taxa. The EMBL accession number for each sequence is listed in Table 1, and the EMBL accession number for the sequence alignment is ds38535 (ITS1) and ds38564 (ITS2). The length ranged from 221 to 223 bp in ITS1, and from 216 to 217 bp in ITS2. A 2-bp indel was found in ITS1 sequences and an 1-bp indel in ITS2 sequences.

The G+C contents of the sequences varied from 59% to 62% for ITS1 and from 60% to 65% for ITS2. Twenty four of 47 variable sites in ITS1 and 24 of 39 variable sites in ITS2 were informative for parsimony analysis. Pairwise genetic distances (abbreviated as distance in the following text) estimated by a Kimura two-parameter genetic distance model ranged from 0.0070 to 0.0789 for ITS1, and from 0.0000 to 0.0112 for ITS2, which were similar to those values of other taxa in the Triticeae (Hsiao et al. 1995a).

Variation within individuals

ITS fragments showed polymorphism within an individual of the tetraploid wheats. Three clones from an individual of *T. turgidum* ssp. *dicoccoides* (Tdi4, Tdi7 and Tdi8) were different from one another, and the average distance between them was 0.0422 for ITS1, and 0.0741 for ITS2, which were greater than that among the sect. *Sitopsis* species (0.0237 for ITS1 + ITS2). Three fragments cloned from another individual of the same taxon (Tdi12, Tdi13 and Tdi15) were different from Tdi4, Tdi7 and Tdi8. The distance between Tdi8 and Tdi13 was only 0.0094 (ITS1 + ITS2), and the average distance among Tdi4, Tdi7, Tdi12 and Tdi15 was 0.0173 for ITS1+ITS2. The average distance among the sequences of three clones from an individual of *T. timopheevii* ssp. *araraticum* (Tar2, Tar3 and Tar4) was 0.0374 for ITS1+ITS2. However, sequence variation between two clones from *T. timopheevii* ssp. *timopheevii* (Tti1 and Tti2) was very low, only 0.0070 for ITS1+ITS2.

Phylogenetic relationships of the diploid species

With *T. caput-medusae* as an outgroup, parsimony analysis of the diploid species using the branch and band search yielded four most-parsimonious (PM) trees with 64 steps. In the consensus tree (Fig. 1), *T. monococcum*

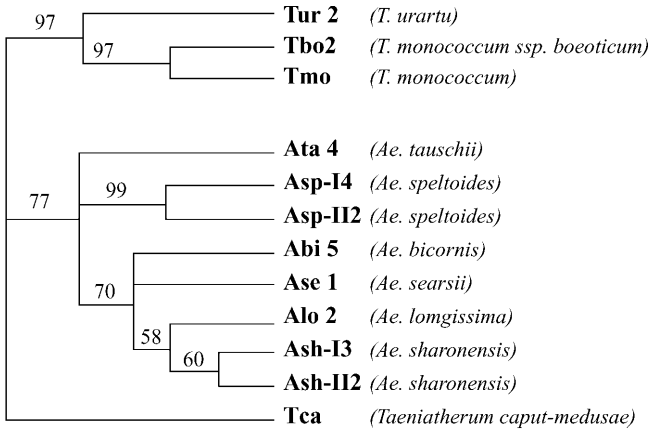

Fig. 1 The strict consensus tree generated four maximum-parsimonious trees of ten diploid taxa based on the ITS1+ITS2 sequences employing PAUP 3.1.1 using the branch-and-bound search. *T. caput-medusae* is an outgroup; *numbers* above branches are bootstrap values of 500 replications. Tree length = 64 steps; CI = 0.875; RI = 0.849

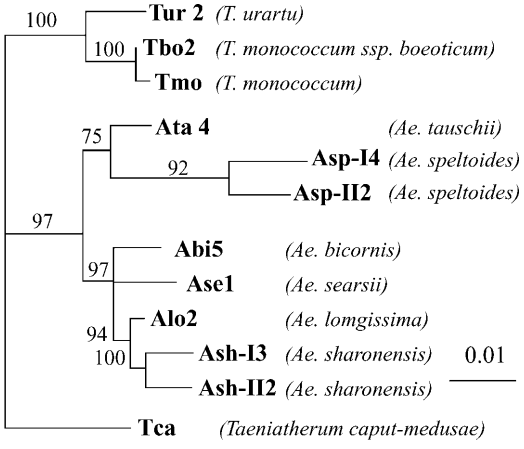

Fig. 2 The maximum-likelihood tree of ten diploid taxa based on the ITS1+ITS2 sequences employing TREE-PUZZLE 5.0 using the Hasegawa-Kishino-Yano model. *T. caput-medusae* is the outgroup; *numbers* above branches are percent values of 1,000-times quartet-puzzling steps. Log L without clock = –1,024.64

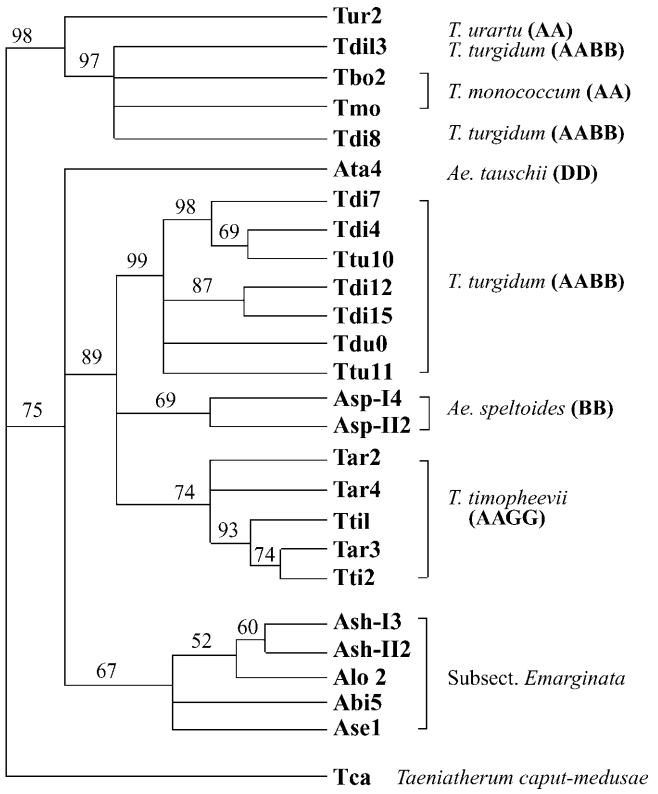

Fig. 3 The strict consensus tree from 33 parsimonious trees of 15 diploid and tetraploid taxa based on ITS1+ITS2 sequences employing PAUP 3.1.1 using the heuristic search. *T. caput-medusae* is the outgroup; *numbers* above branches are bootstrap values of 500 replications. Length = 124 steps

ssp. *monococcum*, *T. monococcum* ssp. *boeoticum* and *T. urartu* constitute a monophyletic group and form a sister group to the clade containing *Aegilops* species. Two subsections of *Aegilops* sect. *Sitopsis*, i.e. subsect. *Emarginata* with four species (*Ae. searsii*, *Ae. longissima*, *Ae. sharonensis* and *Ae. bicornis*), and subsect. *Truncata* with one species (*Ae. speltoides*), did not form sister groups to each other. The species in subsect. *Emarginata* formed a monophyletic group. The general topology of the NJ tree and the ML tree was identical to that of the PM tree except for the position of *Ae. tauschii*. In the ML tree, *Ae. tauschii* formed a sister group to *Ae. speltoides* with a supported value of 75% (Fig. 2). In the above three trees, the relationships among *Ae. tauschii*, *Ae. speltoides* and subsect. *Emarginata* were not well-resolved. When *Secale cereale* was used as an outgroup, the topology of the phylogenetic trees were very similar to that of the above trees, and the bootstrap values of some clades were lower than those in the above trees (data not shown).

Phylogenetic relationships among diploid and tetraploid species

Parsimony analysis of both diploid and tetraploid species generated 33 most-parsimonious trees with 124 steps. The consensus tree showed that the general topological relationships among the diploids have not been changed after adding tetraploid species (Fig. 3). Two different types of ITS sequences were found in *T. turgidum*. One type (Tdi8 and Tdi13) formed a highly supported monophyletic group with *T. monococcum* and *T. urartu*, and the other type (all the other sequences from *T. turgidum*) consisted of a monophyletic group with a 99% bootstrap value. *Ae. speltoides*, *T. timopheevii* and *T. turgidum* (except Tdi13 and Tdi8) compose a clade with a 89% bootstrap value. In the trees generated by the NJ and ML methods, the general topological relationships were the same as those in the tree by the PM method with slight variation at the position of *Ae. speltoides*, which was the sister group to *T. timopheevii* in the ML and NJ trees

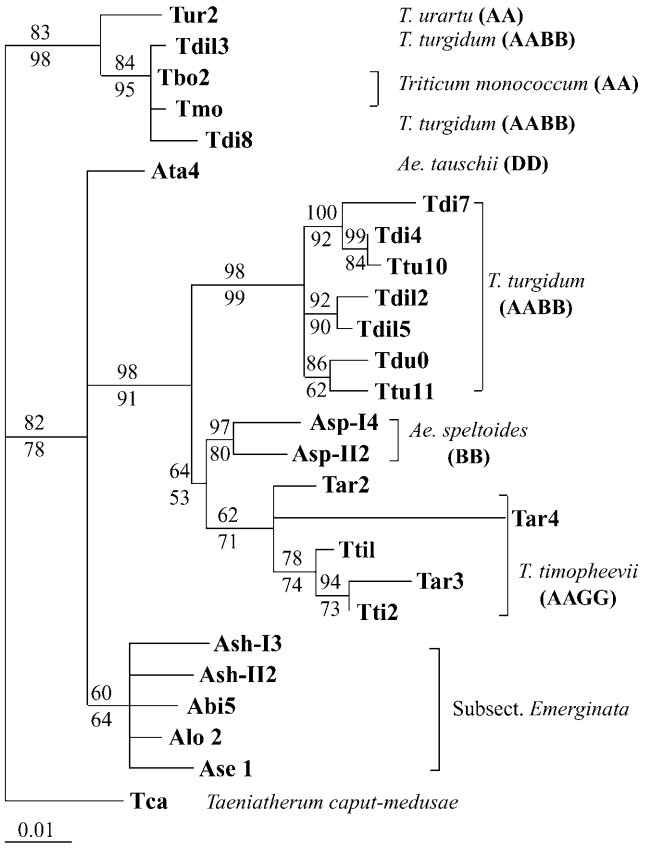

Fig. 4 The maximum-likelihood tree of 15 diploid and tetraploid taxa based on ITS1+ITS2 sequences employing TREE-PUZZLE 5.0 using the Hasegawa-Kishino-Yano model. The transition/transversion parameter is 2.13, and the model of rate heterogeneity is uniform. *T. caput-medusae* is the outgroup; log L without clock = –1,459.84; numbers above branches are percent values of 1,000-times quartet steps. *Numbers* below branches are bootstrap values of 500 replications based on the Neighbor-joining method employing MEGA with the Kimura two-parameter model; gap sites and missing information data were removed from the subset data

(Fig. 4). As with the tree of the diploid species, the position of *Ae. tauschii* has not been well-resolved.

Discussions

The relationship between subsect. *Truncata* and subsect. *Emarginata* in sect. *Sitopsis*

Aegilops sect. *Sitopsis* is divided into two subsections, *Truncata* and *Emarginata*. This systematic treatment has been supported by molecular evidence such as the RFLP pattern of nuclear DNA (Sasanum et al. 1996), repeated nucleotide sequences (Dvorák and Zhang 1992), sequence variation at single-copy loci (Dvorák et al. 1998), and chloroplast DNA (ctDNA) (Miyashita et al. 1994). In this study, the result was not consistent with the results mentioned above. The average distance for ITS1 and ITS2 between subsects. *Truncata* and *Emarginata* was more than that between *Ae. tauschii* and the species in subsect. *Emarginata*. In PM, NJ and ML trees, *Ae. speltoides* of subsect. *Trunacata* was not the sister group to the species in subsect. *Emarginata*. The latter were clustered together forming the sister group to *Ae. tauschii* in the NJ tree. The conflicting result may be due to the fact that the length of the ITS would not be sufficient to reveal the subsectional phylogenetic relationships of *Aegilops* sect. *Sitopsis*.

Origin of the A genome of *T. turgidum* ssp. *dicoccoides*

Comparing the ITS2 sequences of *Aegilops* species with that of diploid wheats, we found a 1-bp deletion at the 19th nucleotide position in the diploid wheats. This enabled us to detect whether these two kinds of ITS fragments existed in tetraploid wheats by direct-sequencing PCR products with the primer “ITS-3” (White et al. 1990). *T. turgidum* ssp. *dicoccoides* had polymorphic bands from that position on the sequencing gel, although the polymorphic bands were very weak. Two types of ITS2 sequences were cloned from *T. turgidum* ssp. *dicoccoides*, one with a 1-bp deletion at the 19th position (Td 8) and the other without the deletion (Tdi4 and Tdi7). It was also found that the sequence of one clone from *T. turgidum* ssp. *dicoccoides* (Tdi8) shared a very high similarity with that from *T. monococcum* ssp. *boeoticum* (Tbo2). The first possibility was that the DNA of *T. turgidum* ssp. *dicoccoides* might be contaminated with the DNA of *T. monococcum* ssp. *boeoticum*. Therefore, another individual of *T. turgidum* ssp. *dicoccoides* was used to repeat the experiment. Three clones (Tdi12, Tdi13 and Tdi15) were obtained and sequenced, among which Tdi13 had a 1-bp deletion at the 19th position of ITS2 and shared very high similarity with Tdi8 and Tbo2. In the phylogenetic trees (Figs. 3 and 4), Tdi8, Tdi13 (from *T. turgidum* ssp. *dicoccoides*) and *T. monococcum* composed a monophyletic group with 84% bootstrap value. It is apparent that the A genome of *T. turgidum* ssp. *dicoccoides* originated from *T. monococcum*. This result is again in conflict with the results based on α-amylase analysis (Nishikawa et al. 1992), the 5S rDNA spacer type and the RFLP analysis of repeated DNA (Dvorák et al. 1993), the RFLP of the genes coding for protein disulfide isomerase (Ciaffi et al. 2000) and the three RbcS subfamilies (Galili et al. 2000), which suggested that *T. urartu* is the donor of the A genome of *T. turgidum* ssp. *dicoccoides* and *T. timopheevii*. Considering that *T. turgidum* ssp. *dicoccoides* originated $2.3–13.5 \times 10^5$ years ago (Mori et al. 1995), it was almost impossible to explain why the distance between Tdi8 or Tdi13 from *T. turgidum* ssp. *dicoccoides* and Tbo2 from *T. monococcum* ssp. *boeoticum* was only 0.0069 and 0.0023, respectively, which were considerably lower than the lowest distance value between *Ae. speltoides* and the B genome (0.0395) (*t*-test, $p < 0.00001$) if *T. monococcum* was considered as the ancestor of the A genome of *T. turgidum*. As Vardi (1973) demonstrated that diploid wheat species could

introgress to tetraploid species by a triploid bridge, the high-sequence similarity between Tdi8 or Tdi13 from *T. turgidum* ssp. *dicoccoides* and Tbo2 from *T. monococcum* ssp. *boeoticum* could be the result of a recent introgression event.

Origin and evolution of the B and G genomes

All cloned ITS sequences from *T. turgidum* (except Tdi8 and Tdi13), *Ae. speltoides* and *T. timopheevii* form a monophyletic group with a bootstrap value greater than 89% in the phylogenetic trees (Figs. 3 and 4). When we selected the six diploid species and one of the two tetraploid wheats for phylogenetic analysis, the tetraploid species and *Ae. speltoides* always form a well-supported clade (data not shown), which suggested that *Ae. speltoides* is the most-likely donor of the B and G genomes to the tetraploid wheats among the five *Aegilops* species used in this study; or that it is most-closely related to the donor if the donor was extinct. This was consistent with the conclusions based on the RFLP analysis of repeated DNA (Dvorák and Zhang 1990), the 18S–26S ribosomal RNA gene loci (Jiang and Gill 1994a), a genome-specific DNA sequence from *Ae. speltoides* (as *Triticum speltoides*, Daud and Gustafson 1996, only for the B genome,), and a low-copy DNA sequence (Blake et al. 1999, only for the B genome). But Galili et al. (2000) and Ciaffi et al. (2000) supported the hypothesis of a polyphyletic origin of the B genome based on the RFLP analysis of three RbcS subfamilies and the protein disulfide isomerase (PDI) gene respectively. This study does not support the polyphyletic theory, because none of seven clones from *T. turgidum* in this study was in the clade with any species of subsect. *Emarginata*.

The relationship among *Ae. speltoides* and the B and G genomes was not resolved in the PM tree, i.e. the relationships of the clades containing *T. turgidum*, *T. timopheevii* and *Ae. speltoides* could not be determined (Fig. 3). These results indicate that *T. turgidum* and *T. timopheevii* might have simultaneously originated from a common ancestral tetraploid species or be derived from two hybridization events but within a very short interval time.

Concerted evolution of ITS repeats in tetraploid wheats

Concerted evolution is the tendency of the different genes in a gene family or cluster to evolve in concert. It is one of the characteristics of repeated ribosomal DNA sequences (Zimmer et al. 1980; Hillis et al. 1991). Nuclear rDNA repeats undergo rapid concerted evolution in many taxa, which enables scientists to sequence pooled PCR products of ITS directly (Baldwin et al. 1995). In the family Poaceae, ITS regions of many diploid species were sequenced with the methods mentioned above, and a high degree of identity among repeats within an individual was observed (Hsiao et al. 1995a). Wendel et al. (1995) found similar concerted evolution in tetraploid cotton. However, different types of ITS repeats were found within individuals of some species in *Bromus* (Poaceae), especially in polyploid species (Ainouche and Bayer 1997), and high polymorphism of repeats within an individual was also found in the 5S RNA genes of many diploid species of the Triticeae (Kellogg and Appels 1995) and that of cottons (Cronn et al. 1996).

Table 2 The average genetic distances of ITS1+ITS2 sequences among subsect. *Emarginata*, *Ae. speltoides*, and the B genome and G genome of tetraploid wheats based on the Kimura two-parameter model; missing and delete sites were removed

Item	*Ae. speltoides*	B genome	G genome
Subsect. *Emarginata*	0.0412	0.0468	0.0459
B genome	0.0395	0.0173[a]	0.0459
G genome	0.0339	0.0459	0.0374[b]

[a] The average genetic distances among Tdi4, Tdi7, Tdi12 and Tdi15 from two individuals of *T. turgidum* ssp. *dicoccoides*
[b] The average genetic distances among Tar2, Tar3 and Tar4 from an individual of *T. timopheevii* ssp. *araraticum*

In this study, different ITS sequences from an individual were only found in the tetraploid species. The phylogenetic relationships of tetraploid taxa based on ITS sequences suggested that polymorphism would have resulted not only from the variation between two different genomes but also from the same genome, e.g. the B or G genome. A mean distance among the four clones from the B genome of *T. turgidum* ssp. *dicoccoides* (Tdi4, Tdi7, Tdi12 and Tdi15) was only 0.0173 (Table 2), evidently less than that between *Ae. speltoieds* and the B genome, which was 0.0395 (Table 2) (*t*-test, $p < 0.00001$), showing that these repeats were not independent in evolution but were affected by a concerted force or by molecular drive.

Phylogenetic trees (Figs. 3, 4) showed that three clones (Tar2, Tar3 and Tar4) from an individual of *T. timopheevii* ssp. *araraticum* were derived from the G genome, but a mean divergence among them was 0.0374, about equal to that between *Ae. speltoides* and the G genome of *T. timopheevii* (0.0339, Table 2) (*t*-test, $p > 0.5$). Although we could not estimate the general level of homogenization of ITS repeats in tetraploid wheats based on a few clones in this study, it is most-likely that some ITS repeats in the G genome evolved almost independently and were not affected by concerted evolution.

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