Phylogenetic relationships of some wild wheat species based on the internal transcribed spacer sequences of nrDNA

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The cultivated wheat, *Triticum aestivum*, is one of the most important staple crops in the world. There has been great interest in the determination of ancestral diploid genome donors of *T. aestivum* – an allohexaploid, because of agronomic significance. Southeastern Turkey in Karacadag District of the Diyarbakir province, exhibits great genetic diversity in terms of Triticeae family plants, where wheat was originally domesticated. In order to better understand the phyletical relationships between *T. aestivum* and its possible ancestral genome donors, tissue samples were collected from 38 individuals belonging to four species of *Triticum* and two subspecies of *Aegilops speltoides*, distributed throughout the above-mentioned geographical area. The ITS1, ITS2 and 5.8S rDNA repeat segments from these plants were sequenced and the nucleotide sequences were phyletically analysed. The results postulated that *Triticum monococcum* var. *boeoticum* is likely to be an a genome donor of both, *Triticum dicoccoides* (AABB) and *Triticum aestivum* (AABBDD). The results further support *Ae. speltoides* as a possible B genome source.

Keywords: *Aegilops speltoides*, ITS1-2, molecular phylogeny, 5.8S rDNA, *Triticum aestivum*.

WHEAT is the world's most widely cultivated crop plant followed by rice and maize. It is the staple food crop for about 35% of the human population, providing about 20% of the caloric intake[1]. Many studies have been carried out to elucidate the evolutionary relationships of the wild and domesticated species belonging to the genera *Triticum* L. and *Aegilops* L.[2–4]. Due to its close relationships with *Aegilops*, the cultivated wheat *Triticum* has attracted attention for a long time.

The genus *Triticum* comprises of a series of diploid, tetraploid and hexaploid forms, the polyploids having arisen by amphiploidy between *Triticum* species and diploid species of the genus *Aegilops*[5]. The wheat group, *Aegilops–Triticum*, is a taxonomically and phylogenetically complex group[6]. The origins of A and B genomes of tetraploid and hexaploid wheat have been the subject of considerable controversy.

Earlier studies in the Poaceae have focused on morphology, anatomy, taxonomy, physiology, cytology, genetics and crop improvement. They have provided important information, but data based on these studies are not enough to assess the true relationships between these species. Phylogenetic constructions proposed for the *Triticum* and *Aegilops* species based on these characters are poorly resolved and differ widely in topology[6]. Therefore, there has been considerable disagreement on the classification and assessing phylogeny between *Triticum* and *Aegilops*.

Nuclear DNA data provide valuable information in the phylogenetic study of plants, and the internal transcribed spacer (ITS) regions of the nuclear ribosomal DNA (nrDNA) have been shown to be a valuable source of evidence to resolve phylogenetic relationships in many angiosperm groups[7–9]. ITS1 separates 18S and 5.8S, while ITS2 separates 5.8S and 26S. These regions can be readily amplified by PCR and sequenced using universal primers[10]. Because of their different rates of evolution, ITS regions have become the favoured markers in evolutionary studies at different taxonomic levels. Because the nrDNA subunits have high copy numbers, DNA isolation and sequencing are easy. In spite of their high copy numbers, rRNA genes are highly homogeneous within a genome and combined with differential rates of evolution among component subunits and spacer regions[11]. Therefore, nrDNA subunits, especially ITS regions, are ideal genomic regions for phylogenetic studies.

This article presents a molecular phylogeny of some *Triticum* and *Aegilops* species inferred from the sequence data of the entire ITS region of the nuclear rDNA.

Materials and methods

Source of materials

Thirty-eight individuals belonging to four species of *Triticum* and two subspecies of *Aegilops speltoides* representing four genomes were studied. The seed samples

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were germinated and grown in pots in a glasshouse. For herbarium specimens, materials were already available in our collection. In both cases, the selection of localities was made taking into account different ecological and climatic zones of Turkey (Table 1). The names, and genomic constitution of these materials are listed in Table 2. GenBank accession numbers of the DNA sequences are shown in Table 3.

Total DNA extraction and amplification of its region

Total genomic DNA was isolated from fresh, young leaves as described by Doyle and Doyle[12]. Total genomic DNA was visualized and estimated using UVP gel documentation tool. Double-stranded DNA of the complete ITS region (including ITS1, 5.8S and ITS2) was amplified with primers ITSL (5′-TCGTAACAAGGTTTCCGTAGGTG-3′)[13] and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′)[10]. Amplification was carried out in 100 µl reaction volume with 10 µl of 10× PCR buffer (10 mM Tris-HCl, pH 9.0, 50 mM KCl and 0.1% Triton X-100), 8 µl of 10 mM dNTP mixture, 14 µl of 25 mM $MgCl_2$ (ranging from 2.0 to 4.5 mM), 5 µl of dimethyl sulphoxide (DMSO), 2 µl of each of the primers (20 µM), 0.5 unit *Taq* DNA polymerase (Promega), and approximately 200 ng template DNA. Depending on the species, 2.0–4.5 mM MgCl2 were used. PCRs were performed on Biometra T-personal thermocycler and the amplification profile was 35 cycles of 93°C for 35 s, 49°C for 35 s, and 72°C for 0.5–2 min (depending on the product size), preceded by an initial denaturation at 95°C for 3 min, followed by a final extension at 72°C for 7 min.

DNA sequencing

Purified PCR products were sequenced using Sequenase Version 2.0 PCR Product Sequencing Kit (USB Corp.). ITS2 (R) (5′-GCTGCGTTCTTCATCGATGC-3′) and ITS3 (F) (5′-GCATCGATGAAGAACGCAGC-3′) were used as sequencing primers[10]. Radioactively labelled fragments were then visualized by autoradiography after separation in 6% polyacrylamide gel in 1× TBE buffer at 70 W (constant power).

Sequence analyses

ITS sequences were aligned with CLUSTAL W multiple sequence aligment program[14]. The phylogenetic relationships among haplotypes were reconstructed using maximum parsimony (MP) and neighbour-joining (NJ) algorithms[15] implemented in PAUP*4.0b10. ITS sequence of *Amblyopyrum muticum* from GenBank (accession number AJ301799) was used as an outgroup. The parsimony analyses were carried out with branch and bound search approach. Strict and 50% majority consensus trees were constructed from multiple, equally parsimonious trees. The hierarchical Likelihood Ratio Test (hLRT) and the Akaika Information Criterion (AIC) implemented in the computer program MODELTEST version 3.06 were used to identify the most appropriate model of DNA substitution for our data[16]. The model test was carried out both with the outgroup and without the outgroup to obtain the NJ tree with the highest bootstrap values. For the data including the outgroup, the HKY model[17] with gamma correction of 0.0151 and the TrN model[18] with the frequency of invariable sites set at 0.8239 were selected by the hLRT and the AIC estimates respectively. For the data without the outgroup, the HKY model and the TrN model with the frequency of invariable sites set at 0.7572 were selected by the hLRT and the AIC estimates respectively. For the NJ trees, the highest bootstrap values were obtained for the TrN model with the frequency of invariable sites set at 0.8239, and this tree is presented. Relative stability of NJ and MP trees was assessed with bootstrap analysis using 10,000 and 1000 replicates respectively. Bootstrapping of the MP tree was carried out using the branch and bound approach.

Results

Characteristics of its region

Thirty-eight individuals representing six taxa collected from different ecological and climatic locations of Turkey were sequenced for ITS1, ITS2 and 5.8S subunits of nrDNA. The results showed 11 haplotypes among 38 individuals sequenced according to the sequence variations (Table 1). The aligned data matrix resulted in 606 base pairs (bp). A total of 37 (6.10%) variable sites were observed and 28 of these were informative for the parsimony analyses. The length of the entire ITS region of *Triticum* species varied from 601 to 603 bp; the ITS1 region ranged from 222 to 225 bp and the ITS2 region ranged from 216 to 217 bp. The 5.8S subunit, flanked by the two ITS spacers, was the most conserved region; it was 162 bp long in all *Triticum* species. No nucleotide difference was observed among 5.8S sequences of *Triticum* species. The entire length of ITS regions of the plants from *Ae. speltoides* spp. *speltoides* and *Ae. speltoides* spp. *ligustica* ranged from 600 to 601 bp; the ITS1 and ITS2 regions were 222 and 216 bp respectively. These two regions were found to be entirely identical in all individuals of these two *Aegilops* taxa. The length of the 5.8 subunit varied between 162 and 163 bp. Unlike *Triticum* species, three nucleotide changes were found in two individuals of *Ae. speltoides* spp. *speltoides*. It was observed that only small variations in ITS fragments existed among all individuals of the six taxa, suggesting that the ITS region is relatively conserved. Small length

Table 1. Geographical and climatical data for 38 accessions

Species	Location	Haplotype designation	Latitude/ longitude	A titude (m)	Mean temperature (°C)			Mean annual rainfall (mm)	Mean humidity at 02:00 pm (%)	Soil*
					Annual	August	January			
Triticum aestivum	Baliseyh-Kirikkale	T.aestivum	39°59′N31°51′E	1300	10.3	21.8	–1.1	397.3	42	B
T. monococcum var. *boeoticum*	Beytepe-Ankara	T.mono.1	39°52′N32°44′E	900	11.2	22.3	–0.6	402.2	48.2	C
T. monococcum var. *boeoticum*	Viransehir to Botas 14 km	T.mono.1	37°20′N39°46′E	630	17.3	29.7	4.7	372	40	B
T. monococcum var. *boeoticum*	Hilvan to S. Urfa 2, 5 km	T.mono.2	37°33′N38°55′E	590	15.9	29.7	3.7	469.7	37	Rb
T. monococcum var. *boeoticum*	Karamuz Village-Diyarbakir	T.mono.3	37°36′N39°07′E	600	15.9	29.7	3.7	469.7	37	Rb
T. monococcum var. *boeoticum*	Viransehir to S. Urfa 5 km	T.mono.4	37°13′N39°39′E	600	17.3	29.7	4.3	372	40	Rb
T. monococcum var. *boeoticum*	Hilvan 15 km NW (S. Urfa)	T.mono.4	37°45′N38°46′E	575	15.9	29.7	3.7	469.7	37	Rb
T. monococcum var. *boeoticum*	Haymana-Ankara	T.mono.5	39°37′N32°40′E	1100	9.3	18.4	–2	413.6	59	Rb
T. monococcum var. *boeoticum*	Kilis to Camatli Village 200 m	T.mono.6	36°20′N37°29′E	650	17.2	29.8	3.3	349.2	42	B
T. urartu	Between Oguzeli-Ekizkoyun (Gaziantep)	T.urartu	36°52′N37°30′E	650	15.3	27.3	3.5	423.8	38	Rb
T. urartu	Caltalsu Village (Sahinbey-Gaziantep)	T.urartu	36°50′N37°23′E	700	15.8	27.1	2.7	381.1	44.5	B
T. urartu	Oguzeli to Yukari Guneyse Village 3 km	T.urartu	36°51′N37°90′E	630	15.3	27.3	2.7	381.1	44.5	R
T. urartu	Hilvan to Diyarbakir 14 km	T.urartu	37°36′N39°07′E	600	15.9	29.7	3.7	469.7	37	R
T. urartu	Karamuz Village (Diyarbakır)	T.urartu	37°46′N40°14′E	720	15.7	30.2	1.7	261.8	41.9	B
T. urartu	AgizhanVillage (Hilvan-S. Urfa)	T.urartu	37°26′N38°55′E	600	15.9	29.7	3.7	469.7	37	Rb
T. urartu	S. Urfa to Suruc 30 km	T.urartu	37°04′N38°31′E	670	18.1	31.1	5.4	305	38.3	Rb
T. dicoccoides	Elmalı Village (Sahinbey-Gaziantep)	T.dicoc.	36°52′N37°22′E	730	15.8	27.1	2.7	381.1	44.5	B
T. dicoccoides	Caltalsu Village (Şahinbey-Gaziantep)	T.dicoc.	36°5′N37°23′E	700	15.8	27.1	2.7	381.1	44.5	B
T. dicoccoides	Siverek to Diyarbakir 2 km	T.dicoc.	37°45′N39°25′E	910	17.2	29.8	3.3	349.2	42	B
T. dicoccoides	Asagikarabahce Village (Diyarbakır)	T.dicoc.	37°48′N39°44′E	1100	15.7	30.2	1.7	261.8	41.9	B
T. dicoccoides	Karamuz Village to Ovabag 8 km	T.dicoc.	37°46′N40°08′E	810	15.7	30.2	1.7	261.8	41.9	B
T. dicoccoides	Viransehir to Botas 10 km	T.dicoc.	37°20′N39°46′E	630	17.3	29.7	4.7	372	40	B
T. dicoccoides	Around Asagidilimli Village (Viranşehir-Diyarbakır)	T.dicoc.	37°17′N39°29′E	670	17.2	29.8	3.3	349.2	42	Rb
T. dicoccoides	Siverek to Diyarbakir 40 km	T.dicoc.	37°54′N39°52′E	900	17.2	29.8	3.3	349.2	42	B
Aegilops speltoides spp. *speltoides*	Nur Mount-Komurler Village 7 km	Ae.spel.1	37°09′N36°47′E	530	16.9	26.8	8.9	676	40.55	RM
Ae. speltoides spp. *speltoides*	Between Nur Mount-Sakcegoz (Gaziantep)	Ae.spel.1	37°23′N36°54′E	760	16.9	26.8	8.9	676	40.55	C
Ae. speltoides spp. *speltoides*	Suruc 1 k NE	Ae.spel.1	36°54′N38°21′E	500	18.1	31.1	5.4	305	38.3	Rb
Ae. speltoides spp. *speltoides*	Kucukakziyaret Village (S. Urfa)	Ae.spel.1	37°19′N38°48′E	650	15.9	29.7	3.7	469.7	37	Rb
Ae. speltoides spp. *speltoides*	Diyarbakir to Mardin	Ae.spel.1	37°51′N40°13′E	650	15.5	29.4	3.8	460	38	B
Ae. speltoides spp. *ligustica*	Yavuzlu to Elbeyli 2 km	Ae.spel.1	36°41′N37°18′E	567	13	27.8	2.7	381.1	44.5	B
Ae. speltoides spp. *ligustica*	Near Atesler Ciftligi (Oguzeli-Gaziantep)	Ae.spel.1	36°49′N37°25′E	680	15.3	27.3	3.5	423.8	38	B
Ae. speltoides spp. *ligustica*	Caltalsu Village (Sahinbey-Gaziantep)	Ae.spel.1	36°50′N37°27′E	700	15.8	27.1	2.7	381.1	44.5	B
Ae. speltoides spp. *ligustica*	Siverek to Diyarbakir 29 km	Ae.spel.1	37°48′N39°37′E	1090	17.2	29.8	3.3	349.2	42	B
Ae. speltoides spp. *ligustica*	Diyabakir 1 km W	Ae.spel.1	37°53′N39°58′E	850	15.7	30.2	1.7	260.3	41.9	B
Ae. speltoides spp. *ligustica*	Viransehir to S. Urfa 5 km	Ae.spel.1	37°13′N39°39′E	600	17.3	30.2	1.7	260.3	41.9	B
Ae. speltoides spp. *ligustica*	Around Karakeci (Viransehir- S. Urfa)	Ae.spel.1	37°27′N39°26′E	810	17.3	29.7	4.7	372	40	Rb
Ae. speltoides spp. *speltoides*	Kilis to Radar 1 km	Ae.spel.2	36°44′N36°06′E	850	16.8	27.7	5.5	341.8	38.3	B
Ae. speltoides spp. *speltoides*	Caltalsu Village (Sahinbey-Gaziantep)	Ae.spel.2	36°50′N37°23′E	700	15.8	27.1	2.7	381.1	44.5	B

*B, Basaltic; C, Calcereous; Rb, Red-brown; RM, Red Mediterranean.

Table 2. Species used in this study and their genomic constitutions

Species	Symbol	Genome 2*n*
Aegilops speltoides spp. *speltoides*	BB (SS)	14
Ae. speltoides spp. *ligustica*	BB (SS)	14
Triticum monococcum var. *boeoticum*	AA	14
Triticum urartu	AA	14
Triticum dicoccoides	AABB	28
Triticum aestivum	AABBDD	42

variations in the ITS1 region were detected only for the individuals of *T. monococcum* var. *boeoticum* among the individuals of the species studied. Sequence variation in the 5.8S subunit was observed only in two *Ae. speltoides* spp. *speltoides* individuals.

The G + C content of the ITS1 region ranged from 59.19 to 60% in individuals of *T. monococcum* var. *boeoticum*. This ratio was 62.16% in individuals of *T. aestivum*, *Ae. speltoides* spp. *speltoides* and *Ae. speltoides* spp. *ligustica*, 61.26% in the individuals of *T. urartu* and 61.71% in individuals of *T. dicoccoides*. The G + C content of the 5.8S subunit was entirely identical in all individuals, 58.28%; except for two *Ae. speltoides* spp. *speltoides* individuals (58.02%). The G + C content of the ITS2 region of the individuals was 60.82% in *T. aestivum* and *T. dicoccoides*, 64.35% in *T. monococcum* var. *boeoticum*, 63.42% in *T. urartu*, and 62.5% in *Ae. speltoides* spp. *speltoides* and *Ae. speltoides* spp. *ligustica* (Table 3).

Pairwise distances within groups varied from 0 to 0.05951 (Table 4). Within *Triticum*, no distance was observed among three haplotype pairs of *T. monococcum* var. *boeoticum* (T.mono.1-T.mono.4, T.mono.1-T.mono.5, and T.mono.4-T.mono.5) because they differed from each other in indels; whereas the greatest distance (0.05951) was between *T. dicoccoides* and *T. urartu*. Within *Ae. speltoides*, distance was less (0.00343). Between *Triticum* and *Ae. speltoides*, the least distance was 0.02354 (between haplotypes T.aestivum–Ae.spel.1) and the greatest distance was 0.04351 (between haplotypes T.mono.3 and Ae.spel.1).

Phylogenetic analyses

The two phylogenetic methods used (MP and NJ) displayed trees with similar topologies (Figure 1 *a* and *b*). Parsimony analysis of the 28 informative sites generated four minimal trees (36 steps, CI = 0.861). Strict consensus tree of four equally most parsimonious trees is presented in Figure 1 *a*, with bootstrap values ranging from 69 and 100%. The MP method discriminated two well-supported major clades. The first one was highly supported (97% bootstrap value) and included *T. aestivum*, *T. dicoccoides*, *Ae. speltoides* spp. *speltoides* and *Ae. speltoides* spp. *ligustica*. This main clade was further divided into two well-defined subclades. The first one was supported by the high bootstrap value (100%) and comprised two *Triticum* species, *T. aestivum* and *T. dicoccoides*. The other subclade was also higly supported (90% bootstrap value) and consisted of two *Aegilops* species, *Ae. speltoides* spp. *ligustica* and *Ae. speltoides* spp. *speltoides*. The second major clade with 98% bootstrap support included all haplotypes of *T. monococcum* var. *boeoticum* which form an unresolved polytomy (supported by relatively low bootstrap value, 69%) as well as *T. urartu* at the basal position. The NJ algorithm resulted in a tree with similar topology (Figure 1 *b*) to that of the parsimony tree. In general, the NJ tree shows the same well-supported groups, but the lineage consisting of haplotypes T.mono.1-6 plus *T. urartu* have better support (100% bootstrap value).

Discussion

The polyploids have arisen by amphiploidy between *Triticum* species and diploid species of the genus *Aegilops*. Knowledge about the role of diploid species on the evolution of polyploid wheats is important because of artificial development of synthetic forms.

The wild diploid species have diverged considerably from each other, but they are presumably monophyletic in origin. This divergence is particularly evident morphologically and ecologically. Each diploid species contains a distinct genome[19]. The related chromosomes of the different genomes show little affinity with each other, leading to irregular chromosome pairing in interspecific hybrids. Thus, diploid species show complete sterility and reproductive isolation.

Cytogenetic studies revealed that the polyploid species indicate two evolutionary lineages. *T. turgidum* (AABB), *T. aestivum* (AABBDD) and *T. aestivum* evolved by the hybridization of *T. turgidum* and *T. taushii*, whereas *T. taushii* (*Ae. squarrosa*) is a D genome donor, and *T. timopheevi* (AAGG) and *T. zhukovskyi* (AAAAGG) constitute the other evolutionary lineage. *T. zhukovskyi* evolved by the hybridization of *T. timopheevi* with an einkorn wheat[20].

Two diploid species, *T. monococcum* var. *boeoticum* and *T. urartu* were proposed as the possible A genome donors to tetraploid wheat based on C-banding pattern of chromosomes, isozyme and seed-storage protein profiles[21,22]. Studies related to the immunological properties of seed-storage proteins revealed that the the A genome in the *T. turgidum* lineage was contributed by *T. urartu* and the A genome of *T. timopheevi* was contributed by *T. monococcum* var. *boeoticum*[23]. Nishikawa[24], based on studies with esterase variations, suggested that the A genomes in both lineages were contributed by *T. urartu*. The studies based on α-amylase analysis[25], the 5S rDNA spacer type and the RFLP analysis of repeated DNA[26],

Table 3. Base compositions of ITS1, 5.8S and ITS2 regions of 11 haplotypes and their GenBank accession numbers

Species	ITS1 length	%G + C	5.8S length	%G + C	ITS2 length	%G + C	Total length	%G + C	GenBank accession no.
T.aestivum	222	62.16	162	59.25	217	62.82	601	60.89	AY450258
T.mono.1	223	59.19	162	59.25	216	64.35	601	61.06	AY450259
T.mono.2	224	59.82	162	59.25	216	64.35	602	61.29	AY450260
T.mono.3	223	59.19	162	59.25	216	64.35	601	61.06	AY450261
T.mono.4	225	59.55	162	59.25	216	64.35	603	61.19	AY450262
T.mono.5	224	59.37	162	59.25	216	64.35	602	61.13	AY450263
T.mono.6	225	60	162	59.25	216	64.35	603	61.36	AY450264
T.urartu	222	61.26	162	59.25	216	63.42	600	61.5	AY450265
T.dico	222	61.71	162	59.25	217	60.82	601	60.73	AY450266
Ae.spel.1	222	62.16	163	58.28	216	62.5	601	61.23	AY450267
Ae.spel.2	222	62.16	162	58.02	216	62.5	600	61.16	AY450268

Table 4. Tamura–Nei distance matrix of 11 haplotypes found in the present study

Haplotype	1	2	3	4	5	6	7	8	9	10	11
T.aestivum	–										
T.dico.	0.00167	–									
Ae.spel.1	0.02743	0.02934	–								
Ae.spel.2	0.02354	0.02542	0.00343	–							
T.mono.1	0.05468	0.05693	0.04142	0.03736	–						
T.mono.2	0.05167	0.05388	0.03886	0.03482	0.00170	–					
T.mono.3	0.05692	0.05921	0.04351	0.03942	0.00168	0.00338	–				
T.mono.4	0.05669	0.05898	0.04135	0.03731	0.00000	0.00168	0.00168	–			
T.mono.5	0.05672	0.05900	0.04138	0.03734	0.00000	0.00169	0.00169	0.00000	–		
T.mono.6	0.05079	0.05298	0.03642	0.03239	0.00342	0.00168	0.00514	0.00342	0.00342	–	
T.urartu	0.05726	0.05951	0.04269	0.03832	0.01044	0.01233	0.01222	0.01042	0.01042	0.01228	–

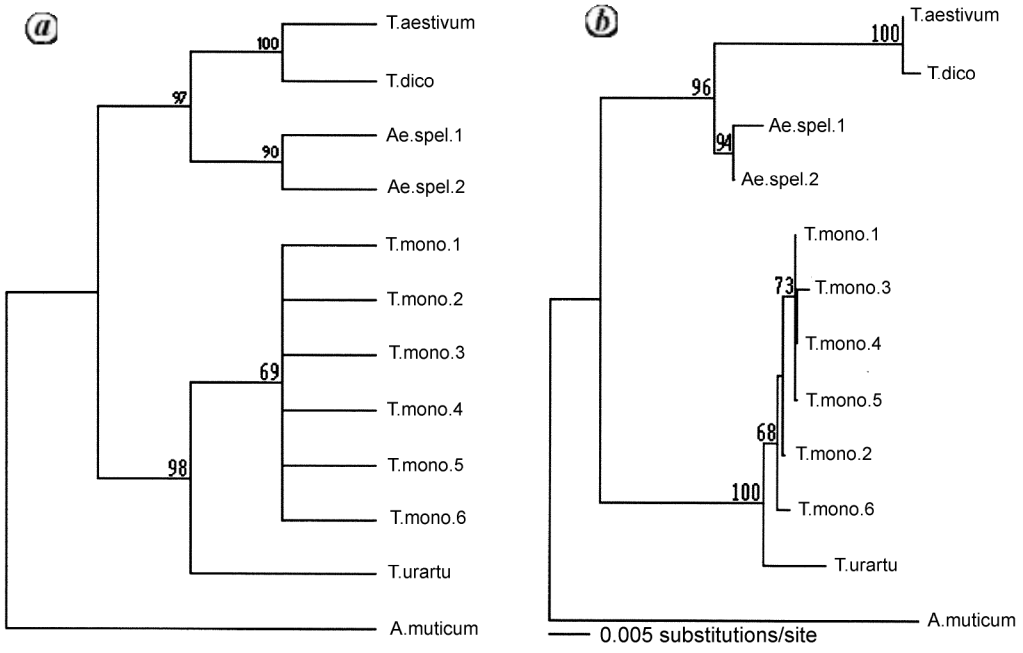

Figure 1. *a*, Strict consensus of four equally parsimonius trees (tree length = 36 steps; CI 0 = 0.861). *b*, Neighbour-joining tree using Tamura–Nei distance estimates. All bootstrap values over 60% are shown.

the RFLP of the genes coding for protein disulphide[27], the three RbcS subfamilies[28], microsatellite analysis[29] and AFLP analysis[30] concluded that the A genomes in both tetraploid wheat are more related to the A genome of *T. urartu* than to that of *T. monococcum* var. *boeoticum*. However, the results based on Tamura–Nei distance

matrix presented in this study are contrary to these reports. The results showed that haplotypes of diploid wheat *T. monococcum* var. *boeoticum* shared higher similarities with hexaploid *T. aestivum* (0.05167–0.05672) and tetraploid *T. dicoccoides* (0.05388–0.05921) compared to *T. urartu*. The genetic distance values between *T. urartu* and the other two polyploid species, *T. aestivum* (0.05726) and *T. dicoccoides* (0.05951) are higher than that of *T. monococcum* var. *boeoticum* (Table 4 and Figure 1 *a* and *b*). According to these results, *T. monococcum* var. *boeoticum* is a possible A genome donor. Zhang *et al.*[31] have also found that the A genome of *T. dicoccoides* originated from *T. monococcum* var. *boeoticum* based on the ITS2 sequences of nuclear ribosomal DNA. However, the distance values between *T. urartu* polyploids and *T. monococcum* var. *boeoticum* polyploids are not significantly different. Low distance values were found between the haplotypes of *T. monococcum* var. *boeoticum* and *T. urartu*. These values ranged from 0.01042 to 0.01233 (Table 4). Since both diploid species have the AA genome, it is not surprising that they show the lowest distance values when compared to the other species investigated.

According to Tamura–Nei distance matrix constituted from the 11 haplotypes, the lowest genetic distance value was found between *T. dicoccoides* and *T. aestivum*, i.e. 0.00167 (Table 4). *T. dicoccoides* has AABB genome and *T. aestivum* has AABBDD genome. The low genetic distance between these species indicates that they might have received A and B genomes from a common ancestor.

There are a number of published reports about the origin of the B genome in tetraploid and hexaploid wheat. However, the donor of the B genome is still a matter of debate[32]. On comparison, it was found that the distances between *Ae. speltoides* subspecies and the other diploid *Triticum* species were significantly higher than those between *Ae. speltoides* subspecies and tetraploid and hexaploid wheat. The genetic distances between the two haplotypes of *Ae. speltoides* (Ae.spel.1 and Ae.spel.2) and *T. dicoccoides* were 0.02934 and 0.02542 respectively. These were near the values for *T. aestivum*, which were 0.02743 and 0.02354 respectively. When we considered the relationship between the two haplotypes of *Ae. speltoides* and *T. urartu*, the values were 0.04269 and 0.03032 respectively, and for the six haplotypes of *T. monococcum* var. *boeoticum*, they ranged from 0.04351 to 0.03642 and from 0.03239 to 0.03942 respectively (Table 4). These results confirm that *Ae. speltoides* is related to the ancestor of the B genome. Many previous studies concerning the B genome donor of polyploid wheat strongly supported this hypothesis[29,31,33].

Low genetic variation is often observed among highly self-pollinated diploid plants such as *Triticum* species. In our study, low polymorphism has been found as expected in diploid species, *Ae. speltoides* spp. *speltoides*, *Ae. speltoides* spp. *ligustica* and *T. urartu*. On the other hand, the level of genetic diversity has been found to be higher in the other diploid species *T. monococcum* var. *boeoticum*, than the others. Although the samples used in this study were collected from different geographic and climatic localities, *T. aestivum*, *T. urartu*, *T. dicoccoides*, *Ae. speltoides* spp. *speltoides* and *Ae. speltoides* spp. *ligustica* had one or two haplotypes. On the other hand, almost all individuals of *T. urartu* sequenced had a unique ITS haplotype (Table 1). The ITS region was found to be relatively conserved as expected. There were small length variations in the ITS1 region for only the individuals of *T. monococcum* var. *boeoticum*. However, there was no length variation among the accessions of the other species studied. Sequence variation of the 5.8S subunit was observed only in two *Ae. speltoides* spp. *speltoides* individuals. This region was entirely identical in the rest of the accessions studied. Goryunova *et al.*[34] also found extremely low variability of the ITS nucleotide sequences among five diploid *Aegilops* species. These suggest a genetic interaction resulting out of pollination between *Ae. speltoides* spp. *speltoides* and *Ae. speltoides* spp. *ligustica*; except for the rarely found pure populations in the *speltoides* accessions collected from the wild, which were not genetically pure individuals and belonged to either *Ae. speltoides* spp. *speltoides* or *Ae. speltoides* spp. *ligustica*[6]. Although these species were morphologically different, they showed similarities with respect to the region studied.

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ACKNOWLEDGEMENT. This study was supported by the Institute of Biotechnology, Ankara University, Ankara, Turkey.

Received 17 September 2008; revised accepted 27 January 2009