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MOLECULAR PHYLOGENY OF *TRITICUM* AND *AEGILOPS* GENERA BASED ON ITS AND *MAT*K SEQUENCE DATA

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Abstract

Understanding the phylogenetic relationship between *Triticum* and *Aegilops* species, which form a vast gene pool of wheat, is very important for breeding new cultivated wheat varieties. In the present study, phylogenetic relationships between *Triticum* (12 samples from 4 species) and *Aegilops* (24 samples from 8 species) were investigated using sequences of the nuclear *ITS* rDNA gene and partial sequences of the *mat*K gene of chloroplast genome. The phylogenetic relationships among species were reconstructed using Maximum Likelihood method. The constructed tree based on the sequences of the nuclear component (*ITS*) displayed a close relationship between polyploid wheats and *Aegilops speltoides* species which provided new evidence for the source of the enigmatic B genome donor as *Ae. speltoides*. Concurrent clustering of *Ae. cylindrica* and *Ae. tauschii* and their close positioning to polyploid wheats pointed the source of the D genome as one of these species. As reported before, diploid *Triticum* species (i.e. *T. urartu*) were identified as the A genome donors and the positioning of these diploid wheats on the constructed tree are meaningful. The constructed tree based on the chloroplastic *mat*K sequences displayed same relationship between polyploid wheats and *Ae. speltoides* species providing evidence for the later species being the chloroplast donors for polyploid wheats. Therefore, our results supported the idea of coinheritance of nuclear and chloroplast genomes where *Ae. speltoides* was the maternal donor. For both trees the remaining *Aegilops* species produced a distinct cluster whereas with the exception of *T. urartu*, diploid *Triticum* species displayed a monophyletic structure.

Key words: *Aegilops*, *Triticum*, Phylogeny, Maximum Likelihood, *ITS*, *mat*K

Introduction

Triticeae (Poaceae) tribe with 330 to 360 (Watson & Dallwitz, 1994) species includes several valuable cereal crops such as wheat, barley, and rye. Sakamura (1918) was the first person who studied the polyploid genome structure of wheat and followed by large number of scientists who were interested in the complex genome compositions of wheat and their related genus *Aegilops* L. (Kihara, 1954; Wang *et al*., 1996). Although, earlier studies (e.g. Stebbins, 1956) proposed that *Aegilops* and *Triticum* L. species should be considered as members of the same genus, van Slageren (1994) proposed that these species belonged to two distinct genera. The genus *Triticum* comprises common or bread wheat [*Triticum aestivum* L. (genome BBAADD, *2n=6x*=42)] and other important cultivated species such as durum, emmer or einkorn wheats. Diploid, tetraploid and hexaploid chromosome numbers with a basic chromosome number n=7 are frequently seen (Sakamura, 1918; Ke *et al*., 2014). Polyploidy is derived through intergeneric hybridizations and polyploidizations resulting in allotetraploids that occurred between species of *Triticum* and *Aegilops* (Dvorak & Zhang, 1990; Caligari & Brandham, 2001; Haider, 2013). Since genus *Aegilops* has been considered as an important gene pool for *Triticum* species, *Aegilops* species attracted the attention of various researchers. Extensive cytological studies have revealed that the genomes of the *Aegilops* species can be classified into seven basic genomes: C, D, M, N, S (B), T and U (Kimber & Tsunewaki, 1988). Kihara (1954) classified these basic genomes into three groups: C-group (C and U genomes), M-group (D, M, N and T genomes) and S-group (S and its modified versions) based on chromosome pairing during meiosis of the inter-specific hybrids. In the current study, several *Aegilops* species including different genome composition such as BB, C^uC^u, $C^uC^uM^bM^b$ were especially selected to figure out positions and evolutionary relationships of these genome compositions compared with that of *Triticum* species. Origin of the B genome is still controversial (Haider, 2013). For example, various authors designate the genome of *Aegilops speltoides* Tausch as S, but not as B (Wang *et al*., 1996). Actually, S genome is used to indicate a group of *Aegilops* species such as *Ae. speltoides*, *Ae. bicornis* (Forssk.) Jaub. et Spach., *Ae. searsii* Feldman et Kislev ex Hammer contained in section *Sitopsis* (van Slageren, 1994). However in the current study, genome of *Ae. speltoides* samples was considered as B to prevent any confusion.

Phylogenetic reconstruction in the Triticeae tribe began with cladistic analyses of both morphological and anatomical characters (Baum, 1983). Molecular information, which was obtained from sequences of both genomic and chloroplast DNA regions has recently provided the basis for phylogenetic reconstruction in grasses at the subfamily and lower levels (Davis & Soreng, 1993; Liang & Hilu, 1996; Sha *et al*., 2008; Dizkirici *et al*., 2013). Different genes from nuclear and chloroplast genomes are utilized to identify the phylogenetic relationships existing between plant species (Golovnina *et al*., 2007). Ribosomal RNA genes (rDNA) are found as parts of repeat units that are arranged in tandem arrays, located at the chromosomal sites known as Nucleolar Organizing Regions (NORs). Each repeat unit consists of transcribed regions (*18S*, *5.8S* and *26S* rRNAs) and non-transcribed spacer (NTS) regions (Al-qurainy *et al.*, 2011). In the transcribed region, internal transcribed spacers (*ITS*) are found on either side of *5.8S* rRNA gene and are called as *ITS1* and *ITS2* (Sharma *et al*., 2002). Their high copy numbers (up to-30000 per cell), relatively small sizes (600-700 bp) and availability of universal

PCR (Polymerase Chain Reaction) primers for easy amplification make them ideal for phylogenetic studies (Baldwin *et al*., 1995).

Chloroplastic *Maturase*K (*mat*K) gene region (coding sequence) is one of the useful regions since it is the most rapidly evolving plastid gene, which provides sufficient information to identify the phylogenetic relationships at the intrageneric level (Young & dePamphilis, 2000). The *mat*K gene is about 1500 base pair (bp) long, located within the intron of the chloroplast gene *trn*K, and functionally might be involved in the splicing group II introns (Neuhaus & Link, 1987). One of the main reasons why this region was preferred to understand the phylogenetic relations between species is its relatively high rate of substitutions compared with other genes used in grass systematics (Liang & Hilu, 1996).

The aim of this study is to refine the phylogenetic structure of the *Triticum* and its close relative *Aegilops* genus by utilizing DNA sequences of both a chloroplast-genome specific gene (*mat*K) and a nuclear-genome specific gene (*ITS*, including the 5.8S gene of the rDNA) and therefore investigate the effects of both genomes on the phylogenetic structure of the investigated genera.

Materials and Methods

Plant materials: *Triticum* and *Aegilops* accessions were obtained from Department of Field Crops, Faculty of Agriculture, Ankara University, where an extensive field survey and sampling of the wild relatives of cultivated wheat were carried out by Özgen (1982). The materials from four *Triticum* species with 12 samples and eight *Aegilops* species with 24 samples were used in the present study. Generally, we have followed the classification system of van Slageren (1994) for the naming of the species in our study. However, ambiguities surrounding the naming of certain species were resolved by referring to the studies of others (Hammer *et al.,* 2011; Kilian *et al.,* 2011). To eliminate possible DNA contamination, each seed sample was germinated and grown *in vitro* under tissue culture conditions (Özgen *et al*., 1998). In addition to our samples, previously identified partial DNA sequence of *mat*K region and complete DNA sequence of *ITS* region of *Triticum* and *Aegilops* species were obtained from the NCBI database and included in our analyses. Species names, their genomic compositions, chromosome numbers, locations of studied materials and GenBank accession numbers are listed in Table 1.

DNA extraction, amplification of *mat*K and *ITS* regions and sequencing: Genomic DNA was extracted from fresh leaves of *in vitro* grown plants by using a modified version of Cetyl trimethylammonium bromide (CTAB) method (Doyle & Doyle, 1987). Genomic DNA was quantified via spectrophotometry and each sample was diluted to 10ng/µL for amplification of the *mat*K and *ITS* regions by PCR. Primer pair ITSL (forward: 5'TCG TAA CAA GGT TTC CGT AGG TG3') and ITS4 (reverse: 5' TCC TCC GCT TAT TGA TAT GC 3'; Hsiao *et al*., 1995) were used to amplify the *ITS* region (*ITS1+ITS2+5.8S*). An amplified product of approximately 600 bp was used for constructing the phylogenetic tree. The primers matKF1 (forward: 5'ACT GTA TCG CAC TAT GTA TCA 3') and matKR3 (reverse: 5'GAT CCG CTG TGA TAA TGA GA 3'; Li *et al*., 1997) were used to amplify approximately 1120 bp at the 5' end of the *mat*K gene. Left part of the region (about 400 bp) was not amplified since 3' end of the gene harbors less variability compared to the 5' (Hilu & Liang, 1997).

All reagents used for DNA manipulations were of molecular biology grade and obtained from Sigma-Aldrich Co. PCR amplification of *ITS* region was performed in a 50 µl volume containing 20 ng template DNA, 5 µl of 10X PCR Buffer (750mM Tris-HCL (pH 8.8), 200 mM $(NH_4)_2SO_4$, 0.1% Tween 20), 3 µl of $MgCl_2$ (25mM), 2 µl of dNTP mixture (10 mM), 2 µl of each primer (10 µM), and 0.2 µl (5u/µl) of Taq polymerase. PCR program for amplifying this region had an initial strand separation step at 97°C for 5 min followed by 30 cycles of denaturation at 94°C for 45 s, annealing at 49°C for 30 s and elongation at 72°C for 45 s and a final extension at 72°C for 10 min. For *mat*K region, amplifications were carried out in 50 µl reaction volume with 5 µl of 10xPCR buffer (750mM Tris-HCL (pH 8.8), 200 mM $(NH_4)_2SO_4$, 0.1% Tween 20), 5 µl of $MgCl_2$ (25mM), 4 µl of dNTP mixture (10mM), 4 µl of each of the primers (10mM), 0.2 µl (5u/µl) Taq DNA Polymerase, and 30 ng template DNA. The PCR program had an initial DNA strand separation step at 94°C for 3 min followed by 35 cycles of denaturation at 94°C for 50 s, annealing at 45°C for 50 s and elongation at 72°C for 60 s and a final extension at 72°C for 10 min.

Amplified products of the *ITS* and *mat*K regions of *Triticum*, and *Aegilops* samples were sequenced with an ABI 310 Genetic Analyzer (PE Applied Biosytem) Automatic Sequencer in the RefGen Biotechnology laboratories (Teknokent, METU, Ankara). To construct the phylogenetic trees, available homologous sequences were obtained from GenBank database and included in the analysis. The DNA sequences of our samples were deposited in GenBank database under the accession numbers of KJ459874-KJ459909 for *ITS* and KC608184-KC608219 for *mat*K region (Table 1).

Phylogenetic analysis: The chromatogram data were visualized by using the Finch TV (Version 1.4.0), developed by the Geopiza Research Team (Patterson *et al*., 2004-2006). The nucleotide sequences were aligned with Clustal W multiple sequence alignment program. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model (Tamura & Nei, 1993) and the phylogenetic trees were constructed by using MEGA version 5 (Tamura *et al*., 2011). One sample from genus *Hordeum* L. within the Triticeae tribe was selected as a potential out-group [for *ITS*: NCBI database accession number AF438195 from *Hordeum vulgare* ssp. *spontaneum*, and for *mat*K: NCBI database accession number HM540019 from *Hordeum marinum* ssp. *marinum*] to construct the Maximum Likelihood phylogenetic trees. Statistical support for each constructed tree was provided by bootstrapping (1000 replications). Total nucleotide length (bp), number of deletion/insertion, conserved and variable sites, and parsimony informative sites were also calculated by MEGA version 5 program (Tamura *et al*., 2011).

Table 1. Names of the plant species, their genome structures, chromosome numbers, locations and altitude informations and GenBank accession numbers.

Species	Genome Structure	Chr. Number	Location (Altitude)	GenBank Acc. No. (*ITS*)	GenBank Acc. No. (*mat*K)
Species in the current study					
Ae. biuncialis Vis.	$C^uC^uM^bM^b$	2n=28	Kahramanmaras (750 m)	KJ459880 KJ459881 KJ459882	KC608190 KC608191 KC608192
Ae. triuncialis L.	C^uC^uCC	2n=28	Ankara (1100 m)	KJ459883 KJ459884 KJ459885 KJ459886	KC608193 KC608194 KC608195 KC608196
Ae. umbellulata Zhuk.	C^uC^u	2n=14	Manisa-Kırkagaç (200 m)	KJ459887 KJ459888 KJ459889	KC608197 KC608198 KC608199
Ae. cylindrica Host	DDCC	2n=28	Ankara (1000 m)	KJ459890 KJ459891 KJ459892	KC608200 KC608201 KC608202
Ae. neglecta Req. ex Bertol.	$C^uC^uM^tM^t$	2n=28	Adana (80 m)	KJ459893 KJ459894 KJ459895	KC608203 KC608204 KC608205
Ae. speltoides Tausch	BB	2n=14	Tunceli (1100 m)	KJ459896 KJ459897	KC608206 KC608207
Ae. speltoides var. *aucheri* Boiss.	BB	2n=14	Kahramanmaras (900 m)	KJ459904 KJ459905 KJ459906	KC608214 KC608215 KC608216
Ae. speltoides var. *ligustica* (Savign.) Fiori	BB	2n=14	Corum (800 m)	KJ459907 KJ459908 KJ459909	KC608217 KC608218 KC608219
T. dicoccon Schrank	AABB	2n=28	Ankara (850 m)	KJ459898 KJ459899 KJ459900	KC608208 KC608209 KC608210
T. boeoticum Boiss.	A^bA^b	2n=14	Ankara (850 m)	KJ459874 KJ459875 KJ459876	KC608184 KC608185 KC608186
T. monococcum L.	A^mA^m	2n=14	Ankara (850 m)	KJ459877 KJ459878 KJ459879	KC608187 KC608188 KC608189
T. spelta L.	BBAADD	2n=42	Ankara (850 m)	KJ459901 KJ459902 KJ459903	KC608211 KC608212 KC608213
Accessions from the NCBI database					
T. monococcum	AA	2n=14	-	AJ301800*	-
T. urartu Thum. ex Gandil.	A^uA^u	2n=14	-	AY450265*	FJ897889*
T. aestivum L.	BBAADD	2n=42	-	DQ981410*	AF164405*
Ae. uniaristata Vis.	NN	2n=14	-	-	FJ897867*
Ae. bicornis (Forssk.) Jaub. et Sp.	S^bS^b	2n=14	-	AF149192*	FJ897863*
Ae. bicornis	S^bS^b	2n=14	-	-	FJ897853*
Ae. sharonensis Eig	$S^{sh}S^l$	2n=14	-	-	FJ897865*
Ae. comosa Sibth. et Sm.	MM	2n=14	-	AF149198*	FJ897859*
Ae. speltoides var. *ligustica*	BB	2n=14	-	-	FJ897876*
Ae. speltoides var. *ligustica*	BB	2n=14	-	-	FJ897885*
Ae. speltoides	BB	2n=14	-	-	FJ897879*
Ae. speltoides var. *speltoides*	BB	2n=14	-	AY450267*	FJ897884*
Ae. speltoides var. *speltoides*	BB	2n=14	-	-	FJ897881*
Ae. speltoides var. *speltoides*	BB	2n=14	-	-	FJ897882*
Ae. tauschii Coss.	DD	2n=14	-	FR716115*	FJ897861*
Ae. tauschii	DD	2n=14	-	-	FJ897888*
Ae. longissima Schweinf. et Muschl.	S^lS^l	2n=14	-	AF149196*	FJ897862*
Hordeum marinum ssp. *marinum* Huds.		2n=14		-	HM540019 *
Hordeum vulgare ssp. *spontaneum* (K.Koch) Thell.		2n=14		AF438195*	-

*DNA sequences of *mat*K and *ITS* regions that were obtained from the NCBI database

Table 2. Estimated molecular diversity parameters based on DNA sequences of *ITS* and *mat*K regions. Numbers of species obtained from NCBI database are indicated by asterisks and they were excluded while calculating the number of variable sites and total lengths.

	ITS			***mat*K**		
	Triticum	***Aegilops***	**Total**	***Triticum***	***Aegilops***	**Total**
Total length (bp)	602/603	602	604	1120	1120	1120
Conserved sites	578	584	568	1106	1109	1099
Variable sites	25	18	36	14	11	21
Parsimony informative sites	25	18	36	11	10	17
Number of indels	3	-	3	-	-	-
Number of sequence	12+3*	24+5*	36+8*	12+2*	24+14*	36+16*

Results

The length of the entire *ITS* region of *Triticum* species varied from 602 (*T. dicoccon* and *T. spelta*) to 603 bp (*T. monococcum*, *T. boeoticum*), while it was 602 bp for all *Aegilops* species (Table 2). The length of *ITS1* was 221-223 bp, *ITS2* was 216-217 bp and the *5.8S* subunit was 164 bp long when aligned sequences were used. No substitutions or indels were detected in the *5.8S* subunit region while almost similar number of variations were observed in *ITS1* (17 substitutions; 2 indels) and *ITS2* subunits (19 substitutions; 1 indel). The alignment of 1120 nucleotides of the *mat*K region indicated that 21 nucleotides were variable, but no insertions or deletions were identified. The lack of indels resulted in identical amplified sequence lengths (Table 2).

Although one belongs to the nuclear (*ITS*), and the other to the chloroplastic (*mat*K) component, the constructed phylogenetic trees consisted of three main clusters: Cluster 1 which is composed of polyploid wheats and *Ae. speltoides* species, whereas Cluster 2, comprised of other *Aegilops* species and finally the Cluster 3 containing the diploid wheats (Figs. 1 and 2).

ITS rDNA gene region: Presence of nine substitutions (some of which were shown in Table 3a) separated *Ae. speltoides* (BB) and its two varieties from other *Aegilops* species (Fig. 1). These species were clustered together with polyploid wheat species with a very high bootstrap value (Fig. 1) and hence designated as Cluster 1.

With the exception of *Ae. speltoides* and its two varieties, all *Aegilops* species were clustered together and this group was named as Cluster 2 due to the common C^u (U) genome among them (Fig. 1). However, *Ae. cylindrica* and *Ae. tauschii** (* denotes the DNA sequence of *Triticum* or *Aegilops* species that were obtained from the NCBI database) -D genome bearing species as well as two indigenous members of the *Sitopsis* section *Ae. bicornis** and *Ae. longissima**-S genome bearing species were also present in this cluster. *ITS* data separated the diploid *T. monococcum* (AA), *T. boeoticum* (AA), and *T. urartu** (AA) from polyploid *T. dicoccon* (BBAA), *T. aestivum** (BBAADD) and *T. spelta* [Synonym: *T. aestivum* ssp. *spelta* (L.) Thell.] (BBAADD) as Cluster 3, based on twenty-one substitutions (some of which were shown in Table 3a). With the exception of the sequence of *T. urartu**, *T. monococcum* and *T. boeoticum* formed a monophyletic group in this cluster.

***mat*K gene region:** Presence of five substitutions (Table 3b) separated *Ae. speltoides* (BB) and its two varieties from other *Aegilops* species (Fig. 2). Also, three of these substitutions (positions of 54, 100, and 564) could be the reason of grouping of *Ae. speltoides* and its two varieties with polyploid *Triticum* samples (*T. aestivum**, *T. spelta* and *T. dicoccon*) at the constructed phylogenetic tree as Cluster 1 (Fig. 2).

Except *Ae. speltoides* and its two varieties, all of the *Aegilops* accessions were clustered in Cluster 2 (Fig. 2). However, *Ae. cylindrica* and *Ae. tauschii**-D genome bearing species, *Ae. uniaristata**-N genome bearing species as well as three indigenous members of the *Sitopsis* section *Ae. bicornis**, *Ae. longissima** and *Ae. sharonensis**-S genome bearing species were also present in this cluster. Cluster 3 included three samples of *T. monococcum* (A^mA^m) and three samples of *T. boeoticum* (A^bA^b) from the current study are noted to be monophyletic with the exception of *T. urartu** (A^uA^u) (Fig. 2).

Discussion

Comparison of the gene analysis (Figs. 1 and 2) indicated that the two data sets were, with few exceptions, congruent. Although cpDNA data (*mat*K) comprised low genetic variation compared to genomic DNA data set (*ITS*), both data included enough genetic information to understand phylogenetic relationships among studied wheat species.

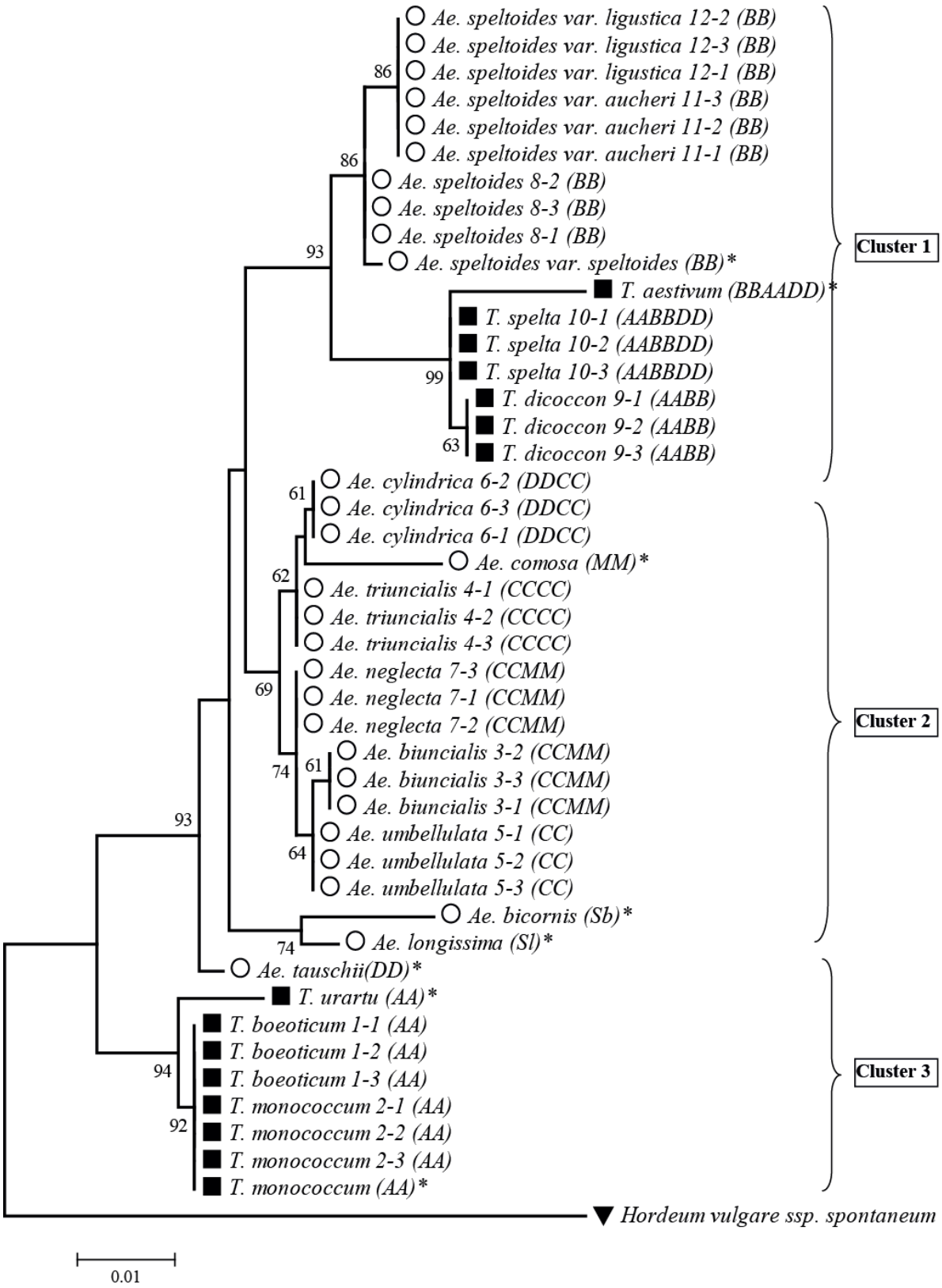

Fig. 1. Maximum Likelihood-phylogenetic tree based on the comparison of DNA sequences of *ITS* region. Genome composition of each species is shown in parenthesis. Numbers at the branch show bootstrap values. (Empty circles: *Aegilops* sp., Filled squares: *Triticum* sp., Filled triangle: *Hordeum vulgare* ssp. *spontaneum* as an out-group, Asterisks: species from which the *ITS* sequences were obtained from GenBank).

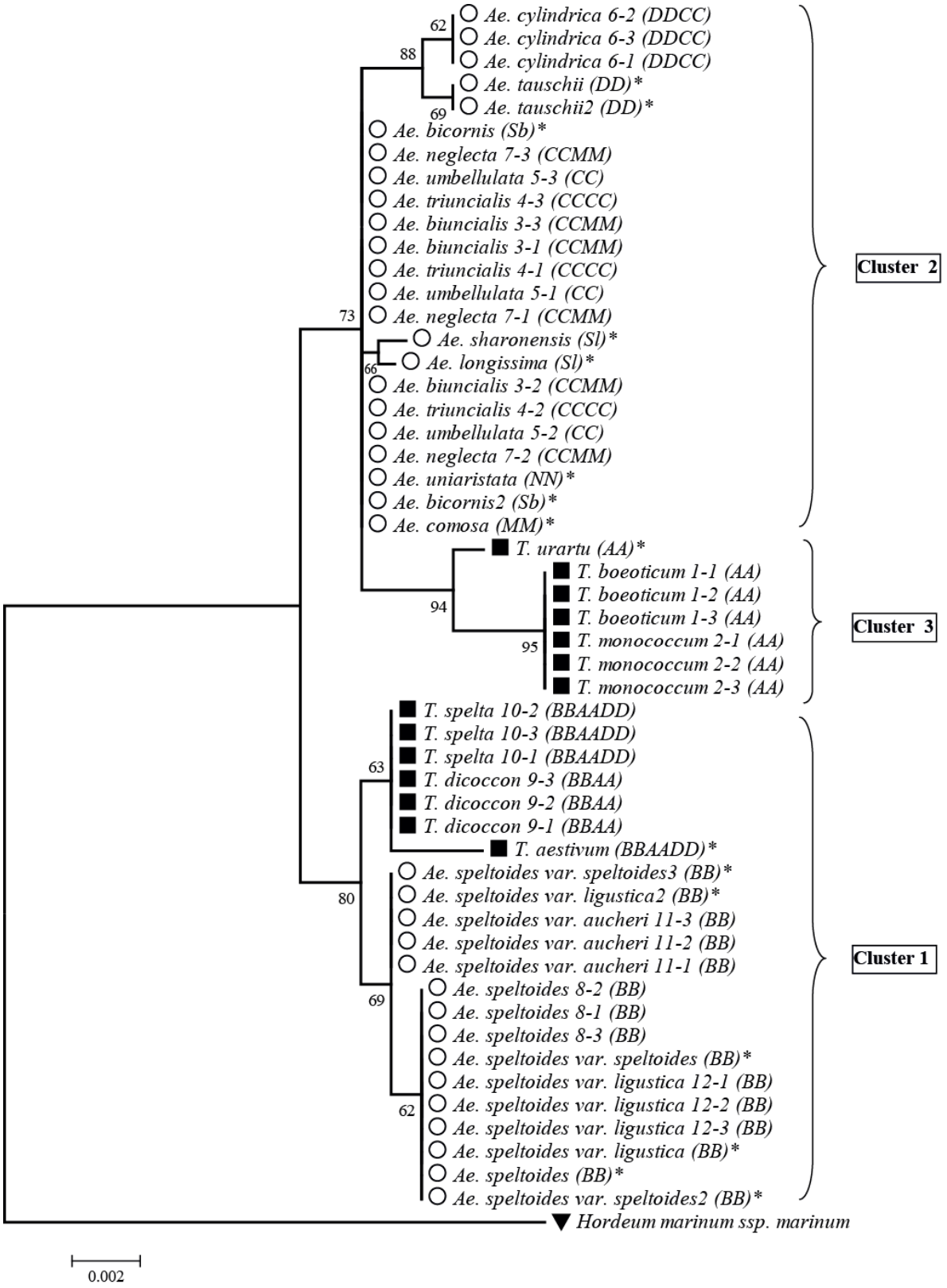

Fig. 2. Maximum Likelihood-phylogenetic tree based on the comparison of partial DNA sequences of *mat*K region. Genome composition of each species is shown in the parenthesis. Numbers at the branch show bootstrap values. (Empty circles: *Aegilops* sp., Filled squares: *Triticum* sp., Filled triangle: *Hordeum marinum* ssp. *marinum*. as an out-group, Asterisks: species from which the partial *mat*K sequences were obtained from GenBank).

Table 3a. Regions of nucleotide substitutions in DNA sequence of *ITS* rDNA region. Only one representative for each species was shown. Numbers above each column depict the positions of the corresponding nucleotide in the whole alignment. Letters in parenthesis denote the genome constitution of the corresponding species. The sequences that were obtained from GenBank database were marked with asterisks.

	26	53	58	59	78	79	101	106	113	117	125	171	173	186	188	193	202	208	219	412	419	428	455	462	478	489	536	549	558	564	566	572	573	574
	ITS1																			ITS2															
T. boeoticum 1-1 (A^bA^b)	**T**	**T**	**T**	**T**	A	T	**T**	**T**	G	T	A	C	G	G	T	T	**A**	**T**	G	-	**T**	**A**	G	G	C	**C**	**C**	A	**C**	**G**	**C**	C	A	A
T. monococcum 2-1 (A^mA^m)	**T**	**T**	**T**	**T**	A	T	**T**	**T**	G	T	A	C	G	G	T	T	**A**	**T**	G	-	**T**	**A**	G	G	C	**C**	**C**	A	**C**	**G**	**C**	C	A	A
T. monococcum (AA)*	**T**	**T**	**T**	**T**	A	T	**T**	**T**	G	T	A	C	G	G	T	T	**A**	**T**	G	-	**T**	**A**	G	G	C	**C**	**C**	A	**C**	**G**	**C**	C	A	A
T .dicoccon 9-1 (BBAA)	C	C	C	C	-	-	C	C	G	**C**	A	C	G	**T**	T	T	G	C	G	A	A	G	**T**	**A**	**T**	**C**	T	**G**	T	T	T	**T**	**T**	**G**
T. spelta 10-1 (BBAADD)	C	C	C	C	-	-	C	C	G	T	A	C	G	**T**	T	T	G	C	G	A	A	G	**T**	**A**	**T**	**C**	T	**G**	T	T	T	**T**	**T**	**G**
T. urartu (A^uA^u)*	**T**	**G**	**T**	**T**	-	-	C	**T**	G	T	A	C	G	G	T	T	**A**	**T**	G	-	**T**	**A**	G	G	C	**C**	**C**	A	**C**	**G**	T	C	A	A
T. aestivum (BBAADD)*	C	C	C	C	-	-	**A**	C	G	T	**A**	C	G	**T**	T	T	G	C	**G**	A	A	G	**T**	**A**	**T**	**C**	T	**G**	T	T	T	**T**	**T**	**G**
Ae. biuncialis 3-1 ($C^uC^uM^bM^b$)	C	C	C	C	-	-	C	C	G	T	**C**	**A**	G	G	T	C	G	C	**T**	A	**T**	G	G	G	C	T	**C**	A	**C**	T	T	C	A	**G**
Ae. triuncialis 4-1 (C^uC^uCC)	C	C	C	C	-	-	C	C	G	T	A	**A**	G	G	T	T	G	C	**T**	A	**T**	G	G	G	C	T	**C**	A	**C**	T	**C**	C	A	**G**
Ae .umbellulata 5-1 (C^uC^u)	C	C	C	C	-	-	C	C	G	T	A	**A**	G	G	T	C	G	C	**T**	A	**T**	G	G	G	C	T	**C**	A	**C**	T	T	C	A	**G**
Ae. cylindrica 6-1 (DDCC)	C	C	C	C	-	-	C	C	G	T	A	**A**	G	G	C	T	G	C	**T**	A	**T**	G	G	G	C	T	**C**	A	**C**	T	**C**	C	A	**G**
Ae. neglecta 7-1 ($C^uC^uM^tM^t$)	C	C	C	C	-	-	C	C	G	T	A	**A**	G	G	T	T	G	C	**T**	A	**T**	G	G	G	C	T	**C**	A	**C**	T	T	C	A	**G**
Ae. speltoides 8-1 (BB)	C	C	C	C	-	-	C	C	**T**	T	A	C	**A**	G	T	T	G	C	G	A	A	G	G	G	C	T	T	A	T	T	T	C	**T**	A
Ae. speltoides var. aucheri 11-1 (BB)	C	C	C	C	-	-	C	C	**T**	T	A	C	**A**	G	T	T	G	C	G	A	A	G	G	G	C	T	T	A	T	T	T	C	**T**	A
Ae. speltoides var. ligustica 12-1(BB)	C	C	C	C	-	-	C	C	**T**	T	A	C	**A**	G	T	T	G	C	G	A	A	G	G	G	C	T	T	A	T	T	T	C	**T**	A
Ae. speltoides var. speltoides (BB)*	C	C	C	**T**	-	-	C	C	**T**	T	A	C	**A**	G	T	T	G	C	G	A	A	G	G	G	C	T	T	A	T	T	T	C	**T**	A
Ae. tauschii (DD)*	C	C	C	**T**	-	-	**C**	C	**T**	T	A	C	G	G	C	C	C	**T**	**T**	A	**T**	G	G	G	C	T	**C**	A	**C**	T	T	C	A	**G**
Ae. bicornis (S^b)*	C	C	**T**	C	-	-	C	C	**T**	T	A	C	G	G	T	T	G	**T**	G	A	**T**	G	G	G	C	T	**C**	A	**C**	T	T	C	A	**G**
Ae. comosa (MM)*	C	**G**	C	C	-	-	C	C	G	T	A	**A**	G	G	T	T	G	C	**T**	A	**T**	G	G	G	C	T	**C**	A	**C**	T	Y	C	A	**G**
Ae. longissima (S^l)*	C	C	**T**	C	-	-	C	C	G	T	A	C	G	G	T	T	G	C	G	A	**T**	G	A	G	C	T	**C**	A	**C**	T	T	C	A	**G**

Table 3b. Regions of nucleotide substitutions in the partial DNA sequence of *mat*K chloroplast DNA region. Only one representative for each species was shown. Numbers above each column depict the positions of the corresponding nucleotide in the whole alignment. Letters in parenthesis denote the genome constitution of the corresponding species. The sequences that were obtained from GenBank database were marked with asterisks.

	54	100	114	282	351	359	417	423	424	537	564	608	611	636	641	750	795	927	1028	1068	1090
T. boeoticum 1-1 (A^bA^b)	**T**	**C**	T	**T**	**T**	C	**C**	**A**	G	G	T	T	T	A	**A**	**T**	**G**	C	**A**	T	A
T. monococcum 2-1 (A^mA^m)	**T**	**C**	T	**T**	**T**	C	**C**	**A**	G	G	T	T	T	A	**A**	**T**	**G**	C	**A**	T	A
T. urartu (A^uA^u)*	**T**	**C**	T	G	**T**	**T**	**C**	**A**	G	G	T	T	T	A	**A**	C	T	C	**A**	T	A
T. dicoccon 9-1 (BBAA)	C	G	T	G	A	C	C	T	G	G	**C**	T	T	A	G	C	T	**A**	C	T	A
T. spelta 10-1 (BBAADD)	C	G	T	G	A	C	C	T	G	G	**C**	T	T	A	G	C	T	**A**	C	T	A
T. aestivum (BBAADD)*	C	G	T	G	A	C	**T**	**G**	**A**	G	**C**	T	T	A	G	C	T	**A**	C	T	A
Ae. uniaristata (NN)*	**T**	**C**	T	G	A	C	C	**A**	G	G	T	T	T	A	G	C	T	C	C	T	A
Ae. umbellulata 5-1 (C^uC^u)	**T**	**C**	T	G	A	C	C	**A**	G	G	T	T	T	A	G	C	T	C	C	T	A
Ae. triuncialis 4-1 (C^uC^uCC)	**T**	**C**	T	G	A	C	C	**A**	G	G	T	T	T	A	G	C	T	C	C	T	A
Ae. neglecta 7-1 ($C^uC^uM^tM^t$)	**T**	**C**	T	G	A	C	C	**A**	G	G	T	T	T	A	G	C	T	C	C	T	A
Ae. cylindrica 6-1 (DDCC)	**T**	**C**	T	G	A	C	C	**A**	G	G	T	T	T	**G**	G	C	T	C	C	**G**	**G**
Ae. biuncialis 3-1 ($C^uC^uM^bM^b$)	**T**	**C**	T	G	A	C	C	**A**	G	G	T	T	T	A	G	C	T	C	C	T	A
Ae. tauschii (DD)*	**T**	**C**	T	G	A	C	C	**A**	G	G	T	T	**A**	**G**	G	C	T	C	C	**G**	A
Ae. tauschii (DD)*	**T**	**C**	T	G	A	C	C	**A**	G	G	T	T	**A**	**G**	G	C	T	C	C	**G**	A
Ae. speltoides 8-1 (BB)	C	G	**C**	G	A	C	C	T	G	**A**	**C**	T	T	A	G	C	T	C	C	T	A
Ae. speltoides var. *aucheri 11-1* (BB)	C	G	T	G	A	C	C	T	G	**A**	**C**	T	T	A	G	C	T	C	C	T	A
Ae. speltoides var. *ligustica 12-1* (BB)	C	G	**C**	G	A	C	C	T	G	**A**	**C**	T	T	A	G	C	T	C	C	T	A
Ae. speltoides var. *ligustica* (BB)*	C	G	**C**	G	A	C	C	T	G	**A**	**C**	T	T	A	G	C	T	C	C	T	A
Ae. speltoides var. *ligustica2* (BB)*	C	G	T	G	A	C	C	T	G	**A**	**C**	T	T	A	G	C	T	C	C	T	A
Ae. speltoides var. *speltoides* (BB)*	C	G	**C**	G	A	C	C	T	G	**A**	**C**	T	T	A	G	C	T	C	C	T	A
Ae. speltoides var. *speltoides3* (BB)*	C	G	T	G	A	C	C	T	G	**A**	**C**	T	T	A	G	C	T	C	C	T	A
Ae. speltoides var. *speltoides2* (BB) *	C	G	**C**	G	A	C	C	T	G	**A**	**C**	T	T	A	G	C	T	C	C	T	A
Ae. speltoides (BB)*	C	G	**C**	G	A	C	C	T	G	**A**	**C**	T	T	A	G	C	T	C	C	T	A
Ae. sharonensis (S^l)*	**T**	**C**	T	G	A	C	C	**A**	G	G	T	**A**	T	A	G	C	T	C	C	T	A
Ae. longissima (S^l)*	**T**	**C**	T	G	A	C	C	**A**	G	G	T	**C**	T	A	G	C	T	C	C	T	A
Ae. bicornis2 (S^b)*	**T**	**C**	T	G	A	C	C	**A**	G	G	T	T	T	A	G	C	T	C	C	T	A
Ae. bicornis (S^b)*	**T**	**C**	T	G	A	C	C	**A**	G	G	T	T	T	A	G	C	T	C	C	T	A
Ae. comosa (MM)*	**T**	**C**	T	G	A	C	C	**A**	G	G	T	T	T	A	G	C	T	C	C	T	A

***ITS* rDNA gene region:** Analyses of the 604 bp sequenced *ITS* region for the 44 samples from *Triticum* and *Aegilops* species demonstrated high rates of substitutions. Phylogenetic relationships between and within studied species were revealed by using 36 (6%) variable sites. Of the three regions of the entire *ITS*, the sequence of *5.8S* rRNA coding region, as expected, was conserved whereas *ITS1* and *ITS2* subunits had 17 and 19 variable sites, respectively**.** Hsiao *et al*. (1995) studied the phylogenetic relationships of 30 diploid species of Triticeae and found that the length of the entire *ITS* region of the studied Triticeae species varied from 596 to 605 nucleotides. Wang *et al*. (2000) studied nine diploid *Aegilops* species by using sequence of *ITS* region and they indicated the length of the region as 601-607 bp. The result obtained from present study confirmed to those of these studies. Wang and his colleagues also found the length of *ITS1* as 221-226 bp, *ITS2* as 215-217 bp and *5.8S* subunit as 164 bp in all the diploid species sequenced. Furthermore, they did not observe nucleotide substitution in *5.8S* subunit. All of these results are in line with our results. In addition to these studies Zhang *et al*. (2002) used sequence of *ITS* region to understand the origin and evolution of tetraploid wheats. They showed the presence of a 2-bp indel in *ITS1* and 1-bp indel in *ITS2* sequences. This result was exactly the same with our results; there was a 2-bp indel in *ITS1* sequences of *T. monococcum* and *T. boeoticum* (AT insertion) and a 1-bp indel (deletion) in *ITS2* sequences of the aforementioned species (Table 3a).

Within the constructed phylogenetic tree (Fig. 1), *Ae. speltoides* (BB) and its two varieties were grouped with polyploid *Triticum* species and designated as Cluster 1. This grouping, with a very high bootstrap value, indicates, *Ae. speltoides* as the enigmatic B-genome donor of polyploid wheats although the controversial nature of the source of the B-genome of polyploid wheats was reported previously (Zhang *et al*., 2002; Petersen *et al*., 2006; Dizkirici *et al*., 2013; Haider, 2013).

Due to its out-breeding nature, *Ae. speltoides* is distinguished from other *Aegilops* species by displaying a broader genetic diversity (Friebe & Gill, 1996; Giorgi *et al*., 2002). Our results also supported this observation. By using RAPD analysis, genetic distance between *Ae. speltoides* and other *Aegilops* species within the *Sitopsis* section, and their closer relations with *T. aestivum* was also reported previously (Salina *et al*., 2006).

Although placed within the same section, separation of *Ae. speltoides* from *Ae. sharonensis, Ae. longissima* and *Ae. bicornis* was also shown by using plant morphology, phytogeography, chromosome pairing and genetic marker studies (Kerby & Kuspira, 1987; Badaeva *et al*., 1996; Giorgi *et al*., 2002; Goncharov *et al*., 2009). Moreover, Giorgi *et al*. (2002) studied phylogenetic relationships among *Aegilops* species belonging to the *Sitopsis* section by using RFLP method and indicated the formation of two clusters, one containing all accessions of *Ae. speltoides* and the second containing the accessions of all other *Sitopsis* species such as *Ae. bicornis*, *Ae. longissima* and *Ae. sharonensis*.

All studied *Aegilops* accessions except for *Ae. speltoides* and its two varieties were grouped within the Cluster 2 (Fig. 1) in the phylogenetic tree and included *Ae. biuncialis* ($C^uC^uM^bM^b$), *Ae. umbellulata* (C^uC^u), *Ae. neglecta* ($C^uC^uM^tM^t$), *Ae. triuncialis* (C^uC^uCC), *Ae. cylindrica* (DDCC), *Ae. comosa* (MM), *Ae. tauschii* (DD), *Ae. longissima* (S^l) and *Ae. bicornis* (S^b). High similarity between polyploid *Aegilops* species containing the C and M genomes (*i.e.* $C^uC^uM^tM^t$ and $C^uC^uM^bM^b$) and the diploid *Ae. umbellulata* (CC) can be explained by their common C^u genome.

Close relation among accessions of *Ae. cylindrica* and *Ae. tauschii* species in Cluster 2 demonstrates that the genome of *Ae. cylindrica* is more similar to the *Ae. tauschii* genome than to the genomes of the other species studied here. This close relation is meaningful because *Ae. tauschii* is putative donor of the D genome for *Ae. cylindrica* (Bakhshi *et al*., 2010; Bordbar *et al*., 2011) and the cytoplasm of *Ae. cylindrica* was also contributed by *Ae. tauschii* (Kimber & Zhao, 1983; Tsunewaki, 1989). This relationship was also observed in our previous study (Dizkirici *et al*., 2013).

The diploid *Triticum* species; *T. monococcum, T. boeoticum*, and *T. urartu* with the genome designation of AA caused a single genomic group called Cluster 3. All genotypes of *T. monococcum* and *T. boeoticum* were clustered as a monophyletic group with a very high bootstrap value. As the A-genome donor of polyploid wheats, *T. urartu* attached to this cluster externally. Even though these three diploid wheats have the AA genome, *T. urartu* was separated from the other two species because of few nucleotide variations seen in the sequence of *ITS* as well as *mat*K regions. Same phylogenetic relation was also demonstrated by different studies (Gulbitti-Onarici *et al*., 2009; Alnaddaf *et al*., 2013).

***mat*K gene region:** Analyses of the 1120 bp sequenced *mat*K gene of chloroplast genome for the 54 samples from *Triticum* and *Aegilops* species demonstrated low rates of substitutions. However, 21 (1.9%) variable sites in the sequences, of which 17 (1.5%) were phylogenetically informative and have contributed enough characters for resolving the phylogeny between and within *Triticum* and *Aegilops* species. Some authors indicated that indels in the sequence of *mat*K gene region are informative to understand phylogenetic relationships between and within families (Johnson & Soltis, 1994; Steele & Vilgalys, 1994; Plunkett *et al*., 1996; Xiang *et al*., 1998). However, still others (Plunkett *et al*., 1997) argued against this and claimed that indels are relatively rare in *mat*K sequences, which is in line with our results.

Although being a chloroplastic marker gene, the phylogenetic tree based on the sequences of the *mat*K gene (Fig. 2) yielded a very similar clustering pattern as that of nuclear *ITS* gene marker.

In this phylogenetic tree, Cluster 1 composed of *T. aestivum, T. spelta* and *T. dicoccon* species together with *Ae. speltoides* species and its two varieties (Fig. 2). This phylogenetic pattern was also noted in previous studies (Golovnina *et al*., 2007; Dizkirici *et al*., 2013) and was not unexpected since *Ae. speltoides* species is not only the B genome donor for all polyploid wheats, but also the

plasmon donor. This means that in the course of hybridization, *Ae. speltoides* as the maternal plant, provided both nuclear genome and plasmon together (Wang *et al*., 1997). Therefore, sequence of *mat*K gene of chloroplast DNA showed a meaningful correlation with the genome composition of the species in phylogenetic analysis. Chen and his coworkers (1975) also proved the co-inheritance of nuclear and chloroplast genomes, by stating that B genome donor, *Ae. speltoides* (BB) provided the chloroplast genome as the maternal parent when tetraploid *T. dicoccon* (AABB) species was produced via possible hybridization with *T. monococcum* (AA). Subsequently, *T. dicoccon* was used as maternal parent in the cross with *Ae. tauschii* (DD), giving rise to the hexaploid wheat *T. aestivum* (BBAADD).

Except for *Ae. speltoides* and its two varieties, all other *Aegilops* accessions including the accessions from the NCBI database [(*Ae. biuncialis* ($C^uC^uM^bM^b$), *Ae. umbellulata* (C^uC^u), *Ae. neglecta* ($C^uC^uM^tM^t$), *Ae. triuncialis* (C^uC^uCC), *Ae. cylindrica* (DDCC), *Ae. uniaristata** (NN), *Ae. comosa** (MM), *Ae. tauschii** (DD), *Ae. sharonensis** (S^l), *Ae. longissima** (S^l) and *Ae. bicornis** (S^b)] were grouped within the Cluster 2 in the constructed tree (Fig. 2). Isolation of *Ae. cylindrica* and *Ae. tauschii* species from the rest of *Aegilops* species in Cluster 2 is meaningful because *Ae. tauschii* is the putative donor of the D genome for *Ae. cylindrica* (Bakhshi *et al*., 2010; Bordbar *et al*., 2011) and the cytoplasm of *Ae. cylindrica* was also contributed by *Ae. tauschii* (Kimber & Zhao, 1983; Tsunewaki, 1989). This relationship was also observed in our previous study (Dizkirici *et al*., 2013).

Three diploid species, *T. monococcum* (Gill & Kimber, 1974), *T. boeoticum* (Zhang *et al*., 2002) and *T. urartu* (Caldwell & Kasarda, 1978) were proposed as the possible A genome donors for some tetraploid wheats and constituted Cluster 3 (Fig. 2). All accessions of *T. monococcum* and *T. boeoticum*, with the exception of *T. urartu*, formed a monophyletic group with a very high bootstrap value. This result might be interpreted as *T. monococcum* being closely related to *T. boeoticum* and the chloroplast genomes of both diploid wheat species were differentiated from that of *T. urartu* (Dvorak *et al*., 1988).

Conclusion

Our study provided new evidence for clarifying the phylogeny of *Aegilops* and *Triticum* genera. The source of the controversial and enigmatic B genome in polyploid wheats was inferred as *Ae. speltoides*. In addition, *Ae. speltoides* species were also found to be the chloroplast donors for polyploid wheats. Therefore, our results supported the idea of coinheritance of nuclear and chloroplast genomes where *Ae. speltoides* was the maternal donor of the polyploid wheats.

Detailed understanding of *Triticum* and *Aegilops* phylogenetic relationships is very important to figure out the origins of wheat, estimate diversity and develop better breeding programs. By combining a large number of *Aegilops* and *Triticum* accessions, the relationship between these genera will become clearer. However, additional molecular studies with more diverse markers and species will be required to clarify the ambiguities surrounding the phylogeny of these important genera.

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References

Alnaddaf, L.M., M.Y. Moualla and N. Haider. 2013. Genetic relationships among *Aegilops* L. and *Triticum* L. species based on the internal transcribed spacer sequences of nrDNA (ITS). *Asian J. Agric. Sci.,* 5(5): 108-117.

Al-qurainy, F., S. Khan, M.A. Ali, F.M. Al-hemaid, M. Tarroum and M. Ashraf. 2011. Authentication of R*uta graveolens* and its adulterant using internal transcribed spacer (ITS) sequences of nuclear ribosomal DNA. *Pak. J. Bot.*, 43(3): 1613-1620.

Badaeva, E.D., B. Friebe and B.S. Gill. 1996. Genome differentiation in *Aegilops*. 1. Distribution of highly repetitive DNA sequences on chromosomes of diploid species. *Genome,* 39: 293-306.

Bakhshi, B., M.J. Aghaei, M.R. Bihamta, F. Darvish and E. Zarifi. 2010. Ploidy determination of *Aegilops cylindrica* host accessions of Iran by using flow cytometry and chromosome counting. *Iranian J. Bot.,* 16 (2): 258-266.

Baldwin, B.G., M.J. Sanderson, J.M. Porter, M.F. Wojciechowski, C.S. Campbell and M.J. Donoghue. 1995. The *ITS* region of nuclear ribosomal DNA: a valuable source of evidence on angiosperm phylogeny. *Ann. Missouri Bot. Gard.,* 82: 247-277.

Baum, B.R. 1983. A phylogenetic analysis of the tribe Triticeae (Poaceae) based on morphological characters of the genera. *Canad. J. Bot.,* 61 (2): 518-535.

Bordbar, F., M.R. Rahiminejad, H. Saeidi and F.R. Blattner. 2011. Phylogeny and genetic diversity of D-genome species of *Aegilops* and *Triticum* (Triticeae, Poaceae) from Iran based on microsatellites, *ITS*, and *trn*L-F. *Pl. Syst. Evol.,* 291: 117-131.

Caldwell, K. and D. Kasarda. 1978. Assessment of genomic and species relationship in *Triticum* and *Aegilops* by PAGE and differential staining of seed albumins and globulins. *Theor. Appl. Genet.,* 52: 273-28.

Caligari, P.D.S. and P.E. Brandham (Eds.) 2001. *Wheat taxonomy: the legacy of John Percival*. Linnean Special Issue 3. Linnean Society, London, pp. 190.

Chen, K., J.C. Gray and S.G. Wildman. 1975. Fraction 1 protein and the origin of polyploid wheat. *Science,* 190: 1304-1306.

Davis, J.I. and R.J. Soreng. 1993. Phylogenetic structure in the grass family (Poaceae) as inferred from chloroplast DNA restriction site variation. *Amer. J. Bot.,* 80: 1444-1454.

Dizkirici, A., C. Kansu, S. Onde, M. Birsin, M. Ozgen and Z. Kaya. 2013. Phylogenetic relationships among *Triticum* L. and *Aegilops* L. species as genome progenitors of bread wheat based on sequence diversity in *trn*T-F region of chloroplast DNA. *Genet. Resources and Crop Evol.,* 60 (8): 2227-2240.

Doyle, J.J. and J.L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.,* 19: 11-15.

Dvorak, J. and H-B. Zhang. 1990. Variation in repeated nucleotide sequences shed light on the phylogeny of wheat B and G genomes. *Proc. Natl. Acad. Sci. U.S.A.,* 87: 9640-9644.

Dvorak, J., P.E. McGuire and B. Cassidy. 1988. Apparent sources of the A genomes of wheats inferred from polymorphism in abundance and restriction fragment length of repeated nucleotide sequences. *Genome,* 30: 680-689.

Friebe, B. and B.S. Gill. 1996. Chromosome banding and genome analysis in diploid and cultivated polyploid wheats. In: Jauhar P.P. (Ed.), *Methods of genome analysis in plants*. CRC Press, Boca Raton, FL, USA, pp. 39-60.

Gill, B. and G. Kimber. 1974. Giemsa C-banding and the evolution of wheat. *Proc. Natl. Acad. Sci. U.S.A.,* 71: 4086-4090.

Giorgi, D., R. D'ovidio, O.A. Tanzarella and E. Porceddu. 2002. RFLP analysis of *Aegilops* species belonging to the Sitopsis section. *Genet. Resources Crop Evol.,* 49: 145-151.

Golovnina, K.A., S.A. Glushkov, A. G. Blinov, V.I. Mayorov, L.R. Adkison and N.P. Goncharov. 2007. Molecular phylogeny of the genus *Triticum* L. *Pl. Syst. Evol.,* 264: 195-216.

Goncharov, N.P., K.A. Golovnina and E.Y. Kondratenko. 2009. Taxonomy and molecular phylogeny of natural and artificial wheat species. *Breeding Sci.,* 59: 492-498.

Gulbitti-Onarici, S., C. Sancak, S. Sumer and S. Ozcan. 2009. Phylogenetic relationships of some wild wheat species based on the internal transcribed spacer sequences of nrDNA. *Curr. Sci.,* 96: 794-800.

Haider, N. 2013. The origin of the B-genome of bread wheat (*Triticum aestivum* L.). *Russ. J. Genet.,* 49: 263-274.

Hammer, K., A.A. Filatenko and K. Pistrick. 2011. Taxonomic remarks on *Triticum* L. and x *Triticosecale* Wittm. *Genet. Resources Crop Evol.,* 58: 3-10.

Hilu, K.W. and H. Liang. 1997. The *mat*K gene: sequence variation and application in plant systematics. *Amer. J. Bot.,* 19: 830-839.

Hsiao, C., N.J. Chatterton, K.H. Asay and K.B. Jensen. 1995. Phylogenetic relationships of the monogenomic species of the wheat tribe, Triticeae (Poaceae), inferred from nuclear rDNA (internal transcribed spacer) sequences. *Genome,* 38: 221-223.

Johnson, L.A. and D.E. Soltis. 1994. *mat*K DNA sequences and phylogenetic reconstruction in Saxifragaceae S-Str. *Syst. Bot.,* 19: 143-156.

Ke, Z, J. Qian-tao, Z. Xiao-wei, L. Xiu-jin, D. Shou-fen, W. Yu-ming, L. Andre, L. Zhen-xiang and Z. You-liang. 2014. Differences in starch composition and physiochemical properties are influenced by granule types in wheat and its relatives. *Pak. J. Bot.,* 46(6): 2239-2248.

Kerby, K. and J. Kuspira. 1987. The phylogeny of the polyploidy wheats *Triticum aestivum* (bread wheat) and *Triticum turgidum* (macaroni wheat). *Genome,* 29: 722-737.

Kihara, H. 1954. Considerations on the evolution and distribution of *Aegilops* species based on the analyser-method. *Cytologia,* 19: 336-357.

Kilian, B.K., E.M. Mammen, R. Sharma, A. Graner, F. Salamini, K. Hammer and H. Ozkan. 2011. *Aegilops*. In: *Wild crop relatives: genomic and breeding resources-Cereals*. (Ed.): Kole, C. Berlin Heidelberg: Springer Verlag, pp. 1-76.

Kimber, G. and K. Tsunewaki. 1988. Genome symbols and plasma types in the wheat group. In: Miller, T.E. and R.M.D. Koebner (Eds.), *Proceedings of the 7th International Wheat Genetics Symposium*. Cambridge, UK: Institute of Plant Science Research, Cambridge Laboratory, pp. 1209-1210.

Kimber, G. and Y.H. Zhao. 1983. The D genome of the Triticeae. *Canad. J. Genet. Cytol.,* 25: 581-58.

Li, J.H., A.L. Bogle and A.S. Klein. 1997. Interspecific relationships and genetic divergence of the disjunct genus Liquidambar (Hamamelidaceae). *Rhodora,* 99: 229-241.

Liang, H. and K.W. Hilu. 1996. Application of the *mat*K gene sequences to grass systematics. *Canad. J. Bot.,* 74: 125-134.

Neuhaus, H. and G. Link. 1987. The chloroplast $tRNA^{LYS(UUU)}$gene from mustard (*Sinapis alba*) contains a class II intron potentially coding for a maturase related polypeptide. *Curr. Genet.*, 11: 251-257.

Özgen, M. 1982. Buğday X *Aegilops* Melezlerinde Sarıpasa (*Puccinia striiformis* West.) Dayanıklılığın Kalıtımı Üzerinde Araştırmalar. PhD Thesis. Ankara: Ankara University.

Özgen, M., M. Türet, S. Altınok and C. Sancak. 1998. Efficient Callus Induction and Plant Regeneration from Mature Embryo Culture of Winter Wheat (*Triticum aestivum* L.) Genotypes. *Pl. Cell Rep.,* 18: 331-335.

Patterson, J., B. Chamberlain and D. Thayer. 2004-2006. Finch TV Version 1.4.0.

Petersen, G., O. Seberg, M. Yde and K. Berthelsen. 2006. Phylogenetic relationship of *Triticum* and *Aegilops* and evidence for the origin of the A,B, and D genomes of common wheat (*Triticum aestivum*). *Molec. Phylogen. Evol.,* 39: 70-82.

Plunkett, G.M., D.E. Soltis and P.S. Soltis. 1996. Evolutionary patterns in Apiaceae: inferences based on *mat*K sequence data. *Syst. Bot.,* 21: 477-495.

Plunkett, G.M., D.E. Soltis and P.S. Soltis. 1997. Clarification of the relationship between Apiaceae and Araliaceae based on *mat*K and *rbcL* sequence data. *Amer. J. Bot.,* 84: 565-580.

Sakamura, T. 1918. Kurze Mitteilung uber die Chromosom-enzhalen und die Verwandtshaftsverhältnisse der *Triticum* Arten. *Bot. Mag. Tokyo,* 32: 151-154.

Salina, E.A., Y.K Lim, E.D. Badaeva, A.B. Scherban, I.G. Adonina, A.A. Amosova, T.E. Samatadze, T. Y. Vatolina, S.A. Zoshchuk and A.R. Leitch. 2006. Phylogenetic reconstruction of *Aegilops* section Sitopsis and the evolution of tandem repeats in the diploids and derived wheat polyploids. *Genome,* 49: 1023-1035.

Sha, L., R. Yang, X. Fan, X. Wang and Y. Zhou. 2008. Phylogenetic analysis of *Leymus* (Poaceae: Triticeae) inferred from nuclear rDNA *ITS* sequences. *Biochem. Genet.,* 46: 605-61.

Sharma, S., S. Rustgi, H.S. Balyan and P.K. Gupta. 2002. Internal Transcribed Spacer (ITS) sequences of ribosomal DNA of wild barley and their comparison with ITS sequences in common wheat. *Barley Genetics Newsletter,* 32: 38-45.

Stebbins, G.L. 1956. Taxonomy and the evolution of genera with special reference to the family Gramineae. *Evolution,* 10: 235-245.

Steele, K.P. and R. Vilgalys. 1994. Phylogenetic analyses of Polemoniaceae using nucleotide sequences of the plastid gene *mat*K. *Syst. Bot.,* 19: 126-142.

Tamura, K. and M. Nei. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molec. Biol. Evol.,* 10: 512-526.

Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei and S. Kumar. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molec. Biol. Evol.,* 28(10): 2731-9.

Tsunewaki, K. 1989. Plasmon diversity in *Triticum* and *Aegilops* and its implication in wheat evolution. *Genome,* 31: 143-154.

van Slageren, M.W. 1994. Wild wheats: a monograph of *Aegilops* L. and *Amblyopyrum* (Jaub. & Spach) Eig. (Poaceae). ICARDA/Wageningen Agr. Univ. Pap., 94 (7): xiii-512.

Wang, G.Z., N.T. Miyashita and K. Tsunewaki. 1997. Plasmon analysis of *Triticum* (wheat) and *Aegilops*: PCR–single-strand conformational polymorphism (PCR-SSCP) analyses of organellar DNAs. *Proc. Natl. Acad. Sci. U.S.A.,* 94: 14570-14577.

Wang, G.Z., Y. Matsuoka and K. Tsunewaki. 2000. Evolutionary features of chondriome divergence in *Triticum* (wheat) and *Aegilops* shown by RLFP analysis of mitochondrial DNAs. *Theor. Appl. Genet.,* 100: 221-231.

Wang, R.R.C., R. von Bothmer, J. Dvorak, G. Fedak, I. Linde-Laursen and M. Muramatsu. 1996. Genome symbols in the Triticeae. *In*: Wang, R.R.C., K. B. Jensen and C. Jaussi (Eds.), *Proceedings of 2nd International Triticeae Symposium*. Forage and Range Research Laboratory, U.S.D.A., Logan, Utah, USA, pp. 29-34.

Watson, L. and M.J. Dallwitz. 1994. *The Grass Genera of the World*. Ed. 2. Oxon: CAB International

Xiang, Q.Y., D.E. Soltis and P.S. Soltis. 1998. Phylogenetic relationships of Cornaceae and close relatives inferred from *matK* and *rbcL* sequences. *Amer. J. Bot.,* 85: 285-297.

Young, N.D. and C.W. dePamphilis. 2000. Purifying selection detected in the plastid gene *matK* and flanking ribozyme regions within a Group II intron of nonphotosynthetic plants. *Molec. Biol. Evol.,* 17: 1933-1941.

Zhang, W., L.J. Qu, H. Gu, W. Gao, M. Liu, J. Chen and Z. Chen. 2002. Studies on the origin and evolution of tetraploid wheats based on the internal transcribed spacer (*ITS*) sequences of nuclear ribosomal DNA. *Theor. Appl. Genet.,* 104: 1099-1106.

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