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RAPD ANALYSES OF SOME WILD *TRITICUM* L. AND *AEGILOPS* L. SPECIES AND WHEAT CULTIVARS IN TURKEY

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Phylogenetic relationships among wild *Triticum* and *Aegilops* species, bread wheat (*Triticum aestivum*) cultivars, and durum wheat (*T. turgidum*) cultivars were investigated using random amplified polymorphic DNA (RAPD) technique. Fourteen RAPD primers generated 328 polymorphic bands in 22 wheat species/cultivars which have the same or different genomes. DNA fragment size ranged from 290 bp to 2570 bp. In the RAPD analysis, wild *Triticum* and *Aegilops* species clustered together and were separated from all other wheat cultivars based on their genome constitution. *T. monococcum* and *T. boeticum* were closer to *Aegilops* species than to other wheat cultivars. *T. turgidum* cultivars were genetically less diverse than *T. aestivum* cultivars. RAPD markers specific to the D and U genomes were detected. There was a weak correlation between RAPD data and pedigree records of the cultivars sharing common ancestor(s). The results suggest that RAPD analysis can be used to distinguish wild *Triticum* and *Aegilops* species, and wheat cultivars. In addition, RAPD technique can be used to develop genome-specific markers.

Key words: *Triticum*, *Aegilops*, wild wheat species, durum and bread wheat cultivars, RAPD analysis, phylogenetic relationships, genomes.

INTRODUCTION

The genera *Aegilops* L. (goatgrasses) and *Triticum* L. (wild and cultivated wheats) are taxonomically placed in the tribe Triticeae of the Poaceae. The value of wild wheat relatives as a genetic resource for wheat cultivar improvement depends on the amount of genetic variability. *Aegilops* is characterized as a Mediterranean-Western Asiatic element, and its center of diversity follows the central part of the Fertile Crescent. The genus *Aegilops* contains 22 species comprising both diploids and polyploids, which originated from the center of origin (Kimber and Feldman, 1987; van Slageren, 1994; Wang et al., 2000).

DNA-based techniques (RFLP, QTL, RAPD, AFLP, SSR, VNTR) are used to evaluate variation at the DNA sequence level (Jones et al., 1997). Random amplified polymorphic DNA (RAPD) has been commonly used for a variety of purposes, including construction of genetic linkage maps (Reiter et al., 1992), identification of cultivars (Nybom, 1994), assessment of genetic diversity in accessions, cultivars and species (Joshi and Nguyen, 1993; Cao et al., 1999; Fahima et al., 1999; Maric et al., 2004; Migdadi et al., 2004), and study of the phylogenetic relationships among species, subspecies and cultivars (Landry et al., 1994) in many plant species including wheat. These applications have also led to the development of species-specific (Chen et al., 1998), genome-specific and chromosome-specific markers (Wang et al., 1995). RAPDs have the advantage that the material is processed by an efficient and inexpensive technique without requiring prior knowledge of the genome (Bhat and Jarret, 1995). RAPD assay has the advantage of being easy to use, requiring a very small amount of genomic DNA without the need for blotting and radioactive detection (Cipriani et al., 1996; Atienzar et al., 2000), and it is moderately reproducible.

This study uses RAPD analysis to estimate the phylogenetic relationships among wild species of *Triticum* and *Aegilops*, and cultivars of *T. aestivum* and *T. turgidum* grown in Turkey.

MATERIALS AND METHODS

PLANT MATERIALS

The wild *Triticum* L. and *Aegilops* L. species and the bread (*T. aestivum* L.) and durum wheat

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TABLE 1. Ploidy level, genome constitution (Kimber and Tsunewaki, 1988; Wang et al., 1997), origin or source, or pedigree of wild wheat species and wheat cultivars used in this study

No.	Species/Cultivar	Ploidy level	Genome constitution	Origin or source in Turkey	Pedigree
1	*T. boeticum*	2X	A	36°46' N, 39°54' E (Şanlıurfa)	–
2	*T. monococcum*	2X	A	36°46' N, 39°54' E (Şanlıurfa)	–
3	*Ae. biuncialis*	4X	UM*	37°14' N, 39°00' E (Şanlıurfa)	–
4	*Ae. biuncialis*	4X	UM	38°45' N, 30°29' E (Afyonkarahisar)	–
5	*Ae. crassa*	4X	DM	36°46' N, 39°54' E (Şanlıurfa)	–
6	*Ae. crassa*	6X	DDM	36°47' N, 39°51' E (Şanlıurfa)	–
7	*Ae. cylindrica*	4X	CD	38°45' N, 30°29' E (İzmir)	–
8	*Ae. geniculata*	4X	UM	36°47' N, 39°51' E (Şanlıurfa)	–
9	*Ae. tauschii*	2X	D	36°47' N, 39°51' E (Şanlıurfa)	–
10	*Ae. triuncialis*	4X	UC	36°42' N, 38°55' E (Şanlıurfa)	–
11	*Ae. triuncialis*	4X	UC	38°27' N, 27°31' E (Afyonkarahisar)	–
12	*Ae. umbellulata*	2X	U	37°14' N, 39°01' E (Şanlıurfa)	–
13	*T. turgidum* cv. Diyarbakır-81	4X	BA	Institute in Diyarbakır	Ld-393//Beladi-116-E/Tehuacan^2/3/Cocorit-71
14	*T. turgidum* cv. Ege-88	4X	BA	Institute in İzmir	Jori-C-69/Anhinga //Flamingo
15	*T. turgidum* cv. Fırat-93	4X	BA	Institute in Diyarbakır	Snipe/3/Jori C-69/ Crane/Ganso/Anhinga
16	*T. turgidum* cv. Kunduru-1149	4X	BA	Institute in Eskişehir	Selection from landraces
17	*T. aestivum* cv. Basribey-95	6X	BAD	Institute in İzmir	Jupateco-73/(sib)Bluejay//Ures-81
18	*T. aestivum* cv. Bezostaya-1	6X	BAD	Institute in Eskişehir	Lutescens17 x Skorospelka2
19	*T. aestivum* cv. Çukurova-86	6X	BAD	Institute in Adana	Bluebird/Kalyansona
20	*T. aestivum* cv. Gerek-79	6X	BAD	Institute in Eskişehir	Mentana/Mayo-48//4-11/3/Yayla-305
21	*T. aestivum* cv. Gönen-98	6X	BAD	Institute in İzmir	II-8156-R/Mara//Bluebird
22	*T. aestivum* cv. Kaşifbey-95	6X	BAD	Institute in İzmir	Hork(Sib)/Yamhill//Kalyansona/Bluebird

* Underlining of symbols indicates substantial modification of the genome concerned.

(*T. turgidum* L.) cultivars used in this study are listed in Table 1. Twelve wild *Triticum* and *Aegilops* species were collected from their natural habitats in July 2003. *The Flora of Turkey* (Davis et al., 1985) was used for identification of wild *Triticum* and *Aegilops* species. The seeds of ten wheat cultivars were obtained from various agricultural research institutes in Turkey.

TABLE 2. Characteristics of primers and detected polymorphisms

Primer	Sequence (5'→3')	Total no. of bands	No. of polymorphic bands	Fragment size (bp) min. - max.
OPA01	CAGGCCCTTC	31	31	350 - 2280
OPA02	TGCCGAGCTG	17	17	450 - 2400
OPA07	GAAACGGGTG	14	14	350 - 1920
OPA08	GTGACGTAGG	24	24	520 - 1660
OPA09	GGGTAACGCC	25	25	470 - 1860
OPA10	GTGATCGCAG	17	17	660 - 1850
OPA11	CAATCGCCGT	39	39	350 - 2570
OPA12	TCGGCGATAG	27	27	400 - 1950
OPA14	TCTGTGCTGG	21	21	560 - 2450
OPA15	TTCCGAACCC	27	27	550 - 2300
OPA17	GACCGCTTGT	18	18	720 - 1790
OPA18	AGGTGACCGT	23	23	360 - 1830
OPA19	CAAACGTCGG	22	22	290 - 1760
OPA20	GTTGCGATCC	23	23	640 - 2320
	Total	328	328	

Ten seeds of each species/cultivar germinated in the dark for two days. The seedlings were grown in daylight for six days at room temperature and then transferred to darkness for two days. The leaf tissues were sampled at the four-leaf stage and immediately frozen in liquid nitrogen.

GENOMIC DNA EXTRACTION AND RAPD ANALYSIS

Genomic DNA was extracted using the cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle, 1990), with minor modifications. The DNA concentration was determined with a spectrophotometer (TU–1880 Double Beam UV-VIS). Polymerase chain reaction (PCR) mixtures of 25 µl contained 25 ng template DNA, 1 U Taq DNA polymerase (Sigma, St. Louis, MO), Taq buffer (10mM Tris/HCl, pH 9.0, and 50 mM KCl) 1.4 mM $MgCl_2$, 50 µm of each: dATP, dCTP, dGTP and dTTP, and 12 ng of a single 10-mer primer obtained from Operon Technologies, Inc. Alameda, USA (Tab. 2). Amplifications were performed in a DNA thermocycler (Uvigene, Uvitech Ltd., UK) programmed for 2 min at 94°C (initial denaturing step), 44 consecutive cycles each consisting of 1 min at 94°C (denaturing), 1 min at 36°C (annealing), 1 min at 72°C (extension), and followed by one cycle for 7 min at 72°C (final extension step). Amplification products were analyzed by electrophoresis on 1.5% agarose gels in 1×TBE buffer, stained with ethidium bromide, and visualized and photographed under UV light (Uvitec).

DATA ANALYSIS

The reproducibility of the selected primers was tested by repeating the PCR amplification at least twice under the same amplification conditions. RAPD data [fragment sizes of all amplification products, estimated from the gel by comparison with a standard molecular weight marker, λ DNA double-digested with *Hind*III and *Eco*RI (Sigma-Aldrich, St. Louis, MO)] were scored as discrete variables, with 1 indicating the presence and 0 the absence of a band. Amplified products were analyzed according to Lynch and Milligan (1994). A pairwise matrix of distances among species/cultivars was constructed for RAPD (14 primers) data using Jaccard's algorithm. The distance data were used to generate neighbor-joining trees after 500 replicate bootstrap tests for the wild *Triticum* and *Aegilops* species and the wheat cultivars. Neighbor-joining trees were generated from the matrix according to Saitou and Nei (1987) using the FreeTree (Pavlíček et al., 1999) and TreeView (Page, 1996) software packages.

RESULTS AND DISCUSSION

Evaluation of phylogenetic relationships is an essential element of germplasm characterization and conservation, needed in order to establish effective breeding programs. In this research, 14 primers producing polymorphisms in the preliminary primer screening against 12 wild *Triticum* and *Aegilops* species, 6 bread wheat cultivars and

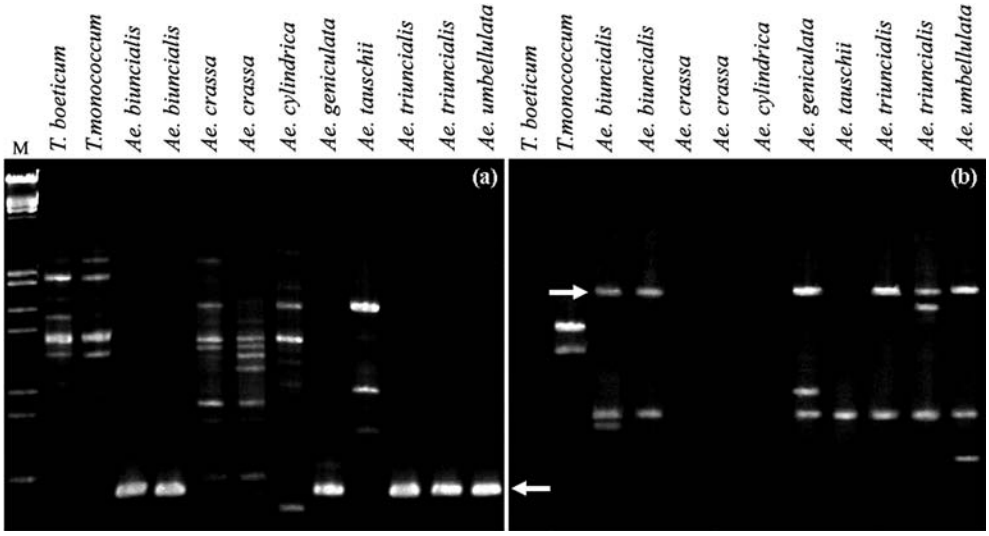

Fig. 1. U-genome-specific markers (arrow) detected in wild *Triticum* and *Aegilops* species for OPA–02 (**a**) and OPA–10 (**b**) primers. M – molecular weight marker.

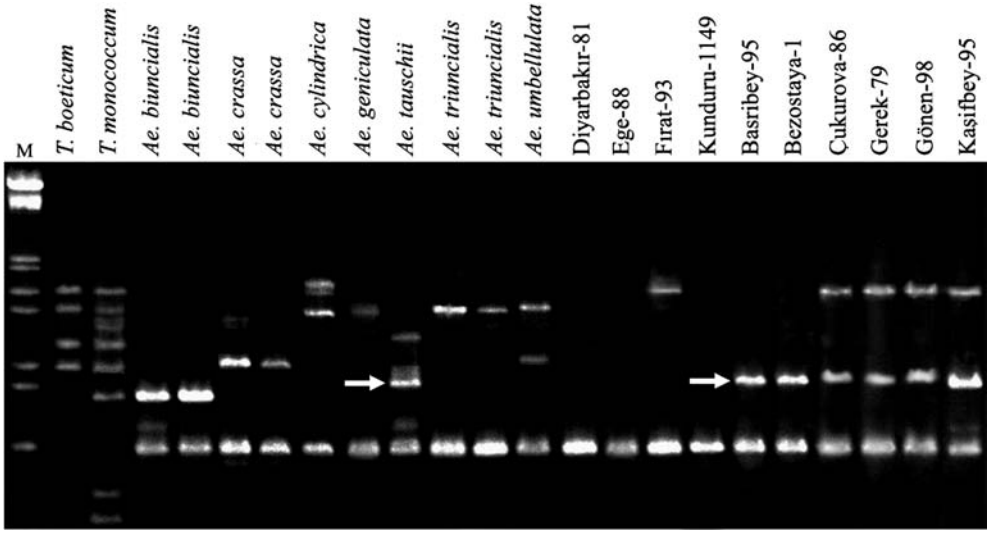

Fig. 2. D-genome-specific marker (arrow) detected in *Ae. tauschii* and bread wheat (*T. aestivum*) cultivars (Basribey–95, Bezostaya–1, Çukurova–86, Gerek–79, Gönen–98 and Kasifbey–95) for OPA–19 primer. M – molecular weight marker.

4 durum wheat cultivars were used in assessing the phylogenetic relationships (Tab. 2). The scored products were in the range of 290–2570 bp in wild *Triticum* and *Aegilops* species and wheat cultivars. The faint and non-reproducible bands were not taken into consideration. In all species/cultivars, 328 polymorphisms were detected by 14 primers, an average of 23.4 polymorphisms per primer. All of the bands were polymorphic in the wild *Triticum* and *Aegilops* species and wheat cultivars (Tab. 2). This high level of polymorphism is in accordance with several previously published results (He et al., 1992; Joshi and Nguyen, 1993; Fahima et al., 1999; Maric et al., 2004; Migdadi et al., 2004). He et al. (1992) reported that DNA polymorphism provides an opportunity for pedigree analysis and fingerprinting of developed lines as well as construction of a high-density genetic map of wheat.

In the current RAPD study, wild *Triticum* and *Aegilops* species and wheat cultivars which have ten different genomes and different ploidy levels were analyzed using RAPD technique. The U genome is common to *Ae. biuncialis* (U<u>M</u>), *Ae. geniculata* (U<u>M</u>), *Ae. triuncialis* (U<u>C</u>) and *Ae. umbellulata* (U).

U-genome-specific markers were detected for these *Aegilops* species by OPA–2 and OPA–10 primers (Fig. 1). In addition, OPA–12, OPA–17 and OPA–20 identified D-genome-specific markers for *T. aestivum* (BAD) cultivars (Basribey–95, Bezostaya–1, Çukurova–86, Gerek–79, Gönen–98, Kasifbey–95), but not for wild *Aegilops* species containing the D genome [*Ae. tauschii* (D), *Ae. crassa* 4X (DM), *Ae. crassa* 6X (DDM), *Ae. cylindrica* (CD)]. We suggest that these findings show a certain degree of within-genome-type variation. However, a D-genome-specific marker was identified for *T. aestivum* (BAD) cultivars, and for *Ae. taushii* (D) which is a putative donor of the D genome for common wheat, by primer OPA–19 (Fig. 2). Specific markers could not be identified for other genomes. As a result, reproducible U- or D-genome-specific markers were detected for wild *Aegilops* (with U genome) or bread wheat cultivars, respectively. Cao et al. (1999) reported that the presence or absence of a genome in a polyploid species of Triticeae can be simply tested by RAPD analysis with a selected primer.

In this investigation, pairwise distances among species/cultivars were computed on the basis of polymorphic bands. The genetic distance varied from 0.420 ('Firat–93' vs. 'Ege–88') to 1.000 ('Gönen–98' vs. '*T. boeticum*') (Tab. 3). The range of genetic distance within wild *Triticum* and *Aegilops* species (0.463–0.949) was higher than that of bread wheat cultivars (0.444–0.724) and durum wheat cultivars (0.420–0.618). Wild *Triticum* and *Aegilops* species are important genetic resources because of their wide range of genetic distance, as genetic diversity is important for conserving, evaluating and utilizing wild genetic resources in modern agriculture. On the other hand, the genetic distance within bread and durum wheat cultivars was found to be reduced. This reduction may be the result of breeding programs.

A phylogram was constructed based on Jaccard's distances for 22 wheat species/cultivars (Fig. 3). The species/cultivars grouped into two main clusters. The first cluster comprised three main subclusters of wild *Triticum* and *Aegilops* species including the U genome (*Ae. geniculata, Ae. umbellulata, Ae. triuncialis*, and *Ae. biuncialis*), A genome (*T. monococcum, T. boeticum*), and D genome [*Ae. cylindrica, Ae. tauschii, Ae. crassa* (4X), and *Ae. crassa* (6X)]. RAPD analysis of 112 accessions of several *Aegilops* species divided them into two major groups corresponding to the D and U genomes (Okuno et al., 1998). *Ae. cylindrica* (CD) was more closely related to polyploids *Ae. crassa* than to U genome species (Hedge et al., 2000). Thus, the RAPD results in this study indicate good resolution for distinguishing between wild *Triticum* and *Aegilops* species (except *Ae. cylindrica* and *Ae. tauschii*, because of the weak bootstrap value) based on their genome composition. The second cluster comprised two main subclusters of bread and durum wheat cultivars. Durum wheat cultivars clustered with two bread wheat cultivars (Gönen–98, Çukurova–86). Other bread wheat cultivars (Gerek–79, Basribey–95, Bezostaya–1) clustered together (Fig. 3). Our genetic diversity study in wild *Triticum* and *Aegilops* species and wheat cultivars suggests that *T. turgidum* (BA) cultivars are genetically less diverse than *T. aestivum* (BAD) cultivars. The lower degree of genetic differentiation between tetraploid durum and hexaploid wheat cultivars might be the result of genome constitution. Similar results have been found within or between different wheat cultivars (Chen et al., 1994; Cao et al., 1998; Donini et al., 2000; Martos et al., 2005), with various molecular markers. On the other hand, the numbers of amplified products in the wild *Triticum* and *Aegilops* species were higher than those of wheat cultivars. The high polymorphism found in wild gene resources of cultivated crops can be important for agricultural crop improvement/breeding purposes.

The correlations between RAPD data and pedigree records in this research were close between cv. Ege–88 and cv. Firat–93, cv. Çukurova–86 and cv. Gönen–98 (Tab. 1, Fig. 3). Cao et al. (2002) found a significant correlation between RAPD markers and 29 wheat cultivars pedigrees. In contrast, although cv. Kasifbey–95 has common ancestor(s) with cv. Çukurova–86 and cv. Gönen–98, this correlation was not clearly identified by RAPD analysis as it was weakly supported by bootstrap. In this regard, RAPD may be insufficient to confirm this relationship in bread wheat cultivars because of the complexity of their genomes or deficiencies in the technique employed. Maric et al. (2004) reported a weak correlation between RAPD data and pedigree records for bread wheat cultivars. Some researchers have successfully shown a strong correlation between pedigrees and molecular markers using amplified fragment length polymorphism technique in wheat cultivars (Incirli and Akkaya, 2001; Tyrka, 2002).

Based on the phylogenetic tree, *T. monococcum* (A) and *T. boeticum* (A) are closer to *Aegilops* species (where the A genome is not found) than to durum (BA) and bread (BAD) wheat cultivars (Fig. 3). A similar result was reported for *T. monococcum* by Wang et al. (1997) using PCR-single-strand conformational polymorphism analysis. It is suggested that *T. monococcum* and *T. boeticum* are not the donor of the A genome of polyploid wheats like *T. turgidum* and *T. aestivum* (Dvorák, 1976; Chapman et al. 1976). On the basis of the pairing behavior of the different chromosome sets and analysis of the polymorphisms of repeated nucleotide sequences, the donor of the A genome of

TABLE 3. Matrix of Jaccard's distances amongst the pairs of wild *Triticum* and *Aegilops* species, durum and bread wheat cultivars grown in Turkey

	Species/Cultivar*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	*T. boeticum*	0.000																					
2	*T. monococcum*	0.778	0.000																				
3	*Ae. biuncialis*	0.916	0.835	0.000																			
4	*Ae. biuncialis*	0.901	0.902	0.463	0.000																		
5	*Ae. crassa*	0.914	0.912	0.941	0.901	0.000																	
6	*Ae. crassa*	0.944	0.923	0.928	0.940	0.707	0.000																
7	*Ae. cylindrical*	0.906	0.943	0.909	0.949	0.906	0.887	0.000															
8	*Ae. geniculata*	0.880	0.908	0.819	0.788	0.923	0.908	0.836	0.000														
9	*Ae. tauschii*	0.946	0.914	0.864	0.890	0.836	0.915	0.892	0.911	0.000													
10	*Ae. triuncialis*	0.901	0.872	0.643	0.758	0.876	0.888	0.894	0.744	0.879	0.000												
11	*Ae. triuncialis*	0.961	0.926	0.760	0.757	0.903	0.836	0.859	0.701	0.875	0.645	0.000											
12	*Ae. umbellulata*	0.892	0.861	0.835	0.750	0.905	0.904	0.925	0.734	0.907	0.711	0.716	0.000										
13	Diyarbakır-81	0.968	0.940	0.887	0.886	0.934	0.915	0.907	0.942	0.919	0.915	0.885	0.934	0.000									
14	Ege-88	0.958	0.923	0.901	0.872	0.929	0.928	0.905	0.908	0.945	0.900	0.886	0.904	0.578	0.000								
15	Fırat-93	0.947	0.916	0.854	0.838	0.933	0.932	0.912	0.900	0.921	0.882	0.893	0.897	0.532	0.420	0.000							
16	Kunduru-1149	0.959	0.925	0.877	0.861	0.945	0.930	0.924	0.951	0.947	0.914	0.889	0.931	0.500	0.618	0.554	0.000						
17	Basribey-95	0.957	0.944	0.897	0.910	0.941	0.908	0.881	0.889	0.879	0.897	0.864	0.886	0.614	0.660	0.618	0.696	0.000					
18	Bezostaya-1	0.974	0.927	0.907	0.919	0.933	0.903	0.896	0.927	0.921	0.882	0.847	0.921	0.620	0.705	0.623	0.579	0.510	0.000				
19	Çukurova-86	0.986	0.945	0.926	0.897	0.942	0.909	0.902	0.920	0.944	0.910	0.899	0.953	0.524	0.615	0.519	0.549	0.647	0.547	0.000			
20	Gerek-79	0.934	0.894	0.895	0.881	0.920	0.904	0.897	0.901	0.893	0.871	0.880	0.922	0.627	0.667	0.509	0.719	0.574	0.559	0.607	0.000		
21	Gönen-98	1.000	0.957	0.899	0.897	0.972	0.957	0.902	0.905	0.929	0.934	0.899	0.940	0.591	0.714	0.625	0.655	0.620	0.600	0.444	0.582	0.000	
22	Kaşifbey-95	0.945	0.913	0.954	0.941	0.900	0.882	0.924	0.951	0.932	0.914	0.889	0.955	0.680	0.593	0.627	0.721	0.648	0.672	0.577	0.656	0.724	0.000

**T. turgidum* and *T. aestivum* cultivars ranged from 13 to 16 and 17 to 22, respectively.

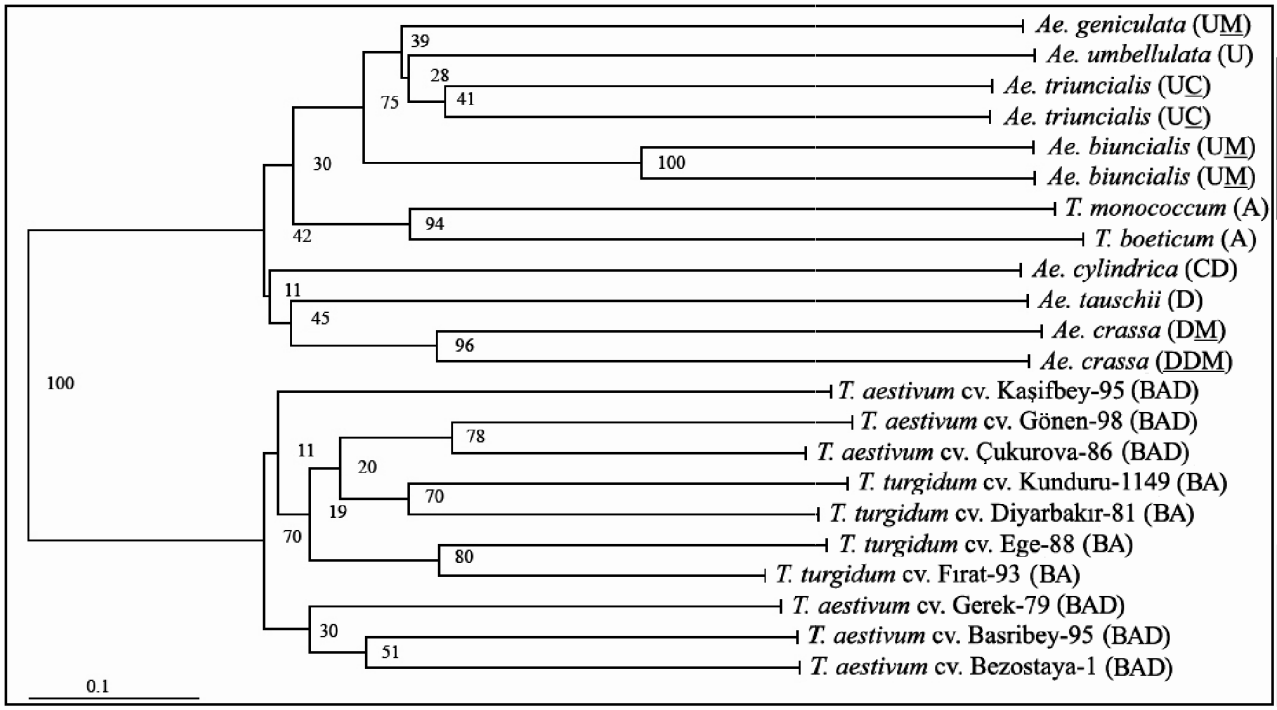

Fig. 3. Cluster analysis of RAPD data of 12 wild *Triticum* and *Aegilops* species, 4 durum wheat cultivars and 6 bread wheat cultivars. Neighbor-joining tree by RAPD method is given as phylogram. Numbers at the forks are the bootstrap percentage values. Scale at bottom left corner of the diagram is the distance scale based on the values in Table 3.

polyploid wheats has been established as *Triticum urartu* (A) (Dvorák, 1976; Chapman et al., 1976; Dvorák et al., 1988; 1993).

Effective conservation of plant gene resources and determination of their phylogenetic relationships are essential for plant breeding. Our results demonstrated that RAPD markers can be of great value in gene bank management for the purposes of identification, measurement of variation, and establishment of genetic distance at inter- and intra-specific levels.

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