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Genetic Relationship of Wild Einkorn Based on Geographical Distribution in Anatolia and Thrace using AFLP Markers

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ABSTRACT

Triticu m monococcum L. ssp *boe oticum* Boiss., is the wild progenitor of domesticated einkorn. High throughput AFLP genetic analysis showed that the domestication of einkorn started in the northern part of the Fertile Crescent, near the Karacadag Mountains, Southeastern Turkey [1]. This study assesses the genetic distribution and the diversity of wild einkorn throughout Turkey, using total of 59 accessions from 22 locations in four different geographical regions. In our study, the four selective combinations of AFLP markers (E+ACC/M+ACT, E+ACC/M+ATA, E+ACT/M+ATA, and E+ATC/M+AAG) resulted in 161 AFLP marker loci. Phylogenetic trees for individual accessions and populations based on geographical regions were obtained using 'PopGen-32' population genetic analysis software. East and Southeast samples were genetically closest to each other among the samples from other regions. The samples from West, Northwest, and Central Anatolia were clustered together.

Key Words: *T. boeoticum*, wild **einkorn**, Turkey, geographical distribution, AFLP, genetic diversity

INTRODUCTION

Genetic improvement of crop plants started with the emergence of agriculture 10,000 years ago. These crops evolved from the wild and cultivated crops by chance, natural selection, or by arrival of new types from distant landscapes [2]. Various supports exist that wheat first grew in Mesopotamia and in the Tigris and Euphrates River valleys in the Middle East nearly 10,000 years ago [3]. Harlan [4] reported in 1981, that Southeast Turkey is the native home of wild einkorn, suggesting that this species might have been domesticated in South Anatolia and then spread into Europe as an agricultural crop [2, 4, 5]. Finally, a large scale genetic analysis revealed that the cultivated form *T. boeoticum*, einkorn (2n=14), originated from the Southeast part of modern Turkey [1]. *T. bo eoticum* still exists in ample amounts in Turkey. In another study, AFLP analysis of a collection of tetraploid wheats indicated that the origin of emmer and the domestication site of hard wheat are also the Southeast Turkey [6].

Amplified fragment-length polymorphism (AFLP) is PCR-based fingerprinting technology [7], in its most basic form; AFLP involves the restriction of genomic DNA, followed by ligation of adaptors complimentary to the restriction sites and selective PCR amplification of a subset of the Amplified fragment-length polymorphism (AFLP) is PCR-based fingerprinting technology [7], in its most basic form; AFLP involves the restriction of genomic DNA, followed by ligation of adaptors complimentary to the restriction sites and selective PCR amplification of a subset of the adapted restriction fragments.

These fragments are visualized on denaturing polyacrylamide gels either through autoradiography or fluorescence methodologies. AFLP technique is abundantly used in genetic diversity studies in plants [1, 6, 8-18].

MATERIALS AND METHODS

Plant materials

The seeds from different regions of Turkey of wild einkorn (*Triticum mono coccum* L. ssp. *boeoticum*) accessions (Table 1) were obtained from Dr. Jan Valkoun, Head, Genetic Resources Unit of ICARDA (http://www.icarda.org/GeneBank.htm).

DNA isolation

DNA samples from each of the accession were isolated from the seedlings grown dark for 10-15 days using 'Qiagen DNeasy Plant Mini Kit' according to instructions of the manufacturers.

AFLP

A single seed from each accession was germinated and the AFLP was performed on this single plant DNA representing each accession. The protocol was based on technique developed by Zabaeu and coworkers [7, 19]. AFLP marker production conditions were the same as previously reported [20]. In selective amplification reactions, four primer sets (E+ACC / M+ACT, E+ACC / M+ATA, E+ACT /
M+ATA, and E+ATC / M+AAG) were labeled with 3000mCi/mmole [γ^{33}P]-ATP (Institute of Isotopes Co., Ltd., Hungary).

Cluster analysis

PopGen-32 software [21] was used for genetic relationship analysis. DNA samples of 59 accessions *Triticum monococcum* ssp. *boeoticum* from different regions were grouped. Scored data were used as input as dominant and diploid data. Homogeneity test, genetic distance, dendrogram, F statistics, Shannon index, gene flow, neutrality test, polymorphic loci, gene frequency, allele number, gene diversity, and effective allele number were applied under Hardy-Weinberg equilibrium [22, 23].

Principal Co-ordinates Analysis (PCoA) was performed using Syntax multivariate data analysis (version 5.1) software [24, 25]. Genetic distance matrixes for both individuals and populations (Nei's unbiased measures of genetic distance) [26] obtained from 'PopGen-32' were modified and used as input for Syntax program to obtain PCoA of individuals and populations.

Table 1. *T. boe oticum* accessions from different locations. (IG and crop numbers are as specified by ICARDA).

(IG) No.	Crop No.	Sample No.	Longitude	Latitude (m)	Altitude (m)	Location
44872	300063	1	E27 31	N41 36	300	Kırklareli
44873	300064	2	E27 17	N41 50	560	Kırklareli
44871	300062	3	E27 36	N41 35	200	Kırklareli
44870	300061	5	E28 02	N41 08	100	Tekirdağ
44860	300051	6	E26 42	N40 20	30	Çanakkale
44863	300054	10	E27 35	N39 38	250	Balıkesir
44864	300055	11	E28 01	N40 03	60	Balıkesir
44867	300058	12	E29 06	N40 20	250	Bursa
44869	300060	13	E29 35	N40 20	625	Bursa
44866	300057	14	E29 06	N40 20	200	Bursa
44868	300059	15	E29 35	N40 20	480	Bursa
44853	300044	17	E26 58	N38 48	15	İzmir
44876	300067	19	E32 27	N40 05	850	Ankara
44879	300070	20	E33 32	N39 32	700	Ankara
44877	300068	22	E32 35	N40 00	580	Ankara
44878	300069	23	E32 28	N39 27	850	Ankara
44880	300071	24	E32 50	N37 49	650	Konya
44881	300072	25	E33 03	N37 17	700	Konya
44815	300006	26	E33 43	N39 22	1,020	Kırşehir
44816	300007	28	E35 59	N38 37	1,200	Kayseri
44819	300010	30	E36 30	N38 48	1,510	Kayseri
44822	300013	31	E36 47	N38 51	1,770	Kayseri
44883	300074	32	E36 02	N38 32	1,150	Kayseri
44884	300075	33	E36 15	N38 40	1,130	Kayseri
44885	300076	34	E36 25	N38 29	1,130	Kayseri
44886	300077	35	E35 36	N38 58	800	Kayseri
44887	300078	37	E37 15	N39 49	970	Sivas
44888	300079	38	E37 30	N39 10	1,590	Sivas
116139	300194	40	E37 31 06	N36 52 51	635	Gaziantep
116136	300191	41	E37 28 31	N36 45 07	530	Gaziantep
116133	300188	42	E37 09	N36 51	640	Gaziantep
116150	300204	46	E37 28 02	N37 19 22	750	Gaziantep
116147	300201	47	E37 14 45	N37 1651	910	Gaziantep
116148	300202	48	E37 19 59	N37 15 11	830	Gaziantep
116153	500624	49	E37 11 57	N36 53 02	840	Gaziantep
116140	300195	50	E37 35 30	N36 49 44	700	Gaziantep
116163	500633	51	E36 57 00	N36 5202	700	Gaziantep
116151	500622	52	E37 11 08	N36 58 06	1,035	Gaziantep
116154	500625	53	E37 13 02	N36 48 19	755	Gaziantep
44897	300088	54	E39 01	N36 52	600	Şanlıurfa
44898	300089	55	E39 00	N36 50	600	Şanlıurfa
44944	300135	56	E39 50	N37 45	1,000	Şanlıurfa
44892	300083	57	E38 49	N37 11	660	Şanlıurfa
46100	600611	58	E37 57	N37 12	615	Şanlıurfa
46152	600663	59	E39 33	N37 40	1,100	Şanlıurfa
44823	300014	60	E37 35	N38 23	1,780	K.Maraş
44850	300041	61	E39 48	N38 19	890	Diyarbakır
44908	300099	62	E38 13	N38 34	675	Malatya
44933	300124	63	E38 13	N38 27	650	Malatya
44825	300016	64	E39 04	N38 37	1,160	Elazığ
44826	300017	65	E39 33	E38 30	1,310	Elazığ
44889	300080	66	E38 40	N38 49	1,100	Elazığ
44890	300081	67	E39 33	N38 30	1,270	Elazığ
44906	300097	68	E39 28	N38 57	850	Tunceli
44907	300098	69	E43 30	N38 33	2,100	Van
44909	300100	70	E44 28	N37 14	1,125	Hakkari

RESULTS AND DISCUSSION

The total bands of 321 were recorded of which 161 were corresponding to polymorphic loci when 4 different selective amplification primer sets were used in this study. Only the presence and the absence of AFLP bands were considered for scoring. The tree obtained with the individual samples of 59 is presented in Table 1. The clustering of individual accessions showed two distinct groups as west (Thrace, Marmara region and Central Anatolia) and east (Southeast and East), I and II, respectively (Figure 1). Single accessions from Tunceli and Kayseri appeared as out-group samples. Group I divided into two major arms I-A and I-B. The upper clade of I-A is all composed of the accessions from Kirklareli of Thrace. In the second arm of 1-A, accessions from the same cities in the Central Anatolia were all grouped as sub-clades of their own. The samples from Tekirdag/Canakkale are being located in Thrace and samples from Bursa/Balikesir from the southwest of Marmara Sea also clustered in this group. Konya and Kirsehir being in the south of the Central Anatolia clustered separately within the group. An accession from Izmir, far west of Turkey, (sample 17, Table 1) is clustered most distantly from all the other ones. As a result, all the accessions fell into subgroups with relative distances to each other almost with perfect colorations to geographical locations. The second arm of Group I, I-B, composed of accessions from the south and east of Central Anatolia, except an accession from Van at the east border (collected from the southwest of Lake Van) and from Cankiri, north of Ankara. The fact that sample from Van clustered within this branch may be due to the location of Van, which is not part of the "Fertile Crescent". It is rather on the northeast of the north edge of Fertile Crescent. Wild einkorn is suggested to be domesticated in the northwest of Fertile Crescent, Karacadag, and Diyarbakir and spread towards both east and west. The fact that the geographical distances from Diyarbakir to Van and Diyarbakir to Malatya are similar and the presence of water sources, a river and a lake, in both of the regions may indicate similar diversification and adaptation. Samples from Malatya, Kayseri and Sivas are on the cross-sections of the ancient roots from east to west, thus they appear as adapted wild einkorn to the region. Group II mostly composed of samples from the southeast and east, except sample number 18, another accession from Izmir, again most distantly linked to Group II, too. This may be because of the reason that the germplasm was displaced by humans during the early spread of agriculture and followed by subsequent naturalization of these lines outside their primary habitats which results in wild lines growing in secondary habitats [6].

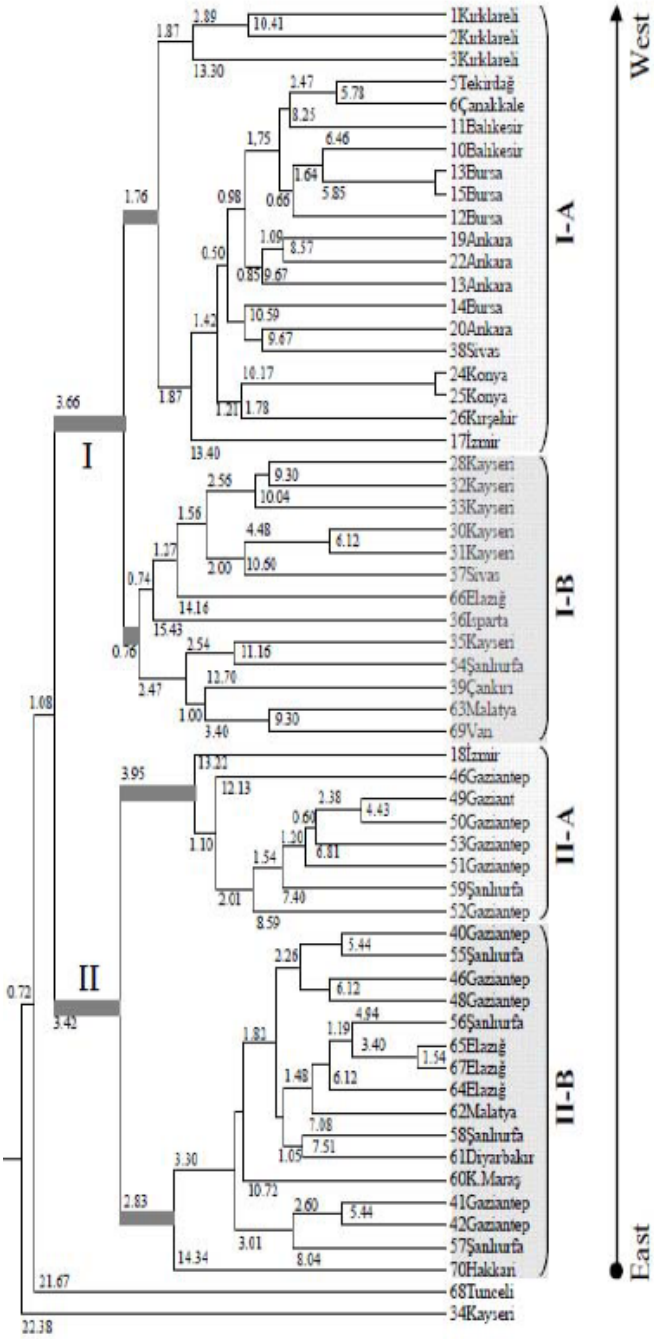

Figure 1. Phylogenic tree of 59 wild individuals based on Nei's [26] unbiased genetic distance and UPGMA modified from NEIGHBOR procedure PHYLIP version 3.5. The numbers on the left of the location names are the sample numbers as in Table 1. The genetic distances are presented in the tree.

Group II was also divided into two arms. The upper arm, II-A, mainly contains samples from Gaziantep except with Sanliurfa sample from a very close proximity to Gaziantep. The lower branch of II-B contains samples from remaining accessions from Gaziantep and the other samples from Southeastern and Eastern Anatolia. Gaziantep accessions appear to be two major type, accessions 40, 46, 48, 41, 42 are distant from the other accessions of Gaziantep. Additionally, Gaziantep samples most likely are the ones having the highest genetic diversity.

Figure 2 and 3 summarize the genetic distance relationships of the accessions analyzed by pooling samples into four major geographical locations (Figure 2C) and 22 sub-locations, cities, (Figure 2A). Cankiri and Isparta samples were not included, since we had only a single accession from each location and they were unexpectedly associated with samples from distant locations. Tunceli accession (location 22) as in Figure 1 appears as an out-group sample. In Figure 2A, samples from Bursa and Balikesir are genetically closer to Ankara than that of Tekirdag and Canakkale, although Balikesir and Bursa geographically much closer to Thrace where Canakale and Tekirdag are located. Nevertheless, Bursa and Balikesir are separated and isolated from Tekirdag and Canakkkale *via* huge inner sea, Marmara. That is why samples from Bursa and Balikesir are genetically closer to samples from geographically distant Ankara. Unlike Figure 1, when all four accessions of Kayseri and 2 accessions of Sivas were brought together as a population from Kayseri, and Sivas (number 10 and 12, respectively) they clustered as a separate clade within the group with samples from south east and east rather than that of Central Anatolia. In this group, samples from southeast form a core to which samples of east are linked to this core. The Gaziantep and Sanliurfa samples are most closely related ones to each other in this tree, to them Elazig and Malatya samples are closely linked. These four locations are in the root of the Firat River (Euphrates). On the other hand, although Diyarbakir (previously shown as the origin of domesticated einkorn) is in close proximity to these cities, it is located on the Dicle River (Tigris). That is why Diyarbakir samples are more distantly linked to the samples from these core locations.

The third group of classifications performed was based on the 4 major geographical regions.

As illustrated in Figure 2C, the genetic identity of wild wheat from Eastern Anatolian and Southeastern Anatolian are very close since they are neighboring each other.

Likely, there is a gradual transition towards Central Anatolian, Marmara and Aegean. All the pair wise distances and similarities are

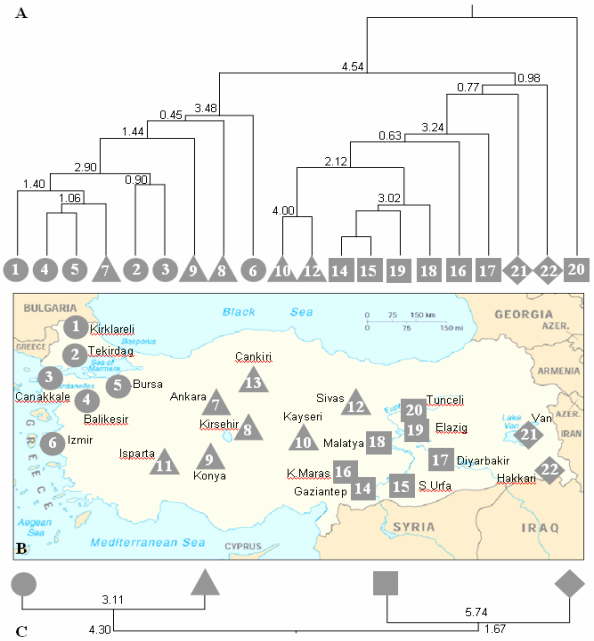

Figure 2. Phylogenic tree of the wild accessions with relative distances from 20 (11 and 13 not included) different locations (**A**) and geographical regions (**C**), and places are indicated in **B** (map), based on Nei's [26] unbiased genetic distance (values are indicated in the trees) and UPGMA modified from NEIGHBOR procedure PHYLIP version 3.5. ● Western, ▲ Central, ■ South East, ◆ East Anatolia. The numbers in the shapes are used to label the locations.

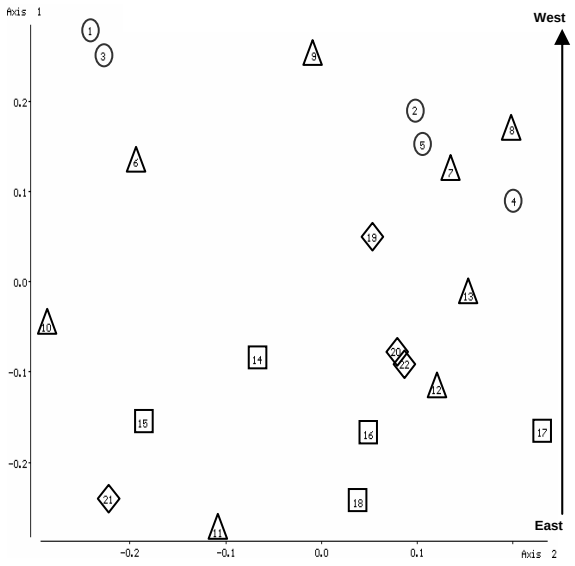

Figure 3. Principal Co-ordinate analysis of *T. boeoticum* subspecies with respect to locations (city) (Axis 1 versus Axis 2) applied on Syntax multivariate data analysis version 5.1. software by using Nei's unbiased measures of genetic distance matrix data.

The locations of cities are the same as in Figure 2. presented in Table 2. The most genetically distant samples are from, as it might be expected, Mediterranean. Mediterranean has much diverse climate and altitude than that of other regions, and may not be favoring the optimum conditions for growth of wheat. The samples of Marmara and Aegean are clustered very close to each other. Central Anatolian samples are located just between southeastern–eastern Anatolian cluster and Marmara-Aegean cluster. This distribution pattern of *T. bo eoticum* samples are expected based on the geographical features of the land and the climates differences.

Table 2. Nei's unbiased measures of genetic identity and distance [26] for populations' genetic relationship analysis.

Geographical Regions	WA	CA	SEA	EA
West Anatolia (WA)	-	0.9398	0.8500	0.8163
Central Anatolia (CA)	0.0621	-	0.9182	0.8676
Southeast Anatolia (SEA)	0.1625	0.0853	-	0.8915
East Anatolia (EA)	0.2030	0.1420	0.1148	-

In addition to the information obtained from phylogenetic trees, the heterozygosity values of the wild einkorn show us that the center of origin of *T. bo eoticum* samples is Southeastern and Eastern Anatolia. The highest heterozygosity values belong to the samples from Eastern and Southeastern Anatolian regions. The lowest heterozygosity value belongs to the samples from Marmara region (western Anatolia) confirming the distribution of *T. bo eoticum* from west to east (Table 4) based on the origin of domestication being in the east.

GST, estimate of gene flow, in practice is used an index of genetic difference among populations [27] similar to FST. The GST values range between 0.1251 and 0.3849 (Table 3) between the populations. This is not a great genetic differentiation or high level of genetic variation as expected, since samples are belonging to same subspecies, *T. boeoticum*.

Table 3. Gene flow, Nm* (above diagonal) and Nei's coefficient of gene variation, GST (below diagonal) estimates.

Geographical Regions	Sample Size	Ht	Hs	Gst	Nm
WA vs CA	27	0.2228	0.1950	0.1251	3.4982
WA vs SEA	38	0.2694	0.2070	0.2316	1.6593
WA vs EA	14	0.2285	0.1406	0.3849	0.7992
CA vs SEA	42	0.2631	0.2288	0.1304	3.3352
CA vs EA	18	0.2276	0.1623	0.2866	1.2445
SEA vs EA	28	0.2290	0.1744	0.2383	1.5979

Standard deviations for Ht and Hs ranges from 0.312-0.317 and 0.0210-0.0250, respectively.

Nm = estimate of gene flow from Gst or Gcs. (Nm = 0.5(1 - Gst)/Gst)

The number of polymorphic loci is: 161

The percentage of polymorphic loci is: 97.58

Table 4. A brief summary of genetic variation statistics results of *T. bo eoticum* samples with respect to regions obtained from PopGen 32 software [26].

Regions	Ian	na	ne	h	I	Nb	% Poly.
WA	12	1.56 (0.50)	1.30 (0.37)	0.17 (0.20)	0.26 (0.28)	93	56.4
CA	16	1.67 (0.47)	1.37 (0.38)	0.22 (0.20)	0.33 (0.28)	111	67.3
SEA	26	1.80 (0.40)	1.40 (0.35)	0.24 (0.19)	0.37 (0.26)	132	80.0
EA	2	1.26 (0.44)	1.18 (0.31)	0.11 (0.18)	0.15 (0.27)	43	26.1
Total	55	1.98 (0.15)	1.46 (0.35)	0.27 (0.17)	0.42 (0.22)	161	97.6

CONCLUSION

From the results of genetic trees, the samples from Marmara, Aegean and Central Anatolian regions are clustered together whereas the samples from Eastern and Southeastern Anatolian regions are clustered separately. The distribution of one species can be monitored easily in genetic relationship analyses. In our study, the distribution pattern of *Triticum monococcum* ssp. *boeoticum* can be easily screened from the dendograms. Both with respect to cities and with respect to regions, the distribution pattern appeared to be from east to west direction. In the dendogram, again samples from locations of west and central Anatolian region are clustered in one branch and samples from east and southeast of Anatolia are clustered together in another with an exception of Van. Our results are very much in accordance with Fertile Crescent being the center of origin of wild einkorn indicating natural distribution starts from southeastern Anatolia and continues to east Anatolia extends towards central Anatolia, Aegean, and Marmara regions (Western Anatolia).

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