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An Assessment of Genetic Diversity in Wild Diploid Wheat *Triticum boeoticum* from West of Iran Using RAPD, AFLP and SSR Markers

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ABSTRACT

The applicability of RAPDs, AFLPs, and SSRs to examine genetic relationships in 36 populations of *Triticum boeoticum* from West of Iran was investigated. A total of 224 (135 polymorphic), 979 (429 polymorphic) and 246 (145 polymorphic) bands/alleles were detected using 14 RAPD primers, 17 AFLP primer combinations and 17 well distributed, mapped SSR markers, respectively. The polymorphic information content (PIC) value was high for SSRs (0.81) but low for RAPDs (0.45) and AFLPs (0.56) reflecting the hypervariability of the first system. AFLPs carried the highest Marker Index (MI) value (14.19), reflecting the high multiplexity ratio of this system. The correlation coefficients of similarity were statistically significant for all the three marker systems employed. UPGMA cluster plots separated the 36 populations into three major groups based on their RAPD fragment similarities, and into two major groups based on their AFLP, SSR and RAPD+AFLP+SSR genotypic similarities. These different marker systems should provide different levels of information, important in the management of germplasm resources. A good level of genetic diversity observed in the populations of Kermanshah and Lorestan Provinces shows that *T. boeoticum* invades a wide range of agroecosystems in the West of Iran.

Keywords*:* AFLPs, Genetic diversity, RAPDs, SSRs, *Triticum boeoticum.*

INTRODUCTION

Einkorn (*Triticum monococcum*) (2n= 2x= 14, nuclear genome constitution AA) is the earliest species of cultivated wheat that was domesticated from its wild progenitor, *T. boeoticum* (Harlan and Zohary, 1996). Primary habitats of *T. boeoticum* occur in the central and eastern parts of the Fertile Crescent (Zohary and Hopf, 2000). Various sites of excavations such as Ali KOSH (Iran), Catal HUYUK and HACILAR (Turkey), from where specimens of *T. monococcum* have been recovered are within the general area of distribution of *T. boeoticum* (Harlan and Zohary, 1996). Heun *et al*. (1997) from an analysis of 288 AFLP marker loci in einkorn and its wild ancestor indicated that a wild group of *T. boeoticum* lines from the Karacadag Mountains (Southeast Turkey) is the likely progenitor of cultivated einkorn varieties.

Little information is available regarding genetic variation in wild wheat relatives from Iran. This country is a main center of distribution of wild wheats (Kimber and Feldman, 1987) with associated compositions of *T. boeoticum* with *Aegilops* spp. as the "richest wheat gene pool" having been found in the region (Tabatabaei and Ramak-Massoumi, 2001). The habitats of wild wheats in the West of Iran (east of Fertile Crescent) are potentially the ideal

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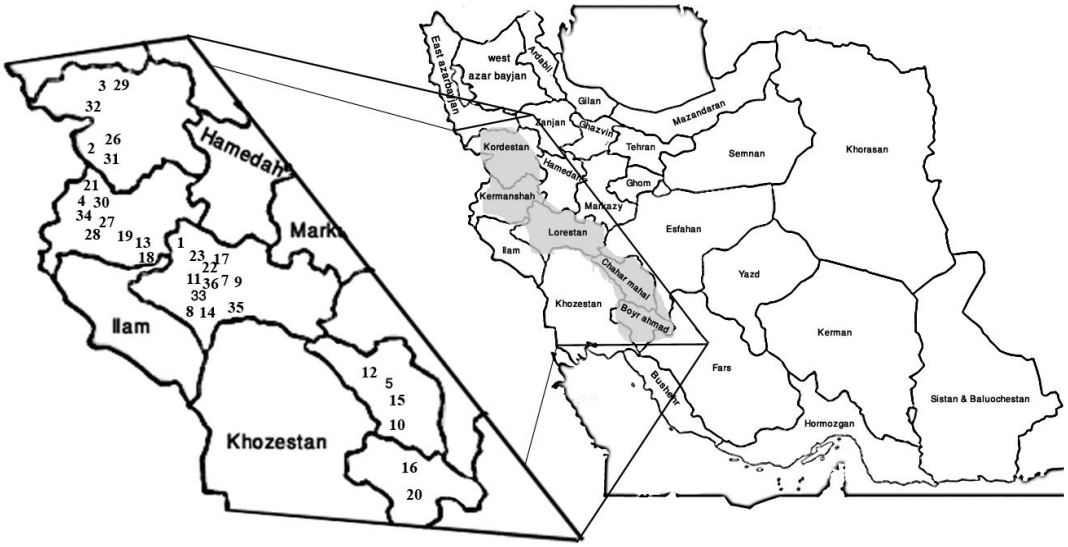

Figure 1. Map of Iran showing the regions from which landraces of *T. boeoticum* were collected for the study.

areas to explore germplasm for suitable genes for introgression into cultivated wheat (van Slageren, 1994). Therefore, it is supposed that the populations of *T. boeoticum* in this region contain high levels of genetic diversity and may provide significant information regarding their potential for breeding purposes. In recent years, based upon morphological examination and a use of the monographs of Gandilyan (1980) and Dorofeev *et al*. (1979), 14 populations of *T. boeoticum,* collected mainly from West of Iran, have been identified (Salimi *et al*., 2005).

Molecular markers provide a good estimate of genetic diversity since they are almost unlimited in number and are not influenced by the environment (Prasad *et al*., 2000). Various DNA markers have been developed, such as RFLPs Restriction Fragment Length Polymorphisms), AFLPs (Amplified Fragment Length Polymorphisms), RAPDs (Random Amplified Polymorphic DNAs) and microsatellites SSRs (Simple Sequence Repeats) which can be employed either separately or in combination, to evaluate genetic diversity. Comparisons of molecular markers for measuring genetic diversity have been carried out in wheat cultivars and related wild species (Bohn *et al*., 1999; Mizumoto *et al*., 2002; Almanza-Pinzon *et al*., 2003; Naghavi *et al*., 2004, Medini *et al*., 2005; Fufa *et al*., 2005; Naghavi *et al*., 2007) but, to our knowledge, no such studies have yet been reported for *T. boeoticum* populations. The main goals of this study were: (1) to evaluate genetic variability among populations of *T. boeoticum* from Iran using RAPD, AFLP and SSR markers and (2) to compare the discriminating capacity and informativeness of the three employed molecular marker systems.

MATERIALS AND METHODS

Plant Material and DNA Extraction

Thirty six populations of *T. boeoticum* (Tb1 to Tb36) were collected from West of Iran (Table 1, Figure 1) during the year 2005. This species is generally distributed along roadsides and margins of fields and sometimes found in the vicinity of *T. urartu.*

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Table 1. Geographical origins from where populations of *T. boeoticum* collected.

No.	Population abbreviation	Origin site	Province	Longitude, E	Latitude, N
1	Tb1	Phirozabad	Lorestan	48° 06'	33° 92'
2	Tb2	Marivan	Kurdestan	46° 13'	35° 30'
3	Tb3	Caghez 1	Kurdestan	46° 19'	36° 20'
4	Tb4	Javanrood 1	Kermanshah	46° 24'	34° 92
5	Tb5	Karimabad	Chaharmehal	50° 60'	32° 40'
6	Tb6	Unknown	-	-	-
7	Tb7	Sepiddasht 1	Lorestan	48° 90'	33° 20'
8	Tb8	Lorestan 1	Lorestan	47° 60'	33° 15'
9	Tb9	Sepiddasht 2	Lorestan	48° 90'	33° 20'
10	Tb10	Lordekan 1	Chaharmehal	50° 70'	31° 49'
11	Tb11	Malavi	Lorestan	47° 60'	33° 19'
12	Tb12	Charmaha l	Chaharmehal	50° 20'	32° 60'
13	Tb13	Norabad	Kermanshah	47° 99'	34° 03'
14	Tb14	Lorestan 2	Lorestan	47° 70'	33° 15'
15	Tb15	Jonkhan	Chaharmehal	50° 52'	32° 10'
16	Tb16	Yasouj	Kohkiloye	51° 55'	30° 75'
17	Tb17	Chaghlvand	Lorestan	48° 49'	33° 60'
18	Tb18	Ilam	Ilam	47° 09'	33° 92'
19	Tb19	Hersin	Kermanshah	47° 75'	34° 12'
20	Tb20	Kohkiloie	Kohkiloye	51° 62'	30° 51'
21	Tb21	Paveh	Kermanshah	46° 23'	35° 08'
22	Tb22	khoramabad	Lorestan	48° 21'	33° 50'
23	Tb23	Aleshtar	Lorestan	48° 20'	33° 91'
24	Tb24	Unknown	-	-	-
25	Tb25	Unknown	-	-	-
26	Tb26	Kamiaran 1	Kordestan	46° 99'	34° 65'
27	Tb27	Ravansar	Kermanshah	46° 60'	34° 62'
28	Tb28	Serabnilofar	Kermanshah	46° 75'	34° 55'
29	Tb29	Caghez 2	Kurdestan	46° 19'	36° 20'
30	Tb30	Javanrood 2	Kermanshah	46° 24'	34° 92'
31	Tb31	Kamiaran 2	Kurdestan	46° 99'	34° 65'
32	Tb32	Gheshlagh	Kurdestan	46° 30'	36° 01'
33	Tb33	Tamrak	Lorestan	47° 61'	33° 17'
34	Tb 34	Javanrood 3	Kermanshah	46° 24'	34° 92'
35	Tb35	Lorestan 3	Lorestan	48° 10'	33° 17'
36	Tb36	Sepiddasht 3	Lorestan	48° 90'	33° 20'

The seeds from each population (mainly from one individual) were mixed and a sample of seven seeds used for DNA extraction. Total genomic DNA was isolated from young leaves of greenhouse-grown plants according to CTAB Protocol (Saghai-Maroof *et al*., 1984) with minor modifications. To reveal the level of genetic variation for each population, DNA from seven plants were bulked and analysed.

Molecular Methods

Fourteen RAPD primers were used (Table 2) according to Williams *et al*. (1990). Polymerase Chain Reactions (PCRs) were carried out in a 25 µl volume, containing 1 unit of *Taq* polymerase, 25 ng of genomic DNA template, 0.2 µM of primer, 2mµ of each dATP, dCTP, dGTP and dTTP, and 2.5 µl of 10X PCR reaction buffer. AFLP analysis was

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Table 2. Arbitrary primers used in RAPD-PCR reactions.

Marker no.	Primer	Sequence	Polymorphic fragments scored
1	UBC84	5' GGG CGC GAG T 3'	10
2	OPAG04	5' GGA GCG TAC T 3'	18
3	OPAG02	5' CTG AGG TCC T3'	8
4	UBC64	5' GAG GGC GGG A3'	13
5	OPH12	5' ACG CGC ATG T 3'	14
6	OPG13	CTC TCC GCC A3' 5'	7
7	OPI01	ACCTGGGCAC3' 5'	7
8	OPI12	5' AGA GGG CAC A 3'	6
9	OPG17	ACG ACC GAC A 3' 5'	12
10	OPI14	TGA CGG CGG T 3' 5'	15
11	OPG16	AGC GTC CTC C 3' 5'	3
12	UBC9	5 'CCT GCG CTT A 3'	8
13	OPA11	5' CAA TCG CCG T 3	10
14	OPH05	AGT CGT CCC C 3' 5'	4
Average			9.64

performed as described by Vos *et al*. (1995) using the enzyme combination *Eco*RI and *Mse*I. Seventeen *Eco*RI and *Mse*I primer combinations with either two or three selective nucleotides on the 3' end of either primer were used for selective PCR amplification (Table 3). Selective amplification was conducted in a total volume of 25 µl reaction mixture containing 50 ng of template DNA, 1X buffer, 200 µM of each of the four dNTPs, 1 unit *Taq*

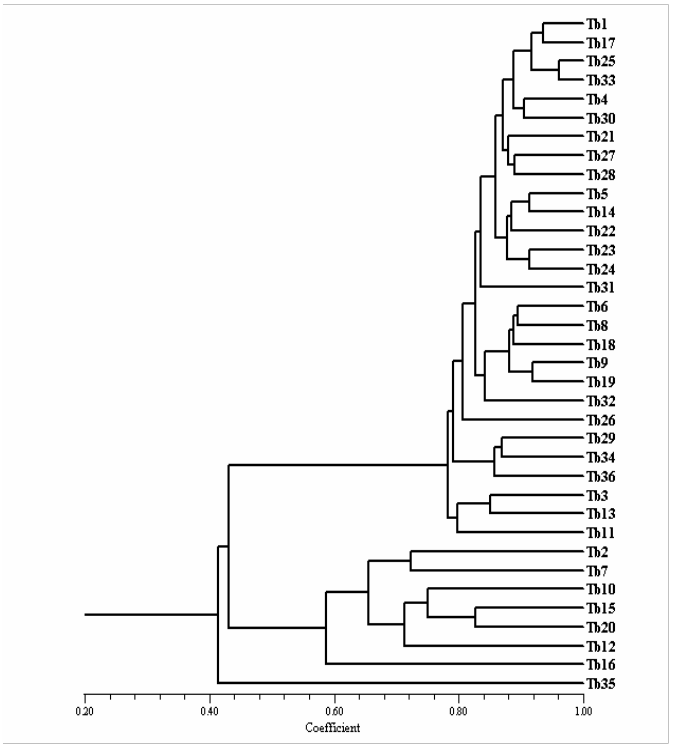

Figure 2. Dendrogram showing the relationships among populations of *T. boeoticum* based on an analysis of 135 bands of RAPD using the Dice similarity coefficient and the UPGMA clustering method. For population abbreviations see Table 1.

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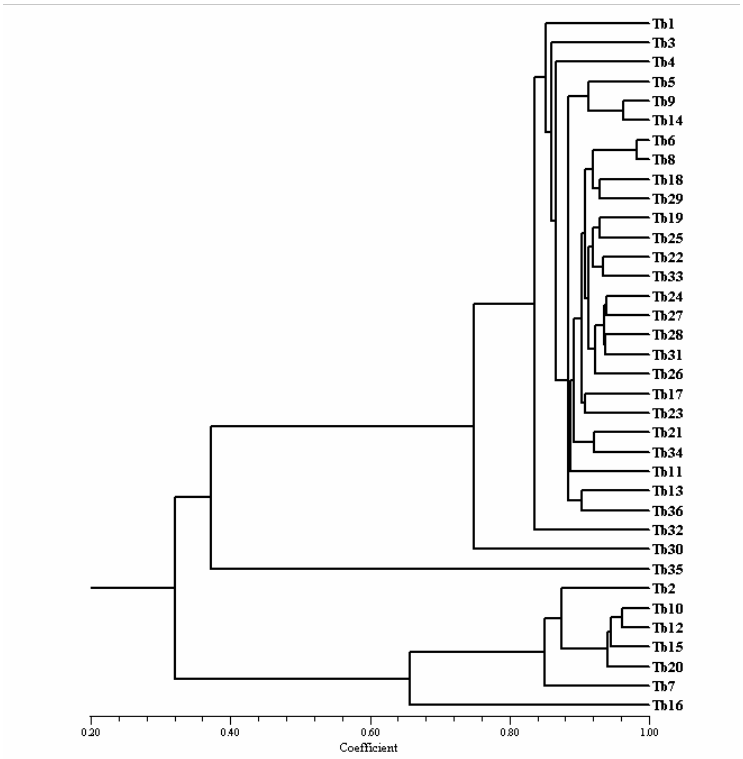

Figure 3. Dendrogram showing the relationships among populations of *T. boeoticum* based on an analysis of 429 bands of AFLP using the Dice similarity coefficient and the UPGMA clustering method. For population abbreviations see Table 1.

DNA polymerase, 2.5 mM $MgCl_2$ and 0.4 µM of each primer. The AFLP loci names were abbreviated according to the standard nomenclature of AFLPs (http://wheat.pw.usda.gov/ggpages/keygeneAFLPs.html). Seventeen SSR primer pairs (Röder *et al*., 1998) were used to assess parental polymorphism (Table 4). PCRs were performed on a BioRad thermocycler (BioRad Laboratories Inc., Hercules, CA, USA) in 25-µl reaction containing the following reagents: 20 ng of template DNA, 200 µM of each of the four dNTPs, *Taq* polymerase buffer, 1 unit *Taq* polymerase, 2.5 mM $MgCl_2$ and 0.25 µM of each of the two primers. Amplification reaction products were separated on 6% denaturing polyacrylamide gels using a Sequi-Gen GT Sequencing Cell 30 cm gel apparatus (BioRad Laboratories Inc., Hercules, CA, USA). The amplified fragments were detected by the silver staining method as described by Bassam *et al*. (1991). Molecular size of the amplified fragments was estimated by 1 kb DNA ladder.

Data Analysis

Each RAPD, AFLP and SSR polymorphic fragment was scored as either present (1) or absent (0) across all populations. Only distinct, well-resolved fragments were scored. The average Polymorphic Information Content (PIC) and the Marker Index (MI) were calculated for each marker system across all polymorphic assay units applying the formulas given by Powell *et al*. (1996). A binary matrix was used to estimate the genetic similarities between pairs, by employing the Dice index (Nei and Li, 1979). Four dendrograms were constructed to express the results of cluster analyses based on RAPD, AFLP, SSR, and RAPD+AFLP+SSR fragments using the Unweighted Pair Grouping Method of

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Table 3. Marker name, selective primer sequence and polymorphic fragments scored of Amplified Fragment Length Polymorphism (AFLP) markers.

Marker no	Marker name [a]	Selective primer sequence [b]	Polymorphic fragments scored
1	E-25/M-33	E-TG/M-AAG	22
2	E-32/M-47	E-AAC/M-CAA	25
3	E-32/M-52	E-AAC/M-CCC	24
4	E-32/M-65	E-AAC/M-GAG	26
5	E-38/M-47	E-ACT/M-CAA	23
6	E-38/-M52	E-ACT/M-CCC	28
7	E-40/M-14	E-AGC/M-AT	20
8	E-41/M-47	E-AGG/M-CAA	29
9	E-41/M-52	E-AGG/M-CCC	23
10	E-58/M-52	E-CGT/M-CCC	19
11	E-64/M-50	E-GAC/M-CAT	33
12	E-76/M-47	E-GTC/M-CAA	24
13	E-76/M-65	E-GTC/M-GAG	28
14	E-78/M-49	E-GTT/M-CAG	38
15	E-82/M-43	E-TAT/M-ATA	19
16	E-82/M-65	E-TAT/M-GAG	29
17	E-82M-49	E-TAT/M-CAG	19
Average			25.2

[a] The AFLP primer combinations names were abbreviated according to the standard nomenclature of AFLPs http://wheat.pw.usda.gov/ggpages/keygeneAFLPs.html).
[b] M: *Mse*I adaptor, E: *Eco*RI adaptor.

Table 4. Location, repeat and number of alleles of Simple Sequence Repeat (SSR) markers used in the study.

Marker no.	Marker name	Location	Repeat	Number of alleles
1	GWM130	7A	((GT)22	7
2	GWM155	3A	(CT)19	4
3	GWM156	5A	(GT)14	10
4	GWM160	4A	(GA)21	11
5	GWM164	1A	(CT)16	5
6	GWM165	4A	(GA)20	9
7	GWM265	2A	(GT)23	9
8	GWM293	5A	(CA)24	9
9	GWM304	5A	(CT)22	5
10	GWM33	1A-1B-1D	(GA)19	9
11	GWM334	6A	(GA)19	8
12	GWM350	7A	(GT)14	6
13	GWM397	4A	(CT)21	3
14	GWM427	6A	(CA)31, (CA)22	12
15	GWM617	2A	(GA)23	17
16	GWM635	7A, 7D	(CA)10 (GA)14	14
17	GWM674	3A	(CT)16CCC(GT)4	7
Average				8.5

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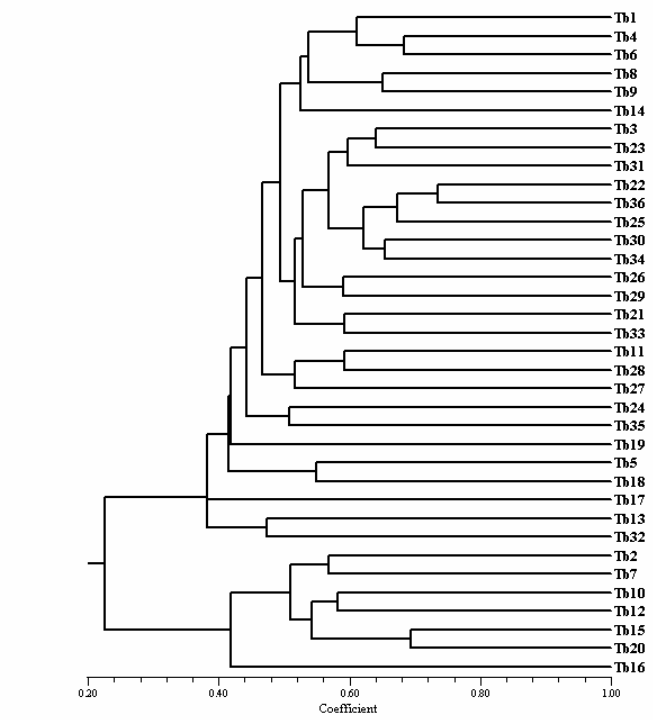

Figure 4. Dendrogram showing the relationships among populations of *T. boeoticum* based on an analysis of 145 alleles of SSR using the Dice similarity coefficient and the UPGMA clustering method. For population abbreviations see Table 1.

Arithmetic Averages (UPGMA). All similarity and cophenetic matrices were tested for pair-wise correlation using Mantel's non-parametric test (Mantel 1967). The purpose of Mantel's test is to verify whether a correlation exists between two matrices by pair-wise comparison of the cells at the corresponding positions. Statistical analysis was carried out using NTSYS-PC software, Version 2.1 (Rohlf 1998).

RESULTS

Molecular Markers Variation

All the three marker systems proved to be highly effective in discriminating the 36 populations analysed. In the RAPD analysis using 14 primers, a total of 224 bands were screened among which 135 were polymorphic (60%) across the 36 populations of *T. boeoticum* (an average of 9.6 bands per primer). The number of polymorphic bands per assay unit ranged from 3 to 18 (Table 2). The 17 AFLP primer combinations generated a total of 979 scorable fragments ranging from 50 bp to 500 bp of which 429 (44%) were polymorphic (Table 5). On average, 25.2 polymorphic bands were amplified by each primer combination. The AFLP primer combinations E78/M49 generated the highest (38 fragments) number of polymorphic bands and the lowest (19 fragments) were generated by primer combinations E82/M49, E82/M43 and E58/M52 (Table 3). Amplification of the SSR markers was performed using 17 primer pairs that produced 147 reproducible fragments, 145 of which were polymorphic (99%) (Table 5). The number of alleles per locus ranged from 3 to 17, with an average of 8.5 alleles per locus. The highest allele number was obtained at the *Xgwm617* locus (Table 4).

A summary of effectiveness of RAPD, AFLP and SSR markers in detecting polymorphism of 36 populations is given in Table 5. The number of scored alleles/bands

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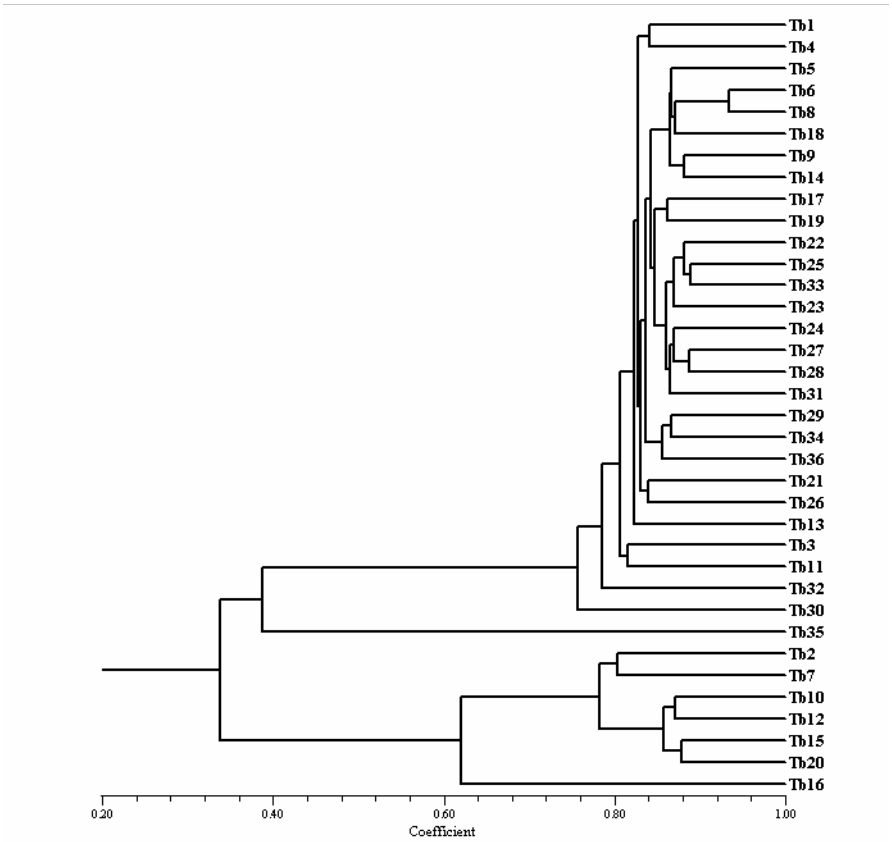

Figure 5. Dendrogram showing the relationships among populations of *T. boeoticum* based on an analysis of 709 bands/alleles of RAPD+AFLP+SSR using the Dice similarity coefficient and the UPGMA clustering method. For population abbreviations see Table 1.

ranged from 147 for SSRs to 977 for AFLPs. The percentage of polymorphic bands/alleles for each assay was not in correlation with the total number of bands/alleles. For example, only 147 alleles were scored for SSRs, which was the lowest number, but 145 alleles were polymorphic (99%). In contrast, 979 AFLP bands were scored, only 44% of which were polymorphic. RAPDs were the intermediate ones with 60% of all bands scored being polymorphic. Across all populations, the average PIC values were different among the three marker systems (Table 5). The average PIC value was highest for SSRs (0.81) but low for RAPDs (0.45) and AFLPs (0.56). MI values were lowest for SSRs (0.80) and highest for AFLPs (14.19).

Estimates of Genetic Similarity

A summary of the genetic similarity estimates between pairs of populations, calculated for each marker system, is shown in Table 6. The results revealed that the average genetic similarities of the populations were lower for SSRs than for RAPDs and AFLPs. Estimates of genetic similarity of RAPDs ranged from 0.26 to 0.96, with an average of 0.67. The estimates revealed by the polymorphic AFLP bands ranged from 0.18 to 0.98 with an average of 0.67. While the genetic similarity values determined from the 145 polymorphic SSR bands had a mean of 0.39 and ranged from 0.04 to 0.73.

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Table 5. Analyses of banding patterns generated by RAPD, AFLP, and SSR assays for 36 populations of *T. boeoticum*.

	Number of assay units	Number of bands/ alleles	Number of bands/ alleles per assay unit	Proportion of polymorphic bands	Number of polymorphic bands per assay unit	Number of loci per assay unit	PIC (SD)[a]	MI
RAPDs	14	224	16	0.60	9.64	16	0.45 (0.12)	4.32
AFLPs	17	979	57.6	0.44	25.2	57.6	0.56 (0.07)	14.19
SSRs	17	147	8.6	0.99	8.52	1	0.81 (0.10)	0.80

[a] Standard deviation.

Table 6. Comparison of genetic similarity estimates obtained from three PCR derived techniques, using Dice coefficient.

	Minimum	Maximum	Mean
RAPDs	0.26	0.96	0.67
AFLPs	0.18	0.98	0.67
SSRs	0.04	0.73	0.39

Table 7. Correlation between cophenetic matrices (above diagonal) and similarity matrices (below diagonal) obtained with three different marker types.

	RAPDs	AFLPs	SSRs
RAPDs	0.96**	0.97**	0.81**
AFLPs	0.93**	0.96**	0.84**
SSRs	0.70**	0.74**	0.86**

** Significant at P< 0.01.

Cluster Analyses

The dendrograms obtained from UPGMA were constructed to express the results of the cluster analyses based, respectively, on RAPD (Figure 2), AFLP (Figure 3), SSR fragments (Figure 4) and RAPD+AFLP+SSR (Figure 5) data. Cluster analyses separated the 36 populations into three major groups based on their RAPD fragment similarities, and into two major groups based on their AFLP, SSR and RAPD+AFLP+SSR genotypic similarities. The UPGMA cluster plots showed distinct levels of divergence depending on the marker employed. All the dendrograms demonstrated a high degree of similarity in the second group (including Tb2, Tb7, Tb10, Tb12, Tb15, Tb16, Tb20), though with some differences in the positioning of some populations at this group. Furthermore, the dendrogram obtained with all marker systems (Figure 5) was more similar to AFLPs than to the dendrograms resulting in RAPDs and SSRs. In other words there are some common associations of populations between the dendrograms of AFLPs and those of RAPDs+AFLPs+SSRs. For instance, populations Tb6 and Tb8, cluster together at the same subgroup for either of marker systems, and also populations Tb9 and Tb14, While RAPD dendrogram showing a greater similarity among populations from the nearby geographical areas than do the AFLP and SSR dendrograms.

Correlation between Measures

The correlation coefficients showed that similarity and cophenetic matrices were statistically significant for all the three marker systems (Table 7). But these correlation coefficients were higher for RAPD and AFLP comparisons (0.97 and 0.93 for comparisons of cophenetic and similarity matrices, respectively) than those obtained for the other comparisons. The calculated correlation coefficient (based on Mantel's test) of 0.96 for RAPDs and AFLPs, and of 0.86 for SSRs verified the goodness of the dendrograms.

DISCUSSION

Molecular markers provide a good estimate of genetic diversity since they are independent of confounding effects by environmental factors (Powell *et al.,* 1996). In this study, three PCR-based systems (RAPDs, AFLPs and SSRs) were employed to investigate the genetic diversity among 36 populations of *T. boeoticum*. Each technique not only differs in principle, but also in the type and extent of polymorphism detected. The levels of polymorphism among the three techniques varied widely, from a maximum of 99% (SSRs) to 60% (RAPDs) and 44% (AFLPs). This high level of polymorphism of SSR data is to be expected because of the unique mechanism responsible for generating SSR allelic diversity by replication slippage (Tautz, 1989). Similar results were observed in wheat (Bohn *et al*., 1999; Donini *et al*., 2000, Naghavi *et al*., 2004; Medini *et al*., 2005; Naghavi *et al*., 2007) where SSRs compared to other marker systems, revealing the highest level of polymorphism. However, although AFLPs do not show the highest level of polymorphism, they can reveal many polymorphic bands in a single assay (Table 5).

The average PIC value for SSRs (0.81) was greater than for RAPDs (0.45) and for AFLPs (0.56) reflecting the hypervariability of the first system (Tautz, 1989). Conversely, we obtained very high MI (14.19) for AFLPs, about eighteen and three times more than the values for SSRs (0.80) and RAPDs (4.32) respectively, revealing a much higher relative information content in comparison to SSR and RAPD markers. These findings are in good agreement with previous germplasm analysis carried out in several crop species (Powell *et al*., 1996; Medini *et al*., 2005; Naghavi *et al*., 2007). The high Marker Index (MI) or diversity

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index is a reflection of the efficiency of markers to simultaneously analyse a large number of bands rather than a reflection of the level of polymorphism detected (Powell *et al*., 1996).

The values of genetic similarity based on SSRs in the present study are much lower than those based on RAPDs and AFLPs (Table 6). Since only the SSRs are multiallelic, they are capable of detecting allelic diversity at a single loci, which is not possible with RAPDs and AFLPs.

In our study, significant associations were observed among molecular markers for both cophenetic and similarity matrices (Table 7), with the highest correlation detected between RAPDs and AFLPs. The lower correlation between SSRs and the other assays (RAPDs and AFLPs), may not be fully unexpected, as Powell *et al.* (1996) reported. He reported that SSRs were well-correlated with AFLPs at the interspecies level, however at the intraspecies level the correlation disappeared, emphasising the uniqueness of the SSR assay. The main reasons for the lower correlation observed between SSRs and RAPDs or AFLPs might be due to the codominant nature of SSR markers, the type of genetic polymorphism detected by the three markers and the number of primers used in different markers (Belaj *et al.*, 2003). Bohn *et al.* (1999) in a study on winter wheat cultivars did not find a common pattern of genetic relationships, using RFLP, AFLP and SSR markers.

Overall, the assessment of genetic diversity estimated by these different marker systems should provide different levels of information important in the management of germplasm resources. Our results suggest that AFLP system generate more data per analysis than other markers, but SSR markers differentiate accessions much better than RAPD and AFLP marker systems.

Our study indicated that the species *T. boeoticum* which is endemic to the major geographic regions of the Fertile Crescent (Heun *et al*., 1997; Salamini *et al.,* 2002) shows a high diversity in the West of Iran, even in geographically close regions (Figures 2-5). As previously reported for *T. urartu* (Moghaddam *et al.,* 2000), also in this study it becomes evident that geographically close regions could be ecologically quite different while conversely, regions which are geographically distant from each other can be very similar in their environmental conditions. In other words, measured relative genetic distances among populations were not completely correlated with geographical distances between places of their origins. We would expect that a greater sampling would be valuable to conclude more precisely the relationship between diversity and geographical origin, therefore, the number of accessions should be increased to more fully reveal the relationship.

Cluster analysis based on RAPDs bears two groups and one outlier (Tb35), which is not part of either group. The dendrograms based on AFLPs and RAPDs+AFLPs+SSRs, with two groups and one outlier (Tb35), are exactly the same as RAPDs. It becomes evident that the two groups for AFLPs and RAPDs are nearly identical as well; only one line is different, and that is probably based only on a few polymorphic bands. The SSR groups are also in fairly good agreement with the other two marker types. Moreover, cluster analysis based on all marker systems (Figures 2-5) separated some seven populations from the others, indicating possible existence of different variants of *T. boeoticum* in the West of Iran, as previously reported by Salimi *et al.* (2005).

A good level of genetic diversity observed in the populations of Kermanshah and Lorestan Provinces is not surprising in view of the wide range of agroecosystems that *T. boeoticum* has successfully invaded in these provinces. This reflects probably both germplasm differences as well as an influence of climatic conditions. All dendrograms, except SSR, separated Tb35 from other populations, indicating the existence of diverse populations in Lorestan Province which can be further confirmed by sampling in more depth.

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In conclusion the study clearly demonstrates that there exists a high diversity among *T. boeoticum* populations in the West of Iran that should be considered along with the diversity observed in Southeast Turkey (Heun *et al*., 1997) to determine the likely progenitor of cultivated einkorn varieties. Moreover, although all the three techniques may provide useful information on the level of polymorphism as well as diversity in *T. boeoticum* populations, but SSR markers differentiate accessions much better than other marker systems.

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بررسی تنوع ژنتیکی گندم های دیپلوئید وحشی (*Triticum boeoticum*) بومی غرب ایران با استفاده از نشانگر های RAPD، AFLP و SSR

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چکیده

در این تحقیق از سه نوع نشانگر RAPD، AFLP و SSR جهت تعیین ارتباط ژنتیکی بین ۳۶ جمعیت*Triticum boeoticum* جمع آوری شده از غرب ایران استفاده گردید. در کل با استفاده از ۱۴ پرایمر RAPD، ۱۷ ترکیب پرایمری AFLP و ۱۷ جفت پرایمر SSR به ترتیب ۲۲۴ باند، ۹۷۶ باند و ۲۴۶ آلل بدست آمد. مقدار اطلاعات چند شکلی (PIC) برای نشانگر های SSR (۰/۸۱) بیشتر از نشانگر های RAPD (۰/۴۵) و AFLP (۰/۵۶) بود که بیانگر تنوع پذیری بالای نشانگر های SSR می باشد. همچنین نشانگر های AFLP بیشترین مقدار (۱۴/۱۹) شاخص نشانگری (MI) را نشان دادند که بیانگر قدرت این نشانگر در شناسایی تعداد زیادی باند در یک واکنش می باشد. همبستگی معنی دار بین ماتریس های تشابه هر سه نوع سیستم نشانگری مشاهده گردید. تجزیه کلاستر ۳۶ جمعیت مورد بررسی را بر اساس نشانگر های RAPD به سه گروه و بر اساس نشانگر های AFLP، SSR و همچنین هر سه نوع نشانگر به طور همزمان به دو گروه تقسیم نمود. هر یک از این نشانگر ها سطوح مختلفی از تنوع را در سطح ژنوم ارایه می دهند که می تواند در مدیریت ژرم پلاسم ذخایر توارثی مفید باشد. همچنین تنوع بالایی در جمعیت های جمع آوری شده از استان های لرستان و کرمانشاه مشاهده گردید که بیانگر گسترش وسیع نمونه های *T. boeoticum* در اکوسیستم های متنوع موجود در غرب ایران می باشد.

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