Short Communication

Study of genetic variation in wild diploid wheat (*Triticum boeoticum*) from Iran using AFLP markers

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Abstract
Little information is available regarding genetic variation in wild wheat relatives from Iran. In this study, genetic diversity of 36 populations of wild einkorn wheat, *Triticum boeoticum,* was studied using amplified fragment length polymorphism (AFLP) primer combinations. Seventeen AFLP primer combinations led to amplify 979 scorable fragments ranging from 50 to 500 bp and of these, 429 (44%) were polymorphic across the 36 populations. The average Dice genetic similarity between *T. boeoticum* populations was 0.67 (range= 0.18-0.98). The dendrogram derived by unweighted pair group method with arithmetic mean algorithm (UPGMA) analysis revealed three main groups. PCO analysis also confirmed subgrouping obtained by cluster analysis. The measured relative genetic distances among accessions was not correlated with geographical distances of places of their origins, indicating that the populations are genetically different. The results demonstrated that AFLP technology is a suitable technique for genetic resource management in *T. boeoticum* populations of these studied origin sites.
Keywords*: Triticum boeoticum*; molecular marker; AFLP; genetic variability.

The genus *Triticum* consists of four distinct groups, including: einkorn (2n=2x=14, AA), emmer (2n=4x=28, AABB), timopheevi (2n=4x=28, AAGG) and common wheat (2n=6x=42, AABBDD). Three species including: *T. monococcum*, wild *T. boeoticum* and *T. urartu* belong to the einkorn wheat group (Mizumoto *et al*., 2002). It was shown that *T. monococcum* was domesticated from *T. boeoticum* and that *T. urartu* was an A genome donor for the polyploidy species of wheat (Dvorak *et al*., 1993; Takumi *et al*., 1993). All of these species have identical nuclear genomes homologous to the A-genome of polyploid wheats (Kihara, 1944). The clear divergence between *T. boeoticum* and *T. urartu* detected in previous reports (Takumi *et al*., 1993; Ciaffi *et al*., 2000, Sasanuma *et al*., 2002) and many taxonomists regard these two taxa as a single biological species (Sharma and Waines, 1981).

In the Near East, primary habitats of *T. boeoticum* occur in the northern and eastern parts of the Fertile Crescent (Harlan and Zohary, 1996). Study of the genetic diversity in this species (including studies involving wheat evolution) may provide significant information regarding their potential for breeding purposes. Various sites of excavations such as Ali KOSH (Iran), Catal HUYUK and HACILAR (Turkey), from where specimens of *T. monococcum* have been collected are within the general area of distribution of *T. boeoticum* (Harlan and Zohary, 1996). Although Iran is one of the main centers of distribution of wild wheats (Kimber and Feldman, 1987) and the associated compositions of *T. boeoticum* with *Aegilops* spp. as the "richest wheat gene pool" has been found in this region (Fakhre-Tabatabaei and Ramak-Massoumi, 2001). But little information is available regarding diversity and distribution of wild wheat populations from Iran. It is supposed that the wild populations of

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Triticum species in this region contain high levels of genetic diversity.

Genetic diversity of the nuclear genome in the einkorn wheat has been well evaluated by the following criteria, isozymes (Jaaska, 1997; Moghaddam *et al*., 2000), seed storage proteins (Ovesna *et al*., 2002), RFLPs (Castagna *et al*., 1994, 1997), random amplified polymorphic DNAs (RAPDs) (Castagna *et al*., 1997; Ovesna *et al*., 2002) and AFLP (Heun *et al*., 1997; Mizumoto *et al*., 2002; Sasanuma *et al*., 2002; Singh *et al*., 2006).

The amplified fragment length polymorphism (AFLP) technology (Vos *et al*., 1995) is a method that combines the advantages of a high detectable number of loci at a time and a high reproducibility. The goal of this study was to evaluate the genetic variability, using AFLP markers, in a collection of *T. boeoticum* populations collected from different geographical regions of Iran. As more is understood about diversity in this species it may be possible to develop insights into identify sources of materials that contain desirable traits for breeding purposes.

Plant material and DNA extraction: Thirty six populations of *Triticum boeoticum* which collected from origin sites of west of Iran, including 6 provinces, (Table 1) were used in this study. Total genomic DNA was extracted from young leaves of greenhouse-grown plants according to the CTAB protocol (Saghai-Maroof *et al*., 1984) with minor modifications. To reveal the level of genetic variation for each population, DNA of seven plants were bulked and analysed.

AFLP analysis: The AFLP analysis was performed as described by Vos *et al*. (1995) with minor modifications. Approximately 250 ng of the isolated genomic DNA per sample was double digested with two restriction enzymes *Eco*RI and *Mse*I and ligated with 5 pmol of *Eco*RI adapter and 50 pmol of *Mse*I adapter. The ligated DNA was preamplified using two primers containing without any selective nucleotide. Selective amplification was conducted in a total volume of 20 µl reaction mixture containing 50 ng of template DNA, 1×buffer, 200 µM of each of the four dNTPs, 1 unit *Taq* DNA polymerase, 2.5 mM $MgCl_2$ and 0.4 µM of each primer with three selective nucleotides. Seventeen primer combinations were selected for the analysis of genetic similarity (Table 2). The reactions were performed in a Perkin-Elmer 9600 thermocycler (Perkin Elmer, Boston, MA, USA), with thermal profile: 94ºC, 30s; 65ºC, 30s; 72ºC, 60s. The amplified DNA product was separated in a 6% denaturing polyacrylamide gel and detected by the silver staining method.

Table 1. Regions of origin from which populations of *T. boeoticum* collected and used in present study.

No.	Population abbreviation[a]	Origin site	Province
1	Tb1	Phirozabad	Lorestan
2	Tb2	Marivan	Kurdestan
3	Tb3	Caghez 1	Kurdestan
4	Tb4	Javanrood 1	Kermanshah
5	Tb5	Karimabad	Chaharmehal
6	Tb6	Unknown	-
7	Tb7	Sepiddasht 1	Lorestan
8	Tb8	Lorestan 1	Lorestan
9	Tb9	Sepiddasht 2	Lorestan
10	Tb10	Lordekan 1	Kohkiloye
11	Tb11	Malavi	Lorestan
12	Tb12	Charmaha l	Chaharmehal
13	Tb13	Norabad	Kermanshah
14	Tb14	Lorestan 2	Lorestan
15	Tb15	Jonkhan	Chaharmehal
16	Tb16	Yasouj	Kohkiloye
17	Tb17	Chaghlvand	Lorestan
18	Tb18	Ilam	Ilam
19	Tb19	Harsin	Kermanshah
20	Tb20	Kohkiloie	Kohkiloye
21	Tb21	Paveh	Kermanshah
22	Tb22	khoramabad	Lorestan
23	Tb23	Aleshtar	Lorestan
24	Tb24	Unknown	-
25	Tb25	Unknown	-
26	Tb26	Kamiaran 1	Kordestan
27	Tb27	Ravansar	Kermanshah
28	Tb28	Serabnilofar	Kermanshah
29	Tb29	Saghez 2	Kurdestan
30	Tb30	Javanrood 2	Kermanshah
31	Tb31	Kamiaran 2	Kurdestan
32	Tb32	Gheshlagh	Kurdestan
33	Tb33	Tamrak	Lorestan
34	Tb 34	Javanrood 3	Kermanshah
35	Tb35	Lorestan 3	Lorestan
36	Tb36	Sepiddasht 3	Lorestan

[a] Tb indicates *Triticum boeoticum*

Data analysis: Polymorphic AFLP fragments were scored as either present (1) or absent (0) across all populations. Only distinct, well-resolved fragments were scored. Binary matrix was used to estimate the genetic similarities between pairs, by employing Dice index (Nei and Li, 1979). These similarity coefficients were used to construct dendrogram using the unweighted pair group method with arithmetic averages (UPGMA) employing the SAHN (Sequential, Agglomerative, Hierarchical, and Nested clustering) from the NTSYS-PC (Numerical Taxonomy and Multivariate Analysis

Table 2. Summary of primer combinations, polymorphic and non-polymorphic fragments of AFLP-data scored.

No	Primer combinations	Total no. of bands	No. of polymorphic bands	No. of non-polymorphic bands
1	Ecs+AGG/Mcs+CAA	58	29	29
2	Ecs+GTC/Mcs+GAG	86	28	58
3	Ecs+ACT/Mcs+CCC	53	28	25
4	Ecs+AAC/Mcs+CCC	46	24	22
5	Ecs+AGG/Mcs+CCC	56	23	33
6	Ecs+GTT/Mcs+CAG	65	38	27
7	Ecs+GAC/Mcs+CAT	56	33	23
8	Ecs+TAT/Mcs+GAG	58	29	29
9	Ecs+AAC/Mcs+GAG	61	26	35
10	Ecs+AAC/Mcs+CAA	55	25	30
11	Ecs+ACT/Mcs+CAA	68	23	45
12	Ecs+TG/Mcs+AAG	48	22	26
13	Ecs+AGC/Mcs+AT	64	20	44
14	Ecs+TAT/Mcs+CAG	47	19	28
15	Ecs+TAT/Mcs+ATA	56	19	37
16	Ecs+GTC/Mcs+CCC	36	19	17
17	Ecs+GTC/Mcs+CAA	66	24	42
Total		979	429	550

System), version 2.02 (Applied Biostatistics) program (Rohlf, 1990). The support values for the degree of confidence at the nodes of the dendrogram were analyzed by 1000 bootstrap resampling using PHYLIP 3.57c computer software (Felsenstein, 1995). Principal co-ordinate (PCO) analysis was also carried out using a square symmetric matrix of Dice similarities between pairs of populations.

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 across the 36 populations. On average, 25 polymorphic bands were amplified by each primer combination. The AFLP primer combinations E-GTT/M-CAG generated the highest (38 fragments) number of polymorphic bands and the lowest (19 fragments) were generated by

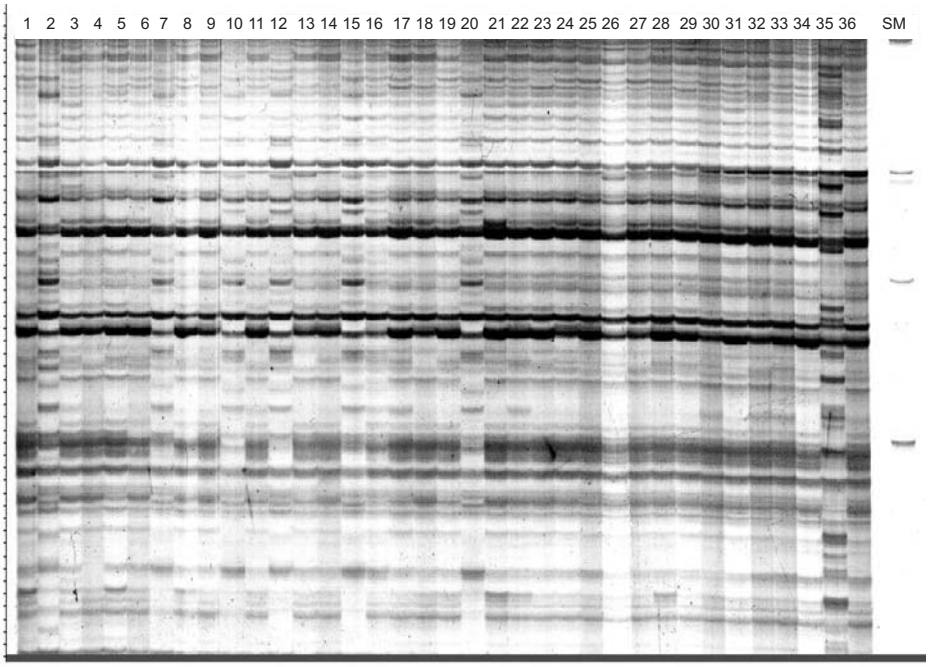

Figure 1. Electrophoretogram of AFLPs generated with primer combination EACT/MCAA in 36 populations of *T. boeoticum*. SM is DNA size markers. For population number see Table 1.

E-TAT/M-CAG, E-TAT/M-ATA and E-GTC/M-CCC primer combinations. These results confirm that AFLP is capable of detecting substantial numbers of polymorphic loci with a relatively small number of primer pairs. The percentage of polymorphic bands for each primer combination did not correlate to the total number of bands. For example, only 36 bands were scored for E-GTC/M-CCC, which was the lowest number, but 19 bands were polymorphic (53%). In contrast, 86 bands were scored for E-GTC/M-GAG, and only 32% of those were polymorphic. Figure 1 shows an example of such a typical AFLP pattern using the E-ACT/M-CAA primer combination. Estimates of genetic similarity of AFLP based on the 429 polymorphic markers between 36 populations of *T. boeoticum* ranged from 0.18 for Tb4 (Javanrood 1) and Tb20 (Kohkiloie), to 0.98 for Tb6 (Unknown) and Tb8 (Lorestan 1) with an average of 0.67 (similarity data are not shown).

The cluster analysis obtained with the UPGMA approach resulted in meaningful groupings of the 36 populations and revealed three main groups (Fig. 2). The clusters 1, 2 and 3 consisted of twenty eight, one and seven populations, respectively, indicating that these populations are somewhat genetically diverse. This grouping of data which is supported by a good bootstrap value, indicating possible existence of different varieties of *T. boeoticum* in the west of Iran as previously reported by Salimi *et al*. (2005).

AFLP analysis also revealed differences in genotype banding patterns between different populations of *T. boeoticum* taken from one region (For instance, Tb7, Tb9, Tb36 from Sepiddasht or Tb4, Tb30, Tb34 from Javanrood 1) indicating a significant level of diversity in *T. boeoticum* germplasm grown in these regions (Fig. 2).

PCO analysis revealed that for AFLP data the first two components of the PCO explained 56 and 8% of the total variation. Although the results of PCO didn't correspond totally to those from cluster analysis, but it confirmed subgrouping obtained by cluster analysis (data are not shown).

In this study, 979 AFLP loci were used to investigate the genetic diversity in the populations of *T. boeoticum.* The amount of polymorphism found in this investigation (44%) was more than what reported in previous studies (Sasanuma *et al*., 2002), reveals that the AFLP technique is a useful method for analysing genetic diversity in *T. boeoticum*.

Pejic *et al*. (1998) reported that 150 polymorphic

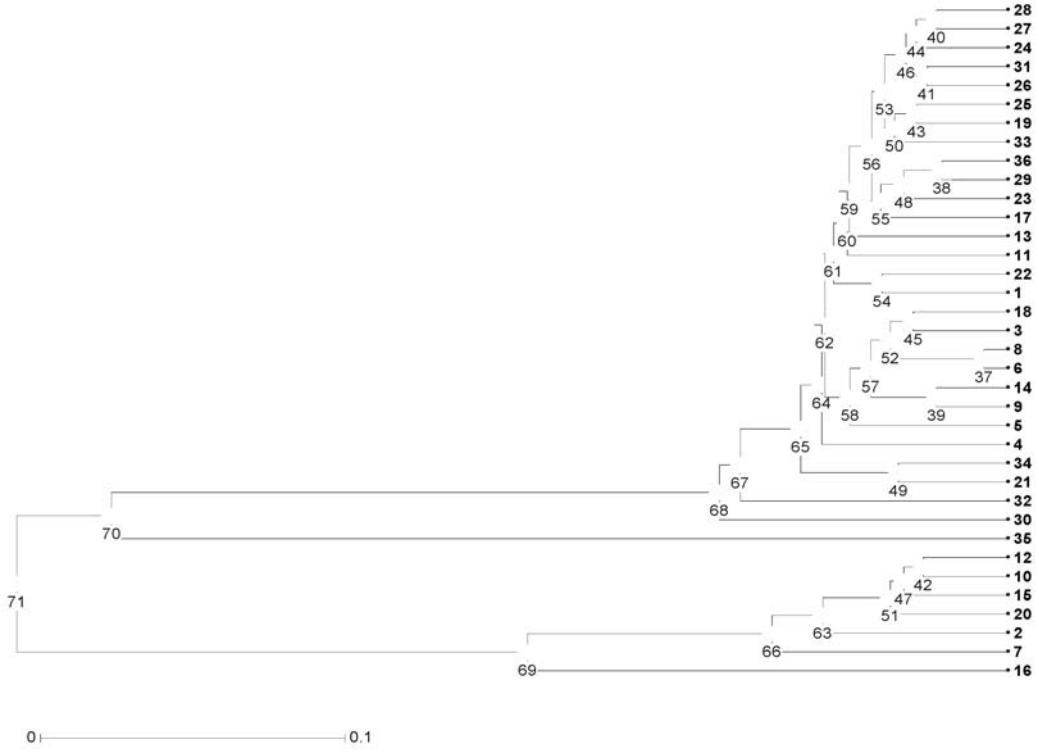

Figure 2. Dendrogram showing the relationships between populations of *T. boeoticum,* obtained using the Dice index and UPGMA. Numbers on the branches are bootstrap values (%) obtained from 1000 replicate analyses. For population number see Table 1.

bands can provide a reliable estimate of genetic similarities among genotypes within a species. In the case of a rapid appraisal of diversity, Singh *et al*. (2006) also recommended more than 200 markers to provide reasonable estimates. In our study, we have shown that AFLP generated 429 polymorphic bands, making it practicable to fingerprint all 36 populations. The level of observed polymorphism using different AFLP markers indicated a large amount of genetic variation among studied *T. boeoticum* populations. This high amount of variation might be attributed to the wide geographical distribution of this species in Iran (Fakhre-Tabatabaei and Ramak-Massoumi, 2001). Contrary to our result, Singh *et al*. (2006) reported very low diversity in *T. boeoticum* populations and this difference can be attributed to utilization of *T. boeoticum* accessions from different geographical origins as well as low number of used accessions in their study.

The average number of polymorphic bands per primer combination was relatively higher than what reported by Mizumoto *et al*. (2002) and Sasanuma *et al*. (2002). These differences might be related to the utilization of different *T. boeoticum* germplasms as well as the used of different primers sequences. Estimates of genetic similarity using genetic fingerprinting data are a useful tool in plant breeding, allowing breeders to make appropriated decisions regarding the selection of germplasm to be used in crossing schemes (Russel *et al*., 1997).

Relative genetic distances between *T. boeoticum* populations, expressed by the dendrogram and PCO analyses, were relatively high. This could be expected for a wild species, indicating that *T. boeoticum* represents a large gene pool. However, measured relative genetic distances among accessions were not correlated with geographical distances of places of their origins. This reflects probably both germplasm differences and influence of climatic conditions as it was also proposed by Ovesna *et al*. (2002). Other effects (e.g. accidental seed transfer with crops) could contribute to the spreading of genotypes to more distant regions (Ovesna *et al*., 2002).

In conclusion, our results indicate that there is high diversity in the populations of *T. boeoticum* in the west of Iran even in geographically close regions. This high level of genetic diversity leads us to think how to conserve and use such variation for the breeding programs and as well as facilitating management of genetic resources. We expect that a more intensive sampling will be valuable in order to find more genetic diversity.

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