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Genetic Variation of Jointed Goatgrass (*Aegilops cylindrica* Host.) From Iran Using RAPD-PCR and SDS-PAGE of Seed Proteins

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Abstract: Genetic variation of 28 populations of jointed goatgrass (*Aegilops cylindrica* Host.), collected from different parts of Iran, were evaluated using both RAPD-PCR and SDS-PAGE of seed proteins. The diversity within and between populations for the three-band High Molecular Weight (HMW) subunits of glutenin pattern were extremely low. Out of 15 screened primers of RAPD, 14 primers generated 133 reproducible fragments which among them 92 fragments were polymorphic (69%). Genetic similarity calculated from the RAPD data ranged from 0.64 to 0.98. A dendrogram was prepared on the basis of a similarity matrix using the UPGMA algorithm and separated the 28 populations into two groups. Confusion can happen between populations with the same origin as well as between populations of very diverse geographical origins. Our results show that compare to seed storage protein, RAPD is suitable for genetic diversity assessment in *Ae. cylindrica* populations.

Key words: *Aegilops cylindrica*, genetic diversity, RAPD, SDS-PAGE

INTRODUCTION

Genetic resources are the gene pool available for breeders and other scientists, and in the *Triticeae* tribe several pools are recognized (Von Botmer *et al.*, 1992; Gill *et al.*, 2006). *Aegilops* is characterized as a Mediterranean-Western Asiatic element and its center of diversity follows the central part of the Fertile Crescent arc in West Asia (Hegde *et al.*, 2002).

Ae. cylindrica Host. (genome formula: CCDD) is an amphiploid, resulting from hybridization between the diploids *Ae. caudata* (CC) and *Ae. squarrosa* (DD). This species is, however, frequent in the Mediterranean area and in the Middle East, which is its center of distribution (Guadagnuolo *et al.*, 2001). This species distributes frequently along roadsides, edges of cultivation, dry hillsides, and grassy steppes, where it tolerates disturbance (van Slageren, 1994). It is known to hybridize spontaneously with wheat (Gandilyan and Jaaska, 1980; Snyder *et al.*, 2000). Naturally formed hybrids between *Ae. cylindrica* and wheat occasionally produce seeds, presumably via spontaneous cross-pollination, which could facilitate gene flow between the D genomes of the two species (Gandilyan and Jaaska, 1980; Guadagnuolo *et al.*, 2001; Galaev and Sivolap, 2005). Therefore, understanding the genetic structure of these troublesome weedy populations may facilitate designing new ways to control them, especially when most other conventional methods of weed control have failed to contain their spread (Donald and Ogg, 1991).

Today, molecular tools are available to evaluate and characterize, to a certain extent, genetic diversity among gene bank accessions. Genetic diversity of *Ae. cylindrica* has been evaluated using morphological data (Dotray and Young, 1993), isozyme analysis (Watanabe *et al.*, 1994; Hegde *et al.*, 2002), protein variation (Wan *et al.*, 2002) and molecular markers (Okuno *et al.*, 1998; Guadagnuolo *et al.*, 2001; Pester *et al.*, 2003). But although many landraces of wheats were collected in Iran and were available before and after the Islamic Revolution, wild wheat and *Aegilops* from Iran are still largely unknown. The objective of this study was to understand the extent and pattern of genetic diversity in 28 populations of allotetraploid wild wheat *Ae. cylindrica*, collected from roadside of Iran, using seed storage protein and RAPD markers.

MATERIALS AND METHODS

Plant material: Twenty eight populations of *Aegilops cylindrica* which collected from origin sites of Iran in 2004, including 10 provinces, (Table 1) were used in this study.

Storage protein analysis: In order to evaluate genetic diversity of high molecular weight subunit of glutenin within and between populations, three samples were taken and analysed from each of 28 populations of *Ae. cylindrica*. Chinies spring cultivar was used as a standard to compare subunit composition. The seed were

Table 1: Abbreviation, origin site and province of populations of *Ae. cylindrica* analyzed

No.	Population abbreviation	Origin site	Province
1	AC1	Hersin 1	Kermanshah
2	AC2	Bognord	khorestan shomali
3	AC3	Shahrood 1	Semnan
4	AC4	Hashrood	Azerbaijan Shargi
5	AC5	Shahrood 2	Semnan
6	AC6	Khoey	Azerbaijan Garbi
7	AC7	Mahabad	Azerbaijan Garbi
8	AC8	Zarine	Kordestan
9	AC9	Salmas	Azerbaijan Garbi
10	AC10	Unknown	-
11	AC11	Hersin 2	Kermanshah
12	AC12	Saghez	Kordestan
13	AC13	Ardabil-Sarein	Ardabil
14	AC14	Divan Dareh	Kordestan
15	AC15	Javanrood	Kermanshah
16	AC16	Mahabad	Azerbaijan Garbi
17	AC17	Shahrood 3	Semnan
18	AC18	Unknown	-
19	AC19	Unknown	-
20	AC20	Unknown	-
21	AC21	Gazvin	Ghazvin
22	AC22	Marand	Azerbaijan Shargi
23	AC23	Mashad 1	Khorasan Razavi
24	AC24	Behshahr	Mazandran
25	AC25	Gheshlagh	Kordestan
26	AC26	Mashad 2	Khorasan Razavi
27	AC27	Hersin 3	Kermanshah
28	AC28	Kamyaran	Kermanshah

crushed after removal of the embryo. The flour was mixed in an extraction buffer of 62 mM Tris-HCL (pH = 6.8) buffer containing 10% (W/V) glycerol, 4% (W/V) Sodium Dodecyl Sulfate (SDS), 0.01% (W/V) bromophenol blue and 4% β -mercaptoethanol. Samples were allowed to stand at room temperature for at least 12 h with occasional vortexing. They were then placed in a boiling waterbath for 2 min and then centrifuged for 5 min at 6500 rpm and 15 μ L of each sample was loaded on the gel. Proteins were fractionated by SDS-PAGE according modified of Laemmli (1970) procedure using stacking gel. Proteins were fractionated using stacking gel containing 4% acrylamide, 0.05% bis acrylamide, 0.1% SDS and 0.00009 M Tris-HCL and separating gel containing 10% acrylamide, 0.13% bis acrylamide, 0.1% SDS and 6.78 M Tris-HCL. Gels were stained overnight with 0.01% (W/V) Coomassie Brilliant Blue R 250 in water and acetic acid (10%) and then destained overnight in water for at least 24 h.

RAPD assay: Total genomic DNA was isolated from young leaves of greenhouse-grown plants according to the CTAB protocol (Saghai-Marooft *et al.*, 1984) with minor modifications. To reveal the level of genetic variation for each population, DNA of five plants were bulked and analysed. A total of fifteen 10-mer oligonucleotides from UBC (University of British Columbia) series were selected according to the number and consistency of amplified fragments. PCR reactions were carried out in a 25 μ L⁻¹ volume containing 1 unit

of Taq polymerase, 5 ng of genomic DNA template, 0.2 μ mol⁻¹ of primer, 2 mM of each dATP, dCTP, dGTP and dTTP, and 2.5 μ L of 10X PCR reaction buffer. Amplifications were performed in a DNA Thermo-cycler (Perkin-Elmer) programmed for 45 consecutive cycles each consisting of 1 min at 92°C, 1 min at 37°C and 2 min at 72°C. Following amplification, the samples were subjected to electrophoresis in 6% acrylamide gels in TAE buffer running at 200 v for 2.5 h. The gels were stained using ethidium bromide and viewed under Ultra-violet light.

Data analysis: Polymorphic RAPD 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 System), version 2.02 (Applied Biostatistics) program (Rohlf, 1998). A principal coordinate analysis (PCO) was also conducted with the same program using the DCENTER and EIGEN procedures.

RESULTS

Protein analysis: The patterns of high molecular weight (HMW) glutenin seed proteins among 28 populations of jointed goatgrass detected that diversity in the three-band HMW glutenin pattern was extremely low. In other words, there was very low diversity within and between studied populations. In previous study, Wan *et al.* (2002) identified 3 subunits bands as 1Cx, 1Cy and 1Dy in all accessions of *Ae. cylindrica*. In this study, we also found 3 subunits bands for all within and between populations except in one sample. In this population from 3 samples analysed, two samples contained 3 subunits, but one of sample showed an extra band at top (1Dy) (Fig. 1).

RAPD analysis: In the RAPD analysis, 15 ten-mer primers were used to amplify all of the genotypes; 14 of these showed informative polymorphic products resolvable by gel electrophoresis. A total of 133 bands were screened (average of 8.9 bands per primer) among which 92 were polymorphic (69%). The number of fragments generated per primer varied between 3 and 16. The highest and the lowest number of polymorphic bands per assay unit were 0 (primer OPA-15) and 13 (primer UB-9), respectively (Table 2). Figure 2 shows an example of such a typical RAPD pattern using the WMS-43 primer.

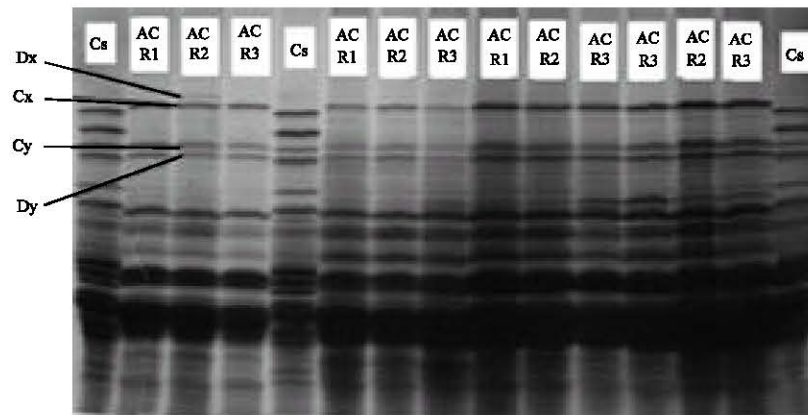


Fig. 1: Example of High Molecular Weight (HMW) subunits of glutenin among some populations of *Ae. cylindrica*. Cs is chines spring; R1, R2 and R3 are three samples taken from each population

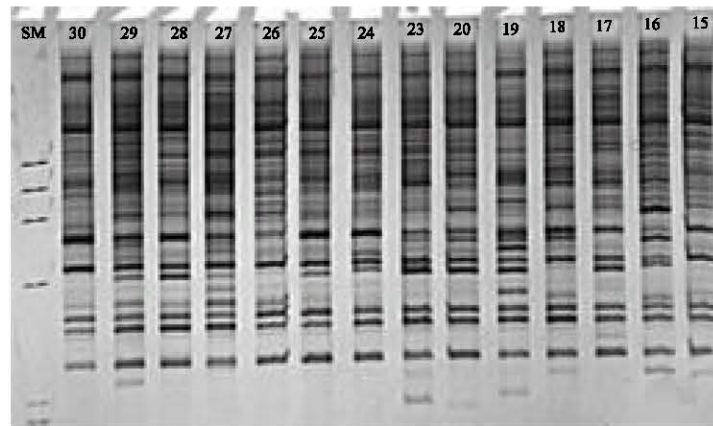


Fig. 2: Example of polymorphism between some populations of *Ae. cylindrica* as revealed by RAPD primer WMS-43

Table 2: RAPD primers with sequence, total number of bands, number of polymorphic bands, percent of polymorphism

No.	Primer name	Primer sequence	Total No. of bands	No. of polymorphic bands	Percent of polymorphism
1	A11	5' CAA TCG CCG T 3'	16	12	75
2	AG-04	5' GGA GCG TAC T 3'	15	11	73
3	AG-02	5' CTG AGG TCC T 3'	14	10	71
4	APG-16	5' AGC GTC CTC C 3'	3	2	66
5	C-15	5' GAC GGA TCA G 3'	7	2	28
6	G-14	5' GGA TGA GAC C 3'	3	2	66
7	H-12	5' ACG CGC ATG T 3'	6	0	0
8	OPA-15	5' TTC CGA ACC C 3'	5	5	100
9	OPA-19	5' CAA ACG TCG G 3'	9	2	22
10	OPM-06	5' CTG GGC AAC T 3'	10	9	90
11	UB-9	5' CCT GCG CTT A 3'	16	13	81
12	UB-13	5' CCT GGG TGG A 3'	6	5	83
13	UB-84	5' GGG CGC GAG T 3'	4	3	75
14	UBC-95	5' GGG GGG TTG G 3'	10	9	90
15	UBC-96	5' GGC GGC ATG G 3'	9	7	77
	Total		133	92	69

Estimates of genetic similarity of RAPD based on 92 polymorphic markers between 28 populations of *Ae. cylindrica* ranged from 0.41 for AC17/AC24, AC13/AC17 and AC12/AC15 to 0.84 for AC20/AC23 with an average of 0.63.

Genetic similarity values were used for cluster analysis through UPGMA, resulting in a dendrogram (Fig. 3). Cluster analysis revealed that populations Ac7 and Ac17 from Mahabad and Shahrod origin sites, respectively were more genetically distinct from the others

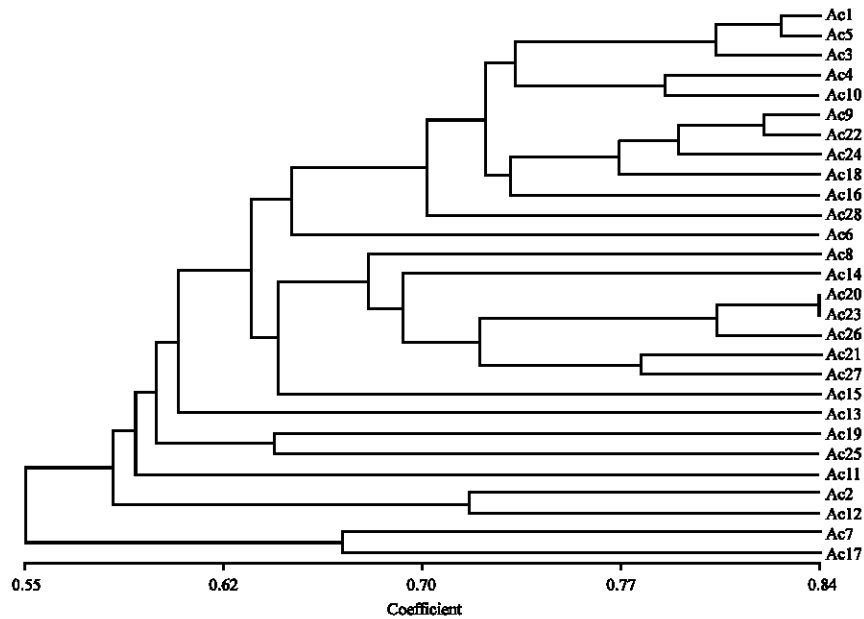


Fig. 3: UPGMA dendrogram showing genetic relationships among the 28 populations of *Ae. cylindrica* used in this study. The dendrogram was constructed based on genetic similarity calculated according to Dice coefficient. For population abbreviations see Table 1

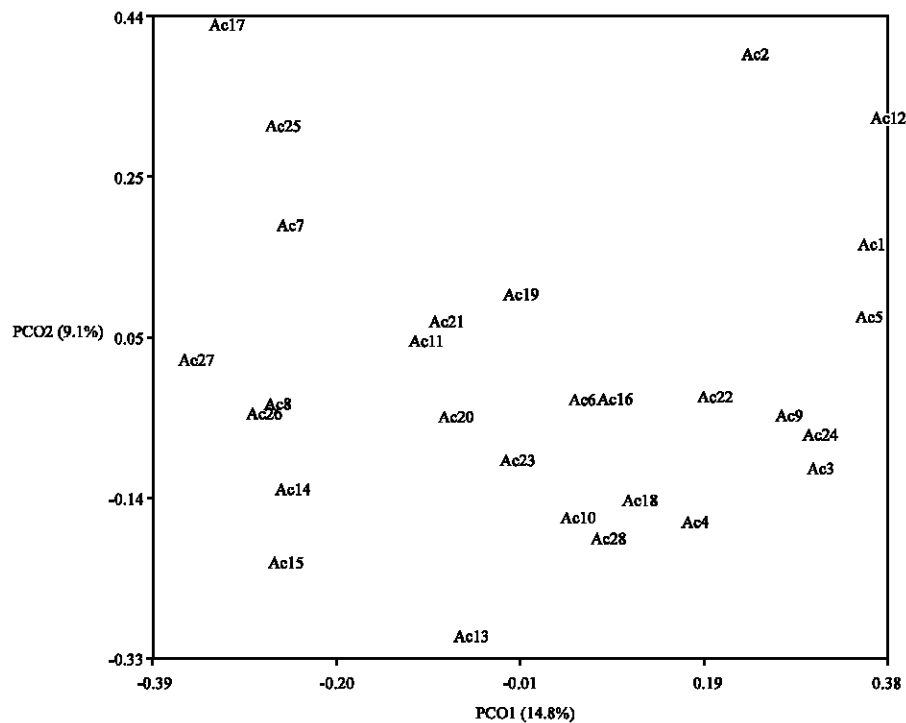


Fig. 4: Patterns of relationships among 28 populations of *Ae. cylindrica* revealed by principal coordinate analysis based on RAPD data. For population abbreviations see Table 1

(Fig. 1) and the most obvious pairs were Ac20 and Ac23. PCO analyses revealed that for RAPD data the first two components of the PCO explained 14.8 and 9.1% of the total variation (Fig. 4). Although the results of PCO didn't

correspond totally to those from cluster analysis, but it confirmed some subgrouping obtained by cluster analysis.

DISCUSSION

In this study, two molecular methods, seed storage protein and RAPD, were used to investigate the genetic diversity in the 28 populations of *Ae. cylindrica*. Seed storage protein analysis showed very low genetic diversity in studied populations. Isozyme analysis also conducted by Watanabe *et al.* (1994) and Hegde *et al.* (2002) revealed little or no variation among accessions of jointed goatgrass. We could find an extra band at top (1Dy) that it was very similar to top band of *Ae. triuncialis*. As this species distributes frequently with *Ae. triuncialis* along roadsides, therefore it is possible that the accession with extra band is a hybrid between *Ae. triuncialis* and *Ae. cylindrica*.

Compare to seed storage protein, RAPD markers revealed more polymorphic fragments. This is expected, as proteins markers reflect only variation in the coding parts of the genome, which is by nature more conservative and thus less polymorphic. While RAPDs can detect variation in both coding and non coding sequences, and the length of the primers allows the amplification of a large number of fragments with a single primer (Guadagnuolo *et al.*, 2001). The RAPD technique has been widely applied in studies of wheat genetic diversity (Cao *et al.*, 1998; Sun *et al.*, 2003; Naghavi *et al.*, 2004; Ahmad Khan *et al.*, 2005) and has shown its usefulness in rapid detection of genetic variation. Although the level of RAPD polymorphism within wheat itself has been reported to be low (Devos and Gale, 1992), but the level of RAPD polymorphism in wild wheat has been found to be higher than that in common bread wheat (Sun *et al.*, 2003).

RAPD amplifications provided the largest set of polymorphic markers. In this study, 92 RAPD loci were used to investigate the genetic diversity in the populations of *Ae. cylindrica*. The amount of polymorphism found in this research (69%) was more than what reported in previous studies (Okuno *et al.*, 1998, Guadagnuolo *et al.*, 2001; Pester *et al.*, 2003). These differences might be related to the utilization of different *Ae. cylindrica* germplasms as well as the used of different primers sequences.

In this research we found that there was little relationship between genetic divergence and geographical origins, so that the populations from similar geographical places (AC1 with AC11 or AC27) belonged to separate clusters. Conversely, populations from different geographical conditions (such as AC7 and AC17) relatively tended to be clustered in one part of the dendrogram.

In conclusion, our results show that compare to seed storage protein, RAPD is suitable for genetic diversity assessment in *Ae. cylindrica* populations and it appears to be the most suitable for this kind of study. This technique has the advantage of requiring very small quantities of template DNA and no prior knowledge of sequencing in the target genome (Pester *et al.*, 2003). Even if the dominance of RAPD markers remains a problem for population genetic studies, it is possible to overcome it, if specific fragments for each taxon or group of taxa compared are produced (Guadagnuolo *et al.*, 2001).

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