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Assessing Genetic Diversity Based on Gliadin Proteins in *Aegilops cylindrica* Populations from Northwest of Iran

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Abstract

Wild wheat progenitors served as a valuable gene pool in breeding perspectives. In this respect, gliadins could be an important tool in assessing genetic variability as protein markers. Thus, genetic diversity of gliadin protein patterns in seventeen populations of *Aegilops cylindrica* collected from northwest of Iran were investigated using acid polyacrylamide gel electrophoresis. Results showed that the highest number of bands in the electrophoregrams were related to the ω type of geliadins. Conversely, the lowest number of bands were pertained to the β type of gliadins. Genetic diversity between populations was greater than within population variation. Assessment of total variation for the three gliadin types indicated that the highest total variation was related to β type while, the lowest one was belonged to ω type. Cluster analysis using complete linkage method divided populations into two separated groups in which genetic diversity does not follow from geographical distribution.

Keywords: *Aegilops cylindrica,* A-PAGE, genetic variation, gliadins

Introduction

Aegilops cylindrica is a winter annual autogamous, allotetraploid (genome formula: $C^cC^cD^cD^c$, 2n=4x=28) grass, with long slender spikes. Studies identified that *A. caudata* L. (2n=2x=14, CC) as the donor of the C^c genome and *A. tauschii* Coss. (2n=2x=14, DD) as the donor of the D^c genome of this species. Its cytoplasm has been also donated from *A. tauschii* (Johnson, 1967; Tsunewaki, 1996). It shares the D genome with wheat, and interspecific hybrids between the two species occur under natural field conditions (Guadagnuolo *et al*., 2001; Mallory-Smith *et al*., 1996; Zemetra *et al*., 1998).

Jointed goatgrass (*A. cylindrica*) has been identified as a useful but under-utilized source for wheat improvement. It has been reported that this species have important traits such as salt tolerance (Farooq *et al*., 1992), Hessian fly resistance (El Bouhssini *et al*., 1998), snow mold resistance and freezing tolerance (Iriki *et al*., 2001).

Gluten, the main storage protein in cereals, comprised of gliadin and glutenin (Carillo *et al*., 1990). Between storage proteins, gliadins, because of easy extraction and analysis by electrophoresis and high level of genetic diversity, attracted many concerns as biochemical markers for estimation of diversity among populations (Kenzewic *et al*., 1998). These subunits with molecular weight of 28-70 kDa (Payne *et al*., 1987) are usually alcohol-soluble monomeric proteins which are divided into four ω γ β and α groups based on their electrophoretic mobility. Among these regions, the ω have low cystein content while the others are cystein-rich, and their amino acid composition is different from other gliadins. The γ-gliadins are different from α- and β-gliadins in the amount of aspartic acid, methionine, proline, phenilalanine, tyrosine and tryptophan (Bietz *et al*., 1977). All gliadins have the low ionic amino acids such as arginine, histidine, lysine, and free carboxylic groups of aspartic acid and glutamic acid. In addition, gliadins can be classified according to their N-terminal amino acid sequence (Gianibelli *et al*., 2001).

In wheat, the genes coding the gliadin proteins are located on the short arms of the first (*Gli-A1, Gli-B1, Gli-D1*) and sixth (*Gli-A2, Gli-B2, Gli-D2*) homoeologous groups. *Gli-1* genes code for all the ω- and most of the γ-gliadins while *Gli-2* genes for all the α-, most of the β-, and some of the γ-gliadins (Metakovsky *et al*., 1984). The γ -45 band of gliadin related to high gluten quality, whereas, the ω-38 and γ-42 bands related to moderate and low gluten quality, respectively (Rashed *et al*., 2007).

Northwest of Iran is centre of origin and diversity for *Aegilops* species including *A. cylindrica*. However, genetic variation of different populations grown in this region is not studied in detail. The aim of present investigation was the study of gliadin proteins diversity in local populations of *A. cylindrica* from northwest of Iran to aid the subsequent studies to introgression from this species and enrich the wheat gene pool.

Materials and methods

Plant material

In this study, 17 populations of *A. cylindrica* collected from the northwest of Iran together with wheat cultivar ('Bezostaya' as standard), were evaluated with five plant (sample per populations) for each population (Tab. 1).

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Protein analysis

Extraction and electrophoretic separation of gliadin proteins was done according to the standard Acid-PAGE method described by Metakovsky and Novoselskaya (1991) with 8.5% gel concentration.

Single grains of each population, after removing embryo by razor blade, were crushed by minimortor and the flour was transferred to Eppendorf tubes, separately. Then, 150 µl of ethanol 70% was added to the flour and the mixture was incubated at room temperature for 30 min. After the centrifuging at 10000 ppm for 5 min, supernatant was transferred to an Eppendorf tube containing the equal volume of sucrose solution in 5.1 mM Al-lactate buffer, pH=3.1. After the adding a minute quantity of pyronine (as marker) to the mixture, 5-7 µl of each ones were loaded into lanes. Electrophoresis was carried out at a constant voltage of 220 V for 10 min, followed by 550 V for 2-3 h at a constant temperature of 14°C.

Data analysis

The data obtained from Acid-PAGE was scored for the presence (1) or absence (0) of the bands and entered as a binary data matrix. Based on the results of electrophoretic band spectra, similarity index of simple matching coefficient was calculated for all possible pair of populations. The similarity matrix thus generated and used to construct the dendrogram using the complete linkage or furthest neighbours method in cluster analysis using NTSYS pc 2.0 software. Nei's (1978) gene diversity within and among populations was estimated using Popgene 1.32 software.

Results and discussion

Results showed that the C3 population had the most gliadin bands, with 16 bands, whereas the least gliadin bands were observed in the C8 population, with 13 bands (Tab. 2). In some of the populations, just a fair band in α region was observed (Fig. 1, Tab. 2). However, Gregova *et al*. (2011) showed that *A. cylindrica* lacks the α region, which is in contrast with our finding. This may be due to different genotypes used in this study or different method for electrophoresis.

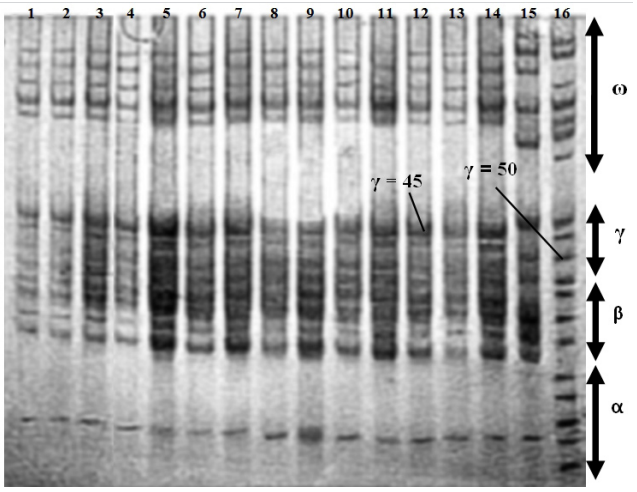

Fig. 1. Example of electrophoretic patterns of gliadin proteins in Bezostaya and the some populations of *A. cylindrica*. lanes 1 to 14 are various populations of *A. cylindrica*. lane 15: landrace wheat and lane 16: Bezostaya (as standard)

In the β region, the highest number of bands were observed in the C3, C4, C6 and C7 populations with five bands, while, the lowest number of bands was observed in the C8 population with three bands. In this region, the highest amount of within population diversity (0.15) observed for the C4 and C9 populations. However, a number of populations did not show any within population diver-

Tab. 1. Locations of different populations of *A. cylindrica* used in this study

No.	Population abbreviation	Province	Latitude	Longitude	Altitude (m)
1	C1	Ardabil	38° 11' 54" N	48° 16' 33" E	1414
2	C2	Ardabil	38° 29' 26" N	48° 25' 55" E	1542
3	C3	Ardabil	38° 25' 37" N	48° 29' 02" E	1430
4	C4	Azarbaijan Shargi	38° 40' 21" N	47° 17' 13" E	1314
5	C5	Azarbaijan Shargi	38° 28' 39" N	47° 04' 12" E	1338
6	C6	Azarbaijan Shargi	38° 03' 56" N	46° 22' 29" E	1497
7	C7	Azarbaijan Shargi	38° 02' 13" N	46° 25' 45" E	1645
8	C8	Ardabil	38° 23' 42" N	47° 16' 55" E	1117
9	C9	Azarbaijan Shargi	38° 25' 30" N	45° 46' 10" E	1355
10	C10	Azarbaijan Shargi	37° 20' 25" N	46° 03' 22" E	1290
11	C11	Azarbaijan Shargi	38° 12' 19" N	45° 51' 10" E	1410
12	C12	Azarbaijan Shargi	38° 10' 32" N	45° 43' 58" E	1426
13	C13	Azarbaijan shargi	38° 31' 08" N	46° 43' 14" E	1648
14	C14	Azarbaijan Garbi	37° 08' 45" N	46° 06' 10" E	1298
15	C15	Azarbaijan Garbi	38° 51' 02" N	45° 13' 28" E	947
16	C16	Azarbaijan Garbi	36° 58' 10" N	46° 06' 10" E	1297
17	C17	Ardabil	39° 36' 24" N	47° 52' 39" E	74
18		Bezostaya (Wheat cultivar)			

Tab. 2. Number of bands and Within population genetic diversity in gliadin bands of *A. cylindrica*

Population abbreviation	Number of bands				Within population variation			
	α	β	γ	ω	α	β	γ	ω
C1	1	4	4	5	0	0	0.075	0.075
C2	1	4	5	5	0	0.075	0.075	0.075
C3	1	5	5	5	0	0.075	0	0
C4	1	5	4	5	0	0.15	0.075	0
C5	1	4	5	5	0	0	0.225	0
C6	0	5	5	5	0	0.075	0	0
C7	0	5	5	4	0	0	0	0
C8	1	3	4	5	0	0	0.075	0
C9	1	4	5	5	0	0.15	0.075	0.075
C10	1	4	5	5	0	0.075	0	0
C11	0	4	5	5	0	0	0.075	0
C12	1	4	4	5	0	0	0.15	0.075
C13	1	4	4	5	0	0	0	0.075
C14	1	4	4	5	0	0	0.075	0.075
C15	1	4	5	5	0	0.075	0.075	0.15
C16	1	4	5	5	0	0	0.075	0
C17	1	4	5	5	0	0.075	0.075	0.075
Mean					0	0.0441	0.0662	0.0397

sity in this region that it would be as a result of selection fixation of some alleles in this respects (Tab. 2).

In the γ region, except for six populations (C1, C4, C8, C12, C13 and C14) which showed four bands, the others showed five bands. The C5 population showed the highest within population diversity (0.225).

In the ω region, all of the populations showed five bands except for the C7 population with four bands in this region. The highest within population diversity (0.15) was observed in the C15 population and other populations did not show any within population variation or a slight variation in this region (Tab. 2). The most bands of the ω region are located in D genome of *A. cylindrica* (Kozub *et al*., 2003, 2004), therefore it can be used in improvement the bread making quality of wheat through chromosome manipolation and hybridization techniques.

It was observed that the mean amount of within population diversity was greater for γ region, even though, was slight for all of regions (Tab 2). This indicating that there was little genetic variation among plants in the same population. However, The genetic diversity among populations was greater than within populations which was greater for the β region (Tab. 3). Assessment of total variation for the three regions indicated that the highest total variation was related to the β region while, the lowest one was belonged to ω region. It can be concluded that there were only a limited variation among northwest Iranian jointed goatgrass populations considering gliadin proteins. This limit intraspecific genetic variation could be useful in accession determination (Tab. 3). Limited genetic diversity in seed storage proteins among different populations was also reported by Farkhari *et al*. (2007) and Vorosvary *et al*. (2000), also Isozyme analysis showed slight or no varation among accessions of jointed goatgrass (Hegde *et al*., 2002; Watanebe *et al*., 1994).

A band similar to γ=45 band, that is related to high quality gluten, was also observed in all of the studied populations (Fig. 1). This was conformed by Sofalian and Valizadeh (2009).

Cluster analysis of 17 *A. cylindrica* populations (Fig. 2) using the similarity matrix of simple matching coefficient with complete linkage or furthest neighbors method separated populations into two groups. The first group included the C1, C4, C5, C10, C11, C12, C13, C14, C16 and C17 populations, whereas other populations aligned as the second group which indicating genetic diversity doesn't follow geographical distribution. Principle coordinate analysis showed that the first three components determined approximately 70% of total variance that it confirmed the cluster analysis grouping, too.

The results of discriminant analysis were completely justified two groups and confirmed the cutting point in cluster analysis. The mantel test between initial similarity matrix and the resulted cophenetic matrix of dendrogram showed that correlation coefficient between the matrices is 0.65, indicating a more or less high efficiency of grouping.

Tab. 3. Among and within population genetic diversity of *A. cylindrica*

Nei's gene diversity	Gliadin band spectra regions		
	ω	γ	β
Within populations	0.0397	0.0662	0.0441
Among populations	0.0699	0.1023	0.1685
Total	0.1096	0.1685	0.2126

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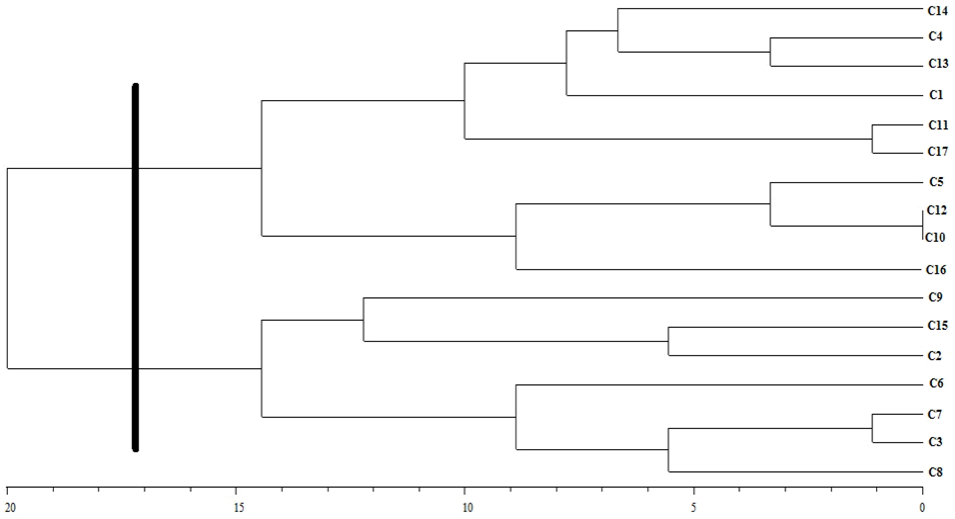

Fig. 2. Dendrogram of 17 *A. cylindrica* populations using complete linkage method in cluster analysis

Conclusions

Results obtained from gliadin patterns as a biochemical marker exhibited low within and among populations genetic diversity in *A. cylindrica* populations from northwest of Iran.

Although this genetic diversity was limited but it could be used in any quality improvement breeding programs. These results can be used for adoption of proper sampling strategies for germplasm conservation of this species. In addition, the most gliadin bands of *A. cylindrica,* at least with regard to mobility and electrophoretic position, shared with those of Bezostaya, from that, it can be used to study the genome affinity of these species.

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