Assessment of Genetic Polymorphism in Durum Wheat Genotypes Based on Electrophoretic Profiles of Gliadin-Coding Loci

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Abstract

The loss of genetic diversity of crops is one of the global problems of agriculture nowadays. Different methods are currently used for evaluation of genetic polymorphism in plants, which include various types of markers (morphological, molecular etc.). Assessment of genetic diversity based on electrophoretic profiles of seed storage proteins is still considered one of the most precise, simple and economical methods in the marker-assisted selection. The genetic diversity of gliadins has been studied among 50 genotypes of *T. durum*, using acid polyacrylamide-gel electrophoresis (Acid-PAGE). In total, 33 bands and 50 gliadin patterns were identified. Twelve different bands and 40 patterns were found in ω-gliadins, 8 polymorphic bands and 31 patterns were determined in γ-gliadins, along with 5 bands and 10 different patterns in β-gliadins, and 8 bands in combination resulted in 42 different patterns in the α-gliadin zone. Fifty patterns were found for each of the *Gli-1* (γ/ω region) and *Gli-2* (α/β region) loci. The genetic diversity index (*H*) was higher for α-gliadins (0.972), followed by ω and γ-gliadins (0.963 and 0.956, respectively), and the lowest value was detected in β-gliadin patterns (0.825). Cluster analysis based on Jaccard coefficient of similarity divided the analyzed collection into four clusters. The data obtained from electrophoretic analysis of gliadins is highly useful for identification of genotypes and selection of genetically distant varieties for breeding.

Keywords: Acid-PAGE; bands; patterns; genetic diversity

Introduction

The study of plant genetic diversity (PGD) is now being considered an important area due to global population explosion with high level of urbanization and decrease of cultivable land area, which put food security in the world under risk. PGD can be assessed and stored in the form of plant genetic resources (PGR) such as gene bank, DNA library etc., which preserve genetic material for long period (Narain, 2000). Genetic erosion, or loss of genetic diversity, in crops caused by the constant use of the same parental genotypes for breeding, is one of the global problems of agriculture nowadays.

Plant genetic diversity can be evaluated with the use of different morphological and molecular markers (Mondini *et al.,* 2009). In wheat, seed storage proteins (gliadins and glutenins) serve as highly efficient markers, because of their codominant inheritance, low-cost methods of identification, stability and independence of the growth conditions (Ruiz & Carrillo, 1993).

Gliadins, alcohol-soluble seed storage proteins, are characterized by high degree of intervarietal polymorphism and can be studied by a standard method of acid electrophoresis (A-PAGE). According to the differences in mobility and intensity of staining, gliadin components can be separated by gel electrophoresis into four subfractions: α (the fastest), β, γ, and ω-gliadins (the slowest). However, some genetic studies suggested that α- and β-gliadins are very similar and only three types of gliadins (α/β, γ and ω) are classified. Due to high polymorphism of gliadins a unique electrophoretic pattern can be observed for almost every cultivar (Sozinov, 1985; Sozinov & Pperelya, 1979). Within some heterogeneous cultivars two or more biotypes can be determined with different gliadin alleles in the same locus. The gliadin pattern does not depend on environmental factors and serves as a specific characteristic of a particular cultivar (Lee & Ronalds, 1967).

Each gliadin-coding locus consists of several tightly linked genes which are always inherited together as a single Mendelian trait. A group of gliadin polypeptides encoded by one locus is called a block of gliadin components (Sozinov, 1985; Sozinov & Pperelya, 1979). Gliadin-coding loci are located on the short arms of chromosomes of homoeological groups 1 (loci Gli-A1, Gli-B1, Gli-A3, Gli-B3 or Gli-B5 in different studies) and 6 (loci Gli-A2, Gli-B2) (Payne *et al.,* 1984; Ruiz and Carrillo, 1993). Gli-1 loci code for the majority of γ-gliadins and the ω-gliadins, while the α/β and some of the γ-gliadins are encoded by the Gli-2 loci. The number of gene copies varies from 25 to 150 copies for α/β -gliadin, from 16 to 39 for γ-gliadin and from 15 to 18 for ω-gliadin (Payne *et al.,* 1984; Ruiz & Carrillo, 1993).

The genetics of gliadins has been studied extensively in common (Metakovsky, 1991; Metakovsky and Branlard, 1998; Metakovsky and Novoselskaya, 1991) and durum (Kudryavtsev *et al*., 1996; Melnikova *et al*., 2012; Melnikova *et al*., 2010; Sadigov, 2015) wheat. Gliadin alleles do not have uniform geographical distribution: certain alleles are rare or absent in some countries and are highly frequent in some others. It is possible that certain alleles have high frequencies because they provide adaptation to the particular agro-climatic conditions. In addition, the allele distribution might be a result of the historical process of national agricultures formation of or a consequence of particular breeding programs (Melnikova *et al*., 2012).

Multiple allelism at gliadin loci allows studying the relations between the allelic state of individual loci and economically important parameters of wheat. The presence of some particular gliadin alleles was shown to be correlated with some qualitative and quantitative characters of the cultivars (Sozinov, 1985).

The present study was aimed to evaluate polymorphism in gliadin-coding loci and to determine genetic relationships among 50 durum wheat cultivars and varieties of Azerbaijan.

Material and methods

Fifty genotypes of durum wheat (*Triticum durum* Desf.) listed in Table 1 were provided by National Gene Bank of Azerbaijan Genetic Resources Institute, Azerbaijan National Academy of Sciences.

Table 1. Samples of durum wheat used for analysis of gliadin electrophoretic profiles

№	Sample	Variety	№	Sample	Variety
G1	BB-FS-0151	v.leucurum	G26	BB-FS-01583	v.aegiptiacum
G2	BB-FS-0159	v. mutico leucurum	G27	BB-FS-015128	v.boeuffi
G3	BB-FS-01512	v.hordeiforme	G28	BB-FS-015144	v.apulicum
G4	BB-FS-01513	v.mutico hordeiforme	G29	BB-FS-015146	v.coerulescens
G5	BB-FS-01516	v.mutico hordeiforme	G30	BB-FS-015133	v.coerulescens
G6	BB-FS-01519	v.murciense	G31	Ag bugda	v.affine
G7	BB-FS-01520	v.murciense	G32	Sari bugda	v. hordeiforme
G8	BB-FS-01526	v.affine	G33	Qaraqilchiq	v.provinciale
G9	BB-FS-01532	v.affine	G34	Qara bugda	v. leucomelan
G10	BB-FS-01533	v.mutico affine	G35	Bozakh	v.hordeiforme

G11	BB-FS-01535	v.erythromelan	G36	Vuqar	v. leucurum
G12	BB-FS-01539	v.melanopus	G37	Arandeni	v.apulicum
G13	BB-FS-01541	v.melanopus	G38	Sherq	v.leucurum
G14	BB-FS-01543	v.mutico melanopus	G39	Khoranka	v.horanoleucurum
G15	BB-FS-01546	v.coerulescens	G40	Sevinj	v. hordeiforme
G16	BB-FS-01551	v.niloticum	G41	Jafari	v.horanoleucurum
G17	BB-FS-01556	v.obscurum	G42	Ag bugda 13	v.leucurum
G18	BB-FS-01557	v.obscurum	G43	Shirvan 3	v.affine
G19	BB-FS-01569	v.alboprovinciale	G44	Mugan	v.leucomelan
G20	BB-FS-01573	v.alexandrinum	G45	Mirbashir 50	v.leucurum
G21	BB-FS-01574	v.reichenbachii	G46	Qaraqilchıq 2	v.apulicum
G22	BB-FS-01576	v.africanum	G47	Tartar	v.provinciale
G23	BB-FS-01579	v.lybicum	G48	Barakatli 95	v.hordeiforme
G24	BB-FS-01580	v.lybicum	G49	Alinja 84	v.leucurum
G25	BB-FS-01589	v.hordeiforme	G50	Qarabag	v.provinciale

The extraction of gliadins has been performed using A-PAGE method (Acid PolyAcrilamide Gel Electrophoresis) of Bushuk and Zillman with modifications proposed by Poperelya and colleagues (Bushuk & Zillman1978; Sozinov & Poperelya 1979). Two grains (from different spikes) of each genotype were tested. Gliadins were extracted from individually ground seeds by adding 250 µL of 70% ethanol. The gel solution contained 8% acrylamide, 0.4% methylenebisacrylamide, 0.1% ascorbic acid and 0.001% $Fe_2(SO_4)_3$ x 7H2O. PAGE was carried out in a 0.005 M glycine acetate buffer solution (pH 3.1) for 4 h at a constant voltage of 450 V. Following electrophoresis, the gel was fixed with 60% trichloroacetic acid (TCA) for at least 20 min and stained overnight in a solution containing 0.04% Coomassie Brilliant Blue R-250 and 60% TCA. Langdon cultivar has been used as a standard for identification of the alleles of gliadin-coding loci.

The presence and absence of each band in the electrophoregrams obtained was coded as "1" and "0", respectively. The genetic distance and similarity were computed with the PAST software (Hammer et al., 2001). Cluster analysis was conducted based on the Jaccard similarity coefficient. The genetic diversity for each gliadin pattern was calculated according to Nei formula (1973) as $H = 1 - \Sigma Pi^2$, in which H is the genetic variation index, and Pi is the proportion of a particular pattern in each group of α, β, γ and ω-gliadins separately. The mean value of H was calculated for all the four groups of gliadins.

Results and discussion

Figure 1 shows electrophoregrams of the genotypes obtained by Acid-PAGE
method. Among the 50 genotypes analyzed, 33 different bands were detected
assuming that the bands with the same relative mobility represent the same protein.
These bands were grouped into patterns at each of the four zones of gel (α-, β-, γ-
and ω-gliadins). Each zone (α, β, γ and ω) was considered as a single locus and
different patterns as allelic variants. The patterns within each gliadin zone were
identified by comparing banding patterns of each genotype with all the other
genotypes. Table 2 presents the number of gliadin bands and patterns and the genetic
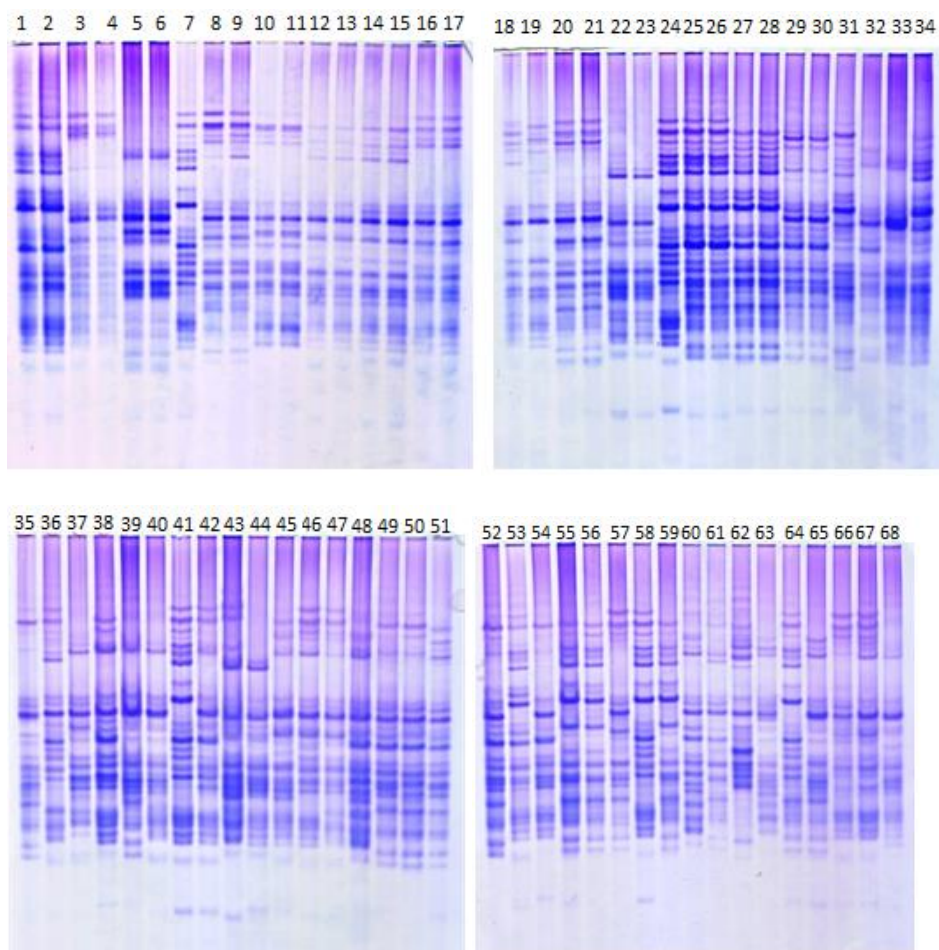diversity in gliadins for the genotypes analyzed.

Figure 1. Electophoregrams of the analyzed *Triticum durum* genotypes.

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A total of 12 different mobility bands and 40 gliadin patterns were identified in the ω-gliadin zone. Bands range from 2 to 10 in this zone. The highest frequency is characteristic of the bands ω-5 and ω-9 (58.82% and 66.67%, respectively). The lowest frequency of occurrence was observed in ω-2 band (3.92%) which has been determined only in two genotypes.

1-2 – G1, 3-4 – G2, 5-6 – G3, 7 – Langdon, 8-9 – G4, 10-11 – G5, 12-13 – G6, 14-15 – G7, 16-17 – G8, 18-19 – G9, 20-21 – G10, 22-23 – G11, 24 – Langdon, 25-26 – G12, 27-28 – G13, 29-30 – G14, 31 – G15, 32 – G16, 33 – G17, 34 – G18, 35 – G19, 36 – G20, 37 – G21, 38 – G22, 39 – G23, 40 – G24, 41 – Langdon, 42 – G25, 43 – G26, 44 – G27, 45 – G28, 46 – G29, 47 – G30, 48 – G31, 49 – G32, 50 – G33, 51 – G34, 52 – G35, 53 – G36, 54 – G37, 55 – G38, 56 – G39, 57 – G40, 58 – Langdon, 59 – G41, 60 – G42, 61 – G43, 62 – G44, 63 – G45, 64 – G46, 65 – G47, 66 – G48, 67 – G49, 68 – G50.

Table 2. Number of bands, patterns, and genetic diversity indices for gliadins in the genotypes of *T.durum*

Genotypes	Number of bands				
	ω	γ	β	α	α + β +γ + ω
1	7	6	3	6	22
2	5	4	3	3	15
3	2	6	4	4	16
4	7	5	4	2	18
5	5	6	3	4	18
6	2	3	3	2	10
7	4	3	4	3	14
8	5	4	3	2	14
9	5	2	3	2	12
10	4	4	4	5	17
11	2	2	5	3	12
12	10	4	5	6	25
13	7	4	5	5	21
14	5	4	4	4	17
15	6	5	5	5	21
16	2	1	4	4	11
17	2	3	5	2	12
18	4	3	5	2	14
19	3	4	3	4	14
20	3	4	4	2	13
21	2	4	3	5	14
22	4	5	3	2	14

23	2	3	3	3	11
24	2	3	3	4	12
25	4	2	3	3	12
26	5	3	3	4	15
27	3	3	3	5	14
28	3	2	2	3	10
29	3	3	2	3	11
30	4	3	3	3	13
31	2	3	4	6	15
32	2	3	4	2	11
33	2	3	4	5	14
34	2	2	4	3	11
35	2	4	3	4	13
36	5	3	2	2	12
37	2	3	3	5	13
38	5	2	3	3	13
39	4	3	2	2	11
40	4	2	2	2	10
41	5	3	3	2	13
42	2	3	3	2	10
43	3	3	3	3	12
44	7	3	4	4	18
45	3	3	3	1	10
46	6	3	4	2	15
47	2	2	3	3	10
48	2	3	2	2	9
49	4	3	2	3	12
50	2	2	4	2	10
Number of bands	12	8	5	8	33
Range of bands	2-10	1-6	2-5	1-6	9-25
Number of patterns	40	31	10	42	50
Genetic diversity indices	0.963	0.956	0.825	0.972	0.929

In the γ-gliadin area, 8 bands were detected, the most frequent bands were γ-5 (66.67%) and γ-6 (60.78%) which were present in 34 and 31 genotypes, respectively. γ-1 and γ-2 bands were observed only in 7.84% of the studied genotypes (four genotypes). In this zone 31 different γ-gliadin patterns were found, each γ-gliadin pattern includes from 1 to 6 bands.

The frequency of individual patterns in the ω- and γ-zones varies from 1.9% to 9.8%. Here, most of the patterns are unique, i.e. specific for a particular genotype. Five bands were determined in the β-gliadin zone. Their combination forms 10 different

patterns, and each pattern contains from 2 to 5 bands. Patterns with three β-gliadin bands dominated being present in 24 out of the 50 genotypes. In the β-zone, band 4 was detected in all genotypes, i.e. this band is monomorphic. The next most common bands are β-3 and β-5 (92.16% and 78.43%, respectively).

In the α-zone, 8 bands were determined which form 42 patterns. The frequencies of the bands vary from 13.73 (band α-2) to 60.78% (band α-5). The genetic diversity index in four zones varies from 0.825 (β-zone) to 0.972 (α-zone). Based on the analysis of the patterns, it was found that the α-zone was characterized by the greatest genetic diversity (the Nei index is 0.972), where 35 of the 42 occurring patterns were unique. The frequency of individual patterns in the α-zone varies from 1.9% to 5.9%. The α-zone is followed by the ω- and γ-gliadin zones (Nei indices are 0.963 and 0.956, respectively). The β-zone has the lowest polymorphism (the genetic diversity index is 0.825), where only one of 10 patterns is unique. The frequency of individual patterns in the β-zone changes from 1.9% to 33.3%.

This variability was higher than that the indices of diversity found in durum wheat landraces from other countries: England, Italy and France with H = 0.676, 0.754 and 0.714, respectively. High level of genetic diversity of the samples analyzed could be caused by the variation of ecological conditions and wide geographical distribution of the genotypes.

In addition, cluster analysis has been conducted based on Jaccard coefficient of similarity (Figure 2). This method of grouping divided the genotypes analyzed into four clusters.

Cluster 1 embodies 15 genotypes. High genetic similarity is observed between genotypes 17 and 18 (Jaccard index of similarity is 0.86), 12 and 13 (index of similarity is 0.84), 13 and 15 (index of similarity is 0.83). The lowest similarity in this cluster is observed between genotypes 44 and 6, 44 and 9 (the indices of similarity are equal to 0.22 and 0.2, respectively).

Cluster 2 unites the largest number of genotypes. It includes 25 genotypes. Here, the highest index of similarity is found between G 28 and G 29 (0.75), 21 and 24 (0.73). The next high index of similarity is equal to 0.71 (between genotypes 31 and 27, 31 and 33). The lowest values for similarity are observed between genotypes 40 and 47, 42 and 28 (the index of similarity is 0.18).

Cluster 3 includes 7 genotypes. G 41 demonstrates high level of similarity with genotypes 38 and 39 (the indices of similarity are 0.857 and 0.846, respectively). The lowest value of similarity in this cluster is observed between genotypes 39 and 26 (0.3).
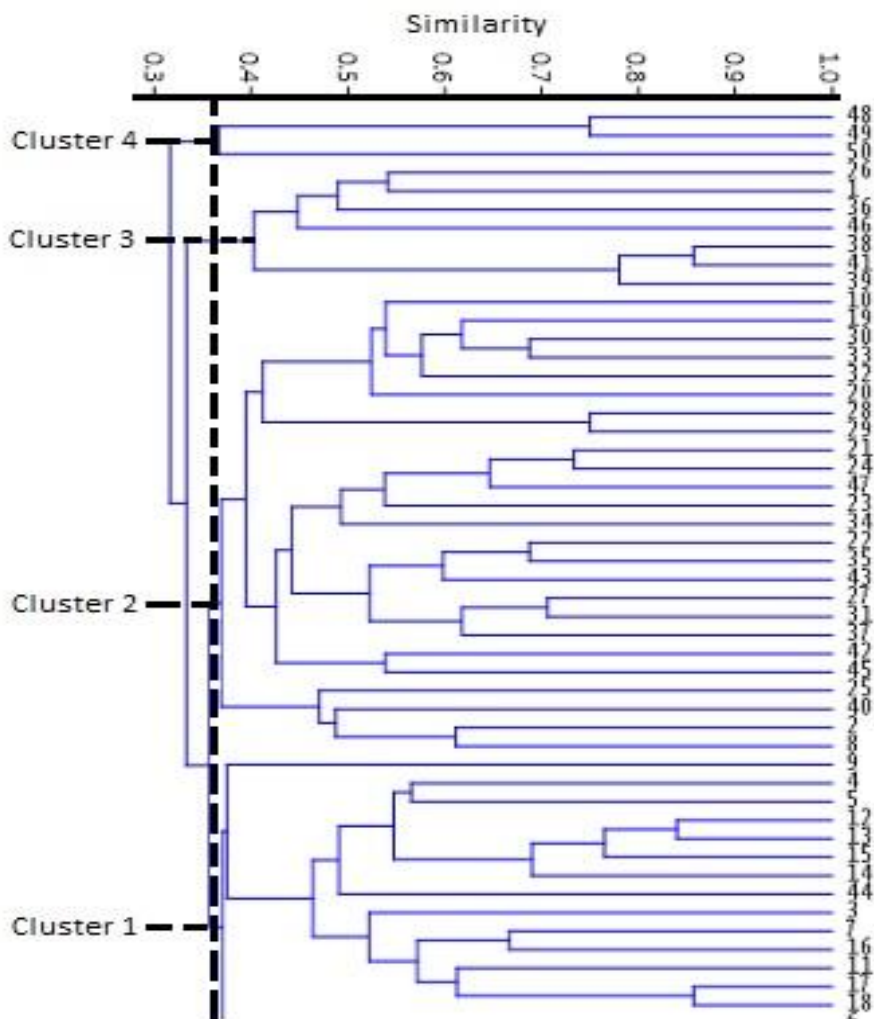
Figure 2. Clustering of the genotypes based on Jaccard coefficient of similarity

Cluster 4 consists of only three genotypes. In this cluster, the highest level of similarity (0.75) is observed between genotypes 48 and 49, while genotype 48 demonstrates the lowest index of similarity with genotype 50 (0,36).

Thus, the data obtained from analysis of electrophoretic patterns and subsequent clustering of the genotypes based on these patterns allows identification of genetically distant samples which can be used for breeding and increase of genetic variation. Because of simplicity, reproducibility and high efficiency, electrophoretic analysis of gliadins in polyacrylamide gel can be used as a powerful method for evaluation of genetic diversity.

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