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Electrophoretic Profiles of Gliadin Subunits to Evaluate Genetic Diversity of Azerbaijan Synthetic Branched Spike Wheat Accessions

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Abstract: The new branched spike forms of wheat were synthesized from the crosses between a complex wheat line 171ACS {*Aegilotriticale* [(*T. durum* Desf. × *Ae. tauschii* Coss.) × *Secale cereale* L. ssp. *segetale* Zhuk.] × *T. aestivum* L. 'Chinese Spring'} (2n = 6x = 42, AABBDD) and durum wheat variety *T. durum* Desf. 'Bereketli-95' (2n = 4x = 28, AABB) and also between a dwarf line 237KACS (*Aegilotriticale* × *T. aestivum* L. 'Chinese Spring') (2n = 6x = 42, AABBDD) and durum wheat variety *T. durum* Desf. 'Caerulescens'. This branched spike forms are distinguished significantly from each other and the other branched spike forms known so far. This study was aimed to analyze the genetic variability of these novel branched spike lines and the other branched spike forms known so far comparatively using the acid-PAGE method. Sixty two polymorphic bands and 69 patterns were identified. Twenty four different motility bands and 24 patterns were found in the ω gliadin region with 10 polymorph bands and 18 patterns for γ-gliadins and 13 bands and 14 different patterns for β-gliadins and 15 bands and 13 patterns for α-gliadins. The combination of these patterns generated 36 and 28 combinations for *Gli-1* and *Gli-2* loci, respectively. The genetic diversity index (H) was higher for ω-gliadins (0.871), followed by γ- and β-gliadins (0.788 and 0.736, respectively) and for α-gliadin patterns (0.593). Extensive polymorphism (H = 0.747) was observed in four gliadin pattern regions for Azerbaijan branched spike wheat accessions. Each genotype had special identifying patterns in the gliadin acid-PAGE analysis and cluster analysis based on Jaccard's similarity coefficients formed eight groups. Because of simple, repeatable and economic analysis, electrophoretic fractionation of gliadins on polyacrylamide gel, it can be used as a powerful method for identifying varieties with similar genotypes.

Key words: Branched spike wheat · Acid-PAGE · Gliadin patterns · Genetic diversity

INTRODUCTION

Genetic diversity has played a vital role in the success of crop improvement. Knowledge of genetic diversity has been successfully used for efficient germplasm management and utilization, genetic fingerprinting and genotype selection [1, 2]. However, there are many methods for estimating genetic diversity. Morphological traits (syn. Phenotypic traits) are commonly used to analyze genetic diversity since they provide a simple way of quantifying genetic variation while assessing genotype performance under normal growing environments. However, morphological traits are limited in number, modified by the environment and may be controlled by epistatic and pleiotropic gene effects [3]. The gliadin protein markers, as primary products of gene expression, are not affected by the plant growth environment and can reveal small changes (e.g. mutations) in accessible to visual examinations. Gliadin alleles are inherited co-dominantly, they have revealed large levels of inter-varietal polymorphism and identification of a genotype is possible immediately by the electrophoretic protein phenotype [4]. Gliadins that are controlled by clusters of linked co-dominant genes located on the short arms of homoeologous group-1 and group-6 chromosomes [5], have so far received little attention. Each cluster codes for a number of proteins (a block) that is inherited as a Mendelian unit [6]. The *Gli-A1* locus, located on the short arm of chromosome 1A, usually controls the synthesis of ω-, γ- and some slow-moving β-gliadins, whereas the *Gli-A2* locus, on the short arm of chromosome 6A, codes mainly for components present in the α region and in some cases, also for one fast-moving β-gliadin [5-7].

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Because of the high levels of polymorphism shown by these proteins, they are useful markers for assessing genetic variation [8] and for genotype identification in wheat species [9, 10].

The main goal of this study was to evaluate genetic diversity of branched spike wheat accessions (*Triticum aestivum* L.) on the basis of their seed storage gliadins by the acid-PAGE method.

MATERIALS AND METHODS

In this analyze 68 wheat accessions obtained from the Genetic Resources Institute, Azerbaijan National Academy of Sciences were evaluated (Table 1). As research materials were used the three accessions of H. Kihara's amphidiploids (AD) produced by crossings of *T. durum* Desf. (2n=28) with *Ae. tauschii* Coss. (2n=14) and having the catalog numbers as k-45918, k-45922 and k-47895 (1-3, respectively; 2n=42), a trigeneric partial amphidiploid or *Aegilotriticale* (4; 2n=42) obtained by Dr Aminov from the hybridization between Kihara's amphidiploid and a rye, *Secale cereale* ssp. *segetale* Zhuk. (5; 2n=14), a soft wheat cultivar *T. aestivum* 'Chinese Spring' (6; 2n=42), a complex wheat line 171ACS derived from the crossing between *Aegilotriticale* and *T. aestivum* 'Chinese Spring' (7; 2n=42), a durum wheat cultivar *T. durum* 'Bereketli-95' (8; 2n=28), the reciprocal hybrids of the first generations obtained from the reciprocal crossings between *T. durum* 'Bereketli-95' and a line 171ACS (9-10; 2n=28-30), three branched spike wheat species as *T. vavilovii* Jakubz. (11; 2n=42), *T. jakubzineri* Udacz. et Schachm. (2n=28) and *T. turgidum* L. (12-13; 2n=28), forty branched spike lines (Figure 1) derived from the crossing between *T. durum* 'Bereketli-95' and a complex line 171ACS (14-53; 2n=28), four normal spiked durum wheat varieties (54-57; 2n=28), one branched spike durum wheat variety (58; 2n=28) and ten branched spike lines (Figure 2) produced by crossing of durum wheat variety (*T. durum* 'Caerulescens') with a dwarf wheat line 237KACS (2n=42) which was obtained from the crossing between *Aegilotriticale* and *T. aestivum* 'Chinese Spring' previously (59-68; 2n=28).

Gliadin patterns were characterized according to the A-PAGE method of Zillman and Bushuk [11] modified by Poperelya [12]. Seeds were individually ground and extracted overnight at room temperature with 0.2 mL of a solution containing 0.9% acetic acid, 18% urea and 0.01% pyronine. Electrophoresis was done at 450 V and 0.16 A for 5 h.

The strategy was followed for all the cultivars and large numbers of different patterns were identified in each group of gliadins (α, β, γ and ω). The exercise was repeated five times to confirm the pattern of genotypes within each group. Since Bezostaya and Anza cultivars were used as checks in each gel, comparison of bond pattern among different genotypes was easy. Homogeneity of each genotype was determined by extracting gliadins from five individual seeds from each sample. The genetic diversity for each gliadin pattern was calculated as per Nei [13] as $H = 1 - \Sigma Pi^2$, in which H is the genetic variation index and $\tilde{N}_i$ 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. The genetic distance and similarity were computed with the SPSS software [14]. Cluster analysis was done based on the Jaccard's similarity coefficients.

Fig. 1: Branched ear lines derived from crossing between *T. durum* 'Bereketli-95' and a complex line 171ACS - a source of a novel branchiness in tetraploid wheats

Table 1: Wheat accessions and their identified α, β, γ and ω gliadin patterns.

Nº Genotypes	Gliadin pattern			
	ω	γ	B	α
1 AD (*T. durum* × *Ae. tauschii*) k-45918	19	1	8	6
2 AD (*T. durum* × *Ae. tauschii*) k-45922	20	16	11	11
3 AD (*T. durum* × *Ae. tauschii*) k-47895	21	1	12	12
4 *Aegilotriticale* [(*T. durum* × *Ae. tauschii*) × *Secale cereale* subsp. *segetale*]	22	12	10	4
5 *S. cereale* subsp. *Segetale*	24	18	14	13
6 *T. aestivum* ‘Chinese Spring’	23	2	6	1
7 171ACS (*Aegilotriticale* × *T. aestivum* ‘Ch. Spring’)	18	15	5	9
8 *T. durum* ‘Bereketli-95’	6	5	7	4
9 *T. durum* ‘Bereketli-95’ × 171ACS	5	4	7	1
10 171ACS × *T. durum* ‘Bereketli-95’	6	4	7	1
11 *T. vavilovii* (branched spike)	1	1	1	1
12 *T. jakubzineri* (branched spike)	2	2	2	2
13 *T. turgidum* (branched spike)	3	2	3	1
14 (‘Bereketli-95’ × 171ACS) branched spike line (2n=28)	4	3	5	3
15 (‘Bereketli-95’ × 171ACS) branched spike line (2n=28)	5	4	3	4
16 (‘Bereketli-95’ × 171ACS) branched spike line (2n=28)	5	4	3	4
17 (‘Bereketli-95’ × 171ACS) branched spike line (2n=28)	5	4	3	4
18 (‘Bereketli-95’ × 171ACS) branched spike line (2n=28)	6	5	7	3
19 (‘Bereketli-95’ × 171ACS) branched spike line (2n=28)	6	5	4	3
20 (‘Bereketli-95’ × 171ACS) branched spike line (2n=28)	7	5	7	4
21 (‘Bereketli-95’ × 171ACS) branched spike line (2n=28)	8	5	4	4
22 (‘Bereketli-95’ × 171ACS) branched spike line (2n=28)	6	5	4	4
23 (‘Bereketli-95’ × 171ACS) branched spike line (2n=28)	13	5	4	3
24 (‘Bereketli-95’ × 171ACS) branched spike line (2n=28)	5	5	4	2
25 (‘Bereketli-95’ × 171ACS) branched spike line (2n=28)	10	5	4	4
26 (‘Bereketli-95’ × 171ACS) branched spike line (2n=28)	10	5	4	4
27 (‘Bereketli-95’ × 171ACS) branched spike line (2n=28)	6	5	4	4
28 (‘Bereketli-95’ × 171ACS) branched spike line (2n=28)	6	4	3	4
29 (‘Bereketli-95’ × 171ACS) branched spike line (2n=28)	6	5	7	4
30 (‘Bereketli-95’ × 171ACS) branched spike line (2n=28)	6	5	7	4
31 (‘Bereketli-95’ × 171ACS) branched spike line (2n=28)	7	13	9	4
32 (‘Bereketli-95’ × 171ACS) branched spike line (2n=28)	11	6	9	4
33 (‘Bereketli-95’ × 171ACS) branched spike line (2n=28)	12	5	4	4
34 (‘Bereketli-95’ × 171ACS) branched spike line (2n=28)	6	5	4	3
35 (‘Bereketli-95’ × 171ACS) branched spike line (2n=28)	6	4	13	3
36 (‘Bereketli-95’ × 171ACS) branched spike line (2n=28)	5	4	13	4
37 (‘Bereketli-95’ × 171ACS) branched spike line (2n=28)	9	4	13	4
38 (‘Bereketli-95’ × 171ACS) branched spike line (2n=28)	5	4	13	4
39 (‘Bereketli-95’ × 171ACS) branched spike line (2n=28)	5	4	13	4
40 (‘Bereketli-95’ × 171ACS) branched spike line (2n=28)	5	4	13	4
41 (‘Bereketli-95’ × 171ACS) branched spike line (2n=28)	5	4	13	4
42 (‘Bereketli-95’ × 171ACS) branched spike line (2n=28)	14	5	13	4
43 (‘Bereketli-95’ × 171ACS) branched spike line (2n=28)	14	5	13	4
44 (‘Bereketli-95’ × 171ACS) branched spike line (2n=28)	14	5	13	4
45 (‘Bereketli-95’ × 171ACS) branched spike line (2n=28)	15	5	13	4
46 (‘Bereketli-95’ × 171ACS) branched spike line (2n=28)	5	4	13	4
47 (‘Bereketli-95’ × 171ACS) branched spike line (2n=28)	16	5	13	3
48 (‘Bereketli-95’ × 171ACS) branched spike line (2n=28)	17	5	13	3
49 (‘Bereketli-95’ × 171ACS) branched spike line (2n=28)	15	5	13	3
50 (‘Bereketli-95’ × 171ACS) branched spike line (2n=28)	5	4	13	4
51 (‘Bereketli-95’ × 171ACS) branched spike line (2n=28)	15	5	13	4
52 (‘Bereketli-95’ × 171ACS) branched spike line (2n=28)	6	7	5	4
53 (‘Bereketli-95’ × 171ACS) branched spike line (2n=28)	6	8	7	4
54 *T. durum* var. *hordeiforme* (normal spiked)	6	5	5	6
55 *T. durum* var. *affine* (normal spiked)	12	9	3	7
56 *T. durum* var. *affine* (normal spiked)	12	14	3	7
57 *T. durum* var. *leucurum* (normal spiked)	6	10	3	5
58 *T. durum* var. *leucurum* (branched spike)	6	11	5	4
59. *T. durum* cv. ‘*Caerulescens*’ × 237KACS (*Aegilotriticale* × *T. aestivum* ‘Chinese Spring’) branched spike line (2n=28)	11	6	3	7
60. *T. durum* cv. ‘*Caerulescens*’ × 237KACS (*Aegilotriticale* × *T. aestivum* ‘Chinese Spring’) branched spike line (2n=28)	11	6	3	7
61 *T. durum* cv. ‘*Caerulescens*’ × 237KACS (*Aegilotriticale* × *T. aestivum* ‘Chinese Spring’) branched spike line (2n=28)	11	6	3	7
62 *T. durum* cv. ‘*Caerulescens*’ × 237KACS (*Aegilotriticale* × *T. aestivum* ‘Chinese Spring’) branched spike line (2n=28)	11	6	3	8
63 *T. durum* cv. ‘*Caerulescens*’ × 237KACS (*Aegilotriticale* × *T. aestivum* ‘Chinese Spring’) branched spike line (2n=28)	11	6	3	4
64 *T. durum* cv. ‘*Caerulescens*’ × 237KACS (*Aegilotriticale* × *T. aestivum* ‘Chinese Spring’) branched spike line (2n=28)	11	6	3	6
65 *T. durum* cv. ‘*Caerulescens*’ × 237KACS (*Aegilotriticale* × *T. aestivum* ‘Chinese Spring’) branched spike line (2n=28)	11	6	3	4
66 *T. durum* cv. ‘*Caerulescens*’ × 237KACS (*Aegilotriticale* × *T. aestivum* ‘Chinese Spring’) branched spike line (2n=28)	11	17	3	9
67 *T. durum* cv. ‘*Caerulescens*’ × 237KACS (*Aegilotriticale* × *T. aestivum* ‘Chinese Spring’) branched spike line (2n=28)	11	6	3	4
68 *T. durum* cv. ‘*Caerulescens*’ × 237KACS (*Aegilotriticale* × *T. aestivum* ‘Chinese Spring’) branched spike line (2n=28)	11	6	3	10

Fig. 2: Branched ear lines produced by crossing *T. durum* 'Caerulescens' with a dwarf wheat line 237KACS (Aegilotriticale × *T. aestivum* 'Caerulescens').

RESULTS AND DISCUSSIONS

Up to 62 different bands were detected assuming that the bands with the same relative mobility represent the same subunit.

Each zone (α, β, γ and ω) was considered as a single locus and the different patterns as allelic variants. The patterns within each gliadin group of α, β, γ and ω were identified by comparing banding patterns of each variety with all the other varieties and a specific number was assigned to each of the patterns as per the modified method of Masouleh [15]. Figure 3 shows representative examples of variation detected using the acid-PAGE gel system.

Among the genotypes studied, 69 different patterns were observed (Table 1). In the ω region, 24 different mobility bands and 24 gliadin patterns were identified. Ideograms of all the different patterns observed are presented in Figure 4. In this area, the most frequent bands were: 12, 14 and 19 (with 98.53% and 97.06%, respectively), whereas ω-gliadin bands 1, 2, 3, 4 and 24 every one was observed in only one genotype. Among the patterns in this region, 6, 5 and 11 ω gliadin patterns had high frequency between studied genotypes. The ω-6, ω-5 and ω-11 patterns were observed in 16, 12 and 11 genotypes, respectively. In this study, the ω gliadin patterns 12, 14, 15 were present in 4.41% genotypes and the ω-gliadin patterns 7 and 10 in 2 genotypes (2.94%).

In total, 10 bands and 18 different patterns were detected in the region of γ. The γ-gliadin bands 5, 10 and 3 had the highest frequencies, whereas γ-gliadin band were studied only 3 genotypes. The most frequent patterns were: 10, 14 and 25 in the region of γ-gliadin. The γ-gliadin patterns 1 and 2 were present in two genotypes, whereas other patterns in this region were observed in one genotype.

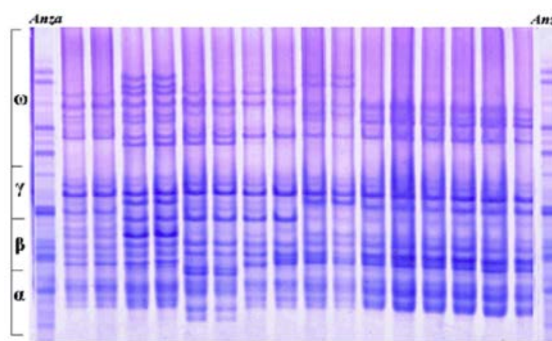

Fig. 3: Electrophoretic patterns of gliadin subunits in wheat accessions.

In the β region 13 bands were observed, of which band 7 (with 95.59%) and 3 (with 80.88%) had the highest frequencies. The lowest frequency band was gliadin band 1, which was detected only in one genotype. In this region gliadin band 5 was monomorph. In the present study β-patterns 13, 3 and 9 were high frequent. The α-gliadin patterns 4, 3, 7 were present in 37, 9 and 5 genotypes, respectively.

The ideogram showed higher variation in the ω-, γ- and β-gliadins than in the α-gliadin (Figure 4). This may be either due to greater staining intensity of the α-gliadin, or the separation of these proteins may not be complete in a 1-D electrophoresis system [16]. Although care was taken to separate all the bands, more than one protein may be present in a band in the region. Since α- and β-gliadins are controlled by genes present on six homological groups and γ and ω gliadins on only one homological group, there may be less polymorphism in gliadins synthesized by the six homological groups, though this should be verified by further genetic studies. Caballero *et al*. [17] and Tanaka *et al*. [18] also reported larger variation in γ- and ω-gliadins than in α- and β-gliadins.

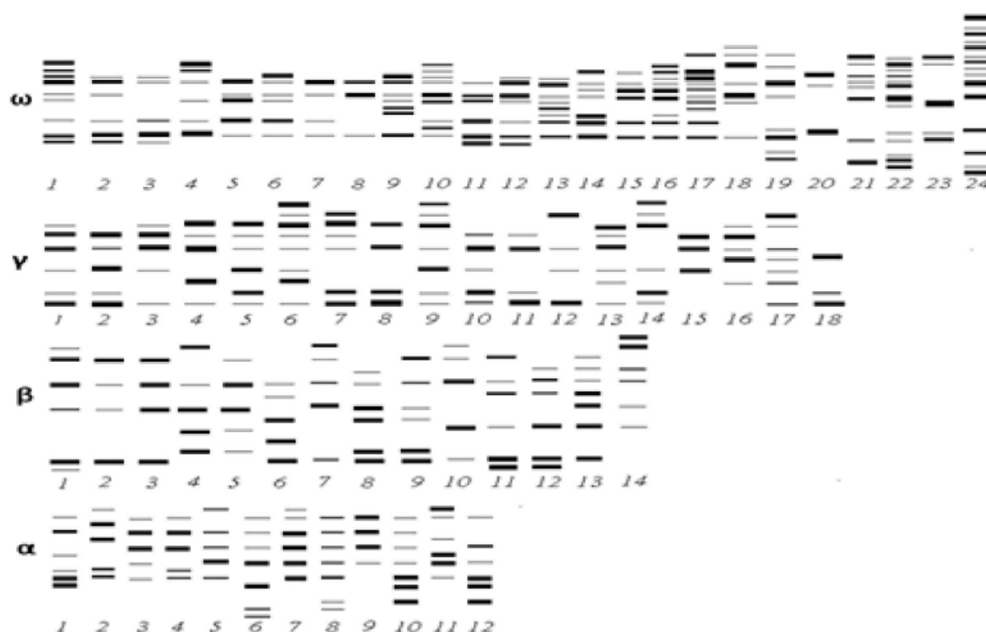

Fig. 4: Ideograms of different gliadins patterns in the α, β, γ and ω regions

Most of γ and ω gliadin genes are located at the *Gli-1* loci [19]. The combination of their different patterns would give different allelic variation for these loci. In our study, we found 36 different combinations. The combination formed by pattern 5 of the ω-gliadins and pattern 4 of the γ-gliadins were most frequent, followed by the combination of patterns γ -6 and ω -11 which appeared in ten genotypes. Thirty-seven combinations were only detected in one accession.

The gene coding for most α- and β-gliadins occur on the *Gli-2* loci [19]. In the present study, 28 different combinations of these gliadins were detected, with the most frequent being pattern 4 of the β-gliadins and pattern 13 of the α-gliadins found in 8.84% accessions. Harsch *et al*. [20] only found 30 different bands when analyzing sixteen spelt cultivars using densitometry methods, whereas Romanova *et al*. [21] detected up to 116 different patterns in 170 lines of spelt.

Seventeen patterns for the ω zone were classified as rare, three with wide distribution appearing in at most two landraces and thirteen that occurred in only one accession each. For the γ zone, two rare and thirteen very rare patterns were found, all with a wide distribution. For the β zone, three rare and eight very rare patterns were found, all with a wide distribution. All of the α-gliadins patterns were rare or very rare, except for the 3 and 4. This finding concurs with the work by Caballero *et al*. [17] and with Spanish spelt wheat accessions.

The variation found in the analyzed gliadin patterns was higher than in the gliadin bands. Sewa *et al*. [16] reported 45 different gliadin bands in 157 Indian wheat genotypes, but they found 147 patterns. Harsch *et al*. [20] found only 30 different bands, when analyzing sixteen spelt cultivars using densitometry methods, whereas Caballero *et al*. [17] detected up to 72 different bands in 403 lines of spelt wheat accessions.

The genetic diversity (H) based on gliadin patterns, observed in this study, was higher (H = 0.747) than in other countries: France H = 0.714 [22]; England and the former Yugoslavia with H = 0.676 and 0.728, respectively [23].

In this study, using Nei's formula the genetic diversity based on the patterns observed was calculated for each of the 4 regions and the ω region proved to have the highest diversity (H = 0.871), followed by γ (H = 0.788) and β (H = 0.736) and the lowest diversity was that of the α region (H = 0.593).

The genotype grouping can be seen in Figure 5. Cluster analysis of gliadin bands using the UPGMA method, as well as Jaccard's similarity coefficients, classified all the genotypes into 8 main groups. Each of clusters 1, 2, 3, 4, 5 and 6 included only one genotype, which these genotypes had specific patterns. Cluster 7 included accessions 13, 11, 10 and 9 which had pattern 1 in the α zone.

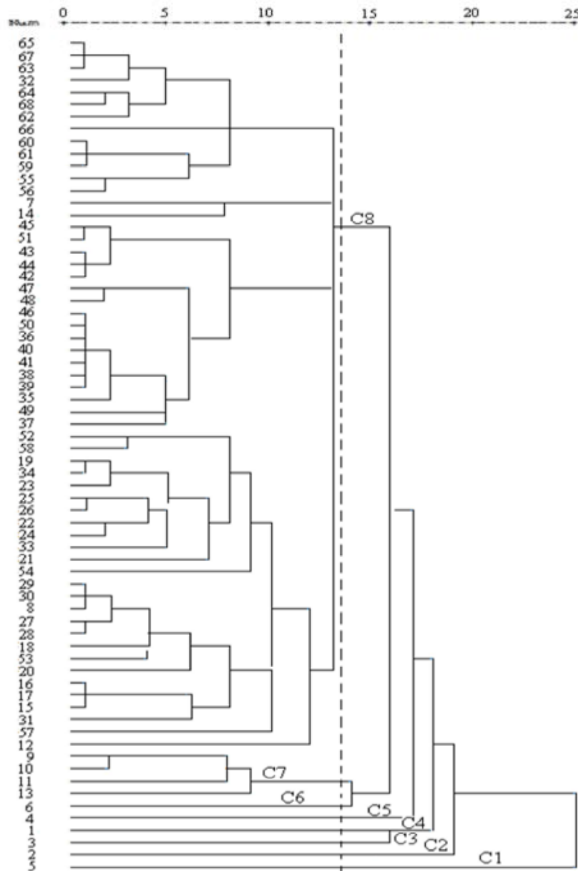

Fig. 5: Dendrogram of wheat accessions from Azerbaijan based on Jaccard´s similarity index, with the six formed clusters (C).

Most of the genotypes grouped in cluster 8. All of the genotypes in this group are branched spike wheat. The highest similarity index was observed between genotypes 63 and 67, with a similarity coefficient of 0.85, followed by genotypes 64 and 68, with a similarity coefficient of 0.81. The lowest similarity index was observed between genotypes 5 and 45, with a similarity coefficient of 0.13. Mean similarity for the total matrix for all genotypes was 0.473. Wegrzyn and Waga [24] evaluated the relationships among 45 cultivars and strains of winter wheat by gliadin and glutenin protein polymorphisms. They found that the cluster analysis showed a considerable variation of the investigated genotypes and the genetic distances between the cultivars ranged from 0.12 to 1.00.

Although many of the genotypes included in the present study had similar parents, gliadin electrophoretic profiles showed that there was considerable diversity among them. Xu *et al*. [25] reported that higher levels of gliadin variation existed in *Triticum turanicum*. Gliadins show the highest level of polymorphism when studied by standard method of acidic electrophoresis [11] and have proved to be useful markers for assessing genetic variation and for genotype identification in different wheat species. The gliadin pattern of wheat genotypes is not affected by the environment and gliadin markers are a simple, inexpensive and powerful tool [11] for assessing the genetic resources of wheat.

From the result of this study it is concluded that the electrophoresis of seed storage proteins used for variety fingerprinting and that methods are reliable, simple, repeatable and economic procedure. It can be utilized by wheat breeders to detect variability among wheat genotypes to identify new sources of variation that could be used in crop improvement programs.

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