Bulgarian Journal of Agricultural Science, 24 (No 6) 2018, 1034–1041

Comparative analysis of genetic diversity of bread wheat genotypes based on protein and DNA markers

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Abstract

Huseynova, E., Gaziev, A., Abbasov, M., Babayeva, S., Haciyev, E., Karimov, A., Sadiqov, H., Rustamov, Kh., & Akparov, Z. (2018). Comparative analysis of genetic diversity of bread wheat genotypes based on protein and DNA markers. *Bulgarian Journal of Agricultural Science*, *24*(6), 1034–1041

Genetic diversity of 32 local and 23 introduced bread wheat (*Triticum aestivum* L.) genotypes was studied using protein and inter simple sequence repeat (ISSR) markers. A total of 26 bands were amplified using 5 ISSR primers. The number of polymorphic bands varied from 1 to 3, averaged 2.2. The average genetic diversity for the whole bread wheat collection based on ISSR data was 0.57. Polymorphism rate of protein markers were superior to ISSR markers. The highest number of patterns was noted at α and ω sections, with 44 and 42 patterns, respectively. Genetic diversity index was high for all studied sections. The maximum diversity was observed at α section (0.968), followed by ω section (0.963). Mantel test did not reveal significant correlation between dendrograms created, based on protein and DNA markers, suggesting that both set of markers explore genetic variation differently. In the ISSR dendrogram some tendencies were observed in grouping of genotypes according to their botanical varieties, while no relationship was found between grouping of genotypes and their origin or botanical variety based on protein markers.

Keywords: wheat; gliadin; protein markers; ISSR markers; genetic diversity

Introduction

The rapid growth of the world population and living standards, acceleration of urbanization processes and climatic changes increase demand for food products all over the world. Wheat constitutes a fundamental nutrient source for about 35% of the world population. Therefore, an improvement of wheat productivity and quality has been considered as a priority all over the world (Bhakti et al., 2009; Khan et al., 2010).

Bread wheat (*Triticum aestivum* L.) is an allohexaploid species (2n = 6x = 42, AABBDD) grown in more than 70 countries of the world and maintains one of the leading places in the food chain (Ray, 2013). In Azerbaijan cultivation of wheat has a long history. A wide range of soil and climatic conditions contributed to the development of rich vegetation, this in turn made possible to consider Azerbaijan as one of the most probable centers of wheat origin. The wheat landraces and their wild relatives spread throughout the country have been well studied and their future conservation and utilization in breeding programs have been provided (Aliyev and Akparov, 2002; Mammadov et al., 2006). In addition to local landraces and breeding varieties, new genetic sources are also introduced to the wheat areas. Effective use of these genetic resources and

hybridization of varieties that are genetically far-reaching are crucial for the proper construction of breeding strategies. Information on genetic diversity and relationships between cultivars are first steps to facilitate their utilization in breeding programs (Soriano et al., 2016). Various markers, including morphological, protein-based, and DNA-based markers have been used to detect genetic diversity in wheat (Sadigov, 1994; Akparov and Mammadov, 2007; Aliyev et al., 2007; Abbasov et al., 2018). Application of more than one marker method in a reliable manner is highly recommended for determination of intra-specific genetic diversity (Jingura and Kamusoko, 2015). Among biochemical markers, seed stock proteins have been widely used due to its simplicity and effectiveness for estimating genetic diversity in wheat (Shuaib et al., 2010; Nemati et al., 2012). Since the 1990s, DNA-based markers have become common tools in genetic evaluation (Jingura and Kamusoko, 2015). The inter simple sequence repeat (ISSR) technique is a PCR based method that is highly effective in plant fingerprinting and phylogenetics studies (Izzatullayeva et al., 2014; Hajiyev et al., 2015; Hasanova et al., 2017; Hajiyeva et al., 2018). ISSR analysis involves amplification of regions between adjacent and inversely oriented microsatellites using di-, tri-, tetra- and pentanucleotide SSR primers, with the advantage that knowledge of the DNA sequence of the target regions is not needed.

The main objective of the current study was to study the genetic diversity of 55 local and introduced bread wheat genotypes based on protein and ISSR markers.

Materials and methods

Plant material

A total of 55 bread wheat genotypes were used in this study (Table 1). Thirty-two of them are local varieties

Table 1. Bread wheat varieties used in the research

No.	Variety	Origin*	No.	Variety	Origin*
1	Gobustan	AZB	29	Aran	AZB
2	Bol bughda	AZB	30	Yegana	AZB
3	Arzu	AZB	31	Tale 38	AZB
4	Birlik	AZB	32	Murov 2	AZB
5	Gurgana 1	AZB	33	Gırmızı gul 1	AZB
6	Garabagh	AZB	34	Starshina	RUS
7	Bezostaja-1	RUS	35	CO970547-7	USA
8	Anza	–	36	Zubkov	KYR
9	Zardabi	RUS	37	MV 06-02	HU
10	Parzivan 1	AZB	38	Gerek	TR
11	Parzivan 2	AZB	39	Gloriya	RO
12	Grekum 75/50	AZB	40	TX96V2847	USA
13	Durdana	AZB	41	Arlin/Yuma	USA
14	Mirbashir 128	AZB	42	MV Dalma	HU
15	Taraggi	AZB	43	Destin	RO
16	Azeri	AZB	44	Dyuopebusa	MOL
17	Akinchi 84	AZB	45	OK00421	USA
18	Giymetli 2/17	AZB	46	Altai	TR
19	Zirve 85	AZB	47	Mima	BG
20	Nurlu 99	AZB	48	LC927/Petja	BG
21	Azametli 95	AZB	49	Sonmez	TR
22	Sheki 1	AZB	50	Steklovidnaya 24	KAZ
23	Ruzi 84	AZB	51	Dalnitskaya	UKR
24	Guneshli	AZB	52	Vita	RUS
25	Shafag	AZB	53	SG-S1915	CZ
26	Saba	AZB	54	Karahan	TR
27	Shafag 2	AZB	55	U1254-7-9-2-1/ TX86A5616//Rina-6	USA
28	Ugur	AZB			

*AZB – Azerbaijan, RUS – Russia, KYR – Kyrgyzstan, HU – Hungary, TR – Turkey, RO – Romania, MOL – Moldova, BG – Belgium, KAZ – Kazakhstan, UKR – Ukraine, CZ – Czech

kindly provided by Genetic Resources Institute of Azerbaijan National Academy of Science and 23 wheat varieties were obtained from CIMMYT (International Center for Corn and Wheat Improvement).

Protein marker analysis

For protein marker analysis two grains of each genotype were used. Storage proteins were extracted with 70% ethanol from a whole wheat grain. One-dimensional polyacrylamide gel (8%) electrophoresis (A-PAGE) was done according to the modified method of Poperelya (1989). PAGE was carried out in a 0.005 M glysine asetat buffer solution (pH 3.1) for 4 h at a constant voltage of 450 V. After the electrophoresis, the gel was fixed with 10% trichloroacetic acid (TCA) for at least 20 min and stained overnight in a solution containing 0.04% Coumassie R-250 and 10% TCA. The allelic variants of gliadin component blocks were identified and designated according to the catalogue compiled earlier (Kudryavchev, 1994; Sadigov, 1994).

ISSR analysis

Genomic DNA was extracted from fresh leaves using CTAB protocol by Doyle and Doyle (1987). PCR reactions for ISSR primers were performed in a 20 μL, containing 2 μL 10x PCR buffer; 2 μL mixture dNTP (5 mM); 1.5 μL $MgCl_2$ (50mM); 2 μL of each primer (15 pmol/μL); 0.1 μL of Taq-polymerase enzyme (1 U/μL) and 2 μL of extracted DNA (50 ng/μL). The Thermal Cycler (Applied Biosystems, USA) was programmed as: pre-denaturation at 94°C for 5 minutes; 35 cycles of – denaturation at 94°C for 1 min, annealing for 45 seconds (temperature depended on the primer used), elongation for 5 minutes at 72°C; the final elongation at 72° C for 10 minutes. PCR products were analyzed by 1.5% agarose gel electrophoresis, following ethidium bromide staining and visualized under UV light using gel documentation system BioRad.

The genetic diversity index (GDI) (Weir, 1990), polymorphism information content (PIC) (Roldan-Ruiz et al., 2000), effective multiplex ratio (EMR), marker index (MI) (Powell et al., 1996), resolution power (RP) and mean resolution power (MRP) (Prevost and Wilkinson, 1999) were calculated for the analyses.

ISSR and protein data were presented in a matrix form of binary (1/0) data. DARwin version 6 software (Perrier and Jacquemoud-Collet, 2006) was used for dendrogram creation with unweighted UNJ tree. Mantel test (Mantel, 1967) was performed to study correlation between dendrograms generated by ISSR and protein data.

Results and discussion

ISSR analysis

Exploiting the variability of wheat landraces and varieties requires previous knowledge of their genetic diversity. Genetic diversity and relationships among 55 bread wheat accessions from different countries was studied using inter simple sequence repeat (ISSR) marker system. The 5 ISSR primers generated a total of 26 bands, with an average of 5.2 bands per primer. The size of the amplicons was in the range of 120-2000 bp. Out of 26 bands 11 were polymorphic (Table 2). The number of polymorphic bands varied from 1 to 3, averaged 2.2. This was similar to Nagaoka and Ogihara (1997) who revealed 3.7 polymorphic bands per ISSR primer and lower than Carvalho et al. (2009) who reported 12.9 polymorphic bands per primer using 18 ISSR primers in 48 wheat accessions. Maximum number of polymorphic bands was scored by IS08 and IS11, while least number of bands was shown by IS18. The present study has shown that % of DNA polymorphism of bread wheat accessions varied between 25 and 60% with mean of 42.2%. The maximum value was noted for primer IS11. Similarly, Altintas et al. (2008) observed 47% polymorphism among 22 bread wheat cultivars using five AFLP and three SAMPL primer pairs.

The marker performance of used ISSR primes were assessed through evaluation of five parameters: PIC, EMR, RP, MRP and MI. The PIC values for the five ISSR primers varied from 0.15 to 0.40 with an average of 0.29. The lowest and highest PIC indices were recorded for primer IS08

Table 2. Polymorphism and genetic diversity parameters of bread wheat genotypes based on ISSR markers

Primer name	Primer sequence 5′~3′	Number of bands	Number of polymorphic bands	Polymorphism, %	GDI	PIC	EMR	MRP	RP	MI
IS08	$GTC(ACC)_6C$	7	3	43	0.64	0.40	1.29	0.42	1.28	0.52
IS10	$(AG)_9C$	4	2	50	0.51	0.28	1.00	0.20	0.80	0.28
IS11	$(AGC)_6G$	5	3	60	0.61	0.35	1.80	0.35	1.06	0.45
IS15	$(AC)_8CG$	6	2	33	0.62	0.27	0.66	0.55	1.10	0.18
IS18	$(GTG)_5$	4	1	25	0.49	0.15	0.25	0.23	0.92	0.04
Mean		5.2	2.2	42.2	0.57	0.29	1.00	0.35	1.03	0.29
Total		26	11							

and IS18, respectively. The range of effective multiplex ratio, which depends on the fraction of polymorphic loci, was 0.25-1.80. The mean EMR for all the primers was 1.0. The primers with higher polymorphism rate had higher EMR values. Thus, primers IS11, IS10 and IS08 with higher polymorphism were also characterized with higher EMR values. The MI values of primers used in the current study ranged between 0.04 and 0.52, the mean was 0.29. The maximum value was observed for the primer IS08 and the minimum for IS18. In the study of 30 wheat accessions using 10 ISSR primers Najaphy et al. (2011) recorded high EMR and MI values which was varied from 1.8 to 12 and from 0.41–3.36, respectively. RP index provides a moderately accurate estimate of the number of genotypes identified by a primer. The average RP and MRP in the current study was 1.03 and 0.35 per primer, respectively. Najaphy et al. (2011) were noted high positive correlation between RP and the number of total bands. The same tendency was also observed in our study; the primers with highest amplicon number (IS08 and IS15) had highest RP values.

The Weir genetic diversity index was further calculated which varied from 0.49 to 0.64. The average genetic diversity for the whole bread wheat collection was 0.57. The moderate and higher values of GDI for the ISSR primers obtained in the current study could be attributed to the diverse nature of the used wheat accessions. The highest GDI was recorded for the primer IS08 followed by IS15 and the lowest value was scored with the primer IS18.

A dissimilarity matrix based on the ISSR fragments was used to establish the level of relatedness among bread wheat genotypes. Pair-wise estimates of dissimilarity coefficient (GD) ranged from 0.05 to 0.67 with an average of 0.36. Four main clusters were detected in the dendrogram generated based on ISSR dissimilarity matrix (Fig. 1).

Most of the studied wheat genotypes (21) were grouped in the Cluster 1, majority (67%) of which was local and seven were introduced genotypes. The Cluster 2 was the most diverse and contained *T. aestivum* genotypes from seven different countries, including Azerbaijan. Out of 32 local varieties only five fell into Cluster 2. These were local varieties mainly released during the 90s years. Three of four Turkish genotypes also placed in Cluster 2. Clusters 3 and 4 mainly contained local genotypes, while introduced varieties constituted homogeneous subcluster within Cluster 3.

The studied bread wheat genotypes represented different botanical varieties. Some tendencies were observed in grouping of genotypes according to their botanical varieties. Thus, out of eight var. *lutescens* accessions five grouped

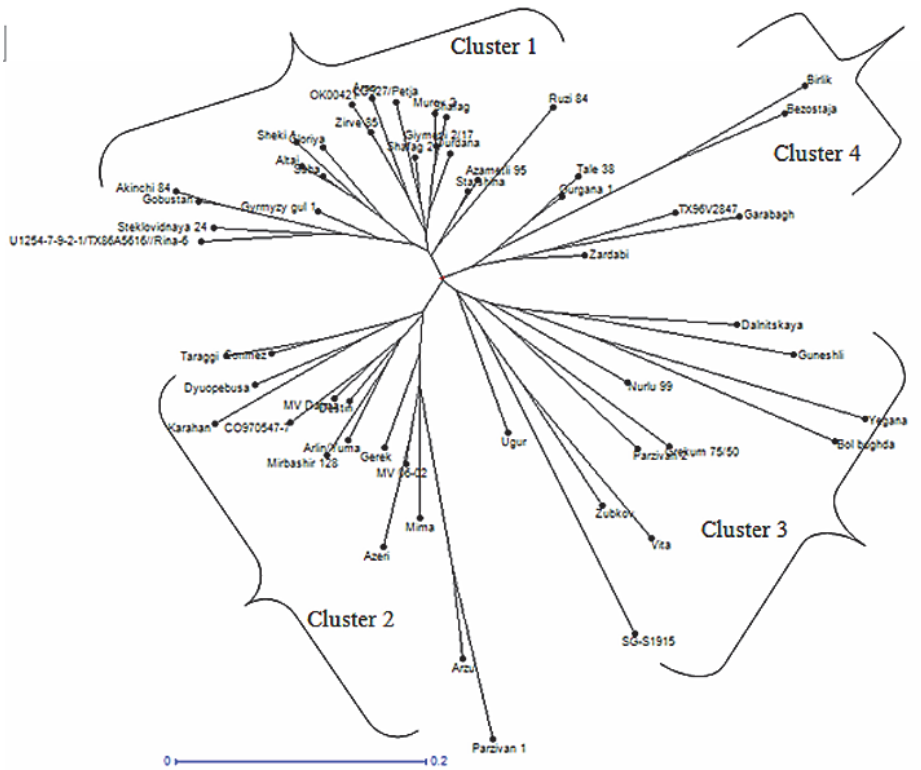

Fig. 1. UNJ tree for 54 bread wheat genotypes based on ISSR data

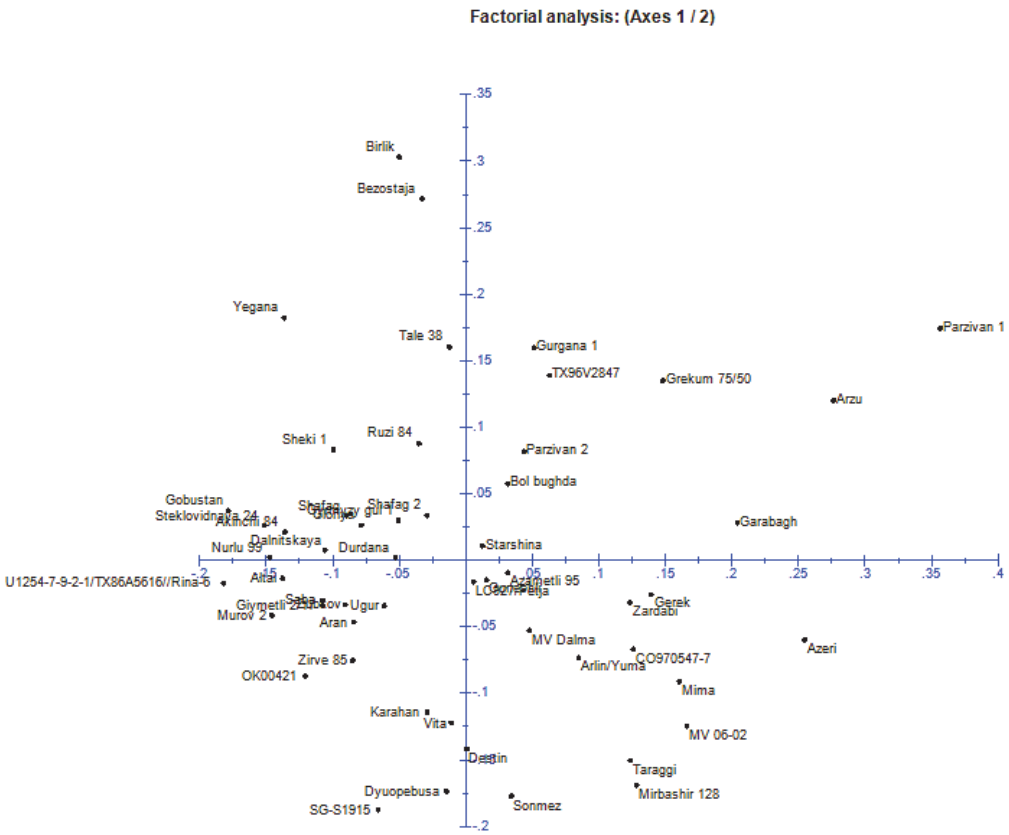

Fig. 2. Scatter plot of bread wheat genotypes using principle coordinate analysis based on ISSR data

into Cluster 1. Despite that var. *graecum* accessions found in all clusters, they were quite close to each other and formed uniform groups within each cluster. On contrary, four var. *psevdoerythrospermum* genotypes included into study were very distinct and scattered among clusters. It should be noted that three of var. *psevdoerythrospermum* accessions formed tight groups together with var. *graecum* genotypes (Akinchi 84 – Gobustan; Tale 38 – Gurgana 1; Garabagh – Zardabi), all of them were local varieties.

The principal coordinate analysis (PCoA) provided a spatial picture of analyzed *T. aestivum* accessions (Fig. 2). PCoA analysis confirmed subgrouping obtained by cluster analysis. The first five principal coordinates explained 50.2% of the total variation. All var. *lutescens* genotypes, except one were placed on left side, while majority of var. *graecum* genotypes scattered in upper part of the PCoA plot. As in the dendrogram genotypes of var. *psevdoerythrospermum* widely distributed and placed apart from each other.

Protein marker analysis

Storage proteins in endosperm of wheat grain represent over 80% of the whole quantity of proteins in grain and widely used in diversity analysis of wheat. Polyacrylamide gel electrophoresis showing the protein banding patterns of studied wheat accessions are given in Fig. 3. High rate of polymorphisms for gliadin subunits were observed in studied *T. aestivum* genotypes. The gliadin subunits were divided into four sections – ω (omega), γ (gamma), β (beta), α (alpha), where ω has the heaviest molecular weight and α has the lightest. In the current study Bezostaja 1 and Anza were used as standard varieties with known gliadin specters. In local and introduced bread wheat varieties most of the variations were observed at ω and α sections, while γ and β sections were less polymorphic.

The number of specters ranged from 18 for ω to 6 for sections γ and β (Table 3). The highest number of patterns was noted at α and ω sections, with 44 and 42 patterns, respectively. β section was characterized with least number of patterns. Genetic diversity index in collection of bread wheat accessions was quite high for all studied sections. The maximum diversity was observed at α section (0.968), followed by ω section (0.963). Seed storage proteins have been widely used for the identification and characterization

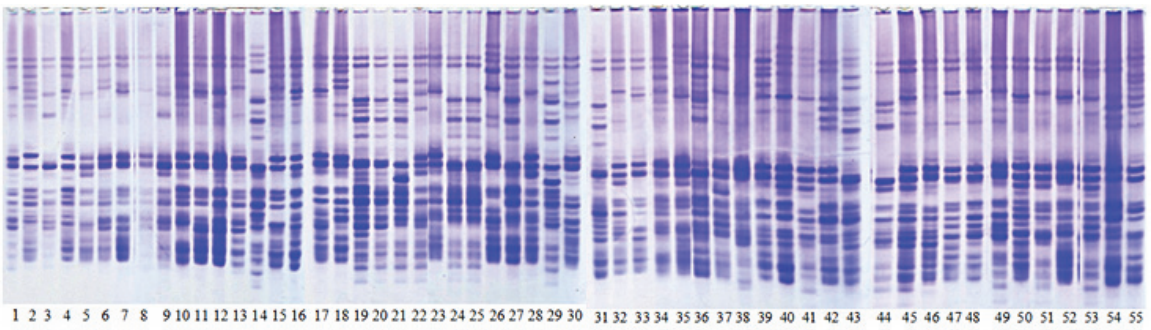

Fig. 3. Polyacrylamide gel electrophoresis of gliadin stock proteins of bread wheat genotypes. The names of the accession are given in Table 1

Table 3. Genetic diversity and polymorphism parameters of bread wheat genotypes based on gliadin subunits

Sections	Number of specters	Number of patterns	Genetic diversity index
α	8	44	0.968
β	6	19	0.862
γ	6	28	0.937
ω	18	42	0.963
Total	38	133	
Mean	9.5	33.3	0.933

of different crops (Chňapek et al., 2013, 2014). Ahmed et al. (2010), Fouda et al. (2011) and Tahir (2014) used protein and DNA markers to assess genetic diversity in wheat and in other crops and reported higher percentage of diversity using RAPD – PCR technique than using SDS – PAGE.

In our study protein markers were superior to ISSR markers for assessing genetic diversity. Similarly, Ahmed et al. (2010) and Hlozáková et al. (2016) reported higher percentage of diversity using seed storage protein than with DNA markers. Tanaka et al. (2003) used polyacrylamide gel

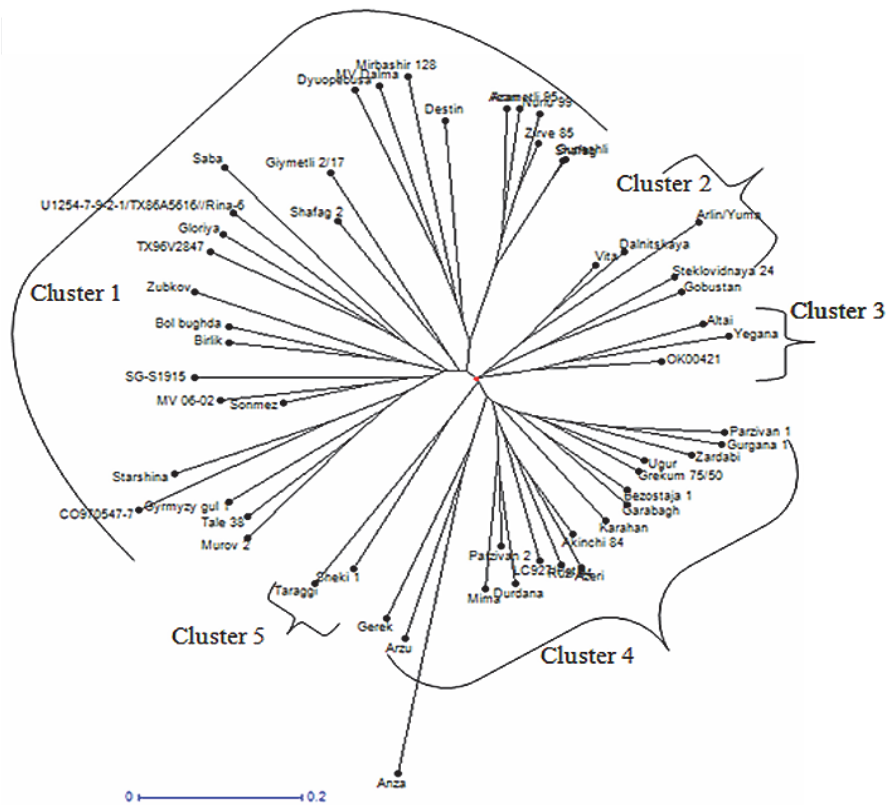

Fig. 4. UNJ tree of bread wheat genotypes based on protein markers

electrophoresis of gliadin subunits to study diversity of 107 Japanese wheat accessions. Forty-six patterns were identified for whole collection. Japanese accessions differ from wheat accessions of other countries.

A dendrogram was constructed based on gliadin data (Fig. 4). The bread wheat genotypes were split into 5 clusters. The distance indices ranged from 0.06 to 0.95, the average was 0.61. Thus, it can be concluded that studied *T. aestivum* varieties were more dissimilar based on protein data rather than ISSR data. We found no relationship between grouping of genotypes and their origin or botanical variety. The Cluster 1 was the largest and contained 49% of all genotypes. The closest genotypes were Gunashli and Shafag, both belonged to var. *graecum*. Cluster 5 contained only two genotypes – Sheki 1 and Taraggi.

The Anza variety used as a standard was the most distinct and formed a long branch within Cluster 4, which indicates the uniqueness of the genotype.

Mantel test did not reveal significant correlation between dendrograms created based on protein and DNA markers. Difference in the results obtained with the ISSR and protein markers is probably due to the different genomic regions studied by the two marker types. In contrast to the ISSR data var. *psevdoerythrospermum* accessions were very close based on protein markers, while var. *lutescens* accessions were widely distributed and could be found in all clusters. However, in both clusters var. *psevdoerythrospermum* and var. *graecum* accessions were neighboring genotypes.

Conclusions

In conclusion, the two genetic marker systems used in this study were differed for effectiveness in assessing genetic diversity and clustering of the genotypes. The lack of correlation between the estimates of dissimilarity computed from the gliadin subunits and ISSR data suggests that both set of markers explore genetic variation differently. The polymorphism rate of gliadin subunits was high, while ISSR loci showed moderate level of polymorphism and diversity. However, ISSR data could more or less differentiate local accessions from introduced ones and group them according to their botanical varieties, whereas no any relationship was revealed based on protein data. Thus, it can be suggested that these marker systems should be used rather as a complementary analysis than instead of each other.

Genetic information obtained from protein and ISSR data can be used to differentiate bread wheat cultivars and can also harmonize the genetic studies generated from morphological traits. Molecular variation assessed in this study can be useful in traditional and molecular breeding programs.

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Received: April 29, 2018; *Accepted*: July 2, 2018; *Published*: December 31, 2018