Allelic diversity of Azerbaijan bread wheat (*Triticum aestivum* L.) by SSR markers

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Abstract: The objectives of this investigation were devoted to studying genetic variation in fifty Azerbaijan wheat accessions from 6 different botanical varieties using simple sequence repeat markers. On the basis of 7 SSR primers used in this work between wheat accessions studied 42 different alleles were observed with an average of 6 alleles per locus. The ranging of polymorphism

information content from 0.428 to 0.772 revealed the existence of rich genetic diversity in Azerbaijan wheat accessions. The highest number of PIC values was calculated in *Xgwm*190, *Xgwm*337, and *Xgwm*261 SSR primers with an average of 0.561. The cluster analysis representing Nei genetic distance index among all samples divided the genotypes into 9 separate groups. The ninth cluster included 12 genotypes, accounting for 24% of all genotypes analyzed. Besides, this group including var. *erythrospermum3* and var. *erythroleucon9*, could not be distinguished based on the 7 microsatellite markers, and it may be due to their sharing of a similar basis of genetic background. It was found that samples of var. *milturum* botanical varieties were located at enough genetic distance from other studied samples. The results of this work clearly indicated that the SSR analysis can be used as a power tool to estimate genotypic similarities, genetic diversity, and fingerprinting of Azerbaijan's local wheat varieties.

Keywords: Bread wheat, botanical variety, microsatellite markers, genetic diversity.

1. Introduction

Wheat (*Triticum* spp.) is one of the three most economically important plants in the world and at the same time its outstanding contribution role for human nutrition and forage supply is non-substitutive (Shewry, 2009). Wheat is grown on 650,000 hectares in Azerbaijan, with a yield of 31.4 centners per hectare and average productivity of 1.9 million tons. Azerbaijan is one of the origins of cereal crops and is rich in wheat and its wild relatives' biodiversity (Eldarov et al., 2015; Mehdiyeva et al., 2021). Some wheat species are particularly important for agriculture; over time, a range of local wheat varieties have been developed, and more recently, a number of forms associated with more intensive agricultural systems have been introduced. The collection, study, and preservation of agricultural crops and their wild ancestors provide the basis for future selective breeding (Akparov and Abbasov, 2019). It was discovered that the distribution of *Aegilops* species in Transcaucasia shows a noticeable decline as one moves from the Caspian Sea towards the Black Sea. Similarly, their presence diminishes when traveling from Nakhichevan (in Azerbaijan) to the

north, toward the Main Caucasian Range. This pattern highlights a significant decrease in the number and diversity of *Aegilops* species across these regions (Eldarov et al., 2015).

Detailed information about the collection and the level of genetic diversity in plant germplasm, as well as determining of genetic relations of breeding materials is the basis of many breeding programs (Donini, 1998). Wheat as an important crop in the world and Azerbaijan with having different genotypes is used in many genetic programs. Thus, in order to use this crop and effectively, the comprehensive study of genetic diversity level and genetic relations of genotypes is inescapable (Mursalova et al., 2015).

Molecular markers have a significant advantage over morphological markers in that they remain stable under various environmental conditions (Ammar et al., 2015). Molecular markers such as Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP), Random Amplified Polymorphic DNA (RAPD), and Simple Sequence Repeats (SSRs) are potential tools for assessing genetic diversity in plant materials (Dar, 2017). Many plant varieties, including PCR-based molecular markers in wheat investigation, especially microsatellite markers, are considered the most important genetic markers (Ma et al., 1996). SSR markers find high polymorphism as compared to other genetic markers by scattering in large numbers along all genomes (Russell et al., 1997). The easy identification of analogue accessions from the view of the point of genetic distance is considered an indicator of their superior features (Archak, et al., 2003). The investigation of the genetic diversity of wheat crops with SSR markers was the subject of numerous studies (Iqbal et al., 2009; Eivazi et al., 2008, Elshafei et al., 2008, Schuster et al., 2009). SSR markers have been used very successfully in the study of genetic diversity of seed gene bank collections of improved wheat germplasm (Börner et al., 2000, Huang et al., 2002) and in the investigation of wild relationships (Li et al., 2000, Hammer et al., 2000) as well as in genetic mapping, Quantitative Trait Locus (QTL) association, population genetics, marker-assisted selection, and evolutionary studies. Thus, studying the genetic diversity and population structure of germplasm collections might help with preservation and genetic improvement strategies.

The objective of this study was to estimate relationship levels among bread wheat varieties of Azerbaijan origin, identification of accessions and detection of marker efficiency on the basis of SSR markers.

2. Materials and Methods

2.1.Plant materials

50 accessions of bread wheat used in the investigation were taken from the collection belonging to the National Genbank of the Genetic Resources Institute of Azerbaijan National Academy of Sciences and their names are listed in Table 1.

2.2.DNA extraction

Genomic DNA was extracted from young leaves with the method suggested by Varadarajan and Prakash (1991). DNA quality and quantity were determined by NanoDrop 2000 (Thermo Scientific) and samples were diluted to a final concentration of 50 ng/µL.

2.3.PCR analysis

In this study, 7 SSR primers were chosen from literature records based on their ability to reveal high levels of polymorphism. Each 20 µL PCR reaction was carried out using 50 ng of template DNA in 20 µL of total reaction volume containing 2 µL of genomic DNA, 2.5 µL of PCR buffer (10 X) containing $MgCl_2$ (15 mM), 1.5 µL of 10 mM dNTPs, 0.2 µL of Taq DNA polymerase (3 U/ µL) and 1.5 µL of each primer (10 µM). Amplification was performed using a T 100^{TM} Thermocycler (Bio-Rad) according to the following program: 5 min at 94 °C predenaturation, then 35 cycles of 1 min at 94 °C, 2 min at 50 °C and 5 min at 72 °C and final extension at 72 °C for 10 min. The amplification fragments were separated by 96 capillary Fragment Analyzer systems of Advanced Analytical Company.

2.4.Statistical analysis

Each band amplified by each primer was scored as present (1) or absent (0) for the fifty genotypes, and the data were entered into a binary matrix as discrete variables.

For each SSR marker, the number of observed alleles was recorded. To measure the informativeness of the SSR markers to differentiate between wheat genotypes, polymorphism information content (PIC), probability identity (PI), effective multiplex ratio (EMR), marker index (MI), discrimination power (D) and resolving power (RP) were calculated. PIC was calculated according to the formula PIC = Σ [2Pi|1-Pi|], where p_i is the frequency of allele for each locus (Mohammadi 2009). EMR is obtained from the equation EMR= np×β, where np is the number of total polymorphic bands and β is the ratio of the number of polymorphic bands to the total number of bands (Powell et al. 1996). MI is estimated from equation MI = EMR × PIC (Saghai et al. 1984); $PI = \Sigma pi^4 + \Sigma\Sigma(2PiPj)^2$ (Paetkau 1995) and D = 1 – PIC, where Pi and Pj represent the frequency of alleles i and j, respectively. RP was calculated using the formula RP=ΣIb, where Ib is band informativeness and Ib= 1- [2 × (0.5 - p)], where *p* is the proportion of genotypes containing the band (Prevost and Wilkinson 1999). A genetic similarity matrix was constructed and Nei's genetic distance (1983) was calculated for each pair of all accessions using the PowerMarker (Liu and Muse 2005). An unweighted pair group method with arithmetic mean (UPGMA) cluster analysis was performed to develop a dendrogram.

3. Results and Discussion

In order to investigate the genetic diversity of the bread wheat accessions originating from the Azerbaijan Republic at the DNA level, 12 various microsatellite primers were used. In our research between selected primers, some primers produced no distinct bands on a smeary background and some of them resulted in very faint bands upon a highly smeared background. As a consequence, 7 informative SSR primers were selected due to their ability to produce polymorphic and unambiguous markers between studied wheat accessions. By using the SSR primers observed alleles in the wheat botanical variety are shown in Table 2. For each microsatellite loci calculated some parameters like the number of alleles, polymorphism information content (PIC), probability identity (PI), effective multiplex ratio (EMR), marker index (MI), discrimination power (D) and resolving

power (RP) are given in table 3. The number of alleles per SSR locus is one of the most important parameters describing polymorphism, in our study the average number of alleles for each locus was 6. Elshafei et al. (2019) in the study of genetic diversity of bread wheat accession using 33 SSR primers have reported 1.36 alleles per each locus. At the same time in previous studies, different results were obtained. In the research works of Khavarinejad and Karimov (2012), the average number of alleles per locus was calculated at 8.44 and 3.4 respectively.

As a result of this research among studied bread wheat from 6 botanical varieties in accordance with Xgwm437, Xgwm261, Xgwm577, and Xgwm190 primers, the maximum number of alleles were 4, 5, 4, and 4, respectively, which were obtained in *var. graecum*. By the Xgwm46 primer, the maximum number of obseallelesllele had been achieved in *var. milturum, var. ferrugineum* and *var.erythroleucon* with 4 alleles. The maximum number of observed alleles by Xgwm389 primers was three which was determined in *var. erythrospermum, var. lutescens* and *ear.erythroleucon*. At the same time, the maximum number of alleles obtained through Xgwm337 was 5 in both of *var. milturum* and *var. erythroleucon* botanical varieties. The current results are proof of the existence of rich genetic diversity in Azerbaijan bread wheat.

The number of alleles detected by a primer ranged from 4 to 7 among the bread wheat. During the investigation rare alleles were found between the studied bread wheat. As criteria, rare allele can be used to provide a reliable identification of genotypes, as well as to protection of breeder's right in breeding programmes. In our research could be found the rare allele through Xgwm261 in Standard Aran, by using Xgwm190 in *var. lutescens, var. erythroleucon* and Standard Aran again, through Xgwm46 in *var. graecum,* through Xgwm389 in *var. milturum*and finally through Xgwm337 in Standard Aran again.

Figure 1. Illustrates a capillary electropherogram of DNA amplification by using the Xgwm-190 SSR marker in some bread wheat botanical varieties.

Figure 1. An example of capillary electrophoregram obtained by Fragment Analyzer machine with primer Xgwm-190; the numbers indicate bread wheat accessions as listed in Table 1.

The observed polymorphism information content range in all of the used primers in this research was variable between 0.428 – 0.672 (Table 2). The results showed among the 7 used microsatellite primers in this research, primers Xgwm190, Xgwm337, Xgwm261 and Xgwm46 with PIC values of 0.672, 0.606, 0.605, and 0.579, respectively, had most PIC between studied wheat botanical varieties, the highest PIC value detected the generic distance between samples better than others, so they can be used as markers to distinguish genetic diversity. In contrast, the Xgwm577 primer with a PIC value of 0.428 showed less PIC.

The probability of Identity (PI) is defined as the probability with which 2 random genotypes display the same SSR profile. The calculated PI value for each locus across all genotypes varied from 0.335 for Xgwm577 to 0.14 in Xgwm190. At the same time, the locus with a low PI value showed a high level of other parameters including Marker Index (MI), Effective Multiplex Ratio (EMR), Discrimination Power (D), and Resolving Power (RP). The MI values ranged between 2 and 4.7. The maximum MI (4.7) was observed for the Xgwm190 locus. The primers that showed higher polymorphism had higher EMR values. This feature varied from 4 to 7 with a mean value of 6. The estimates of RP ranged from 1.08 to 2 with an average of 1.53 per locus.

The moderate values of PIC for the SSR primers could be attributed to the diverse nature of the wheat accessions and also the highly informative SSR markers used in this study. As a result of the investigation, the average PIC value was identified 0.561.

The study of genetic diversity in bread wheat accessions through microsatellite markers was carried out by different researchers in many parts of the world.

In previous studies, Arora et al. (2014) reported the number of observed alleles ranged from 2 to 5 and the PIC value with an average of 0.584 in 319 Indian bread wheat accessions by using 16 microsatellite markers. The high level of PIC value with an average of 0.83 was reported by Sardouie-Nasab et al. (2013) in assessing the genetic diversity of promising wheat (*Triticum aestivum* L.) lines using microsatellite markers. In another study, the number of the allele was determined between 7-11 and the PIC value with an average of 0.79 (Ateş et al., 2012).

In our research, the obtained PIC value showed a higher level of genetic diversity exists within bread-wheat accessions. Thus, the SSR primers Xgwm190, Xgwm337, Xgwm261, and Xgwm46 could be used as informative and most appropriate markers for the assessment of genetic diversity as well as identification of bread wheat accessions.

A dissimilarity matrix was used to determine the level of relatedness among the Azerbaijan bread wheat studied. Cluster analysis for all samples was performed according to Nei's genetic distance following the UPGMA method. It allowed to classify all the genotypes into nine main clusters (Figure 2).

Sample No.3 from *var. ferrugineum* and samples No. 2 and 6 from *var. lutescens* botanical varieties are placed in the first group.

Among the samples in this group, the samples *var. ferrugineum*3 and *var. lutescens*2 as well as the samples *var. ferrugineum*3 and *var. lutescens*6 appeared very close genetically, with a genetic distance index of 0.125 and 0.173, respectively. Samples No.7 from *var. milturum*and and No. 2 from *var. ferrugineum* is separated from other bread wheat accessions and located in a second different cluster. This result shows the genetic distance between these two genotypes and other studied accessions. The value of the genetic distance index for these samples was 0.15.

Following the second cluster also the third and fourth clusters have been consisted of two samples. It was found that in the third cluster, both sample No.2 and No.3 was representative of *var. milturum* botanical variety. Calculated Nei's genetic distance index between these two accessions was 0.15. Sample No.4 from *var. milturum* and No.5 from *var. erythrospermum* botanical variety with a genetic distance index of 0.125 has resided in the fourth group.

The fifth cluster included only three samples from *var. graecum*. In this group, the lowest genetic distance index was studied between *Var.Graecum*2 and *Var.Graecum*4.

The sixth group consisted of 9 genotypes, which made up 18% of all examined bread wheat accessions. In the current cluster, the representative of *var. erythroleucon* (samples No.1, 2, 3 and 7) was more than the other botanical varieties representative. Also samples No.4 and 6 from *var.*

erythrospermum, samples No.5 and 6 from *var. miltur*um and only one sample No.1 from *var. lutescens* are located in this group. One of the most interesting results of this group was founding identical samples *var. erythroleucon*1 and *var. lutescens*1 at all loci tested and were then undistinguishable in our study. At the same time, the furthest genetic distance index (0.2) was determined between *var. erythrospermum*4 and *var. erythrospermum*6.

The seventh cluster consisted of only two *var. graecum*6 and v*ar. ferrugineum*4.

The eighth cluster consisted of a, b, c, and d subgroups. The samples *var. erythrospermum*2, *var. erythroleucon*5, *var. lutescens*8, *var. ferrugineum*5, *var. ferrugineum*7, *var. erythroleucon*6 and *var. graecum*5 is located in the "a" subgroup. Within this subgroup between *var. ferrugineum*5 and *far. graecum*5 samples obtained the furthest genetic distance index (0.15). The samples *var. lutescens*3, *var. lutescens*4, *var. erythrospermum*1 and *var. erythroleucon*4 were resided in the "b" subgroup. The nearest genetic distance was studied between *var.lutescens*3 and *var. lutescens*4, and the furthest genetic distance index was obtained between *var. lutescens* 3 and *var. erythroleucon*4, with genetic distance index of 0.05 and 0.1, respectively. The "c" subgroup included only one sample sampled from *var. erythrospermum8* and therefore this sample belongs to a separate subgroup which indicates the genetic distance of this sample from the other investigated genotypes of the eighth cluster. Samples No.5 and 7 from *var. lutescens* and sample No.6 from *var. ferrugineum* has located in the "d" subgroup. In the current subgroup, the highest genetic distance was found between *var. lutescens7* and *var. ferrugineum*6 with 0.125 value of genetic distance.

In comparison, the 9th cluster contained 12 genotypes which made up 24% of all examined genotypes. In the current cluster 3 accessions No. 3, 7, and 9 from *var. erythrospermum*, three samples No.1, 7 and 8 from *var. graecum*, two accessions No.8 and 9 from *var. erythroleucon*, at the same time from each botanical variety namely *var. miltur*um and *var. ferrugineum* only one accession (No.1), also *Standard Aran*1 and *Standard Aran*2 were the main members of this cluster. Besides, this group including *var. erythrospermum*s3 and *var. erythroleucon*9, could not be

distinguished based on the 7 microsatellite markers, and it may be due to their sharing of a similar basis of genetic background.

In order to determine the distance among the Azerbaijan wheat botanical varieties under study, a UPGMA dendrogram (Figure 3) was constructed based on Nei's genetic distance (1983). As observed, the botanical varieties of wheat such *var. milturum* and *var. graecum* were the most divergent from the other Azerbaijan botanical varieties studied. In fact, the samples from *var. miltur*um and *var. graecum* showed the highest difference. Moreover, *var. ferrugineum*, *var. lutescens*, *var. erythrospermum* and *var. erythroleucon* displayed the highest genetic similarity.

4. Conclusions

Thus, the results showed significant variation in microsatellite DNA polymorphisms among wheat varieties. This study using microsatellite markers revealed considerable genetic diversity among 50 Azerbaijan wheat varieties at the DNA level and identified diverse genotypes for use in breeding programs for wheat improvement. These results suggest that the SSR markers are valuable tools for identification and diversity analysis in wheat genotypes.

Conflicts of Interest: "The authors declare no conflict of interest."

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Table 1

The name of bread wheat accessions originating of Azerbaijan

№	Accessions	№	Accessions
1	*Var. Graecum 1*	26	*Var. Erythrospermum 4*
2	*Var. Graecum 2*	27	*Var. Erythrospermum 5*
3	*Var. Graecum 3*	28	*Var. Erythrospermum 6*
4	*Var. Graecum 4*	29	*Var. Erythrospermum 7*
5	*Var.Graecum 5*	30	*Var. Erythrospermum 8*
6	*Var.Graecum 6*	31	*Var. Erythrospermum 9*
7	*Var. Graecum 7*	32	*Var. Lutescens 1*
8	*Var. Graecum 8*	33	*Var. Lutescens 2*
9	*Var. Milturum 1*	34	*Var. Lutescens 3*
10	*Var. Milturum 2*	35	*Var. Lutescens 4*
11	*Var. Milturum 3*	36	*Var. Lutescens 5*
12	*Var. Milturum 4*	37	*Var. Lutescens 6*
13	*Var. Miturum 5*	38	*Var. Lutescens 7*
14	*Var. Milturum 6*	39	*Var. Lutescens 8*
15	*Var. Milturum 7*	40	*Var. Erythroleucon 1*
16	*Var. Ferrugineum 1*	41	*Var. Erythroleucon 2*
17	*Var. Ferrugineum 2*	42	*Var. Erythroleucon 3*
18	*Var. Ferrugineum 3*	43	*Var. Erythroleucon 4*
19	*Var. Ferrugineum 4*	44	*Var. Erythroleucon 5*
20	*Var. Ferrugineum 5*	45	*Var. Erythroleucon 6*
21	*Var. Ferrugineum 6*	46	*Var. Erythroleucon 7*
22	*Var. Ferrugineum 7*	47	*Var. Erythroleucon 8*
23	*Var. Erythrospermum 1*	48	*Var. Erythroleucon 9*
24	*Var. Erythrospermum 2*	49	*Standart Aran 1*
25	*Var. Erythrospermum 3*	50	*Standart Aran 2*

Table 2

The number of observed alleles by microsatellite markers in studied bread wheat varieties

Botanical variety	Xgwm437	Xgwm261	Xgwm577	Xgwm190	Xgwm46	Xgwm389	Xgwm337
Var. Graecum	4	5	4	4	1	2	2
Var. Milturum	2	3	3	3	4	1	5
Var. Ferrugineum	3	3	2	1	4	2	3
Var. Erythrospermum	3	2	3	3	3	3	4
Var. Lutescens	2	3	2	1	3	3	4
Var. Erythroleucon	2	2	2	1	4	3	5
Standard Aran	2	1	2	1	2	1	1

Table 3

Genetic parameters calculated based on SSR markers in investigated bread wheat

Locus	Number of allele	PIC	PI	EMR	MI	D	RP
Xgwm437	7	0.533	0.245	7	3.73	0.75	1.32
Xgwm261	5	0.605	0.234	5	3.03	0.77	2
Xgwm577	6	0.428	0.355	6	2.57	0.64	1.08
Xgwm190	7	0.672	0.14	7	4.7	0.86	1.92
Xgwm46	6	0.579	0.206	6	3.47	0.79	1.52
Xgwm389	4	0.502	0.281	4	2	0.72	1.28
Xgwm337	7	0.606	0.18	7	4.24	0.82	1.6
Average	6	0.561	0.234	6	3.39	0.76	1.53

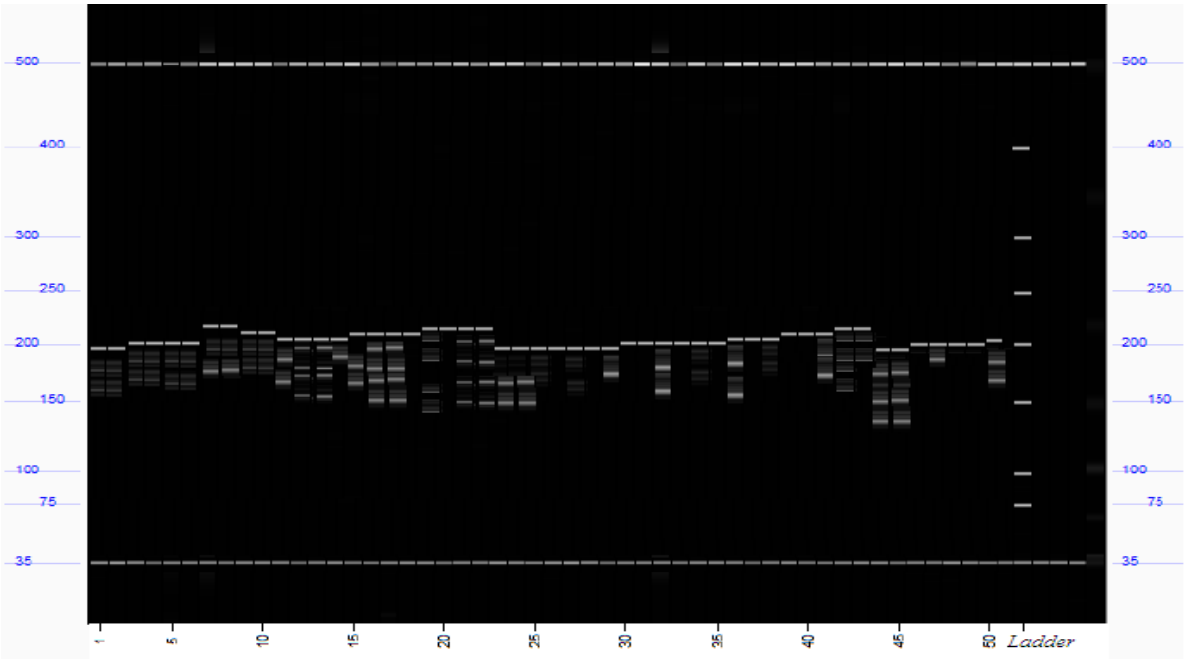

Fig. 1. An example of capillary electropherogram obtained by Fragment Analyzer machine with primer Xgwm-190; the numbers indicate bread wheat accessions as listed in Table 1.

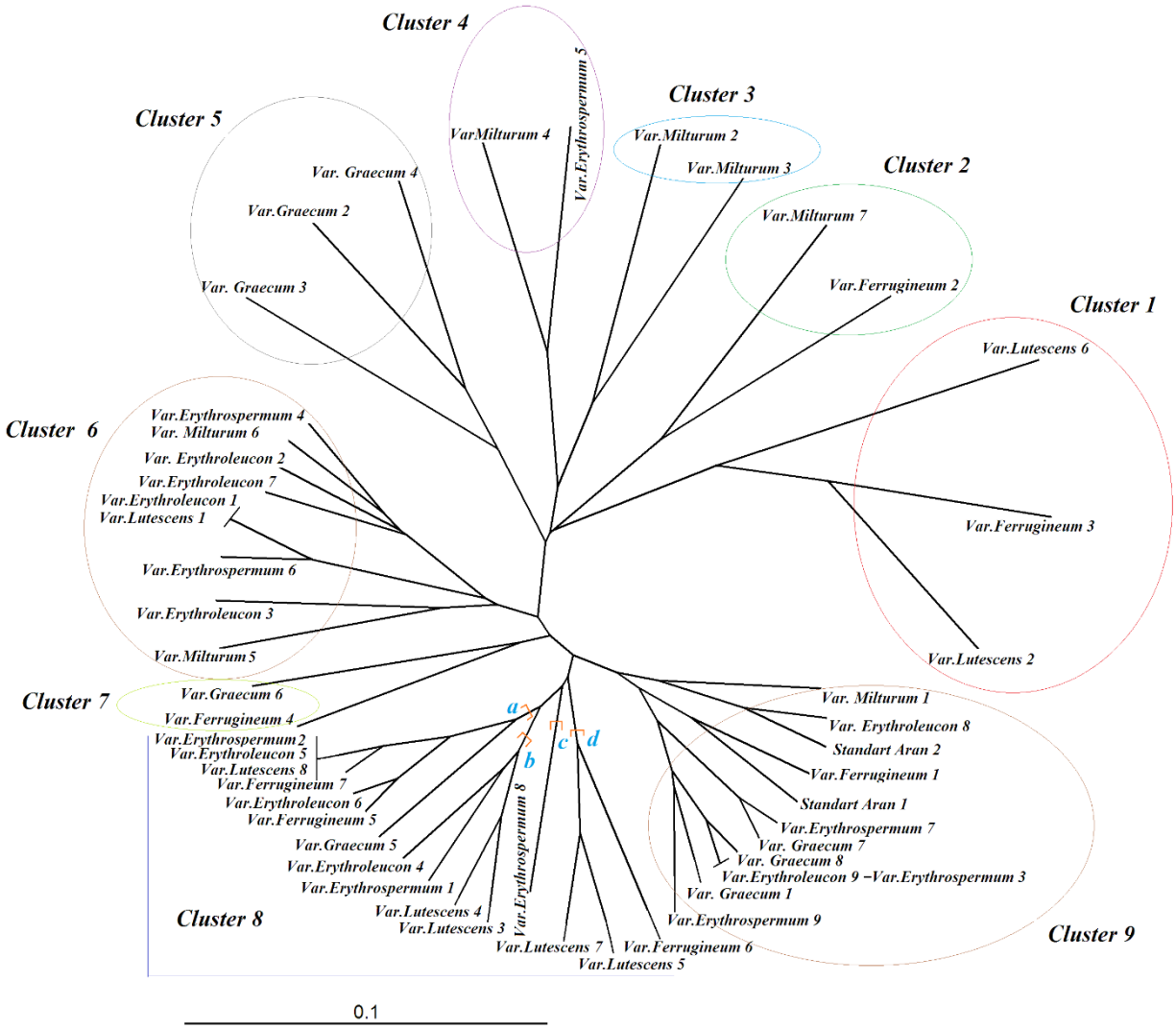

Fig. 2. Dendrogram showing the genetic relationship among Azerbaijan bread wheat accessions. The scale is based on Nei's genetic distance index

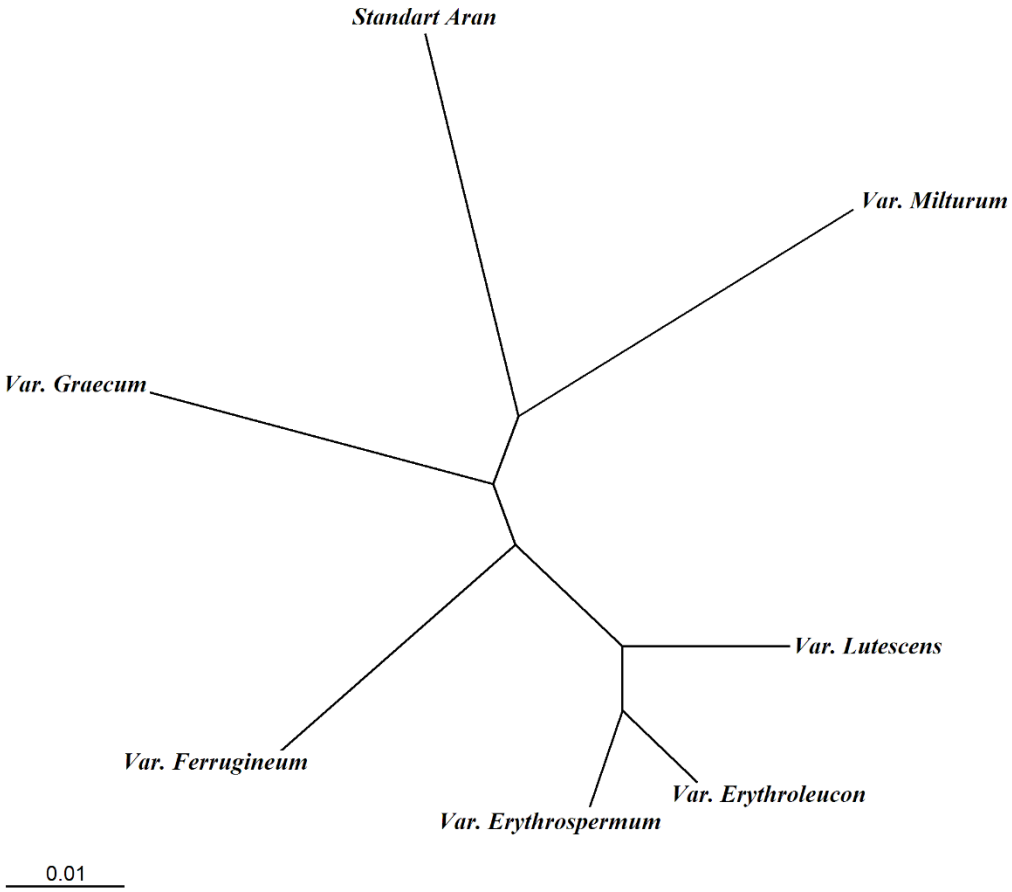

Fig. 3. Dendrogram showing the genetic distance between studied bread wheat botanical varieties on the basis of allele diversity of microsatellite loci