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

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Abstract: The objectives of this research entailed studying genetic variation in 50 Azerbaijani wheat accessions from 6 different botanical varieties using simple sequence repeat (SSR) markers. On the basis of the seven SSR primers used in this work, 42 different alleles were observed among the wheat accessions studied with an average of six alleles per locus. Polymorphism information content (PIC) ranged from 0.428 to 0.772, revealing the existence of rich genetic diversity in Azerbaijani wheat accessions. The highest PIC values were calculated with the *Xgwm*190, *Xgwm*337, and *Xgwm*261 SSR primers with a mean value of 0.561. Cluster analysis representing the Nei genetic distance index among all samples divided the genotypes into nine separate groups. The ninth cluster included 12 genotypes, accounting for 24% of all genotypes analyzed. This group also included var. *erythrospermum* 3 and var. *erythroleucon* 9, which could not be distinguished based on the seven microsatellite markers; this may be due to their sharing of a similar genetic background. Samples of var. *milturum* botanical varieties were located at notable genetic distances from the other studied samples. These findings clearly indicate that SSR analysis can be used as a powerful tool for estimating the genotypic similarities, genetic diversity, and fingerprinting of Azerbaijani local wheat varieties.

Key words: 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 its contributions to human nutrition and forage supplies are irreplaceable (Shewry, 2009). Wheat is grown across 650,000 ha in Azerbaijan, with a yield of 3,14 t/ha and average productivity of 1.9×10^6 t. Azerbaijan is one of the origins of cereal crops and is rich in terms of the biodiversity of wheat and its wild relatives (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 provides 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 (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 germplasms, as well as determination of the genetic relations of breeding materials, forms the basis of many breeding programs (Donini, 1998). Wheat, as an important crop in the world and in Azerbaijan with many different genotypes, is used in many genetic programs. To use this crop effectively, the comprehensive study of genetic diversity levels and genetic relations of genotypes is a necessity (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 repeat (SSR) markers are potential tools for assessing genetic diversity in plant materials (Dar, 2017). For many plant varieties, PCR-based molecular markers in investigations of wheat and

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especially microsatellite markers are considered the most important genetic markers (Ma et al., 1996). SSR markers reveal high polymorphism compared to other genetic markers as a result of scattering in large numbers along all genomes (Russell et al., 1997). The easy identification of analog accessions from the perspective 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 has been the subject of numerous studies (Eivazi et al., 2008, Elshafei et al., 2019; Iqbal et al., 2009; Schuster et al., 2009). SSR markers have been used successfully in the study of the genetic diversity of seed gene bank collections of improved wheat germplasms (Börner et al., 2000; Huang et al., 2002) and in investigations of wild relationships (Hammer et al., 2000; Li et al., 2000), as well as for genetic mapping, quantitative trait locus (QTL) associations, population genetics, marker-assisted selection, and evolutionary studies. Thus, studying the genetic diversity and population structures 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 Azerbaijani origin, identify accessions, and evaluate marker efficiency on the basis of SSR markers.

2. Materials and methods

2.1. Plant materials

The 50 accessions of bread wheat used in this study were taken from the collection belonging to the National GenBank of the Genetic Resources Institute of the Azerbaijan National Academy of Sciences. They are listed in Table 1.

2.2. DNA extraction

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

2.3. PCR analysis

Seven SSR primers were chosen from the literature based on their ability to reveal high levels of polymorphism. Each PCR reaction of 20 µL 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 (10X) 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 T100 thermocycler (Bio-Rad, Hercules, CA, USA) with the following program: 5 min of predenaturation at 94 °C; 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 using a 96-capillary fragment analyzer system (Advanced Analytical Company, Ames, IA, USA).

2.4. Statistical analysis

Each band amplified by each primer was scored as present (1) or absent (0) for the 50 genotypes, and the obtained 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 in differentiating between wheat genotypes, polymorphism information content (PIC), probability identity (PI), effective multiplex ratio (EMR), marker index (MI), discrimination power (D), and resolving power (RP) values were calculated. PIC was calculated according to the formula $PIC = \Sigma [2Pi|1 - Pi|]$, where Pi is the frequency of the allele for each locus (Mohammadi et al., 2009). EMR was obtained from the equation $EMR = np \times \beta$, 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 was estimated with the equation $MI = EMR \times PIC$ (Saghai-Maroof et al., 1984); $PI = \Sigma pi^4 + \Sigma\Sigma(2PiPj)^2$ (Paetkau et al., 1995); and $D = 1 - PIC$. Here, Pi and Pj represent the frequency of allele i and allele j, respectively. RP was calculated using the formula $RP = \Sigma Ib$, where Ib is band informativeness and $Ib = 1 - [2 \times (0.5 - p)]$, where p is the proportion of genotypes containing the relevant band (Prevost and Wilkinson, 1999). A genetic similarity matrix was constructed and Nei's genetic distance (Nei et al., 1983) was calculated for all pairs of accessions using PowerMarker (Liu and Muse, 2005). Unweighted pair group method with arithmetic mean (UPGMA) cluster analysis was performed to develop a dendrogram.

3. Results and discussion

To investigate the genetic diversity of the bread wheat accessions originating from Azerbaijan at DNA level, 12 microsatellite primers were used. In our investigation of the selected primers, some primers produced no distinct bands on a smeared background and some resulted in very faint bands upon a highly smeared background. As a consequence, seven informative SSR primers were selected from the initial 12 due to their ability to produce polymorphic and unambiguous markers between studied wheat accessions. The alleles observed in the wheat varieties using those SSR primers are shown in Table 2. For each microsatellite locus analyzed, the number of alleles and values of PIC, PI, EMR, MI, D, and RP are given in Table 3. The number of alleles per SSR locus is one of the most important parameters describing polymorphism, and in this study, the average number of alleles for each locus was 6. In a study of genetic diversity of bread wheat

Table 1. Studied bread wheat accessions originating from Azerbaijan.

No	Accessions	No	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	Standard Aran *1*
25	var. *erythrospermum 3*	50	Standard Aran *2*

Table 2. Numbers of observed alleles by microsatellite markers in the studied bread wheat varieties.

Botanical variety	*Xgwm*437	*Xgwm*261	*Xgwm*577	*Xgwm*190	*Xgwm*46	*Xgwm*389	*Xgwm*337
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 the investigated bread wheat accessions.

Locus	Number of alleles	PIC	PI	EMR	MI	D	RP
*Xgwm*437	7	0.533	0.245	7	3.73	0.75	1.32
*Xgwm*261	5	0.605	0.234	5	3.03	0.77	2
*Xgwm*577	6	0.428	0.355	6	2.57	0.64	1.08
*Xgwm*190	7	0.672	0.14	7	4.7	0.86	1.92
*Xgwm*46	6	0.579	0.206	6	3.47	0.79	1.52
*Xgwm*389	4	0.502	0.281	4	2	0.72	1.28
*Xgwm*337	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

accessions using 33 SSR primers, Elshafei et al. (2019) reported 1.36 alleles per locus. In other previous studies, different results were obtained. For example, in the study by Khavarinejad and Karimov (2012), the average number of alleles per locus was calculated as 3.4.

As a result of the present study, from among the analyzed bread wheat accessions from 6 botanical varieties in accordance with the *Xgwm*437, *Xgwm*261, *Xgwm*577, and *Xgwm*190 primers, the maximum numbers of alleles were 4, 5, 4, and 4, respectively, which were obtained for var. *graecum*. With the *Xgwm*46 primer, the maximum number of alleles was observed for var. *milturum*, var. *ferrugineum*, and var. *erythroleucon*, each with 4 alleles. The maximum number of alleles observed with the *Xgwm*389 primer was 3, determined for var. *erythrospermum*, var. *lutescens*, and var. *erythroleucon*. The maximum number of alleles obtained with *Xgwm*337 was 5, as observed for both var. *milturum* and var. *erythroleucon*. These results are proof of the existence of rich genetic diversity in Azerbaijani bread wheat.

The number of alleles detected by a primer ranged from 4 to 7 among the bread wheat accessions and some rare alleles were identified. Rare alleles can be used to provide a reliable identification of genotypes, as well as to protect the breeder's rights in breeding programs. We found rare alleles with *Xgwm*261 in 'Standard Aran'; with *Xgwm*190 in var. *lutescens*, var. *erythroleucon*, and 'Standard Aran'; with *Xgwm*46 in var. *graecum*; with *Xgwm*389 in var. *milturum*; and with *Xgwm*337 in 'Standard Aran.'

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

The observed PIC values for all of the primers used in this study ranged between 0.428 and 0.672 (Table 2). Among the seven microsatellite primers used in this work, primers *Xgwm*190, *Xgwm*337, *Xgwm*261, and *Xgwm*46 with PIC values of 0.672, 0.606, 0.605, and 0.579, respectively, yielded the highest PICs for the studied wheat botanical varieties. High PIC values allow for better detection of distances between taxa; thus, markers with high PICs can be used to distinguish genetic diversity. In contrast, primer *Xgwm*577 had a low PIC value of 0.428.

PI is defined as the probability of two random genotypes displaying the same SSR profile. The calculated PI value for each locus across all genotypes varied from 0.335 for *Xgwm*577 to 0.14 for *Xgwm*190. The locus with the low PI value had high values for other parameters including MI, EMR, D, and RP. The MI values ranged between 2 and 4.7. The maximum MI value of 4.7 was observed for the Xgwm190 locus. The primers that showed higher polymorphism had higher EMR values, varying from 4 to 7 with a mean value of 6. Estimates of RP ranged from 1.08 to 2 with a mean of 1.53 per locus.

The moderate PIC values obtained for the SSR primers could be attributed to the diverse nature of the wheat accessions as well as the highly informative SSR markers used in this study. As a result of this investigation, the mean PIC value was found to be 0.561.

Studies of genetic diversity in bread wheat accessions using microsatellite markers have been carried out by different researchers around the world. In previous studies, Arora et al. (2014) reported that the number of observed alleles ranged from 2 to 5 and the mean PIC value was 0.584 for 319 Indian bread wheat accessions using 16 microsatellite markers. A high mean PIC value of 0.83 was reported by Sardouie Nasab et al. (2013) in an assessment of the genetic diversity of promising wheat (*Triticum aestivum* L.) lines using microsatellite markers. In another study, the number of alleles was determined to range from 7 to 11 and the mean PIC value was 0.79 (Ateş Sönmezoğlu et al., 2012).

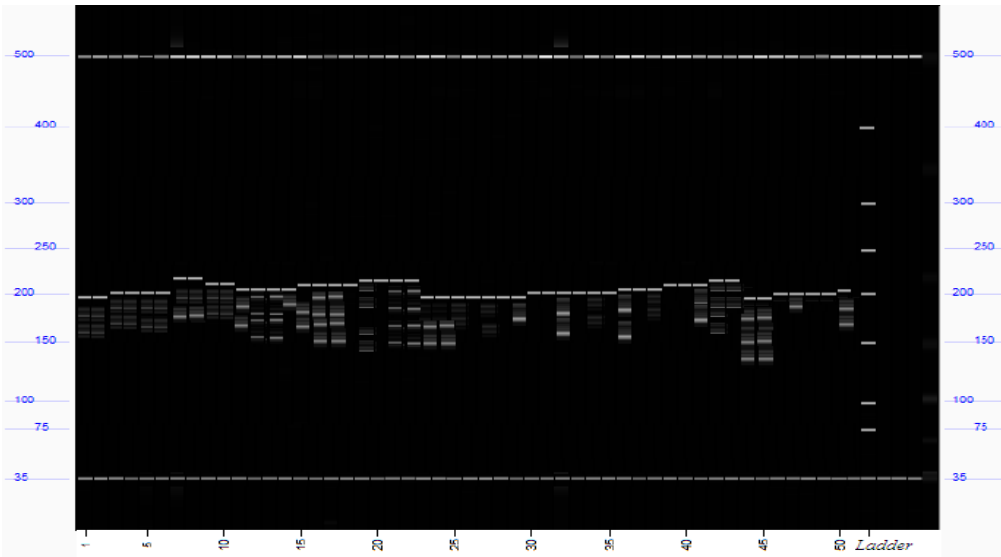

Figure 1. Example of a capillary electropherogram obtained with the fragment analyzer system with primer *Xgwm*190; numbers indicate bread wheat accessions as listed in Table 1.

In our study, the obtained PIC values confirmed that a high level of genetic diversity exists within these bread wheat accessions. Thus, SSR primers *Xgwm*190, *Xgwm*337, *Xgwm*261, and *Xgwm*46 could be appropriately used as informative markers for the assessment of genetic diversity as well as for the identification of bread wheat accessions.

A dissimilarity matrix was created to determine the level of relatedness among the Azerbaijani bread wheat accessions studied. Cluster analysis for all samples was performed according to Nei's genetic distance with the UPGMA method. This facilitated the classification of all the genotypes into nine main clusters (Figure 2).

Sample No. 3 from var. *ferrugineum* and Samples No. 2 and No. 6 from var. *lutescens* botanical varieties were placed in the first group. Among the samples in this group, the samples of var. *ferrugineum* 3 and var. *lutescens* 2 as well as the samples of var. *ferrugineum* 3 and var. *lutescens* 6 appeared very similar genetically, with genetic distance index values of 0.125 and 0.173, respectively. Sample No. 7 of var. *milturum* and Sample No. 2 of var. *ferrugineum* were separated from the other bread wheat accessions and located in a second cluster. This reflects the genetic distance between those two genotypes and the other studied accessions. The value of the genetic distance index for these samples was 0.15.

Following the second cluster, the third and fourth clusters also consisted of two samples each. In the third cluster, both Sample No. 2 and Sample No. 3 were representative of the var. *milturum* botanical variety. Nei's genetic distance index value between these two accessions was calculated as 0.15. Sample No. 4 of var. *milturum* and Sample No. 5 of the var. *erythrospermum* botanical variety with a genetic distance index value of 0.125 were assigned to the fourth group.

The fifth cluster included only three samples of var. *graecum*. In this group, the lowest genetic distance index value was obtained between var. *graecum* 2 and var. *graecum* 4.

The sixth group consisted of nine genotypes, accounting for 18% of all examined bread wheat accessions. In this cluster, var. *erythroleucon* (Samples No. 1, 2, 3, and 7) was represented more than the other botanical varieties. Sample No. 4 and Sample No. 6 of var. *erythrospermum*, Samples No. 5 and No. 6 of var. *milturum*, and Sample No. 1 of var. *lutescens* were included in this group. One of the most interesting results of this group was the finding of identical results for var. *erythroleucon*1 and var. *lutescens*1 at all loci tested, making them undistinguishable in this study. The largest genetic distance index value (0.2) was determined between var. *erythrospermum*4 and var. *erythrospermum*6.

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

The eighth cluster consisted of subgroups a, b, c, and d. The samples of var. *erythrospermum* 2, var. *erythroleucon* 5, var. *lutescens* 8, var. *ferrugineum* 5, var. *ferrugineum* 7, var. *erythroleucon* 6, and var. *graecum* 5 were placed in subgroup a. In this subgroup, var. *ferrugineum* 5 and var. *graecum* 5 had the largest genetic distance index value (0.15). The samples of var. *lutescens* 3, var. *lutescens* 4, var. *erythrospermum* 1, and var. *erythroleucon* 4 were placed in subgroup b. The smallest genetic distance index

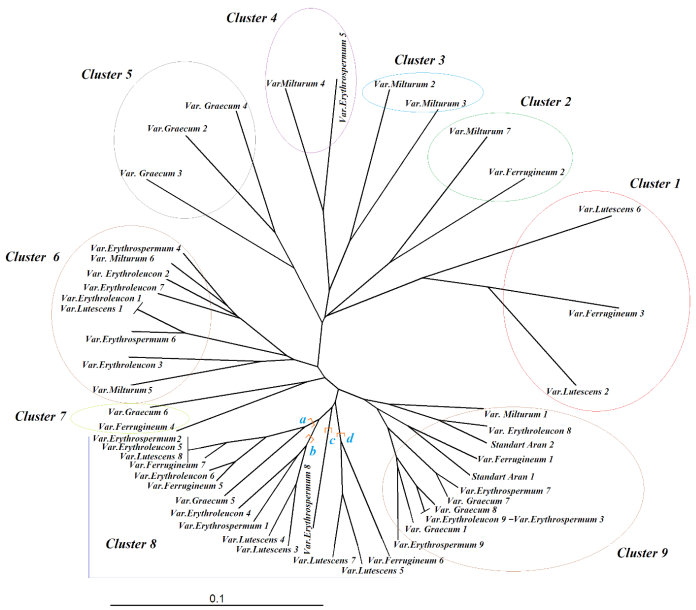

Figure 2. Dendrogram showing the genetic relationships among Azerbaijani bread wheat accessions. The scale is based on Nei's genetic distance index.

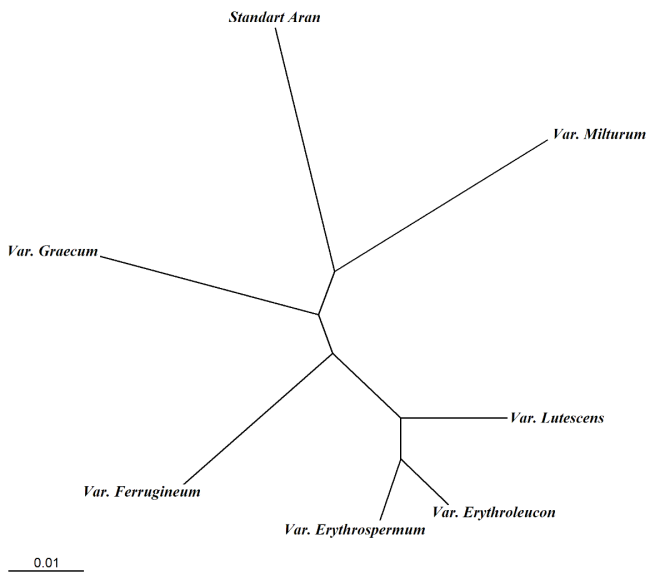

Figure 3. Dendrogram showing genetic distances between studied bread wheat botanical varieties on the basis of allele diversity of microsatellite loci.

value of 0.05 was observed between var. *lutescens* 3 and var. *lutescens* 4, while the largest genetic distance index value of 0.1 was obtained for var. *lutescens* 3 and var. *erythroleucon* 4. Subgroup c included only one sample of var. *erythrospermum*8; therefore, this sample belongs to a separate subgroup, reflecting the genetic distance of this sample from the other investigated genotypes of the eighth cluster. Samples No. 5 and No. 7 of var. *lutescens* and Sample No. 6 of var. *ferrugineum* were placed in subgroup d. In this subgroup, the highest value of the genetic distance index (0.125) was found between var. *lutescens*7 and var. *ferrugineum*6.

The ninth cluster contained 12 genotypes, accounting for 24% of all examined genotypes. The main members of this cluster included Samples No. 3, No. 7, and No. 9 of var. *erythrospermum*; Samples No. 1, No. 7, and No. 8 of var. *graecum*; Samples No. 8 and No. 9 of var. *erythroleucon*; Sample No. 1 of var. *milturum*; Sample No. 1 of var. *ferrugineum*; and 'Standard Aran' 1 and 'Standard Aran' 2. In this group, var. *erythrospermum* 3 and var. *erythroleucon* 9 could not be distinguished based on the seven microsatellite markers, perhaps due to their sharing of a similar genetic background.

To determine the distances among the Azerbaijani wheat botanical varieties analyzed in this study, the UPGMA dendrogram (Figure 3) was constructed based on Nei's genetic distance (Nei et al., 1983). Botanical varieties of wheat such as var. *milturum* and var. *graecum* diverged most from the other Azerbaijani botanical varieties studied. In fact, the samples of var. *milturum* and var. *graecum* showed the highest difference. Moreover, var. *ferrugineum*, var. *lutescens*, var. *erythrospermum*, and var. *erythroleucon* displayed the highest level of genetic similarity.

In conclusion, this study revealed significant variations in microsatellite DNA polymorphism among wheat varieties. The use of microsatellite markers confirmed the existence of considerable genetic diversity among 50 Azerbaijani wheat varieties at the DNA level and identified diverse genotypes for use in breeding programs for wheat improvement. These results suggest that SSR markers are valuable tools for the identification and diversity analysis of wheat genotypes.

Conflict of interest

The authors declare that they have no conflict of interest.

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