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AEGILOPS TAUSCHII GENETIC DIVERSITY USING SSR MARKERS AND MORPHOMETRIC CHARACTERS

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SUMMARY

The genus *Aegilops* L. native species, especially *Aegilops tauschii,* are important sources of numerous beneficial traits that are applicable as donors in bread wheat (*Triticum aestivum* L.) improvement. In this regard, the genetic diversity study of 23 local populations of *Ae. Tauschii,* collected from the southeastern region (Samarkand, Urgut, and Kitab districts) of Uzbekistan, became the presented research's aim, using nine SSR markers and morphometric traits. The SSR analysis amplified 32 alleles, with an average of 3.55 alleles per locus. The average polymorphism information content (PIC) was 0.447, ranging from 0.163 to 0.599, and the expected heterozygosity (He) varied, from 0.007 to 1.557, with an average of 0.850. Genetic distance (GD) indices based on five of nine SSR markers ranged from 0 to 0.789, with a mean of 0.669. The greatest genetic similarity was notable between populations from the Samarkand and Urgut districts (0.789), while the least was evident between the populations of the Samarkand and Kitab districts (0.560). Based on molecular analysis of nine SSR markers, the most informative ones (PIC > 0.5) showed distinction, beneficial to develop genetic passports and determine the genetic homogeneity of local species in *Ae. tauschii.*

Keywords: local ecotypes *Ae. tauschii*, PCR analysis, genetic diversity, DNA microsatellite loci, clustering, heterozygosity, genetic similarity

Key findings: A collection of 23 local accessions of *Ae. tauschii* showed characteristics of a higher level of genetic diversity. Based on molecular analysis, the SSR markers (WSP130, WSP192, and WSP513) occurred highly informative to benefit in developing genetic passports to determine the homogeneity of local species in genus *Aegilops* L.

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INTRODUCTION

Soft wheat (*T. aestivum* L. 2n = 6x = 42, ABD) is the founding staple food and economically important crop in the Republic of Uzbekistan. The manifestation of adaptive properties in cultivated plants weakened due to climate change. This resulted in cultivated cultivars of spring wheat not fully meeting production requirements because of yield instability and complex natural and climatic conditions of the republic (Baboeva *et al*., 2022).

The decline in genetic diversity is a global problem affecting most cultivated crops. However, for bread wheat, as the world's most consumed crop, these processes are in leading position – 65%–84% relative to wild ancestral types (Smith *et al*., 2015; FAOSTAT, 2019). Therefore, expanding the genetic diversity of bread wheat donors in terms of resistance to biotic and abiotic factors is an urgent task requiring the use of wild wheat relatives in different breeding programs (Martynov *et al*., 2015; Arzani and Ashraf, 2017; Shelenga *et al*., 2020; Itam *et al*., 2020; Ghobadi *et al*., 2021). Expanding the genetic diversity of the developed wheat cultivars for resistance to biotic and abiotic stress conditions can succeed by involving the genera *Aegilops* L., *Agropyron* L., *Secale* L., and *Elymus* L. in hybridization programs (Wulff and Moscou, 2014; Khodaee *et al*., 2021).

Aegilops L. is a large genus belonging to the tribe Triticeae, comprising 23 annual species with varying ploidy levels, vital in wheat improvement due to its tolerance to various biotic and abiotic stresses. Particularly, it is beneficial to expand the wheat gene pool and enhance the resistance to diseases, pests, drought, cold, and other environmental stresses (Urazaliev *et al*., 2018; Abbasov *et al*., 2019; Abbas *et al*., 2020). The *Ae. tauschii* (2n=2x=14, DD) consisted with 1219 genes presumably responsible for disease resistance, 485 genes for resistance to abiotic stress factors, 216 genes for cold tolerance, and 14 transcription factors responsible for drought resistance (Wang *et al*., 2013).

Aegilops tauschii, the Tausch's goatgrass or rough-spike hard grass, is an annual grass species. It is native to Crimea, the Caucasus region, Western and Central Asia, Afghanistan, Pakistan, the Western Himalaya, and parts of China. *Ae. tauschii* is part of the tribe Triticeae, along with wheat. It is a diploid (2n=2x=14, DD) goatgrass species, which has contributed the D genome to common wheat. The *Lr42* nucleotide-binding site leucine-rich repeat (NLR) is a resistance gene used in hexaploid wheat, conferring all-stage resistance to leaf rust (Zimin *et al.*, 2016).

The geographical location of the southeastern region of Uzbekistan, with its peculiar climatic conditions, is a unique place of natural habitat for wild relatives of wheat. The said environment comprised five species of the genus *Aegilops* L., which are valuable for identifying potential sources of useful traits in wheat breeding (Djabbarov *et al*., 2023). However, in Uzbekistan, the local *Ae. tauschii* represents a small collection with limited studies on genetic diversity, breeding utility, and molecular levels. Therefore, it seemed relevant to us to assess the genetic diversity of local *Ae. tauschii* in this region using SSR markers and morphological characters. The latest study aimed to assess the genetic diversity of *Ae. tauschii* local populations collected from different localities of the southeastern region of Uzbekistan.

MATERIALS AND METHODS

Plant material and experimental site

A collection of the local populations of *Ae. tauschii* (2n=2x=14, DD), developed in the Department of Genetics and Biotechnology, Samarkand State University named after Sh. Rashidov, was the research sample (Sobirov and Djabbarov, 2021). Detailed information about the collection sites and geographic coordinates of distribution of *Ae. tauschii* samples appear in Table 1. The field research applied Dospehov's (1986) method at the experimental site, Samarkand District, Institute of Genetics and Experimental Biology of the Academy of Sciences, Republic of Uzbekistan, with the laboratory research carried out at the Laboratory of Plant Molecular and Biochemical Genetics.

Table 1. *Ae. tauschii* accessions collected from different localities of Southeastern region of Uzbekistan.

Seed sample collection location		Height above	Geographical coordinates		Number of
Village (V)	District	sea level (m)	Latitude	Longitude	accessions
Agalyk	Samarkand	870	39°55′04.76″	66°89′66.49″	4
Akbuyra	-do-	850	39°51′22.79″	66°88′62.89″	1
Kyzylbash	Urgut	1121	39°23′17.03″	67°00′19.37″	1
Tersak	-do-	1121	39°36′92.53″	66°94′38.18″	4
Aman-Kutan	-do-	1320	39°18′24.29″	66°55′49.11″	1
Varganza	Kitab	855	39°19′72.08″	66°98′36.83″	1
Dzhauz	-do-	1225	39°11′46.08″	67°16′83.92″	2
Reserve	-do-	1375	39°11′23.74″	67°17′35.27″	4
Panji	-do-	719	39°14′80.68″	66°96′07.64″	1
Khazraty-Bashi	-do-	874	39°23′38.40″	67°03′65.46″	4
Total					23

DNA extraction

Total DNA isolation came from leaves of 10–15-day-old seedlings of *Ae. tauschii* grown at the Institute's experimental field using the CTAB method, with some modifications (Saghai-Maroof *et al*., 1984). The present research used nine pairs of SSR primers, generated by Bio-Basic Inc. (20 Konrad Crescent, Markham, ON L3R 8T4, Canada, https://www.biobasic.com).

PCR analysis and electrophoresis

The 25 µL SSR amplification reaction medium included 0.2 mM each dNTP, 250 µM each primer, 1.5 mM $MgCl_2$, 10 mM Tris-HC1 (pH 9.0), 1 U Taq polymerase (Promega), and 50-100 ng of DNA being tested. Amplification continued in the following mode: initial denaturation of double-stranded DNA – 3 min at 94 °C; 35 cycles: 94 °C for 30 s; annealing – 1 min at 50 °C, 55 °C, or 60 °C (annealing temperature – depending on the SSR primers used in the analysis); elongation – 2 min at 72 °C, and the final stage – elongation 10 min at a temperature of 72 °C. PCR ran on a BioRad thermal cycler (BioRad Laboratories Inc., Hercules, CA, USA).

Fractionation of amplification products employed electrophoresis on a 6% denaturing polyacrylamide gel (PAGE), and after staining with ethidium bromide, visualized under UV light using a Sequi-Gen GT Sequencing Cell 30 cm gel apparatus (BioRad Laboratories Inc., Hercules, CA, USA).

Statistical analysis

The indicators of genetic diversity—the total number of alleles (Na), expected heterozygosity (He), and observed heterozygosity (Ho) — using the PowerMarker v 3.51 program (Liu and Muse, 2005). The calculation of information content index of PIC (polymorphism information content) used the formula:

$$PIC_j = 1 - \sum_{i=1}^{n} P_i^2,$$

For statistical programs, the PowerMarker used helped calculate allele frequencies, cluster analysis, DARwin 6.0 for constructing an unweighted neighbor joining tree (Gascuel, 1997; Perrier and Jacquemoud-Collet, 2006), and Nei (Pop Gene32, UPGMA = Unweighted PairGroup Methods) for determining genetic distances and similarities.

RESULTS

Polymorphism of SSR markers

The *Ae. tauschii* local collection comprised 23 accessions, collected from various administrative localities of the southeastern

Table 2. PCR fragments in different species of *Ae. tauschii* obtained using SSR primers.

Primers	Number of fragments									Total
	WSP006	WSP044	WSP107	WSP130	WSP156	WSP190	WSP192	WSP325	WSP513	
Total	2	3	3	4	3	5	4	2	6	32.0
Polymorphic	1	2	2	3	2	4	3	1	6	24.0
Polymorphic (%)	50.0	66.7	66.7	75.0	66.7	80.0	75.0	50.0	100	75.0
Monomorphic	1	1	1	1	1	1	1	1	0	8.0
Monomorphic (%)	50.0	33.3	33.3	25.0	33.3	20.0	25.0	50.0	0	25.0

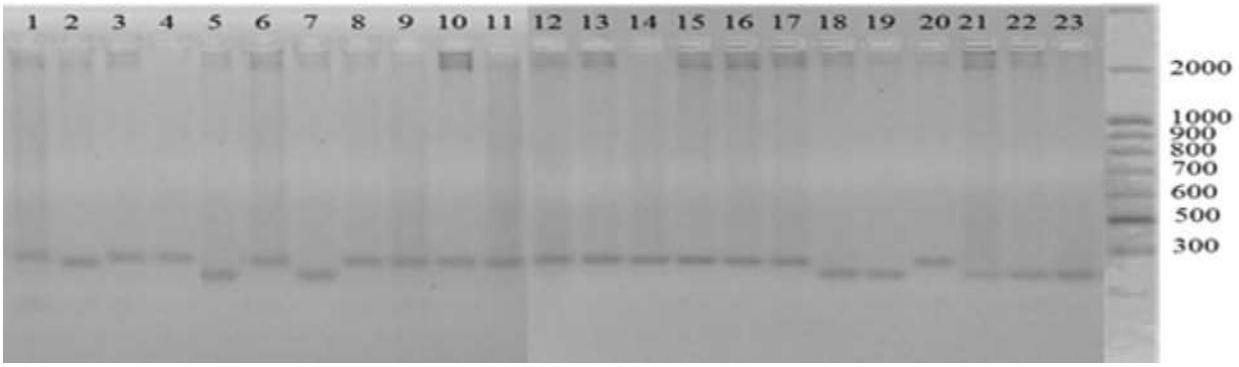

Figure 1. Electropherogram of the SSR spectrum of 23 *Ae. tauschii* populations by using WSP190 primer (gel fragment).

region of Uzbekistan, underwent studies by using nine pairs of SSR primers. The PCR fragments showing the polymorphism of *Ae. tauschii* local populations by microsatellite markers are visible in Table 2.

The SSR analysis identified 32 fragments, in which 24 (75.0%) turned out to be polymorphic, and eight (25%) were monomorphic. The number of DNA fragments amplified by one primer ranged from two (WSP006) to six (WSP513). The maximum number of polymorphic fragments (6) was notable for the primer WSP513, while the minimum for marker WSP006 (2).

Overall, in local populations of *Ae. tauschii* from the southeast region, the average number of fragments per marker was consistent with data obtained for *Ae. tauschii* from other regions of the world (Yu *et al*., 2021). It indicated a high level of diversity in *Ae. tauschii* populations in this region. The electropherogram showing the polymorphism of local populations of *Ae. tauschii* by microsatellite markers is available in Figure 1. Each electrophoretic profile also corresponds to an individual *Ae. tauschii* population. Based on SSR analysis, the data showed each population had characteristic and non-repetitive PCR fragments. However, in some cases, more than one fragment was distinct in one sample, accompanied by the presence of intraspecific heterogeneity.

The molecular analysis revealed the two SSR primers WSP190 and WSP192 showed fragments with a molecular weight of 800 and 1000 bp, respectively. The polymorphism of 75%–80% was evident, with the fragments recorded with a molecular weight of 300, 500, 700, 800, 900, and 1000 bp, and even above 100% polymorphism. The most polymorphic primers included WSP107, WSP130, WSP190, WSP192, and WSP513.

PCR amplification and genetic diversity

Calculating the genetic parameters of microsatellite loci also assessed inter- and intrapopulation diversity of the *Ae. tauschii*

local populations. The molecular genetic analysis revealed 24 polymorphic alleles using nine pairs of SSR markers, and their length varied from 121 to 196 bp (Table 3). Among 23 *Ae. tauschii* accessions, the observed number of alleles (Na) at different loci varied from two (WSP006) to six (WSP513) and averaged 3.55 per locus. The average effective number of alleles (Ne) was 0.850, ranging from 0.007 to 1.557.

The ratios for expected heterozygosity (He) and observed heterozygosity (Ho) ranged from 0.007 to 1.557, with an average of 0.850, and 0.537 (WSP325) to 1.294 (WSP107), with an average of 0.921, respectively. The average polymorphism information value (PIC) was 0.447, ranging from 0.163 to 0.599. Among the nine SSR markers, the most effective and informative were those with PIC values ≥ 0.5. In the total population, the Shannon diversity index (I) varied from 0.693 (WSP107) to 1.220 (WSP006), with an average of 0.940 (Table 4).

Table 3. Characteristics of the SSR primers used.

Primers	Forward and reverse direction (5`-3`)	Annealing temperature (°C)	Length (bp)	Chromosome
WSP006	CGT ATC ACC TCC TAG CTA AAC TAG AGC CTT ATC ATG ACC CTA CCT T	55	196	4B
WSP044	GTT GAG CTT TTC AGT TCG GC ACT GGC ATC CAC TGA GCT G	60	176	7B
WSP107	ATT AAT ACC TGA GGG AGG TGC GGT CTC AGG AGC AAG AAC AC	60	188	4B
WSP130	AGC TCT GCT TCA CGA GGA AG CTC CTC TTT ATA TCG CGT CCC	60	121	7A
WSP156	CCA ACC GTG CTA TTA GTC ATT C CAA TGC AGG CCC TCC TAA C	60	279	5A
WSP190	GTG CTT GCT GAG CTA TGA GTC GTG CCA CGT GGT ACC TTT G	60	253	5D
WSP192	GGT TTT CTT TCA GAT TGC G CGT TGT CTA ATC TTG CCT TGC	60	232	5D
WSP325	TTT CTT CTG TCG TTC TCT TCC C TTT TTA CGC GTG AAC GAC G	60	138	6D
WSP513	ATC CGT AGC ACC TAC TGG TCA GGT CTG TTC ATG CCA CAT TG	60	146	4B

Table 4. Genetic diversity parameters of *Ae. tauschii* populations based on SSR markers.

Primers	Na	Ho	He	PIC	I I
WSP006	2	0.692	0.450	0.599	1.220
WSP044	3	1.117	0.902	0.461	0.797
WSP107	3	1.294	0.422	0.163	0.693
WSP130	4	0.848	1.231	0.503	0.902
WSP156	3	0.723	0.419	0.575	0.927
WSP190	5	1.129	1.557	0.586	1.180
WSP192	4	0.891	0.438	0.371	0.753
WSP325	2	0.537	0.007	0.221	0.827
WSP513	6	1.062	0.458	0.548	1.190
Average	3.55	0.921	0.850	0.447	0.940
Total alleles	32				

Note: Number of observed alleles (Na); Observed heterozygosity (Ho); Expected heterozygosity (He); Polymorphism information content (PIC); Shannon information index (I).

Table 5. Nei's genetic distance among the populations of *Ae. tauschii* based on the SSR analysis.

Populations	*Ae. tauschii* (S)	*Ae. tauschii* (U)	*Ae. tauschii* (K)
Ae. tauschii (Samarkand-S)	-	0.789	0.560
Ae. tauschii (Urgut-U)	0.789	-	0.658
Ae. tauschii (Kitab-K)	0.560	0.658	-

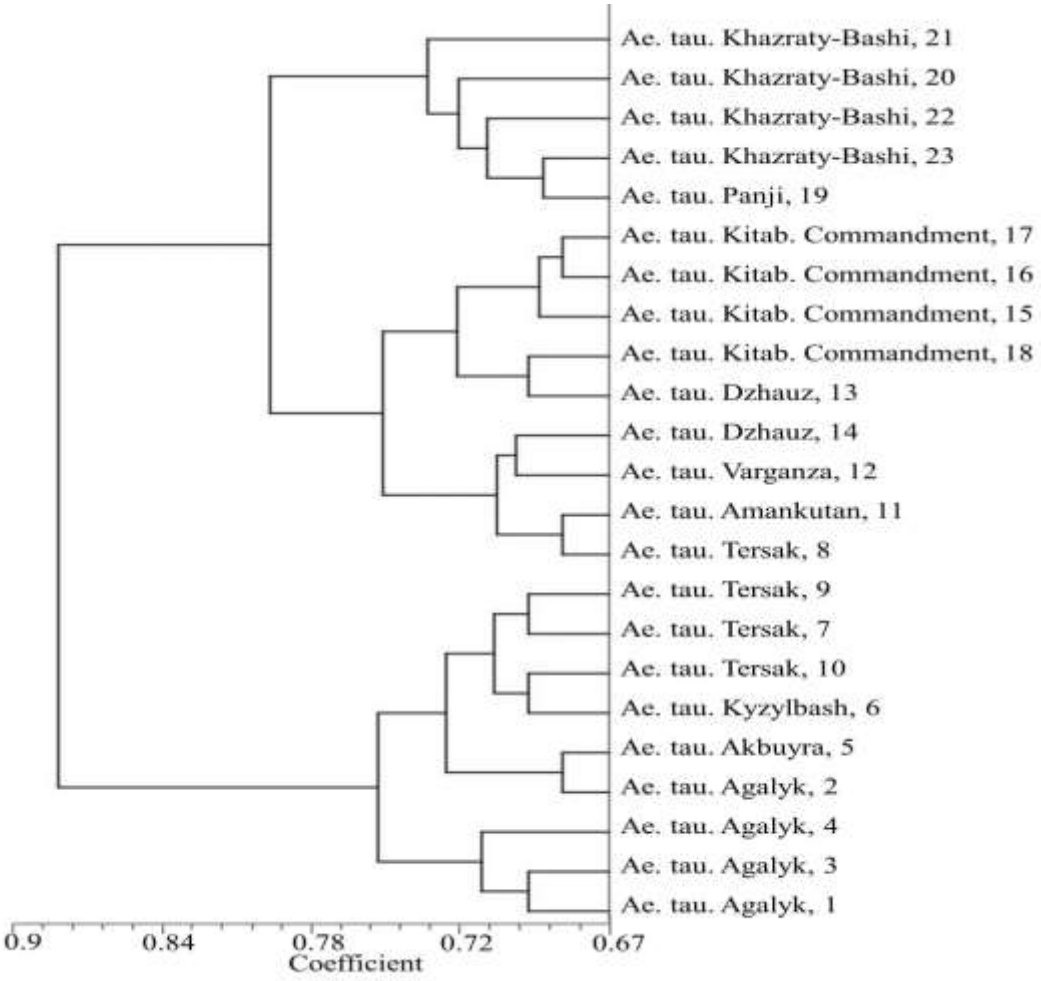

Figure 2. Dendrogram of phylogenetic relationships of *Ae. tauschii* based on nine SSR markers.

Cluster analysis

The SSR analysis helped study the genetic diversity of the *Ae. tauschii* local populations and identify genetic distances among the different populations. The genetic distance (GD) level for nine SSR markers among the *Ae. tauschii* populations varied from zero to 0.789, with an average value of 0.669. The largest value of genetic distance came from between the populations of the Samarkand and Urgut districts (GD = 0.789), while the smallest genetic distance was notable between the populations of the Samarkand and Kitab (GD = 0.560) districts (Table 5).

A dendrogram construction relied on the UPGMA method using data obtained from nine SSR markers based on the UPGMA method, grouping the *Ae. tauschii* 23 populations into three clusters (Figure 2). The dendrogram reflects the genetic distances among the analyzed local populations of *Ae.*

tauschii that revealed a relationship among the genotypes belonging to three different districts (Samarkand, Urgut, and Kitab of Uzbekistan) (Figure 2). Thus, cluster I comprised the genotypes 1-5 (accessions obtained from the Samarkand district), while the second cluster included 6-11 (landraces from the Urgut district).

The cluster III consists of accessions 12-23 procured from the Kitab district of Uzbekistan. Apparently, on specific clustering of local populations of *Ae. tauschii* by ecological and geographical origins, one can conclude the discriminatory analysis based on SSR markers. These accurately reflect the genetic similarity of genotypes belonging to these districts of the southeastern region of Uzbekistan. Among the studied populations of *Ae. tauschii*, the highest genetic diversity was prominent among the accessions obtained from Samarkand and Kitab districts, characteristically indicating 75% of their polymorphism (Table 2).

DISCUSSION

Wild relatives of wheat are potential sources of valuable genetic material used in bread wheat improvement. Knowledge about the genetic diversity of wild wheat relatives is crucial for their conservation and utilization (Salehi *et al*., 2018; Elhadi *et al*., 2021; Ghobadi *et al*., 2021; Gaurav *et al*., 2021). *Aegilops* comprising wheat wild relatives is a large genus of the *Triticeae* tribe. It includes 23 annual species with different ploidy levels, which have the potential sources of economically valuable traits beneficial in wheat improvement programs (Tuler *et al*., 2015; Kishii, 2019). Therefore, in *Ae. tauschii* population, the identification of allelic diversity is of vital importance for effective breeding and expansion of the genetic base in common wheat (Mahjoob *et al*., 2021).

The need to analyze polymorphism in those populations at the genetic level will reliably distinguish and identify the plant genetic resources. The most promising approach for analyzing genetic diversity seems to be the use of molecular genetics methods based on the analysis of DNA polymorphism (RAPD, AFLP, SSR, and SNP), which further allows for obtaining an individual characteristic of each genotype with a DNA profile (Bushakra *et al*., 2015; Lee *et al*., 2015; Lacis *et al*., 2017; Zurn *et al*., 2018).

Genetic material comprising 23 local accessions of *Ae. tauschii* was also under scrutiny for the identification of alleles using nine SSR primers. In total, 32 alleles identified had the number of alleles per locus varying from two to 3.55. The maximum number of alleles (6.0) appeared for the locus in primer WSP513. Overall, the average number of alleles per locus (3.55) in local populations was consistent with data obtained for *Ae. tauschii* in other regions of the world, indicating a high level of diversity. Yu *et al.* (2021) examined the distribution, genetic diversity, and population structure of *Ae. tauschii* populations in major wheat growing regions in China. They reported the total number of effective alleles amplified by 17 SSR loci was 80, ranging from 1.7 to 11, with an average of 4.706 per locus.

The analysis established the polymorphism information content (PIC) for all the studied SSR markers (Table 4). The mean PIC value of 0.447 was quite consistent with those reported in other studies. Moradkhani *et al*. (2015) probed the different *Aegilops* species using 10 microsatellite markers, and showed PIC values for all SSR markers varied from 0.345 to 0.375, with an average of 0.367. Abbas *et al*. (2020) reported the maximum PIC value was 0.63, with a mean of 0.20, and the maximum allele frequency was 1.00, with an average of 0.88. However, these results imply an abundant genetic polymorphism exists in *Ae. tauschii* accessions. Similarly, in other studies, the average PIC value in *Ae. tauschii* accessions was consistent with the present results (Moradkhani *et al*., 2015; Abbas *et al*., 2020; Yu *et al*., 2021).

Moreover, in 46 populations of *Commelina communis* using 12 SSR markers, the obtained average PIC value was 0.20 (Yang *et al*., 2018). In the presented study, while assessing the genetic diversity and structure of 23 local populations of *Ae. tauschii* using nine SSR markers obtained 32 alleles, with an average of 3.55 alleles per primer. The

average polymorphism information content (PIC) and expected heterozygosity (He) for the entire population were 0.447 and 0.850, respectively. The genetic distance (GD) index based on nine SS markers ranged from zero to 0.789, with a mean of 0.669. Abbas *et al*. (2020) reported, based on assessment of genetic diversity of six *Aegilops* L. species from Azerbaijan and Georgia using five SSR markers, 39 alleles occurred, with an average of 7.8 alleles per primer.

Microsatellite analysis using nine SSR primers identified 24 (75%) polymorphic loci, which collectively amplified 32 alleles, ranging from 121 to 279 bp in size. Depending on the locus, the number of alleles varied from two to six, with an average of 3.55 alleles. The effective number of alleles (Ne) ranged from 0.007 to 1.557, with an average of 0.850, and the PIC (polymorphic information content) index value ranged from 0.163 to 0.599, with an average of 0.447 (Table 4). It was slightly lower than results reported by Abbas *et al*. (2020). The degree of polymorphism can vary significantly depending on the number of genotypes studied and their genetic diversity. Tuler *et al*. (2015) declared the transferability of SSR markers depends on the conservation of SSR regions and the primer annealing sequence. Thus, the highest percentage of transferability of nine SSR markers indicates the high conservation of these regions and close phylogenetic relationship among the studied local accessions of *Ae. tauschii*.

Based on the SSR analysis, the study of genetic diversity of the *Ae. tauschii* accessions had the genetic distances among the genotypes identified. A phylogenetic tree constructed demonstrated the clustering of local *Ae. tauschii* accessions in the southeastern region of Uzbekistan (Figure 2). Genetic distance (GD) indices based on nine SSR markers ranged from 0.560 to 0.789, with an average of 0.669. The highest genetic similarity was evident between *Ae. neglecta* and *Ae. triuncialis* (GD = 0.26), and the lowest between *Ae. neglecta* and *Ae. tauschii* (GD = 0.66).

The dendrogram reflects the genetic distances among the analyzed local accessions of *Ae. tauschii*, indicating relationships among the genotypes of Samarkand, Urgut, and Kitab districts of Uzbekistan (Figure 2). Thus, the cluster I prevail the Samarkand samples, while cluster II owned the accessions of Urgut. Cluster III grouped the accessions from the Kitab district. The genetic structure among the 23 *Aegilops* accessions was similar to the cluster analysis. Abbasov *et al*. (2019) stated the genetic distance indices based on five SSR markers ranged from zero to 0.83, with an average value of 0.47 in the *Aegilops* L. species from Azerbaijan and Georgia, and were consistent with the presented results.

From Table 4, using nine pairs of SSR primers detected the greatest diversity for microsatellite markers, and according to Nei, the highest values of genetic diversity were notable (0.00–1.557). This confirms these SSR markers were the most informative for studying the genetic diversity in local accessions of *Ae. tauschii* (Al-Tamimi and Al-Janabi, 2019; Ali *et al*., 2023)

CONCLUSIONS

For the first time, the genetic diversity study in local populations of *Ae. tauschii* in southeastern region of Uzbekistan materialized. In *Ae. tauschii* populations, genetic variability has been distinct for number of alleles per locus and genetic diversity and heterozygosity indices. Based on molecular analysis, the most informative markers, WSP130, WSP190, WSP513, WSP130, WSP190, and WSP513 as selected, can be favorable for DNA certification and determination of the genetic homogeneity of *Ae. tauschii* local species in Uzbekistan.

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