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AEGILOPS L. GENETIC DIVERSITY IN SOUTHWESTERN REGION OF UZBEKISTAN

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SUMMARY

As wheat donors, wild species of the genus *Aegilops* L. play a vital role in practical breeding to improve wheat production because of their strong relationship and wide genetic diversity. Using nine simple sequence repeat (SSR) markers helped assess the genetic diversity in 96 collected samples of four species belonging to the genus *Aegilops,* i.e., *Aegilops tauschii Coss* (D), *Ae. cylindrica Host* (CD), *Ae. crassa Boiss* (DDM), *and Ae. triuncialis* L. (UC). The said collection came from 21 sites of various expeditions located in three regions of Southwestern Uzbekistan (Samarkand, Urgut, Kitab, and Shakhrisabz regions). Generally, 102 distinct alleles were found, with an average of 11.33 alleles per primer. The total number of species-specific amplicons was 35. The polymorphism detected varied from 28.6% (for the WSP107 primer) to 77.0% (for the WSP130 and WSP192 primers). The mean values of polymorphism information content (PIC) and expected heterozygosity (Ho) for all samples were 0.675 and 0.527, respectively. Based on nine SSR markers, on average, the genetic distance indices (GD) varied from 0.63 to 0.77. The highest genetic similarity (GD = 0.77) recorded occurred between the species *Ae. crassa* and *Ae. cylindrica*, whereas the least (GD = 0.48), between *Ae. cylindrica* and *Ae. triuncialis* with their taxonomic classification. Genus *Aegilops* samples from the same region often attain an identical subgrouping, which might be due to relatedness by genetic parameters. The gene pool of native species of the genus *Aegilops* from the Southwestern region of Uzbekistan may provide suitable alleles for wheat improvement and adaptation in the future.

Keywords: *Aegilops* L., A*e. tauschii, Ae. crassa, Ae. cylindrica, Ae. triuncialis,* genetic diversity, D-genome, SSR primers

Key findings: Results revealed from nine SSR markers a marker WSP513 was the most polymorphic. In the genus *Aegilops* L., the species *Ae. tauschii* showed the highest genetic diversity, and samples collected from Khazraty Bashi (21) enunciated the foremost polymorphism among the studied regions.

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INTRODUCTION

Aegilops is a large genus of the tribe *Triticeae*, including 23 annual species (11 diploid, eight tetraploid, and four hexaploid species) with diverse ploidy (Konstantinos and Bebeli, 2010). These *Aegilops* species have main distribution in the Mediterranean and Southwestern and Central Asia (Kilian *et al.,* 2011), with centers of origin in Georgia, Armenia, Azerbaijan, and Northern Iran (Hammer *et al.,* 2001; Van-Slageren, 1994), and Cyprus, Lebanon, Israel, Syria, Iraq, Southeast Turkey, Southwest Iran, and Northwest Jordan (Lelley *et al.,* 2000). These diverse regions have high concentrations of the *Aegilops* species, which are also well-adapted compared with other genera. Some *Aegilops* species even had involvement in the wheat evolution (*Ae. tauschii* – the D-genome donor and of great importance in the origin of common wheat); thus, potentially beneficial in wheat improvement programs (Konstantinos and Bebeli, 2010; Tilman *et al*., 2011; Reynolds *et al.,* 2012; Ogbonnaya *et al.,* 2013; Kishii, 2019; Abbas *et al.,* 2020).

Aegilops and Gossipum species often have a wide variety of the desired traits characterizing them, which provides an invaluable gene pool for resistance to biotic and abiotic stressors in wheat breeding (Saghai-Maroof *et al.,* 1984; Alnaddaf *et al.,* 2012; Martynov *et al.,* 2015; Arzani and Ashraf, 2017; Elbashir *et al.,* 2017; Itam *et al.,* 2020; Shavkiev *et al.,* 2022, 2023; Makamov *et al.,* 2023; Kurbanbaev *et al.,* 2023). For cereal resistance to biotic stresses, 20% of genes were found in various species of the genus *Aegilops* (Roder *et al*., 1995). Several *Aegilops* species are also distinct with exceptional yield and quality traits, such as, growth, large ears, and the high content of microelements and gluten in the grains (Kavrakova, 2009; Buronov *et al*., 2023).

Molecular analysis can better suit the study of the genetic diversity of *Aegilops* species and facilitate the complex trait association and the selection of suitable donors for breeding purposes (Aliyev e*t al.,* 2007; Hajiyev *et al.,* 2015). In this regard, DNA markers have breeders' wide use of these to assess the *Aegilops* intraspecific genetic diversity. Like the SSR markers, molecular markers have proven the most effective because of their polymorphism, reproducibility, co-dominance, and simplicity (Roder *et al.,* 1995). These markers are chromosome-specific, with an extensive use of breeders for identifying the helpful genes (Fufa *et al*., 2005), assessing genetic diversity (Ehtemam *et al.,* 2010; Masoumi *et al.,* 2012; Hajiyev *et al.,* 2015; Henkrar *et al.,* 2016; Vieira *et al.,* 2016), and phylogenetic relationships in various crops, including wheat and its wild relatives (Liu *et al.,* 2016; Luo *et al.,* 2017; Abbasov *et al.,* 2018). However, despite long-time research on the genetic diversity of *Aegilops* species, each species requires additional information on their distribution and diversity in different agroecological zones.

The southwest of Uzbekistan is a mountainous region within which various crop plant species are prevalent, including diverse species of the genus *Aegilops* (Sobirov and Djabbarov, 2021; Chorshanbiev *et al.,* 2023). The area has a unique natural habitat for wild relatives of wheat, wherein five species of the genus *Aegilops* occupy a prominent place. This region has limited study of the *Aegilops* species on their genetic diversity and breeding utility. Given these facts, the local populations of *Aegilops* species should have much consideration as potential sources of helpful alleles for wheat improvement and adaptation programs.

Therefore, it seemed relevant to assess the genetic diversity of *Aegilops'* local species grown in different natural and climatic conditions of the southwestern region of Uzbekistan using SSR markers since it is the first time in this region that research on the genus *Aegilops* using SSR markers will happen. The presented study sought to assess the genetic diversity of the genus *Aegilops* collection comprising 96 specimens of four local species distributed in the southwestern region of Uzbekistan, using nine SSR markers.

MATERIALS AND METHODS

Plant material

The breeding material comprised a collection of 96 samples representing four diverse species

of the genus *Aegilops* L. belonging to the southwestern region of Uzbekistan. The samples' collection occurred during the route reconnaissance expeditions in 2021. The collected 96 specimens consisted of the following: 1) 23 (1-23) belonged to *Aegilops* L. species *Ae. tauschii*, 2) 11 (24-34) from *Ae. crassa*, 3) 28 (35-62) from *Ae. cylindrica*, and 4) 34 (63-96) from the species *Ae. triuncialis*. Details of collection points are available in Figure 1 and Table 1.

DNA isolation

Total genomic DNA isolation from the tissues of fresh leaves of 10–15-day-old seedlings used the CTAB method with minor modifications (Saghai-Maroof, 1984). Using an ultraviolet photometer measured the quantity and quality of the DNA. The study utilized nine pairs of SSR markers (generated by Bio-Basic Canada Inc., China). For polymorphism analysis, evaluation engaged the genus *Aegilops* L. accessions (Table 2).

PCR analysis

The reaction medium for SSR-amplification with a volume of 25 µl included 0.2 mM of each dNTP (Syntol, Russia), 250 µM of each primer, 1.5 mM $MgCl_2$ (Syntol, Russia), 10 mM Tris-HC1 (pH = 9.0), 1 unit of Taq-polymerase (Syntol, Russia), and 50–100 ng of the researched DNA. Amplification continued with the following mode: initial denaturation of double-stranded DNA - 3 min at 94 °C; 35 cycles: 94 °C - 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 the temperature of 72 °C (Abugalieva and Turuspekov, 2009; Abugalieva *et al*., 2010; Sultanov *et al.,* 2022). The PCR ran on a BioRad thermal cycler (BioRad Laboratories Inc., Hercules, California, USA).

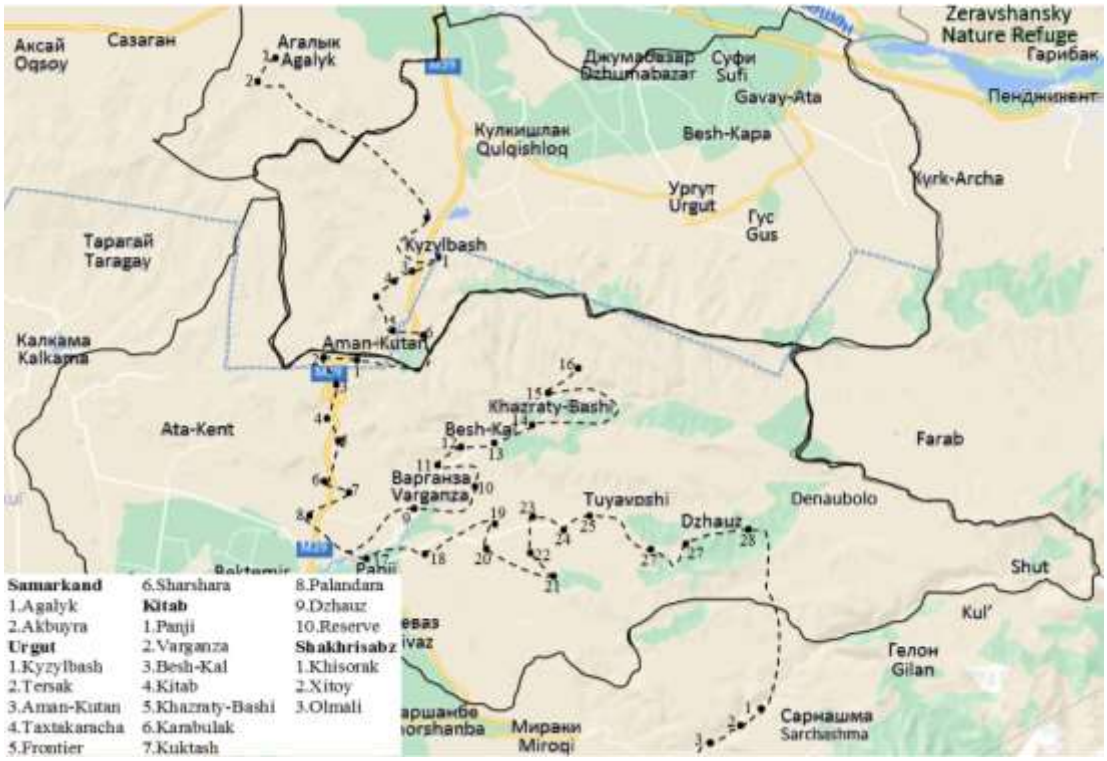


Figure 1. Map of the route-reconnaissance survey to collect a sample of seeds of species of the genus *Aegilops* (numbers of collection points correspond to those presented in Table 1).

Table 1. Genus *Aegilops* accessions belonging to four local species used for the analysis.

Collection place	Accessions	Geographical coordinates	
		Latitude	Longitude
Ae. tauschii, 2n=14 (D)			
Samarkand region Samarkand district. Agalyk village, h=870	1-4	39°55′04.76″	66°89′66.49″
Samarkand region Samarkand district. Akbuyra village, h=850	5	39°51′22.79″	66°88′62.89″
Samarkand region Urgut district. Kyzylbash village, h=1121	6	39°23′17.03″	67°00′19.37″
Samarkand region Urgut district. Tersak village, h=1121	7-10	39°36′92.53″	66°94′38.18″
Samarkand region Urgut district. Amankutan village, h=1320	11	39°18′24.29″	66°55′49.11″
Kashkadarya region Kitab district. Varganza village, h=855	12	39°19′72.08″	66°98′36.83″
Kashkadarya region Kitab district. Dzhauz village, h=1225	13-14	39°11′46.08″	67°16′83.92″
Kashkadarya region Kitab district. Kitab geological reserve, h=1375	15-18	39°11′23.74″	67°17′35.27″
Kashkadarya region Kitab district. Panji village, h=719	19	39°14′80.68″	66°96′07.64″
Kashkadarya region Kitab district. Khazraty Bashi village, h=874	20-23	39°23′38.40″	67°03′65.46″
Ae. crassa, 2n=42 (DDM)			
Samarkand region Samarkand district. Agalyk village, h=870.	24-25	39°55′04.76″	66°89′66.49″
Samarkand region Samarkand district. Akbuyra village, h=850	26	39°51′22.79″	66°88′62.89″
Samarkand region Urgut district. Kyzylbash village, h=1121	27	39°23′17.03″	67°00′19.37″
Samarkand region Urgut district. Tersak village, h=1121	28-29	39°36′92.53″	66°94′38.18″
Samarkand region Urgut district. Amankutan village, h=1320	30	39°18′24.29″	66°55′49.11″
Kashkadarya region Kitab district. Varganza village, h=855	31	39°19′72.08″	66°98′36.83″
Kashkadarya region Kitab district. Kitab geological reserve, h=1375	32	39°11′23.74″	67°17′35.27″
Kashkadarya region Kitab district. Panji village, h=719	33	39°14′80.68″	66°96′07.64″
Kashkadarya region Kitab district. Khazraty Bashi village, h=874	34	39°23′38.40″	67°03′65.46″
Ae. cylindrica, 2n=28 (CD)			
Samarkand region Samarkand district. Agalyk village, h=870.	35-37	39°55′04.76″	66°89′66.49″
Samarkand region Samarkand district. Akbuyra village, h=850	38	39°51′22.79″	66°88′62.89″
Samarkand region Urgut district. Kyzylbash village, h=1121	39	39°23′17.03″	67°00′19.37″
Samarkand region Urgut district. Tersak village, h=1121	40-43	39°36′92.53″	66°94′38.18″
Samarkand region Urgut district. Amankutan village, h=1320	44	39°18′24.29″	66°55′49.11″
Kashkadarya region Kitab district, Besh-Kal village, h=785	45	39°19′72.08″	66°98′36.83″
Kashkadarya region Kitab district. Varganza village, h=855	46-49	39°19′72.08″	66°98′36.83″
Kashkadarya region Kitab district. Dzhauz village, h=1225	50-51	39°11′46.08″	67°16′83.92″
Kashkadarya region Kitab district, Kitab geological reserve, h=1375	52-55	39°11′23.74″	67°17′35.27″
Kashkadarya region Kitab district surrounding Kitab town, h=770	56	39°19′65.25″	66°90′68.54″
Kashkadarya region Kitab district. Kuktash village, h=915	57	39°17′71.26″	67°07′55.20″
Kashkadarya region Kitab district. Palandara village, h=815	58	39°16′22.07″	66°98′77.85″
Kashkadarya region Kitab district. Panji village, h=719	59	39°14′80.68″	66°96′07.64″
Kashkadarya region Kitab district. Khazraty bashi village, h=874	60-62	39°23′38.40″	67°03′65.46″
Ae. triuncialis, 2n=28 (UC)			
Samarkand region Samarkand district. Agalyk village, h=870.	63-66	39°55′04.76″	66°89′66.49″
Samarkand region Samarkand district. Akbuyra village, h=850	67-68	39°51′22.79″	66°88′62.89″
Samarkand region Urgut district. Kyzylbash village, h=1121	69	39°23′17.03″	67°00′19.37″
Samarkand region Urgut district. Tersak village, h=1121	70-71	39°36′92.53″	66°94′38.18″
Samarkand region Urgut district. Amankutan village, h=1320	72	39°18′24.29″	66°55′49.11″
Samarkand region Urgut district. Taxtakaracha village, h=1650	73	39°28′48.88″	65°82′25.25″
Samarkand region Urgut district the border between Kitab h=1620	74	39°28′48.88″	65°82′25.25″
Samarkand region Urgut district. Sharshara h=1610	75	39°28′48.88″	65°82′25.25″
Kashkadarya region Kitab district. Besh-Kal village, h=785	76	39°19′72.08″	66°98′36.83″
Kashkadarya region Kitab district. Varganza village, h=855	77-78	39°19′72.08″	66°98′36.83″
Kashkadarya region Kitab district. Dzhauz village, h=1225	79-82	39°11′46.08″	67°16′83.92″
Kashkadarya region Kitab district. Kitab geological reserve, h=1375	83-85	39°11′23.74″	67°17′35.27″
Kashkadarya region Kitab district. Karabulak village, h=735	86	39°23′38.40″	67°03′65.46″
Kashkadarya region Kitab district, surroundings Kitab town, h=770	87	39°19′65.25″	66°90′68.54″
Kashkadarya region Kitab district. Kuktash village, h=915	88	39°17′71.26″	67°07′55.20″
Kashkadarya region Kitab district. Palandara village, h=815	89	39°16′22.07″	66°98′77.85″
Kashkadarya region Kitab district. Panji village, h=719	90	39°14′80.68″	66°96′07.64″
Kashkadarya region Kitab district. Khazraty Bashi village, h=874	91-93	39°23′38.40″	67°03′65.46″
Kashkadarya region Shakhrisabz district. Khisorak village, h=820	94	38°53′51.11″	67°15′42.82″
Kashkadarya region Shakhrisabz district. Xitoy village, h=850	95	38°52′29.21″	67°18′24.21″
Kashkadarya region Shakhrisabz district. Olmali village, h=790	96	38°87′35.35″	67°30′89.28″

Table 2. Characterization of nine SSR markers used to assess the genetic diversity of 96 *Aegilops* landraces.

Primers	Forward and reverse direction (5`-3`)	Annealing temperature °C	Base pair length	Chromosome
WSP006	F: CGTATCACCTCCTAGCTAAACTAG R: AGCCTTATCATGACCCTACCTT	55	196	4B
WSP044	F: GTTGAGCTTTTCAGTTCGGC R: ACTGGCATCCACTGAGCTG	60	176	7B
WSP107	F: ATTAATACCTGAGGGAGGTGC R: GGTCTCAGGAGCAAGAACAC	60	188	4B
WSP130	F: AGCTCTGCTTCACGAGGAAG R: CTCCTCTTTATATCGCGTCCC	60	121	7A
WSP156	F: CCAACCGTGCTATTAGTCATTC R: CAATGCAGGCCCTCCTAAC	60	279	5A
WSP190	F: GTGCTTGCTGAGCTATGAGTC R: GTGCCACGTGGTACCTTTG	60	253	5D
WSP192	F: GGTTTTCTTTCAGATTGCG R: CGTTGTCTAATCTTGCCTTGC	60	232	5D
WSP325	F: TTTCTTCTGTCGTTCTCTTCCC R: TTTTTACGCGTGAACGACG	60	138	6D
WSP513	F: ATCCGTAGCACCTACTGGTCA	60	146	4B

Fractionation of amplification products proceeded by electrophoresis in 6% denaturing polyacrylamide gel (PAAG), followed by staining in ethidium bromide visualized under ultraviolet using a 30 cm Sequi-Gen GT Sequencing Cell gel apparatus (BioRad Laboratories Inc., Hercules, California, USA).

Statistical analysis

Indicators of the genetic diversity assessment, i.e., the total number of alleles (Na), expected heterozygosity (He), observed heterozygosity (Ho), and polymorphism (PIC), used the PowerMarker 3.51 program (Liu and Muse, 2005). PowerMarker software also helped formulate the allele frequencies. Cluster analysis and creating an unweighted neighbor union tree also employed the DARwin 6.0 software package (Gascuel, 1997; Perrier and Jacquemoud-Collet, 2006). The statistical program also served various methods for determining genetic distances and genetic similarity according to Nei (PopGene32, UPGMA - Unweighted Pair Group Method with Arithmetic mean). Based on the binary matrices in the PAST 3.16 program, the Dice genetic similarity coefficients among the samples attained calculations (Hammer *et al*., 2001).

RESULTS

SSR marker polymorphism

The use of methods of monolocus analysis and SSR-marking, most commonly used, helped to characterize and compare the levels of genetic diversity of the *Aegilops* collection comprising four local species. Selecting 96 genotypes for the analysis represented 21 places of expeditionary collections located in three agricultural regions of the southwestern region of Uzbekistan. The selection of genotypes continued based on their representation of the entire initial diversity in terms of origin and botanical species.

The result of SSR-analysis of 96 *Aegilops* genotypes identified the 102 fragments, wherein 65 (63.7%) were polymorphic, and 37 (36.3%) were monomorphic. The number of DNA fragments amplified by one primer ranged from 7 (WSP107) to 17 (WSP513). Using the primer WSP513 obtained the maximum number of polymorphic fragments, whereas the minimum by primer WSP107. Figure 2 shows the results of a fragment analysis of four species of the genus *Aegilops* applying the SSR-primer WSP190. The SSR spectrum of samples of *Aegilops* species obtained used the primer

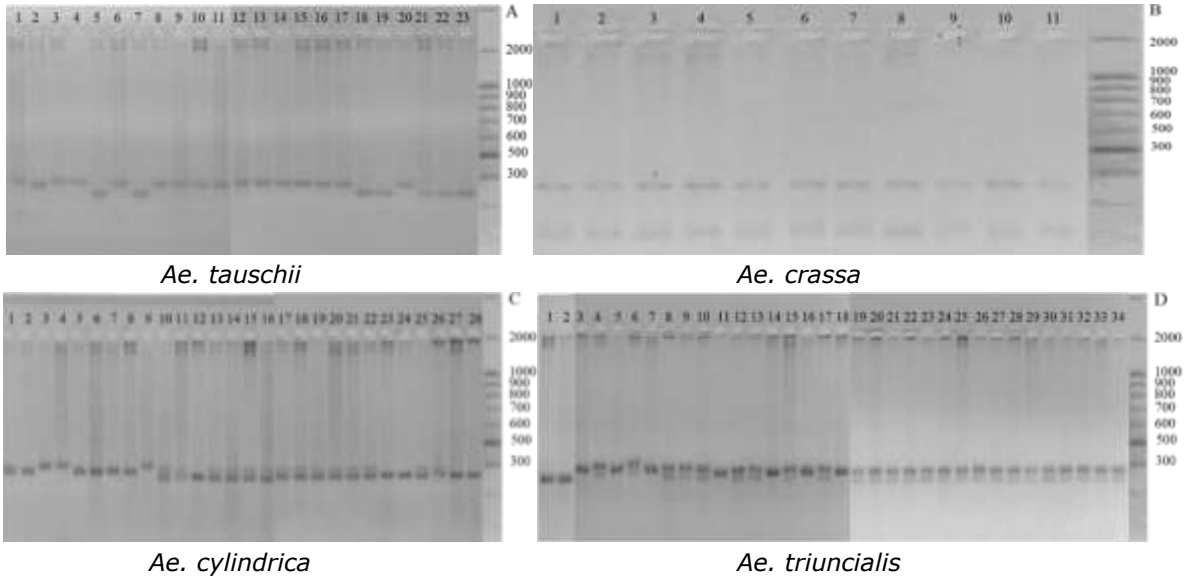


Figure 2. The SSR spectrum of the landraces of *Aegilops* species obtained using primer WSP190 (gel fragment). The rightmost lane is the molecular weight marker.

WSP190. It can also be apparent in the data that each species had different characteristics and non-repeating PCR fragments.

The SSR spectrum, using the primer WSP190 for species *Ae. tauschii, Ae. crassa, Ae. cylindrica,* and *Ae. triuncialis,* collected from various geographic locations in the southwestern region of Uzbekistan, showed inter- and intraspecific DNA polymorphism. Among the studied species, the most polymorphic were the genotypes of the species *Ae. tauschii*, with the SSR-primer WSP190 identifying six PCR fragments in 23 accessions. It indicates that 75% of the landraces of this species turned out to be polymorphic for this primer (Figure 2-A). In 11 accessions of the species *Ae. crassa*, one monomorphic fragment appeared (Figure 2-B). In addition, the use of primer WSP190 in 28 populations of the species *Ae. cylindrica* also identified one monomorphic portion (Figure 2-C). For 34 accessions of the species *Ae. triuncialis,* three PCR fragments emerged (Figure 2-D). Unlike other species, the species *Ae. cylindrica* and *Ae. triuncialis* by SSR primers revealed the lowest level of genetic variability by holding the minimum polymorphism (57.1%), respectively.

Thus, using nine SSR primers revealed a clear intraspecific difference in all the studied species of the genus *Aegilops.* One must also note that primers WSP006 and WSP044 in the assessed *Aegilops* collection have only two fragments each, and primers WSP130, WSP156, and WSP192 for individual genotypes and species owned three portions each. Identifying more than three PCR fragments surfaced in primers WSP192 and WSP513 per species of the genus *Aegilops* (Table 3). For nine SSR primers, 75% polymorphism was evident in the species *Ae. tauschii*, which might be related to its adaptive capabilities. Interestingly, the results obtained for the species *Ae. crassa* utterly differed from the data obtained from other species. It might be due to this species being less variable, regardless of the growth place.

The PCR fragments in different species of the genus *Aegilops* obtained using nine pairs of SSR primers appear in Table 3. As seen from the data, genotypes of the species *Ae. cylindrica* with primers WSP006, WSP107, and WSP190, and in the species *Ae. triuncialis* with primer WSP107 were 100% monomorphic. However, the species *Ae. crassa* revealed a

Table 3. PCR amplification and genetic parameters of SSR markers in the four species of genus *Aegilops.*

Locus	*Na, Number allelic*	Genus/species												Ho	*H*	PIC	I
		Ae. tauschii			*Ae. crassa*			*Ae. cylindrica*			*Ae. triuncialis*						
		Total	polymorph	monomorph	Total	polymorph	monomorph	Total	polymorph	monomorph	Total	polymorph	monomorph				
WSP006	8	2	1	1	3	2	1	1	0	1	2	1	1	0.004	0.69	0.63	1.27
WSP044	8	3	2	1	0	0	2	3	2	1	2	2	0	0.601	0.74	0.70	1.37
WSP107	7	3	2	1	1	0	1	2	0	2	1	0	1	0.000	0.75	0.69	1.40
WSP130	13	4	3	1	5	5	0	2	1	1	2	1	1	1.000	0.68	0.64	1.28
WSP156	12	3	2	1	5	3	2	2	1	1	2	1	1	1.508	0.72	0.67	1.32
WSP190	13	5	4	1	1	0	1	2	0	2	5	1	4	1.012	0.73	0.68	1.35
WSP192	13	4	3	1	2	0	2	5	5	0	2	2	0	0.003	0.72	0.67	1.32
WSP325	11	2	1	1	5	5	0	2	1	1	2	1	1	0.010	0.73	0.68	1.35
WSP513	17	6	6	0	6	2	4	2	2	0	3	3	0	0.602	0.75	0.71	1.39
Total alleles	102													4.74	6.51	6.07	12.05
Average	11.33													0.527	0.72	0.67	1.34

low level of genetic variability, i.e., 60.7% polymorphic and 39.3% monomorphic loci, which indicates an average level of intraspecific variability according to the SSR analysis. Unlike other species, the accessions of the species *Ae. cylindrica* collected in the Samarkand region indicated having 100% polymorphic loci with primer WSP192.

In these species, the highest level of diversity may correlate with plasticity and a wide range of intraspecific variability in different habitats. The microsatellite analysis also showed that the SSR primers WSP190 and WSP192 have fragments with 800, 1000, and 1200 bp and were not polymorphic, but sections with a length of 380, 500, 750, 770, and above 1000 bp turned out to be polymorphic. The primers WSP192 and WSP513 exhibited 100% polymorphism. It is also visible from the data presented in Table 3 that the species *Ae. triuncialis* recorded with 57.1% of polymorphic loci and in two other species, i.e., *Ae. tauschii* and *Ae. crassa* recorded 70% and 60.7% polymorphic loci, respectively. The results indicated that the level of variability in *Aegilops* species with D-genome was approximately the same.

PCR amplification and genetic diversity

Using nine SSR markers, generally amplified 102 alleles (Table 3). The number of alleles (Na) in the total sample ranged from seven (WSP107) to 12 (WSP513) and averaged 11.33 alleles per locus. The interval of expected heterozygosity (Ho) ranged from 0.000 (WSP107) to 1.508 (WSP156) and averaged 0.527. The Nei genetic diversity index (H) ranged from 0.68 (WSP130) to 0.75 (WSP107, WSP513), with an average value of 0.72. The most genetic diversity emerged for primers WSP044 and WSP513, whereas the least for WSP130 and WSP006. The PIC values of each marker locus ranged from 0.63 (WSP006) to 0.71 (WSP513) and averaged 0.68. The highest PIC value resulted from the primer WSP513 locus and the least for primer WSP006. At the same time, all the microsatellite loci had a PIC value above 0.6. The Shannon diversity index (I) in the total sample ranged from 1.27 (WSP006) to 1.40 (WSP107) and averaged 1.34.

The alleles' diversity analysis of nine microsatellite loci showed that all SSR markers produced species-specific amplicons and

Table 4. Nei's genetic distance indices between species of the genus *Aegilops* based on SSR analysis.

	Ae. tauschii	*Ae. crassa*	*Ae. cylindrica*
Ae. crassa	0.616		
Ae. cylindrica	0.608	0.768	
Ae. triuncialis	0.566	0.762	0.480

identified 35 species-specific amplicons. At the same time, the most number of alleles (15) resulted in the species *Ae. tauschii*, followed by 12 in *Ae. crassa,* eight in *Ae. cylindrica*, and five in the species *Ae. triuncialis*. However, it is noteworthy that the species *Ae. triuncialis* for SSR marker WSP044 differed by two independent alleles. Meanwhile, the primer WSP044 had the highest number of alleles and distinguished the three species, but it did not produce amplification products in the species *Ae. crassa* (Table 3).

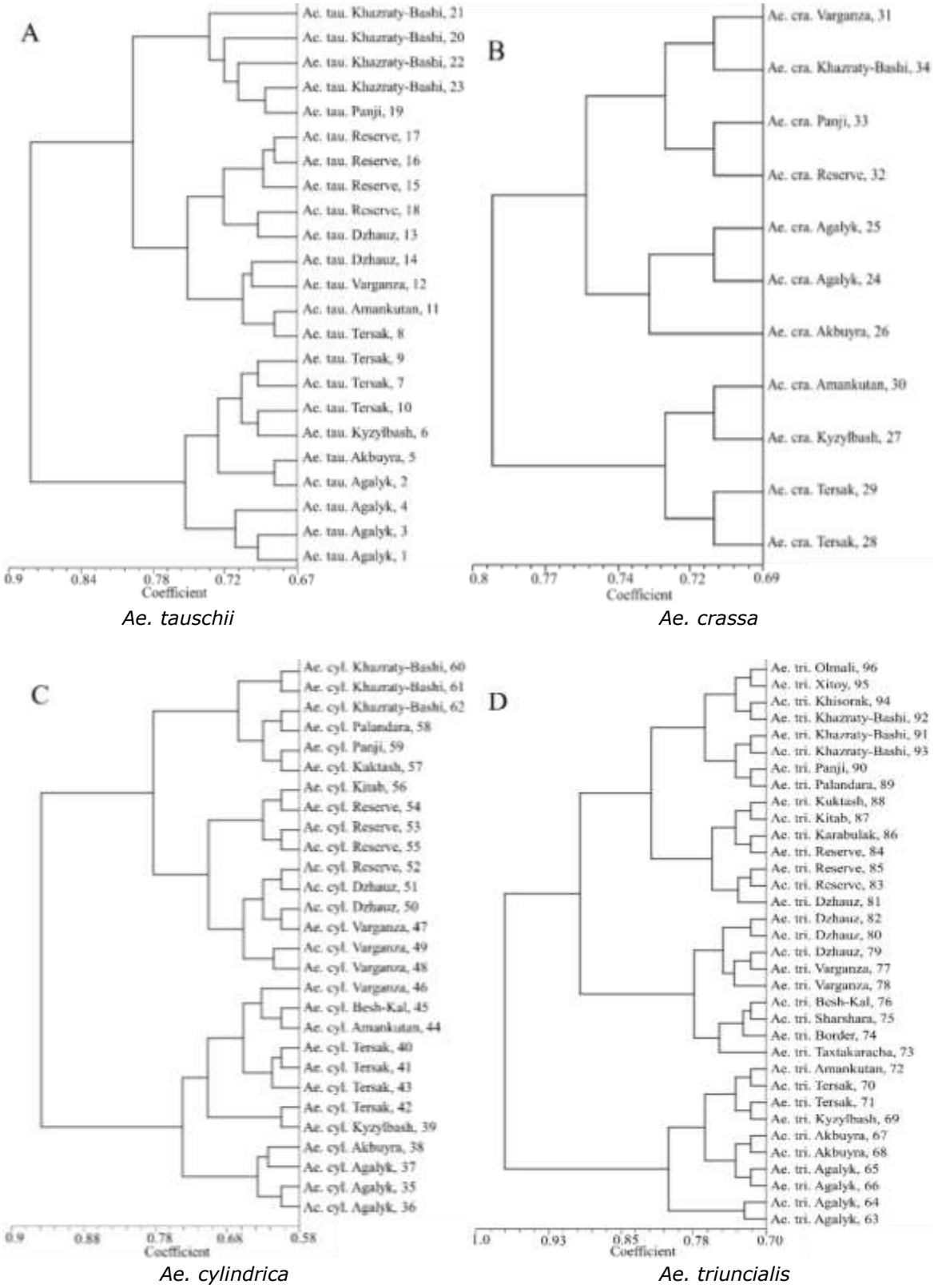
Cluster analysis

The genetic distance (GD) index for SSR markers among all the paired combinations of samples varied from zero to 0.93, with an average value of 0.79. The study obtained the highest distance value among the genotypes 2 (*Ae. tauschii*), 38 (*Ae. cylindrica*), and 64 (*Ae. triuncialis*). However, the obtained smallest value was among various pairs of genotypes of the species *Ae. crassa*. Among the studied species, the genetic similarity was prominent between the two species *Ae. crassa* and *Ae. cylindrica* (GD = 0.77), while least between the species *Ae. cylindrica* and *Ae. triuncialis* (GD = 0.48) (Table 4).

A dendrogram constructed from the data of nine SSR markers based on the UPGMA method grouped 96 genotypes into 10 clusters (Figure 3). All four *Aegilops* species studied were significantly distinguishable and formed distinct clusters and subclusters (Figure 3). Based on cluster analysis (Figure 3-A), 23 genotypes of the species *Ae. tauschii* attained dividing into three groups (I-III). Cluster group I included genotype numbers 1-4 (Agalyk population), number 5 (Akbuyra population) of Samarkand region, and numbers 6-11 (Tersak, Kyzylbash, and Amankutan population) of District Urgut, Uzbekistan. Group II cluster included the genotype numbers 12-23 from the District Kitab, Uzbekistan (Table 1).

Apparently, due to the specific clustering of the species *Ae. tauschii* by ecological and geographical origins, a conclusion may indicate the results of discriminatory analysis based on the use of SSR markers relatively accurately reflect the levels of genetic similarity of the genotypes belonging to this species and collected from different places in the southwestern region of Uzbekistan. However, one must note that the environments of the studied species showed the highest genetic diversity in the species *Ae. tauschii*, which characteristically indicates 75% polymorphism of this species (Table 3).

The genetic similarity analysis showed that among the genotypes of the species *Ae. tauschii* collected from various places in the southwestern region of Uzbekistan, no significant clustering occurred according to the location of their growth, as evidenced by the uniform polymorphism of this species. Genetic similarity analysis calculations based on SSR markers for the species *Ae. crassa* revealed the relative characteristics of differentiation. The genetic distance (GD) index by SSR markers among the genotypes of the species *Ae. crassa* ranged from 0 to 0.80, with an average value of 0.74. The highest distance value obtained emerged between genotype 27 (GD = 0.78), while the lowest genetic similarity (0.68) was among various pairs of genotypes from the District Kitab (Table 4). Based on the cluster analysis, the 11 genotypes of the species *Ae. crassa* gained division into different groups (Figure 3-B). The first cluster group I included genotype numbers 24-26 (population Agalyk, Akbuyra) of the District Samarkand and numbers 27-30 (populations of Kyzylbash, Amankutan) of the District Urgut. Group II clusters included genotype numbers 31-34 (populations of Varganza, Khazraty Bashi, and Panji) of the District Kitab (Table 1).



Ae. tauschii *Ae. crassa*

Ae. cylindrica *Ae. triuncialis*

Figure 3. Dendrogram of 96 accessions of four native species of the genus *Aegilops* based on nine SSR markers.

Cluster analysis revealed a closer genetic relationship occurred among some populations from the Districts Urgut and Kitab, which were also neighboring provinces; hence, an assumption for an existing genetic relationship among the said districts' populations. It may be due to these agricultural areas' genetic changes associated with soil and climatic conditions. Similarly, the SSR analysis showed that among all the studied genotypes of the species *Ae. crassa* has a lower level of intraspecific diversity (60.7% of polymorphic loci), indicating an average level of intraspecific variability of this species (Table 3).

The dendrogram obtained from nine SSR markers made it possible to isolate the species *Ae. cylindrica* into two clusters, within which the samples have the highest genetic similarity. Based on molecular genotyping for nine SSR markers an outcome showed relatively higher genetic diversity in the species *Ae. cylindrica*, which was consistent with previous findings by Moradkhani *et al*. (2015) and Pour-Aboughadareh *et al*. (2017). The genetic similarity analysis based on SSR markers revealed a high level of intraspecific variability for the genotypes of the species *Ae cylindrica* populations. The maximum intraspecific similarity in this species was 0.90, whereas the minimum was 0.58, with an average value of 0.76 (Table 4). The farthest distance value resulted in genotype number 39 (GD = 0.89), with the smallest (0.68) among the genotype pairs collected from the District Kitab (Table 4).

The species *Ae. cylindrica,* represented in the collection by 28 samples from the Samarkand, District Urgut of the Samarkand region, and the District Kitab of the Kashkadarya region, were divided into two groups, within which the genotypes have a substantial genetic similarity. The first cluster group I included 10 genotype numbers 35-38 (populations of Agalyk and Akbuyra) and numbers 39-44 (populations of Kyzylbash, Tersak, and Amankutan) of the Districts Urgut and Samarkand. However, the second cluster, further subdividing into two subclusters, grouped 18 accessions (populations from Varganza, Khazraty Bashi, and Dzhauz) of the District Kitab.

Cluster analysis showed that populations from the same agricultural area also combined in the same group, and their further division into groups showed diversity. Such differentiation of species *Ae. cylindrica* generally corresponds to the geographical origin of the accessions. The populations most distant from each other (Districts Kitab and Samarkand) formed separate groups, and the populations closest to each other of the Districts Samarkand and Urgut came together into one group.

Based on molecular genotyping by SSR primers, results showed different degrees of intra- and interspecies polymorphism in the species *Ae. triuncialis* and *Ae. triuncialis* (Figure 3-D). The said polymorphism was evident in the collection of 34 landraces from 21 diverse locations in the southwestern region of Uzbekistan and characteristic of a significant intraspecific diversity level. Species *Ae. triuncialis* appeared as one common species in various agricultural zones of Southwestern Uzbekistan.

Based on the coefficient of genetic similarity, the species *Ae. triuncialis* sustained division into four groups (I-IV). The first cluster included 10 accessions (collected from Agalyk and Akbuyra) of the District Samarkand and the populations of Kyzylbash, Tersak, and Amankutan of the District Urgut. The second cluster included nine accessions (collected from Varganz and Besh-Kal). The third cluster contained seven accessions (collected from Karabulak and Kuktash). The fourth cluster comprised eight samples (collected from Khazraty Bashi, Panji, and Palandara) of the District Kitab. The maximum value of the Dice genetic similarity index among all the accessions of the species *Ae. triuncialis* was 1.00 (observed for sample number 13 [Agalyk population from the District Samarkand]), while the minimum was 0.89 (sample number 87 from District Kitab). According to the SSR analysis, the Dice genetic similarity index values among the accessions of the entire sample were higher than for an individual population.

DISCUSSION

Wheat's wild relatives are potential sources of valuable genetic material for its improvement. Among the wild relatives, *Aegilops* is the largest genus of the tribe Triticeae, which includes 23 annual species with diverse levels of ploidy and are promising sources of economically valuable traits that can benefit wheat improvement programs (Reynolds *et al.,* 2012; Tuler *et al.,* 2015; Yang e*t al.,* 2018; Kishii, 2019). The SSR markers have wide usage to assess the genetic diversity of genus *Aegilops* species and to purposefully use its gene pool in the breeding process (Noormohammadi *et al.,* 2014; Tang *et al.,* 2015; Henkrar *et al.,* 2016; Liu *et al.,* 2016; Luo *et al.,* 2017; Pandian *et al.,* 2018; Itam *et al.,* 2020). These research works emphasized the role of introducing wild relative genes into the existing crop cultivars that are of great value for global food security (Brozynska *et al*., 2015; Redden, 2015; Buronov *et al*., 2023).

Genetic diversity studies of genus *Aegilops* species using molecular genetics tools based on the analysis of DNA polymorphism (RAPD, AFLP, SSR, and SNP) allow obtaining an individual characteristic of a separate genotype - a DNA profile. Based on long-term studies of the genetic diversity of 20 samples of five unique species of the genus *Aegilops-Triticum* using 10 SSR primers, findings showed a higher number of alleles per locus (Moradkhani *et al*., 2015). In addition, Naghavi *et al*. (2009) assessed the genetic diversity of 52 genotypes of diploid wheat *Tr. boeoticum*, using 21 microsatellite markers, and the average number of alleles per locus was 13.0 (varying from three to seven). In the existing genetic diversity study of four species of the genus *Aegilops,* i.e., *Ae. tauschii, Ae. crassa, Ae. cylindrica,* and *Ae. triuncialis* using nine pairs of SSR markers, the number of alleles (Na) in the total sample ranged from four (WSP107) to 12 (WSP006) and averaged 7.78 alleles per locus, which is consistent with the findings of Moradkhani *et al*. (2015). Saeidi *et al*. (2006) assessed the genetic diversity of Iranian populations of *Ae. tauschii* using 13 microsatellite markers, with 66 alleles amplified with a mean PIC of 0.65.

In the presented study, by assessing 96 accessions of four diverse species of the genus *Aegilops*, the maximum PIC value was 0.70 with a mean value of 0.68, which was 0.03 more than the value reported by Naghavi *et al*. (2009). Similarly, the genetic diversity of 46 *Commelina communis* populations using 12 SSR markers gained assessing with a mean PIC value of 0.20 (Yang *et al.,* 2018). Pour-Aboughadareh *et al.* (2017) and Roder *et al.* (1995) also reported the variability of the PIC values that occurred dependent on the content (GT), the number of alleles per locus, and the type of motifs. In the pertinent study, nine SSR markers used comprised four microsatellite loci primers, viz., WSP006, WSP044, WSP130, and WSP513 that were the most polymorphic, which can help identify the species *Ae. tauschii, Ae. triuncialis,* and *Ae. crassa*. The SSR marker WSP006 revealed 67% of polymorphic fragments (*Ae. triuncialis),* 80% (*Ae. tauschii*), and 67% (*Ae. crassa*), as well as, 75% with primer WSP130 that were either species-specific or accession specific, to discriminate the two and three species.

Despite the different numbers of studied samples for each species, research further explored the genetic diversity at the species level. In this study, species *Ae. triuncialis*, represented by 34 accessions, had a higher level of genetic diversity. The species *Ae. tauschii,* represented by 23 samples, the third species in terms of volume, was characteristic of the highest level of genetic diversity. The extensive variety of the D genome in the species *Ae. tauschii* had confirmation with AFLP markers (Dvorak *et al*., 1998; Lelley *et al*., 2000). As for the polyploid species, *Ae. crassa* and *Ae. cylindrica,* with a D genome, also have a lower level of intraspecific diversity. The low disparity of the species *Ae. cylindrica* was consistent with previous findings by Goryunova *et al*. (2004). In contrast to the latest results, Moradkhani *et al*. (2015) and Pour-Aboughadareh *et al*. (2017) identified a relatively higher diversity of the species *Ae. cylindrica* compared with other species based on SSR and SCoT markers. The D-genomic groups of the plants had less intraspecific polymorphism characterization than most polyploid species with a U genome. The RAPD

analysis showed no difference between the ploidy of two species of the genus *Aegilops;* however, these species were very similar morphologically, with some researchers not even considering them as two independent species (Goryunova *et al.,* 2004).

The promising results showed that accessions of the species *Ae. crassa* have a closer level of genetic similarity to *Ae. tauschii* than with *Ae. cylindrica,* which seems related to the fact that *Ae. cylindrica*, in addition to the D genome, also has a C genome. The polyploid species *Ae. triuncialis* does not have a D genome but has a C genome and a U genome, wherein this species forms a single cluster with *Ae. cylindrica*, which also appeared in the dendrogram of this relevant study. There is also evidence in the literature that the species *Ae. cylindrica* and *Ae. triuncialis* have a mutual C genome, confirmed by the presence of a shared cluster according to RAPD analysis (Kavrakova, 2009). According to recent results, in contrast to the species with the D genome, representatives of the species with the U genome *(Ae. triuncialis*) were characteristic of a significant level of intraspecific diversity. The species *Ae. triuncialis* proved one of the most widespread with a high level of genetic diversity. Clustering based on genetic distance was consistent with the taxonomy. The dendrogram obtained from nine pairs of SSR markers made it possible to identify all the studied species of the genus *Aegilops* at the level of a cluster or subcluster, which proved consistent with the findings of Pour-Aboughadareh *et al*. (2017).

All the accessions of the *Aegilops'* four species sustained division into three groups, i.e., a) genotypes collected from Agalyk and Akbuyra, with both of these points included in the foothill zones of the Samarkand region, b) genotypes from the Urgut mountainous district, and c) genotypes collected from various agricultural centers of the District Kitab. The District Kitab is geographically adjacent to the Samarkand region and has locations in the eastern part of the Kashkadarya region on the spurs of the Zeravshan and Gissar ranges. The main territory of these districts lies on the Kitab-Shakhrisabz lowland. The altitudinal boundary here has a contrasting presentation from the lowest (700 masl) to the highest (2000 masl) farming zones.

Two trends can be distinct in the genetic diversity of local species of the genus *Aegilops*. Firstly, the use of SSR markers to identify phylogenetic relationships among the species and intraspecific polymorphism of representatives of the genus *Aegilops* in the flora of the Districts Samarkand, Urgut, and Kitab, Uzbekistan, showed variations among the genotypes. It may be due to differences in soil and climatic conditions in the areas where the landraces were collected. However, all three districts are characteristic of a continental arid climate. The second trend was the joint grouping of the genotypes collected from Samarkand and Urgut. An analysis of the influence of longitude, latitude, and height of sampling districts on clumping within clusters showed that these parameters do not affect the genetic diversity of the studied collection. Consequently, the latitudes and heights above sea level of the Districts Samarkand, Urgut, and Kitab vastly differed. Moghaddam *et al*. (2000, 2014) reported the genetic diversity of *Triticum-Aegilops* and *Triticum urartu* Thum using SSR, ISSR markers, and isoenzymes, those similar geographically distant regions in terms of their conditions can lead to a joint grouping of geographically distant populations.

According to current results, the grouping of accessions of different species of the genus *Aegilops* from varied agricultural zones of the southwestern region of Uzbekistan according to SSR markers occurred by species, subspecies, areas, longitude, latitude, and altitude, revealing no influence on the intraspecific grouping of landraces. For more information, clustering samples from the same agricultural area continued into the same subcluster. Grouping analysis showed that within the same district, genetic parameters closely correlate to the geographical origin of the samples, and the joint grouping of specimens from different areas within the region was most likely due to the distribution of populations in similar climatic conditions. As a rule, the populations of the Samarkand region showed a distant genetic relationship with populations from the Districts Urgut and Kitab, which might refer to long-term evolution

and genetic variations associated with local climatic conditions. As a result of SSR marking, estimating the genetic diversity of local populations of four species of the genus *Aegilops* originating from Southwestern Uzbekistan ensued, establishing the degree of their polymorphism and genetic diversity in terms of the allelic composition of microsatellite loci.

CONCLUSIONS

Central Asia is one of the centers of origin of the genus *Aegilops* species. Uzbekistan also lies in this region, with the southwestern zone mainly mountainous and has a unique plant genetic treasure. Thus, a collection comprising 96 accessions of four local species of the genus *Aegilops* ensued from 21 expedition sites located in four agricultural regions, mostly mountainous in Southwestern Uzbekistan. As a result of SSR marking, determining the degree of genetic diversity and similarity of the genomes of these landraces for the first time also attained evaluation. The degree of polymorphism and genetic diversity of microsatellite loci collected by allelic composition was evident. The gene pool of native species of the genus *Aegilops* from the southwestern region of Uzbekistan can provide valuable alleles for wheat improvement and adaptation programs.

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