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GENETIC ANALYSIS AND MOLECULAR CHARACTERIZATION OF THE WHEATGRASS (*AGROPYRON CRISTATUM* L. GAERTN.) IN SOUTH-EAST KAZAKHSTAN

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SUMMARY

Assembly, genetic analysis, and preservation of wheatgrass (*Agropyron cristatum* L. Gaertn.) diversity is an important task in improving the said crop under the environmental conditions of the Republic of Kazakhstan. The presented study evaluated and isolated various wheatgrass species and their populations for breeding and developing promising cultivars based on the genetic principles to improve the degraded pasture situation in South-East Kazakhstan from the perspective of global climate change. For the succeeding investigations, collection of the wheatgrass landraces from various regions of Kazakhstan ensued, as well as from accessions already conserved in the gene pool, and gained evaluation under the environmental conditions of South-East Kazakhstan. In performance-based screening, 20 wheatgrass cultivars identified underwent further genotyping by using inter-simple sequence repeat (ISSRs) polymerase chain reaction markers. The pertinent study significantly contributes to understanding the *Agropyron* species' genetic structure and molecular characteristics. Using seven ISSR primers helped study the kinship and genetic polymorphism of the five *Agropyron* species collected from diverse locations. The ISSR primers were extremely informative for determining the *Agropyron's* genetic diversity and interspecific similarity. Moreover, they come highly recommended for an in-depth study comparing the DNA sequencing of the *Agropyron* populations available in the National Center for Biotechnology's database.

Keywords: Wheatgrass (*Agropyron cristatum* L. Gaertn.), genetic diversity, perennial grasses, drought resistance, DNA electrophoresis, phylogenetic tree

Key findings: The study contributed to understanding the *Agropyron* species' genetic structure and molecular characteristics. The seven ISSR primers investigated the affinity and genetic polymorphism of five *Agropyron* species collected from various locations. The ISSR primers were also highly informative in determining the Agropyron's genetic diversity and interspecific resemblance.

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INTRODUCTION

The interest in drought-resistant and salt-resistant perennial forage crops is vastly relevant and demanding in the territory of the Republic of Kazakhstan due to climate change and increasing aridization (Yessimbek *et al*., 2022; Yesmagulova *et al.*, 2023). Currently, acclimatizing plants from wild flora into the existing domesticated crops in various natural and climatic zones of Kazakhstan continues to expand the diversity of cultivated grasses and increase the productivity of pastures and hayfields (Kalibayev *et al*., 2021; Nugmanov *et al*., 2022a, b). Wheatgrass (*Agropyron cristatum* L. Gaertn.) is a plant that combines a relatively high yield of green mass and seeds, as well as longevity, drought resistance, and excessive forage qualities (Didenko *et al*., 2021).

The wheatgrass nature and its use can be illustrative of its unique ecological and biological possessions, such as high productivity, valuable forage quality, drought and heat resistance, low temperatures and relative salt resistance, productive longevity (10–15 years or even more), low demand of soil fertility, and the symbiotic microflora on its roots accede to absorb nitrogen from the air (Ainebekova *et al*., 2022). All these allow the wheatgrass crop to occupy a stable position in arid land forage production and to serve as an effective phytomeliorant to increase the productivity of degraded soils (Didenko *et al*., 2021).

Wheatgrass provides early spring pasture forage, characterized by a good after-grass protein, which easily digests nutrients, and a full set of essential amino acids and high protein content under arid conditions. Overall, there are 17 types of wheatgrass in nature, i.e., fairway crested wheatgrass (*Agropyron cristatum* [L.] *Beav*.), crested wheatgrass (*A. pectiniforme* Roem et Schul.), *A. cristatum* subsp. *pectinatum* [Bieb] Tzvel., *A. imbricatum* Roem et Schult, Kazakhstan (Karatau), Kazakhstan wheatgrass (*Agropyron cristatum* subsp. *kazachstanicum* Tzvel.), and desert wheatgrass (*A. desertorum* [Fisch] Schult, unlike the crested wheatgrass, it further spreads into the desert steppes). Outside the Commonwealth of Independent States, it also exists in Dzungaria in the form of Siberian wheatgrass (*A. sibiricum* [Wild] P.B. Agrost.), Don wheatgrass (*A. tanaiticum* Nevski.), Kerch wheatgrass (*A. cimmericum* Nevski.), and sand wheatgrass (*Adgorugop cristatum* subsp. *sabulosum* Lavr). Of all these wheatgrass types, the fairway crested wheatgrass (*Agropyron cristatum* L.) is most known as one of the first ones to begin vegetation, which is drought- and frost-resistant (Ainebekova and Erzhanova, 2022).

The genetic potential of the wheatgrass is not optimal since numerous wheatgrass cultivars recommended for cultivation have unstable forage and seed productivity, as well as less resistance to abiotic stress conditions in Kazakhstan due to varied origins and lack of proper selection through genotype by environment interactions (Meiirman and Yerzhanova, 2015; Dzyubenko *et al*., 2017).

In this regard, the collection, screening, and preservation of wheatgrass diversity is an imperative task. Therefore, the wheatgrass population collection’s comprehensive study of biological and economic valuable features is relevant. The presented research pursued the evaluation and isolation of various wheatgrass (*Agropyron G.*) species and their populations for breeding certain cultivars using several genetic principles and performance-based selection methods to improve the degraded pastures in South-East Kazakhstan in the context of global climate change.

MATERIALS AND METHODS

Experimental location and procedure

The experimental research ran in 2020–2022 at the field stations of the Kazakh Scientific Research Institute of Animal Husbandry and Forage Production, Limited Liability Partnership (LLP), Koldy Village, Almaty region, Kazakhstan. The wheatgrass collection used for the study comprises 300 samples with varied morphological and ecotypic characteristics. All the accessions' seeds remained in the gene pool of the Forage Production Laboratory, with their viability tested through various germination tests, ranging from 12% to 75%. The wheatgrass is one of the most winter-hardy plants among the perennial cereal grasses. The collected wheatgrass landraces' comparison used the standard *Agropyron pectinatum* L. or the cultivar Taukumsky. The measurement of plant height started in the phase of cutting ripeness.

The wheatgrass collection nursery, having 300 landraces, began establishment from April 6 to 10, 2020, manually, with a row spacing of 60 cm. Overall, the plot area was 3 m^2 without repetition, and the seeding rate was 8–10 kg ha^{-1}. The formation of the nursery, descriptions, observations, and records proceeded and maintained according to the methodology of breeding perennial grasses (Kosolapov *et al.*, 2012) and the morphological traits' study according to the World Flora Online (WFO) classifier (World Flora Online, 2023). The study used five subspecies of the *Agropyron Gaertn*. Genus, i.e., *A. desertorum* (Fisch. et Link Schult. et Schult. f.), *A. fragile (Roth)* Candargy, *A. cristatum* ssp. cristatum (L.) Beauv*, A. imbricatum,* and *A. sibiricum* ([Willd] Beauv.).

Genetic analysis of *Agropyron Gaertn* continued using microsatellite markers. DNA extraction from fresh wheatgrass leaves collected during tillering used the phenol-chloroform method (Sharp *et al.*, 1988). For these loci, the oligonucleotides' synthesis followed the sequences described in past studies (Shirvani *et al.*, 2013; Kondratskaya *et al.*, 2017). The genetic diversity parameters, such as frequency and the number of alleles, heterozygosity, Shannon index, probability of identity (PI), frequency of zero alleles, principal component analysis (PCA), and genetic distance employed the GenAlEx version 6.5 (Peakall and Smouse, 2012) and Popgene version 1.32 (Yeh *et al*., 1999). Construction of the phylogenetic tree based on the standard Nei genetic distance values engaged the MEGA-X (Kumar *et al*., 2018) based on the neighbor-joining principle (Saitou and Nei, 1987). Bayesian analysis using the Markov chain Monte Carlo (MCMC) algorithm ensued in the STRUCTURE software (Falush *et al.*, 2003). The statistical processing of the experimental material ran the SAS application software package (SAS Institute Inc., U.S.) (Dospekhov, 2011).

RESULTS

Results of the study of wheatgrass landraces carried out from 2020 to 2022 revealed that the tilling capacity was mainly dependent on the genotype species, growing conditions, and the age of the herbage. Generally, the crested wheatgrass specimens exceeded the standard cultivar Taukumsky (*Agropyron pectinatum* L.) for tillering capacity. Through the study years, on average, the best were the wild-growing landraces obtained from Russia (k-564872) and China (k-315160, 449389), which had 171.0 to 180.0 tillers per bush. However, in the wheatgrass standard cultivar Taukumsky, the average stem numbered 137.0 per bush.

The studies further revealed that in the collection, the tallest wheatgrass genotypes were the crested wheatgrass (k-325185, k-345583), Siberian wheatgrass from Russia (k-45158), hybrid (crested × desert) from the USA (k-48559), crested wheatgrass from Mongolian People's Republic (MPR) (k-46878), and desert wheatgrass from Turkey (k-340060) and Australia (k-316121), ranging from 71 to 79 cm.

During the study years of the wheatgrass collection, the samples' leafiness ranged from 27% to 41%. Forty-nine landraces had average leafiness, with an indicator range of 33%–38%. The genotypes from China (k-44939) and Russia (k-345583) had the

Table 1. Measurement of DNA purity on a spectrophotometer on 20 samples.

Sample name	Nucleic Acid (ng/uL)	A260/A280	A260/A230
Sample 1	165.744	1.796	2.124
Sample 2	102.586	1.782	2.123
Sample 3	250.845	1.790	2.140
Sample 4	139.782	1.758	1.900
Sample 5	103.736	1.760	1.958
Sample 6	113.209	1.765	2.003
Sample 7	64.846	1.730	1.747
Sample 8	159.359	1.767	2.004
Sample 9	203.278	1.661	1.869
Sample 10	211.781	1.787	2.210
Sample 11	165.092	1.784	2.192
Sample 12	205.280	1.782	2.102
Sample 13	136.887	1.766	1.992
Sample 14	274.015	1.791	2.150
Sample 15	217.190	1.788	2.138
Sample 16	136.528	1.778	2.107
Sample 17	133.463	1.778	2.129
Sample 18	58.456	1.734	1.851
Sample 19	109.409	1.769	2.150
Sample 20	123.546	1.753	1.973

Table 2. The degree of polymorphism of primers used for the molecular genetic study of wheatgrass samples.

Primers	Number of markers	Number of polymorphic markers	Polymorphism level (%)
UBC-807	10	8	80.00
UBC-808	15	12	80.00
UBC-812	15	7	53.33
UBC-817	21	15	71.43
UBC-818	18	12	66.67
UBC-827	19	14	73.68
UBC-836	7	3	42.86

maximum leafiness (50%–60%), while the standard cultivar only had 38%. Leafiness is a rather variable feature. However, in the whole wheatgrass collection, the coefficient of variability ranged from 24.5% to 33.9%.

The results of the DNA purity measurement appear in Table 1. DNA electrophoresis ran with seven inter simple sequence repeat (ISSR) primers, i.e., UBS807, UBS808, UBS812, UBS817, UBS818, UBS827, and UBS836. The degree of polymorphism is in Table 2.

Genetic polymorphism of *Agropyron* species

The *Agropyron* species' analysis and assessment of genetic polymorphism through seven ISSR primers used genotyping to determine the genetic characteristics of such species. A total of 207 loci resulted in all samples. The largest number of polymorphic loci was in the wheatgrass population of the species *A. cristatum* (186 fragments), and the smallest number in the populations of the

Table 3. Indicators of genetic diversity of 5 *Agropyron* species.

Species	P	P (%)	Na	Ne	H	I
Agropyron cristatum	186	89.86	1.8986± 0.3027	1.3258± 0.3149	0.2083± 0.1613	0.3354± 0.2180
Agropyron desertorum	145	70.05	1.7005± 0.4592	1.3146± 0.3168	0.1991± 0.1711	0.3127± 0.2451
Agropyron fragile	89	43.00	1.4300± 0.4963	1.2985± 0.3753	0.1716± 0.2049	0.2518± 0.2962
Agropyron pectinatum	163	78.74	1.7874± 0.4101	1.3100± 0.3137	0.1982± 0.1642	0.3173± 0.2288
Agropyron imbricatum	89	43.00	1.4300± 0.4963	1.2963± 0.3729	0.1708± 0.2041	0.2510± 0.2952
All species	206	99.52	1.9952± 0.0695	1.3336± 0.3019	0.2165± 0.1516	0.3524± 0.1979

P = number of polymorphic loci, P % = percentage of polymorphic loci, NA = observed number of alleles, NE = effective number of alleles, H = Nei's gene diversity (1973), I = Shannon Information Index.

species *A. fragile* and *A. imbricatum* (89 fragments). In 186, 145, 89, 163, and 89 snippets, 89.86%, 70.05%, 43.00%, 78.74%, and 43.00% were polymorphic in the species, i.e., *A. cristatum, A. desertorum, A. fragile, A. pectinatum,* and *A. imbricatum,* respectively. The notable number of alleles ranged from 1.8986 ± 0.3027 (*A. cristatum*) to 1.4300 ± 0.4963 (*A. fragile* and *A. imbricatum*). The average observed number of alleles was 1.9952 ± 0.0695 for the entire *Agropyron species.* The indicators of genetic diversity are available in Table 3.

Main coordinates of *Agropyron* species

A multidimensional approach helped study the relationship between five species of wheatgrass sprouts. The first two principal coordinate analyses (PCA) accounted for 53.4% and 5.24% of the total variance based on a pairwise population matrix of genetic distance. PCA also showed clear differences among the *Agropyron* species; for example, *A. desertorum* differed from *A. fragile, A. cristatum*, and *A. pectinatum* in PCA1 and PCA2. In addition, *A. imbricatum* was distinct from *A. fragile, A. cristatum*, and *A. pectinatum*. As a result of the PCA, the *Agropyron* species had groups of three separate clusters, i.e., the first cluster includes *A. fragile, A. cristatum*, and *A. pectinatum*. The second cluster has *A. imbricatum*, while the third cluster with the species *A. desertorum.* The genetic relationship emerges in Figure 1.

Phylogenetic analysis of *Agropyron* species

Evaluation of the evolutionary relationships of *Agropyron* species used the neighbor-joining method. The phylogenetic tree’s construction occurred based on Nei's genetic distance. The species *A. imbricatum* separates from all other wheatgrass species, while the remaining population groups in one cluster. However, *A. desertorum* was closer to the species *A. fragile* compared with *A. cristatum* and *A. pectinatum*. The wheatgrass species *A. cristatum* exhibited closeness to *A. imbricatum* compared with the other species (Figure 2).

Evolutionary relationships of *Agropyron* species by geographical origin

The evolutionary relationships of *Agropyron* species studied by geographical origin used the method of unweighted paired groups with arithmetic averages. Analysis of the phylogenetic tree showed that the *Agropyron* species have no obvious grouping by species (Figure 3).

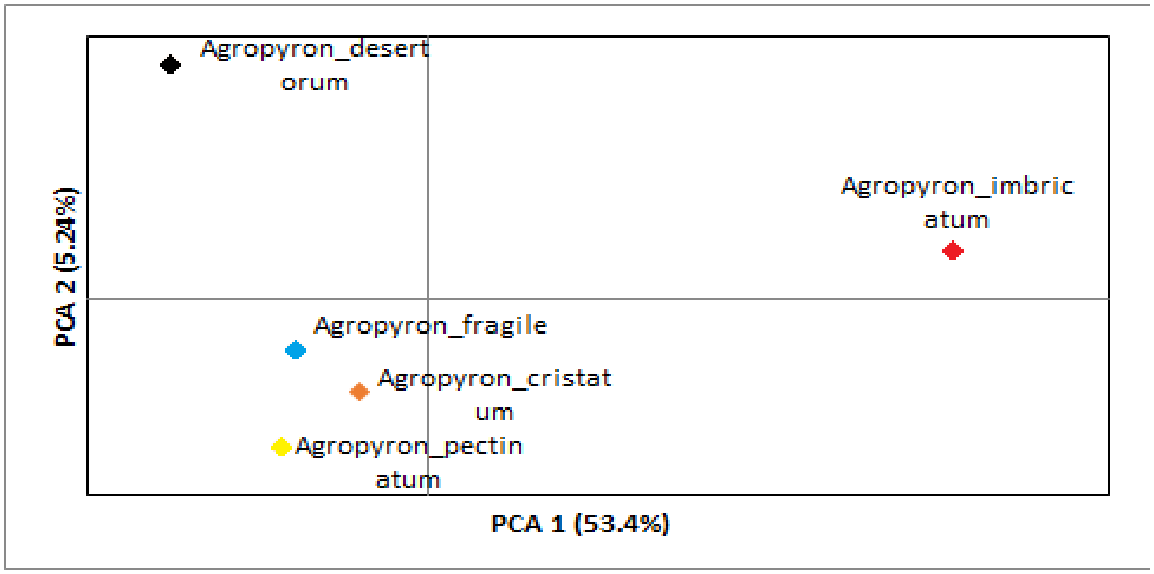

Figure 1. Genetic relationship of five *Agropyron* species by ISSR loci based on PCA.

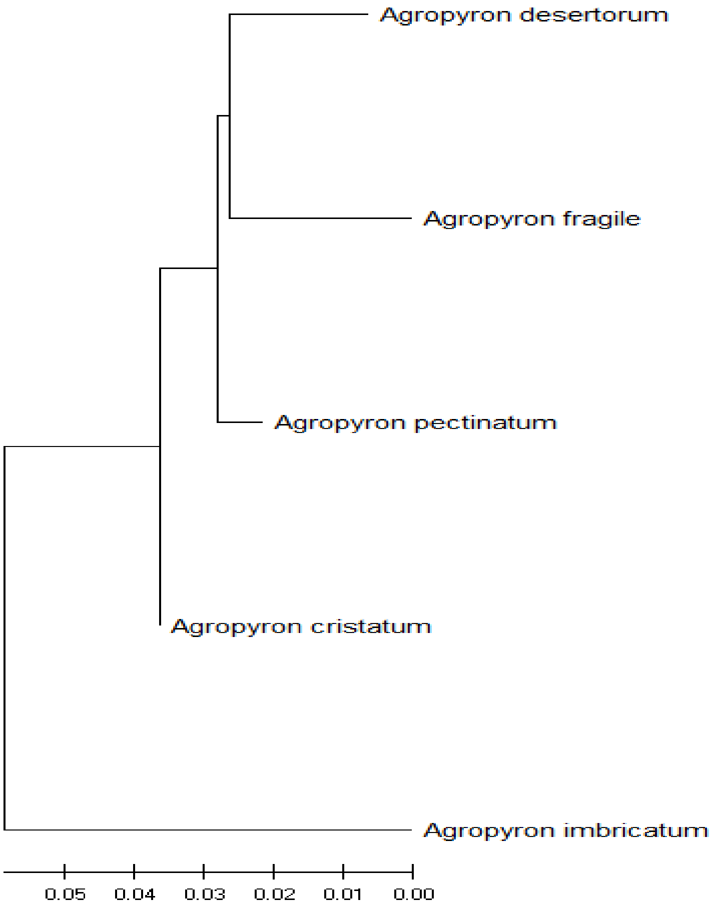

Figure 2. Phylogenetic relationship of five *Agropyron* species by ISSR loci based on the neighbor-joining method.

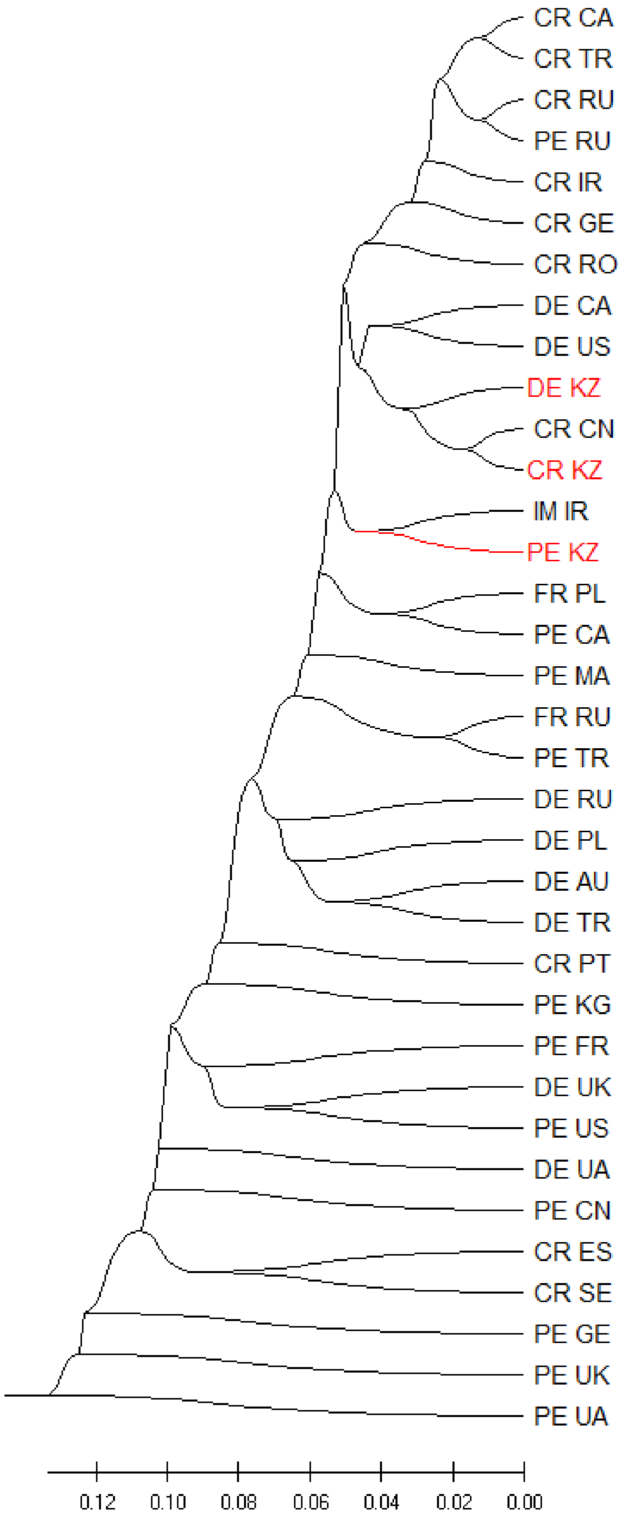

Figure 3. Phylogenetic tree of five species by geographical origin.

DISCUSSION

Kazakhstan is the richest place for wheatgrass species diversity worldwide (Didenko *et al*., 2021; Ainebekova and Erzhanova, 2022; Ainebekova *et al*., 2022). The relevant research ran on the wheatgrass collection (300 samples) belonging to five different species, i.e., crested wheatgrass (*A. pectinatum* [Bieb.] Beauv.), fairway crested wheatgrass (*A. cristatum* [L.] Bea uv.), *A. imbricatum*, desert wheatgrass *(A. desertorum* [Fisch. et Link] Schult. et Schult. f.), and Siberian wheatgrass *A. fragile*, obtained from various ecological and geographical origins of Kazakhstan, Russia, and Ukraine. The complex of economically valuable signs and properties of wheatgrass also identified 21 wild samples and cultivars in the study.

Yield is the main indicator of the economic value of any forage plant. Together with qualitative indicators, it determines the economic efficiency of sowing (Kenebayev *et al*., 2022; Nogayev *et al*., 2022). In the valuable study, the average yield of green mass in samples of *A. pectiniforme* obtained from China (k-315360), Russia (k-325185 and k-311600), and the Kurdai cultivar from Kyrgyzstan, showed stable results and had 720 to 769 g m^2, amounting to 123% and 131% of the standard value.

Three Kazakh species of *Agropyron* attained close grouping on the phylogenetic tree. *A. desertorum* of Kazakh origin grouped with Canadian and American species *A. desertorum* in the first subcluster (Figure 3). It attributed to the fact that the initial material of the wheatgrass samples collected in Kazakhstan came in 1934 from the All-Union Research Institute of Plant Breeding (VIR) by the plant growing center in Pullman, Washington (Bukhteeva *et al*., 2016). However, the Kazakh species *A. cristatum* was very close to the Chinese species. Also notable was the Kazakh species *A. pectinatum* grouping in the same subcluster as the Iranian species *A. imbricatum* (Figure 3). The identified genotypes of the wheatgrass were of great interest for further breeding, especially for use in hybridization, to obtain the initial hybrid material with valuable traits. Researchers seek to use plants capable of growing well in extreme soil and climatic conditions, especially under water-scarce conditions. As a forage crop, the wheatgrass (*Agropyron* L. Gaertn.) is most adaptable to these absolute conditions with better production (Absattar *et al.*, 2019; Didenko *et al*., 2021).

CONCLUSIONS

The study contributes to understanding the *Agropyron* species' genetic structure and molecular characteristics collected from different locations. The study was limited to seven ISSR primers (UBS807, UBS808, UBS812, UBS817, UBS818, UBS827, and UBS836). However, the ISSR primers proved extremely informative for determining the genetic diversity and interspecific affinity of *Agropyron* species and their populations. Recommendations for future studies are the in-depth work based on DNA sequencing and comparing with other types of *Agropyron* DNA sequences found in the National Center for Biotechnology Information (NCBI) database.

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