



## Study of Wild Wheatgrass (*agropyron gaertn.*) for the Genetic Diversity and Breeding Potential of Forage Grasses

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### ABSTRACT

In the context of climate change and increasing aridization in Kazakhstan, the main problem is the preservation of perennial drought and salt resistant forage crops for the development of agriculture. Wheatgrass is a type of plant that combines a high yield of green mass and seeds with longevity, drought resistance, and high forage qualities (*Agropyron Gaertn.*). This study aims to investigate the collection, preservation, and genetic analysis of wild wheatgrass samples to enhance the collection of gene pools for the development of high-yielding, drought-resistant forage crops suitable for arid and semiarid regions in Kazakhstan. A total of 270 samples from five *Agropyron* species were collected and analyzed. Genetic analysis revealed significant polymorphisms, with many unique genetic loci identified across different species. The morphological study revealed that several samples, particularly those from Russia and China, presented superior traits, including increased bushiness, plant height, and water retention capacity. The identified high-yield and drought-resistant samples are promising candidates for breeding programs to improve forage crop productivity in arid regions. These findings underscore the importance of *ex situ* conservation and the need for continuous genetic resource documentation to ensure sustainable agricultural development.

**Keywords:** Wheatgrass; Species; Collection; Gene pool; Molecular genetic analysis

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### INTRODUCTION

The cultivation of wheatgrass and its nature are determined by its unique ecological and biological properties, which include its high feed value, productivity, resistance to drought, heat, and low temperature, salt resistance, productive longevity (10–15 years or more) and low need for soil fertility (Ainebekova et al., 2023).

This allows wheatgrass to occupy a stable position in arid forage production and could be used as an effective revegetation crop to increase the productivity of degraded soils. This method is especially valuable because it provides early spring pasture feed under arid conditions, good nutrient digestibility, a full set of essential amino acids, and a high protein content (Robins and Jensen, 2020).

Plant genetic resources are fundamental for ensuring global food security and economic development. *Ex-situ*

conservation is the most significant and widespread method for preserving plant genetic resources (Haroon et al., 2023). To do this, gene banks have been created, where various collections of genetic resources are preserved *ex-situ* (Matys & Markevich, 2022).

The forage crop varieties collected and those obtained through natural selection constitute the material basis for creating high-yielding varieties and protecting state food security. Special attention is given to the formation, study, and evaluation of collected varieties for breeding (Haroon et al., 2022).

Wheatgrass (*Agropyron Gaertn.*) belongs to the family of grasses (*Poaceae*), one of the largest families important in the composition of vegetation cover and human economic activity, as their distribution area covers the entire globe, excluding territories covered with ice (Derevyannikova, 2020). Three wheatgrass species are

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common in Kazakhstan: *Agropyron pectinatum*, desert wheatgrass (*Agropyron desertorum*), and Siberian wheatgrass (*Agropyron fragile*) (Ainabaev, 2013).

Wheatgrass species are native to moderately cold meadows and sandy soils of Eurasia (Li & Dong, 1993) and were first introduced to Canada in 1911 (Chen et al., 2013). Wheatgrass species used as crops for arid conditions are well-studied in Kazakhstan, the USA, Canada, and Russia (Koryakina & Kohegina, 2021). Compared with other perennial forage crops, including brome, ryegrass, melilot, sainfoin, and medick (Humphries et al., 2018), wheatgrass is characterized by resistance to drought, heat, and frost. In global warming, scientific interest in this culture is growing (Kalibayev et al., 2021; Didenko et al., 2023).

Wheatgrass, which has high adaptive ability and forage value, is widely distributed in seeded hayfields and pastures in Kazakhstan. As the main forage crop, it interests breeders and researchers (Mustafina, 2019). The multilocus DNA labeling of wheatgrass via randomly amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR) primers allows us to differentiate genotypes and to develop unique profiles. On the basis of the obtained multilocus RAPD/ISSR spectra, genetic passports were compiled for the first time for the studied wheatgrass samples (Kondratskaya et al., 2017).

This study aimed to investigate the collection, preservation, and genetic analysis of wild wheatgrass

samples to enrich the gene pool for the development of high-yield, drought-resistant forage crops tailored to arid and semiarid regions of Kazakhstan.

## MATERIALS & METHODS

From 2021-2022, the LLP "Kazakh Research Institute of Livestock and Fodder Production" conducted route expedition surveys in the Zhambyl, East Kazakhstan, and Abai regions. Foci of wild wheatgrass species were established, confined to mountainous regions: Zhambyl region: Kordai district (Sulutor, Kishi Sulutor, Kordai, Kalguty, Zhana Turmys, Shorgo), Lugovoye, Merken district (Aktogan, mountains toward the border), Zhambyl, Zhanatas, and Karatau following established routes covering mountainous, submontane, and steppe zones (Fig. 1); Tarbagatai massif: Abai region: Mailin, Tarbagatai, Aksuat, and Kokpekty; East Kazakhstan region: Akzhar, Zaisan, Kabanbai, Kalbatau, Glubokoe, and Ust-Kamenogorsk (Fig. 2). We used nurseries from previous years and collection nurseries (2021--2023) and seed material collected during the expedition. The research was conducted at the Kolda station of the LLP "Kazakh Research Institute of Livestock and Fodder Production" in the submontane semidesert zone of the Almaty region. The climate is continental, the annual precipitation ranges from 200--300 mm, and sharp temperature fluctuations are observed in individual months and seasons.

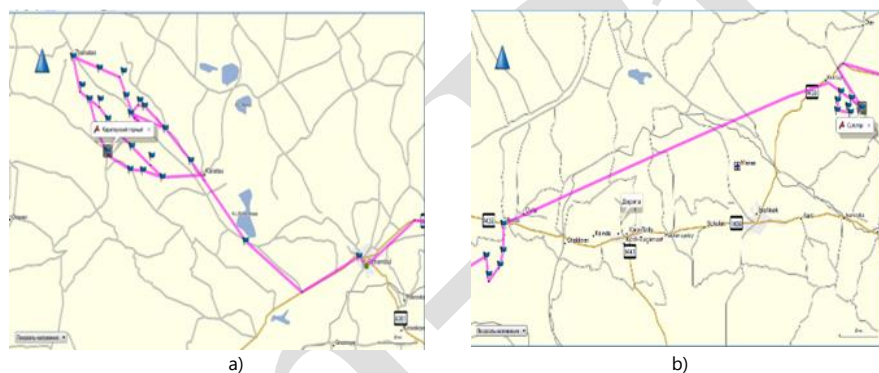


Fig. 1: (a and b). The expedition route in the Zhambyl region

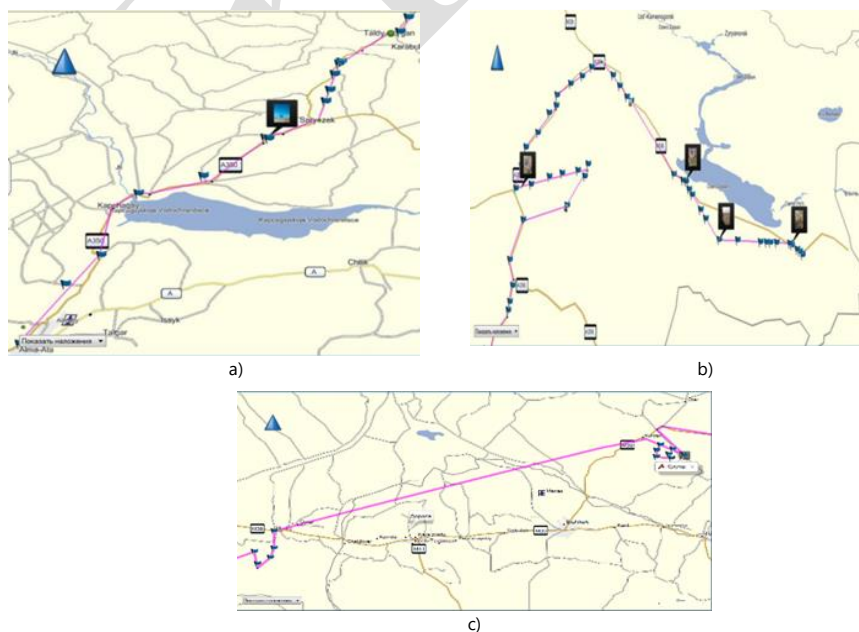


Fig. 2 (a-c): The expedition routes in the Abai and East Kazakhstan regions

### Morphological Analysis

Phenological observations were carried out following the All-Russian Research Institute of Plant Industry method. The experiments were performed in triplicate; the plot length was 1 m<sup>2</sup>. Sowing was carried out at the optimal time, on April 14, 2021. Spring regrowth was noted when seedlings appeared, and the leaves acquired an intense shade of green. The intensity of spring growth in grasses was estimated by eye on a nine-point scale: 1 – very weak (height significantly below the standard); 3 – weak (height below the standard); 5 – average (height close to the standard); 7 – strong (standard height); and 9 – strong (height above the standard).

Plant height in the cultivation variant for green mass was measured before cutting and for seeds, in the phase of mass flowering or at the beginning of seed maturation at five plot sites (Kosolapov et al., 2012; State Commission for Crops of the Republic of Kazakhstan, 2002). Earing was noted when an inflorescence appeared on 1/3 of the total length of the upper leaf; the beginning was recorded with 10% of earing plants, and the mass earing was recorded with approximately 75%. The beginning of flowering was recorded when the flower glumes opened, and their anthers appeared outside 10–20% of the plants. Mass flowering was recorded for 60–70% of the flowering plants. For grasses, a sign of the end of seed formation and the onset of waxy ripening is a change in ear color from green to yellow. With slight shaking or compression of the inflorescence, the seeds fall out.

### Physiological Analysis

The physiological study was conducted following the Methodological Guidelines for determining the drought resistance of grain crops by changing the parameters of the water regime. To determine the water retention index, artificial wilting of leaves and ears isolated from the plant was used; four to five leaves from four repetitions were taken for each sample. The leaves were placed in plastic bags and weighed in the laboratory on torsion scales. Water retention capacity:  $a = B - b / A * 100\%$ , where  $a$  is the water retention capacity,  $B$  is the raw mass before wilting,  $b$  is the raw mass after wilting, and  $A$  is the absolute water content. The leaves were cut before sunrise, weighed, saturated for 2 hours, and weighed again. The residual water deficit is  $b = B1 - B2 / B1 * 100\%$ , where  $B2$  is the initial crude mass and  $B1$  is the mass after saturation (Udovenko, 1984).

Microsatellite markers were used for genetic analysis. We used the phenol–chloroform method to extract DNA from fresh wheatgrass leaves collected during bushing (Tan & Yiap, 2009). For these loci, we performed oligonucleotide synthesis via DNA sequences (Shirvani et al., 2013; Kondratskaya et al., 2017). To construct a phylogenetic tree based on the standard values of Nei's genetic distances, we used MEGA-X software via the neighbor-joining method (Kumar et al., 2018). Bayesian analysis via the Markov chain Monte Carlo (MCMC) algorithm was performed via STRUCTURE software (Falush et al., 2003).

We performed molecular genetic analysis using DNA isolated from the samples. We compiled a tree diagram via the nearest neighbor method to identify the genetic

relationships between the varieties and determine their genetic structure. The phylogenetic tree was constructed on the basis of Nei's genetic distance (Shirvani et al., 2013).

To identify genetic polymorphisms and establish phylogenetic relationships, we performed fragment analysis of the genome via seven ISSR primers via polymerase chain reaction (PCR). The ISSR primers are informative in terms of genetic diversity and interspecific affinity. To carry out molecular genetic analysis, we selected 105 samples (Fig. 3).

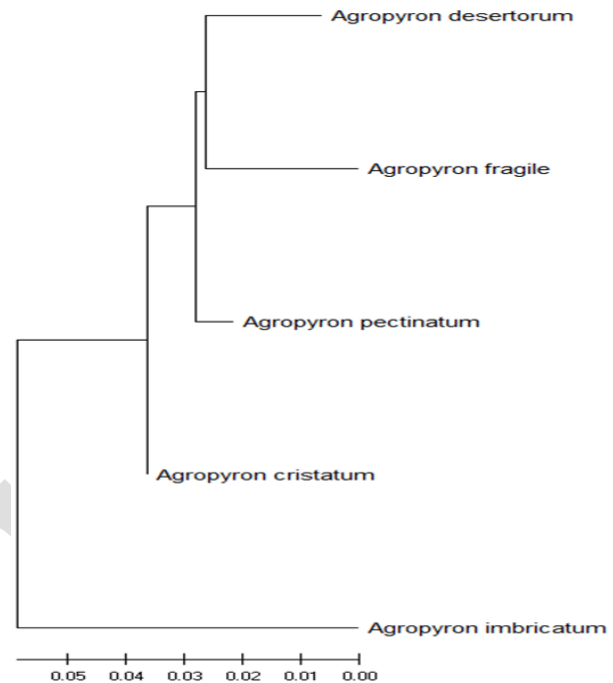


Fig. 3: Phylogenetic relationships of five *Agropyron* species according to ISSR loci via the neighbor-joining method

### Statistical Analysis

Statistical processing of the results to ensure the reliability of the data was performed via the Dospekhov method (Dospekhov, 2012). An important task in organizing information is improving storage and processing methods. We used a database management system to track each sample's movement in the gene bank. To automate this work, we used AC-DB software, which is based on MS VFP 5.0 software, and established the basic principles of creating ICARDA and VIR information systems. We also used international descriptors for crops and methodological guidelines for databases on plant genetic resources (Esimbekova et al., 2006).

## RESULTS

During the survey of the Zhambyl and East Kazakhstan regions, samples of different ecotypes and species of forage grasses were collected from a total of 99 samples, including black medick (*Medicago lupulina*): 29, wheatgrass (*Agropyron* sp.): 36, Volga fescue (*Festuca valesiaca*): 16, Pamirian winterfat (*Krascheninnikovia ceratoides*): 9, feathergrass (*Stipa*): 2, couchgrass (*Elymus* sp.): 4, and *Leymus* sp.: 3. Samples were collected in expeditions (Table 1).

**Table 1:** Sample collection expeditions

Expedition Number	Location	Coordinates	Altitude (m)	Terrain	Associated Vegetation	Plant Height (cm)	Business	Ear Type
01-02	Sulutor village, Kordai district, Zhambyl region	N=45° 19750 E=075° 05505	1195	mountainous, submontane, stony, dark chestnut, dry soil	Artemisia, grasses, medicinal plants	dried 40	average	ellipsoid ear, light yellow and lance-shaped seeds
03-06	Sulutor village, Kordai district, Zhambyl region	N=45° 19750 E=075° 05505	1195	mountainous, submontane, stony, dark chestnut, dry soil	Artemisia, sage, Leymus, Volga fescue, medicinal plants	50	good	crested leaves, erect bush, seeds
14	Kordai district, Zhambyl region	N=43° 21617 E=075° 01521	1010	mountainous, submontane, stony, dry soil	Medicinal plants, club-rush	25	average	crested ear, erect bush, thin and average seeds
01-04	Mailina village, Ayagoz district, Kazakhstan region	N=47° 57096 E=080° 30019	3608	mountainous, submontane, stony, dark chestnut, dry soil	Artemisia, grasses, medicinal plants	dried 30	average	ellipsoid ear, light yellow and lance-shaped seeds
06-09	Tarbagatai village, Ayagoz district, Kazakhstan region	N=47° 59641 E=080° 40462	1400	mountainous, submontane, chestnut soil	Leymus, Volga fescue	40	good	comb-shaped ear, erect bush, thin and average seeds
12-13	Aksuat district, Abai region	N=47° 48230 E=081° 17465	1400	mountainous, submontane, stony, dry soil	Medicinal plants, club-rush	25	average	crested ear, erect bush, thin and average seeds
16	Akzhar village, Tarbagatai district, East Kazakhstan region	N=47° 33396 E=083° 37945	2828	mountainous, chestnut soil and chernozem	Feather grass, shrubs, medicinal plants	25	average	crested ear, erect bush, thin and average seeds
20-25	Zaisan district, Kazakhstan region	N=47° 72713 E=083° 49685	740	mountainous, chestnut soil and chernozem	Volga fescue, feather grass, shrubs, medicinal plants	25	average	crested ear, erect bush, thin and average seeds
30-34	Kabanbai village, Tarbagatai district, East Kazakhstan region	N=48° 32415 E=084° 43198	2828	mountainous, chestnut soil and chernozem	Volga fescue, Artemisia, feather grass, shrubs, medicinal plants	25	average	crested ear, erect bush, thin and average seeds
35-36	Kokpekty village, Abai district	N=48° 75354 E=082° 36070	1400	mountainous, chestnut soil and chernozem	Volga fescue, Artemisia, feather grass, shrubs	25	average	erect bush, thin and average seeds
42-43	Kalbatau village, Abai district	N=49° 27138 E=081° 51826	1400	mountainous, chestnut soil and chernozem	Volga fescue, Artemisia, feather grass, shrubs	30	average	erect bush
47-52	Glubokoe village, Kazakhstan region	N=50° 27138 E=082° 51826	1400	mountainous, chestnut soil, chernozem, mountain meadow soil	Volga fescue, brome, Artemisia, medick, timothy, clover	30	average	erect bush

Figure 4 consists of two screenshots, (a) and (b), showing the database interface for wheatgrass gene pool storage. Both screenshots are for the accession '81690' of *Agropyron cristatum* from the origin country 'TUR'.

Screenshot (a) displays the 'Active collection (seeds)' section with fields for Weight (28.10), Seeds (14050), Year (2015), Location ID (KSRIAHP), Viability (%), and Test date (2005/01/13). It also shows 'Vegetatively propagated' and 'In-vitro' sections with fields for Year, Planting site, Location ID, and In-vitro location. A 'Stock notes' field contains 'анакт.кхпр' and a 'Safety duplication site' field contains 'VIR'. The 'Type of germplasm storage' is 'семена'.

Screenshot (b) displays the 'Conservation' section with fields for Conserved by (KSRIAHP), Receipt date, Donor, and Country (don). The 'Sample characteristics' section includes Sample type (WI), Status (PO), Seasonality (P), and Habitat. The 'Herbarium specimen' section has fields for N and A. The 'Field collecting' section includes Collector's number, Coil date, Site code, Country (KAZ), Province (Almaty province), Site (Алматынская область, Жамбылский район, Аулыский округ Караастек), Altitude (3344), Latitude (43 08838), and Longitude (76 03982). A table at the bottom lists identifying names and numbers with columns for 'What', 'Where used', and 'Pref'.

**Fig. 4 (a and b):** The database of the wheatgrass gene pool storage

Documenting plant genetic resources is important when working with the plant gene pool. A total of 1,500 samples representing 147 varieties were documented, 467 were assigned to the research material, and 882 were wild. By status, the samples were assigned to the population. The donors of the collection were two countries that submitted collections from 20 countries of origin (Table 2).

Fig. 4 shows the database of the passport data and data storage of the wheatgrass gene pool via descriptors developed by ICARDA to document plant genetic resources for food and agriculture (PGRFA). A total of 1,500 wheatgrass samples stored in the LLP "Kazakh Research Institute of Livestock and Fodder Production" gene pool were inventoried and documented.

A total of 207 loci were observed in all samples. The greatest number of polymorphic loci was found in the *A. cristatum* population (186 fragments), and the smallest number was found in *A. fragile* and *A. imbricatum* (89 fragments). Among the 186, 145, 89, 163, and 89 fragments, 89.86, 70.05, 43.00, 78.74, and 43.00% were polymorphic in *A. cristatum*, *A. desertorum*, *A. fragile*, *A. pectinatum*, and *A. imbricatum*, respectively. The number of alleles ranged from  $1.8986 \pm 0.3027$  in *A. cristatum* to  $1.4300 \pm 0.4963$  in *A. fragile* and *A. imbricatum*. The average number of alleles was  $1.9952 \pm 0.0695$  (Table 3).

When a tree diagram of genetic similarity was constructed, the collection differentiated varieties according to their origin and main economically valuable characteristics. The evolutionary relationships of *Agropyron* species were

**Table 2:** Documentation of the wheatgrass gene pool by the main passport descriptors

Numbers of the national catalog of the wheatgrass gene pool (NC), KRILFP			
Descriptors	Field code	Definition of the field code	Number of samples, units
Type of development	P	perennial	1,500
Population type	CV	crop variety	147
	RM	research material	467
	WI	wild	882
	WE	weed	2
	LS	local selection	2
Status	PO	population	1,500
Donor	RUS	Russia	1,251
	KAZ	Kazakhstan	249
Country	RUS	Russia	587
	KAZ	Kazakhstan	501
	KGZ	Kyrgyzstan	4
	TUR	Turkey	95
	CAN	Canada	22
	FRA	France	5
	IRN	Iran	151
	PRT	Portugal	5
	DEU	Germany	7
	ROM	Romania	14
	AUS	Australia	4
	POL	Poland	6
	USA	United States of America	18
	UKR	Ukraine	7
	CHN	China	60
	MAR	Morocco	7
	GBR	Great Britain	3
	ESP	Spain	2
	SWE	Sweden	1
-	Yugoslavia	1	
Species		<i>Agropyron cristatum</i>	423
		<i>Agropyron pectinatum</i>	485
		<i>Agropyron desertorum</i>	277
		<i>Agropyron fragile</i>	161
		<i>Agropyron imbricatum</i>	154
Herbarium	N	None	0
Stored at KRILFP	KIZ	KRILFP	1,500
Storage status	A	Accepted, stored	1,500
Storage	t=+4-+6°C	3-20 g	1,500

studied by geographical origin via unweighted paired groups with arithmetic mean values. This study resulted in the development of a technology for identifying and certifying *Agropyron* samples based on molecular analysis via ISSR markers.

In southeastern Kazakhstan, *Agropyron* was studied in the gene pool's collection nursery on the basis of its main economically valuable and biological characteristics. The study included 270 collection samples, which were characterized morphologically and ecotypically. The viability of seeds stored in the feed production laboratory gene pool, where germination ranged from 12 to 75%, was determined. At the Kolda station, the collected samples were compared with the standard *A. pectinatum* (Taukumsky variety).

Bushiness depends on the species (genotype), growing conditions, and herbage age. The collection samples exceeded the standard in terms of bushiness. The best samples were wild-growing samples from Russia (collection 564872) and China (collections 315160 and 449389), which had from 172 to 180.0 stems/bush on average. The standard has a total number of 131.0 stems/bush on average.

Plant height was measured during sloping ripening. The samples of crested wheatgrass (collections 325185 and 345583), Siberian wheatgrass from Russia (collection

325158) and China (collection 449389), and desert wheatgrass from Turkey (collection 340060) and Australia (collection 316121) were tall, with heights of 71–79 cm.

The leaf coverage of the samples ranged from 45–60%. The average leaf coverage was found in 18 samples (33–38%). The samples from China (collection 449389) and Russia (collection 345583) had the maximum leaf coverage (50–60%), whereas the standard had a leaf coverage of 47%. Leaf coverage is a variable feature. The coefficient of variability in the collection ranged from 24.5 to 33.9%.

Yield is the main indicator of a forage plant's economic value. Together with qualitative indicators, it determines the economic efficiency of sowing. The average yields of the green mass in samples of *A. pectiniforme* from China (collection 315360) and Russia (collections 325185, 311621) and the Kurdaisky variety from Kyrgyzstan were stable, ranging from 720--774.5g/m<sup>2</sup>, which was 123–132% greater than the standard yield (Table 3).

Studying the drought resistance of the wheatgrass source material collected in expeditions is important for the development of drought-resistant varieties. At different stages of ontogenesis, plants react differently to drought, and critical periods in which plants are most affected by drought have been established.

We investigated several features of the water regime of *A. pectiniforme* and *A. desertorum* populations in southeastern Kazakhstan. Experiments with wheatgrass were carried out during earing, flowering, and milk ripening with 15 populations of different geographical origins (Kazakhstan, Russia, Turkey, Kyrgyzstan, China, etc.).

Mass assessment methods for drought resistance are needed, allowing for comprehensive physiological characterization. On the basis of these indicators, we established different types of drought resistance (Table 4). We established critical periods when plants are most affected by drought. For grasses, drought is most dangerous during stem elongation, spikelet differentiation, and flowering. The decrease in drought resistance begins with the appearance of staminate tubercles in the spikelets of the middle part of the ear and ends with fertilization.

As noted by the N.I. Vavilov Research Institute of Plant Industry Scientists (Dzyubenko et al., 2017), the resistance of wheatgrass species to drought is based on their ability to quickly change their nature, norms, and speed of physiological reactions to drought and adaptability, which allows them to avoid drought.

Intensive bushing occurs in autumn and early spring, and the development of plants begins under snow. At this time, plants use the moisture that has entered the soil due to winter precipitation. By the time of the onset of hot weather, plants enter the earing phase. When studying several features of the water regime of ecological and geographical groups of wheatgrass for drought resistance, we used the following indicators: the water retention capacity of leaves isolated from plants and the residual water deficit.

The samples differed more in water retention capacity if the leaves lost at least 40–50% of their total water content. The optimal wilting time is 5 hours.

**Table 3:** Indicators of the genetic diversity of *Agropyron* species

Species	P	P %	na	Ne	h	I
<i>Agropyron cristatum</i>	186	89.86	1.8986±0.3027	1.3258±0.3149	0.2083±0.1613	0.3354±0.2180
<i>Agropyron desertorum</i>	145	70.05	1.7005±0.4592	1.3146±0.3168	0.1991±0.1711	0.3127±0.2451
<i>Agropyron fragile</i>	89	43.00	1.4300±0.4963	1.2985±0.3753	0.1716±0.2049	0.2518±0.2962
<i>Agropyron pectinatum</i>	163	78.74	1.7874±0.4101	1.3100±0.3137	0.1982±0.1642	0.3173±0.2288
<i>Agropyron imbricatum</i>	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

Note: \*P=number of polymorphic loci, \*P%=percentage of polymorphic loci, \*na=observed number of alleles, \* ne=effective number of alleles, \* h=Nei gene diversity (1973), \* I=Shannon information index.

**Table 4:** The best drought-resistant samples according to average yield of green mass from 2021--2023

No.	Species, variety	Origin	Productivity of the green mass		Bushiness, units/bush	Height, cm	Leaf coverage, %
			g/m <sup>2</sup>	% to the standard			
No.	Sample name	Origin					
	Variety, standard	KRILFP	585.5	-	131	59	47
370652	<i>Agropyron pectiniforme</i>	Russia	639.5	109	140	64	50
314927	<i>Agropyron desertorum</i>	Russia	650.0	111	133	65	56
316121	<i>Agropyron desertorum</i>	Australia	652.1	111	145	64	53
315068	<i>Agropyron pectiniforme</i>	Russia	662.0	113	154	66	54
340060	<i>Agropyron desertorum</i>	Turkey	668.8	114	101	69	52
345583	<i>Agropyron pectiniforme</i>	Russia	673.0	115	154	70	58
325185	<i>Agropyron pectiniforme</i>	Russia	683.4	117	104	72	56
311621	<i>Agropyron pectiniforme</i>	Russia	706.3	121	158	75	47
315360	<i>Agropyron pectiniforme</i>	China	720.9	123	180	66	50
325185	<i>Agropyron pectiniforme</i>	Russia	760.5	130	145	64	56
311600	<i>Agropyron pectiniforme</i>	Russia	774.5	132	156	72	53
315160	<i>Agropyron pectiniforme</i>	Russia	733.4	125	125	71	54
449389	<i>Agropyron pectiniforme</i>	China	630.0	108	172	75	60
564872	<i>Agropyron desertorum</i>	Russia	648.0	111	176	77	60
564878	<i>Agropyron desertorum</i>	Russia	618.0	106	123	70	56
31872	Kurdaisky variety	Kyrgyzstan	769.0	131	118	69	47
27790	<i>Agropyron desertorum</i>	Russia	598.0	102	130	60	45
Least significant difference (LSD)05			105.1				

After wilting, repeated weighing was carried out, and the dry mass of the leaves was determined. The water retention capacity was determined by the dynamics of deepening drought (stem elongation, earing, flowering, and milk ripening) (Table 4).

The best indicators of water retention were noted in desert wheatgrass samples from Russia (collections 314927 and 315068), Australia (collection 316121), and Turkey (collection 340060) and ranged from 12-14% (Table 5).

Wheatgrass species respond strongly to additional moisture due to atmospheric precipitation. In the under-resourced non-irrigated lands of the Almaty region with 320 mm of precipitation, the productivity of the dry mass was 54c/ha, and with 200mm of precipitation, it was only 8.4c/ha. Drought resistance is provided by physiological properties (for example, the protoplasm's high viscosity) and plants' ability to develop during cold periods.

## DISCUSSION

The results of this study provide information on the collection, preservation and genetic analysis of wild wheatgrass (*Agropyron Gaertn.*) samples with the goal of enhancing the gene pool and developing high-yield, drought-resistant forage crops suitable for arid and semiarid regions in Kazakhstan. In the context of improving the climate resistance of wheat by using its wild relatives, our study agrees with the review of Kumar et al. (2022), who highlighted the application of gene introgression into wheat.

The polymorphism displayed by *Agropyron* sp. highlights its genetic variation, which is an important factor in the development of climate-resistant crops in arid

regions. Our study revealed that, compared with other samples, *A. cristatum* is a rich source of genes and alleles due to its high population of polymorphic loci and alleles. Our data agree with the work of Zwyrtková et al. (2022), who identified 159 polymorphic simple-sequence-repeat (SSR) markers in their samples. Our data also agree with the works of Ainebekova et al., (2023), who carried out a genetic analysis and molecular characterization of wheatgrass (*Agropyron cristatum* L.Gaertn.) in southeastern Kazakhstan.

During our study, we used simple sequence repeats (ISSR) to assess genetic diversity between intraspecific wheatgrass species. According to the study by Safari et al. (2022), the ISSR marker was more efficient in the determination of genetic diversity between intraspecific species. We also observed that the wild-growing wheatgrass samples from Russia and China were exceptional and presented the greatest plant height, bushiness, leaf coverage and yield. These data agree with the work of Ainebekova et al., (2023), who reported that these traits are essential for thriving in arid regions and that they can be harnessed in genetic introgression to develop climate-resistant qualities in other forage crops.

Our assessment of the drought resistance results (Table 4) revealed that between 2021 and 2023, the wild Kurdaisky variety of wheatgrass was more resistant to drought because of its crop yield. This logical relationship between crop yield and drought resistance is consistent with the work of Dietz et al. (2021). Owing to unstable global climate conditions, we recommend that more tests be carried out to obtain updated information on favorable wheatgrass species in later years.

Our study revealed that in critical stages of plant growth, such as flowering, there was a significant reduction

**Table 5:** Water retention capacity of wheatgrass leaves, %

No.	Sample name	Origin	Time of wilting, hours	5	24	5	24	5	24	5
				Phases			stem elongation	ear formation	flowering	milk ripeness
	Taukumsky variety (standard)	KRILFP	Group of stable populations	13	37	17	50	30	65	53
370652	<i>Agropyron pectiniforme</i>	Russia		12	39	13	55	35	65	52
314927	<i>Agropyron desertorum</i>	Russia		12	35	15	50	30	67	48
316121	<i>Agropyron desertorum</i>	Australia		13	35	17	54	29	65	44
315068	<i>Agropyron desertorum</i>	Russia		14	35	19	48	26	67	42
340060	<i>Agropyron desertorum</i>	Turkey		12	35	18	46	24	69	47
345583	<i>Agropyron pectiniforme</i>	Russia	Populations	13	39	30	60	28	73	52
325185	<i>Agropyron pectiniforme</i>	Russia		16	42	23	60	31	69	65
311621	<i>Agropyron pectiniforme</i>	Russia		20	40	28	62	36	70	52
315360	<i>Agropyron pectiniforme</i>	China		18	42	24	63	24	70	64
325185	<i>Agropyron pectiniforme</i>	Russia		27	43	30	60	35	75	62
311600	<i>Agropyron pectiniforme</i>	Russia		21	45	28	55	26	70	60
449389	<i>Agropyron pectiniforme</i>	China	Group of unstable	22	40	25	60	37	73	55
564872	<i>Agropyron desertorum</i>	Russia		23	46	23	61	35	70	62
564878	<i>Agropyron desertorum</i>	Russia		16	44	36	59	40	59	56
31872	Kurdaisky variety	Kyrgyzstan		14	38	25	58	24	59	45
27790	<i>Agropyron desertorum</i>	Russia		28	51	28	61	39	75	72

in the drought tolerance of grass. These data are consistent with those of Hahn et al. (2021), who reported varying resistance rates/drought resistance rates in different grasses throughout the growing season, and this phenomenon increased during the reproductive phenological stage when the growth rates were highest. The data we obtained on the relationship between the physiological responses of wheatgrass species and drought resistance also support the explanations of Koryakina and Kochegina (2021), who suggested that drought resistance in wheatgrass is driven primarily by the ability to rapidly alter physiological responses.

In the course of this research, it was possible to select certain samples of wheatgrass that had the highest drought tolerance and potential for good yield.

These samples, especially those from Russia - collection 370652, 314927 - and China - collection 315360, have shown promise for replication in breeding programs with the purpose of increasing the productivity and resistance of forage crops in the arid conditions of the Republic of Kazakhstan. Our results corresponded with the conclusions drawn by Ainebekova et al., (2023).

Although our study produced positive outcomes, it has several limitations in the area of gene introgression. Field testing of drought-resistant wheatgrass species yields a number of very valuable data; however, it cannot even closely represent the complex physiological mechanisms that form the foundation for the trait, and this scenario leaves room for future research. We propose that further research be focused on drought resistance mechanisms at the molecular level and that advanced genetic tools be integrated into breeding programs. By implementing this approach, we contribute to attaining food security and ensuring sustainable agricultural development amidst current climatic changes.

## Conclusion

The expedition was conducted to collect seeds of forage grasses in the Zhambyl, East Kazakhstan, and Abai regions. Foci of wild species such as medickgrass, wheatgrass, Volga fescue, Pamirian winterfat, wild rye, and feather grass were established. Information management on the crop genetic resources was standardized, and documentation/digitalization was carried out on 1,500

wheatgrass samples.

The genetic structure and molecular characteristics of five *Agropyron* species from the LLP "Kazakh Research Institute of Livestock and Fodder Production" gene bank were analyzed. As a result of this study, the technology of identifying and certifying *Agropyron* samples via molecular analysis with ISSR markers was developed. Considering the important role of the correct choice of source material in the creation of new wheatgrass varieties in the laboratory of feed production of the LLP "Kazakh Research Institute of Livestock and Fodder Production", we studied the collection material represented by cultivated and wild forms originating from various ecological and geographical regions in Kazakhstan and other countries.

Among the 270 samples from the collection nursery of wheatgrass sown in 2021, four were distinguished by the combination of economically valuable signs: 314927, 315068, 316121, and 340060. They are used in breeding work as sources of high yields of green mass, dry matter, and seeds, with good cold and drought resistance.

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**Author's Contribution:** NM, AS, KK, AT, KS, KM, EK, BA, SY, and MY conceived and designed the study. KK and AT performed the experiments, while KS and KM conducted laboratory analyses. NM supervised the project and coordinated the experimental work. EK and BA performed statistical analyses of the collected data. SY and MY prepared the first draft of the manuscript. All authors critically revised the manuscript and approved the final version.

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