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RESEARCH ARTICLE
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Results of the Mobilization of Genetic Resources of Wild Forage Grasses and their Importance for Introduction and Breeding

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ABSTRACT

This study focused on the mobilization and enrichment of Kazakhstan's forage genetic resources through dedicated collection missions conducted in 2024 across the Almaty, Jambyl, and Turkestan regions, which represent ecologically diverse zones of the southeastern republic. A total of 52 accessions of wild forage species were collected, including 30 belonging to the genus *Medicago*, along with several other valuable forage taxa. Detailed accession-level morphological and phenological traits were recorded during collection. Greenhouse evaluations of the Medicago subset revealed considerable variation in emergence and early development. Germination rates ranged from 50 to 100%, with a median of approximately 90%. Key developmental stages were chronologically documented: cotyledon emergence occurred between 29 December and 29 February, simple leaf development between 5 January and 11 March, and the appearance of the third true leaf between 1 February and 27 March. The duration of early development differed markedly among accessions, with a maximum inter-accession gap of 55 days and intra-accession variation of 23–38 days, indicating substantial phenological diversity that may contribute to stress avoidance and ecological adaptability. Field revisits to original collection sites revealed very low natural emergence rates (approximately 2–3%) in wild *Medicago* populations, likely attributable to hard-seededness and innate dormancy mechanisms. This emphasizes the importance of integrating seed-technology interventions with genetic improvement to enhance establishment rates under field conditions. Across surveyed habitats, wild *Medicago* species were most prevalent in mesic microhabitats and exhibited several adaptive traits; such as pronounced epicuticular wax, vigorous branching, and compact racemes—that are of considerable value for breeding under increasing heat, drought, and salinity stress. All collected accessions have been deposited in medium-term seed storage for preservation, and they will support ongoing greenhouse and field evaluations. Collectively, these genetic resources represent a strategically important donor pool for climate-resilient pre-breeding, germplasm enhancement, and the development of improved alfalfa and related forage crops in Kazakhstan.

Keywords: Forage crops, Wild relatives, Adaptivity, Expeditionary collection, Collections.

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INTRODUCTION

Biological diversity has significant ecological, socio-economic, cultural, educational, recreational, and aesthetic value. Recognizing the enduring importance of biodiversity for the evolution and preservation of life in the biosphere, it is essential to maintain and expand it for the benefit of all humanity (Xu et al., 2021; Pathirana & Karimi, 2022). Forages serve as a crucial livestock feed resource globally, especially for numerous smallholder farmers who depend on natural pastures and grasslands to sustain their livestock systems. This resource enables ruminants to transform inedible feed into milk and meat, supplying vital nutrients necessary for human health, growth, and cognitive development (Hanson & Ellis, 2020; Ochar & Kim, 2023). The importance of forage genetic resources can be overestimated (Esimbekova & Mukin, 2021). Hanson et al. (2020) highlighted that due to the limited availability of tropical breeding programs of

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limited species coverage, forage germplasm remained the foundation for selecting and developing new feeds, and for such endeavors to be realizable, tropical and subtropical forage genetic resources were particularly essential. Tucak et al. (2021) also reported that numerous genetic studies on forages have highlighted the significance, potential and benefits of employing divergent genetic resources (crop wild relatives, landraces, and wild populations) in breeding strategies aimed at enhancing forage legume cultivars, particularly in the context of global climate change, which poses the foremost challenge for the future breeding of forage crops. Previous studies have shown that for the case of climate change, timely response is required, and this requires creating adaptable forage crop varieties that can not only withstand the current environmental crisis but also, in some way, benefit from it (Kostenko et al., 2021). Public and private plant breeders in response to climate change, are working on new, more competitive cultivars on the market, and to achieve that, they need diverse plant genetic resources (PGR). According to the studies by Kalibayev et al. (2021), no nation is self-sufficient in plant genetic resources, and all nations require access to germplasm from other countries and regions. As a result, most PGRs are stored in 1625 gene banks around the world. They gather CWRs and other plant materials that exhibit features that breeders are interested in, therefore their purpose is not just to collect and maintain diverse germplasm but also to provide resources to breeders (Perić et al., 2024). Some forage plants have been cultivated for a long time and are widely used in various forms for animal feed, including forage, hay, haylage, silage, and others.

One way to increase the productivity and sustainability of feed production is to introduce new plant species and forms into the culture, selected from the wild phytodiversity (United Nations, 2015; FAO, 2019). Generally, wild relatives and traditional landraces serve as invaluable reservoirs of adaptive genetic variation (Meiirman et al., 2024), harboring numerous beneficial traits—including diverse forms of resistance to both biotic and abiotic stresses—that remain critical for enhancing the resilience of modern cultivars to current and future climatic challenges (Bohra et al., 2021). For several decades, crop wild relatives (CWRs) have played a pivotal role in improving plant performance, and their contribution to global agriculture is substantial. It is estimated that CWRs generate approximately USD 186.3 billion annually for the global economy through the genetic enhancement of crops.

A major proportion of the economically valuable traits introgressed from CWRs relates to resistance against diseases and pests. Notable historical examples underscore the immense value of these genetic resources. For instance, the incorporation of resistance to late blight *[Phytophthora infestans* (Mont.) de Bary] into cultivated potato represents one of the most significant advancements in crop protection. Likewise, the introgression of stem rust resistance—particularly genes contributed by the wild wheat progenitor *Aegilops tauschii*—played a transformative role in boosting wheat yield and stability during the global “Green Revolution” (Bohra et al., 2021). These cases emphasize the indispensable role of CWRs in sustaining agricultural productivity and ensuring food security under intensifying climate pressures. Perić et al. (2024) also reported that the use of common wild relatives and landraces can reduce the loss of genetic diversity observed in cultivated alfalfa and also expand the narrowed genetic base with useful genetic variations. Wild species, unlike domesticated ones, have not been subjected to strict anthropogenic selection pressure and have evolved to adapt to extreme weather conditions. They further explained that to achieve adapted genotypes to abiotic stress such as drought and achieve outstanding production, introducing drought-resistant alleles into elite cultivars can be considered.

In alignment with this research, the 2020 ICARDA annual reports highlight several programs dedicated to genetically crossing wild relatives to achieve elite cultivars. The DIIVA-PR Project focused on developing new varieties derived from crop wild relative crosses, assessing their response to climatic stresses across these locations, and introducing them to farmers through a participatory varietal selection strategy. The ICARDA Global Barley Breeding program developed new wild-relative-derived genotypes with increased nutritional value (ICARDA, 2021). However, only a few dozen species have specific prospects for introduction into culture (IPCC, 2019; FAO, 2021). Therefore, there is a significant shortage of information related to biological diversity, as well as a need to develop scientific, technical, and organizational capacities for a common understanding of this problem, planning, and implementation of relevant measures. However, wild forms remain a treasure trove of knowledge about the evolutionary path traveled by the species and a source of genetic elements that determine the species' resistance to sudden fluctuations in weather and climate.

By their origin, cultivated plants are divided into three groups: the ancient group, the weed plants on arable land, and the youngest group. Representatives of the youngest group of cultivated plants, including most forage grasses (alfalfa, clover, brome, orchard grass, timothy, and others), are still found in the wild. Among this group of plants, many have not yet attracted the attention of breeders, but could become more useful for humans if they were cultivated (Convention on Biological Diversity, 2022). Despite the recognized importance of crop wild relatives (CWRs) for forage improvement, there remains a significant gap in the systematic mobilization, ecological assessment, and phenotypic characterization of wild forage grasses and legumes in Kazakhstan—particularly in the southern regions where biodiversity hotspots are under increasing anthropogenic pressure. Existing studies provide fragmented information on the distribution and adaptive traits of these species, but comprehensive expedition-based assessments linked with controlled greenhouse evaluations are largely lacking. To address this gap, the present study aimed to:

(1) conduct targeted expeditions across diverse ecological zones of southern Kazakhstan to collect and document wild forage species;

(2) assess the morphological, ecological, and phenological characteristics of collected accessions under field and greenhouse conditions; and

(3) identify promising wild germplasm with adaptive traits relevant for future forage breeding, conservation, and pre-breeding programs.

MATERIALS & METHODS

Kazakhstan represents one of the richest centers of global forage diversity, containing more than 70 species belonging to 29 genera. In 2024, targeted expeditions were conducted to survey natural habitats and collect seeds of wild perennial and annual forage crops across the Almaty (Jambyl district), Jambyl (Merki, Ryskulov, Jambyl districts), and Turkestan (Tulkibas, Kentau, Saryagash districts) regions. These areas span foothill–steppe and semi-arid landscapes and are known for their high ecological heterogeneity. More than 2,000 km of routes were traversed, and all sampling sites were georeferenced using an ASUS-7 tablet equipped with Navitel GPS software (Fig. 1). For each site, ecological conditions—including soil type, vegetation composition, slope exposure, soil moisture, terrain profile, and co-occurring plant communities—were recorded following the protocols of Tsvelev (1976) and Shamsutdinov et al. (1993). During the expeditions, a total of 52 accessions were collected. Mature seeds were sampled whenever possible; otherwise, whole plants with intact root systems were transplanted to the Institute's nursery for regeneration. Taxonomic identification of collected species relied on "Grasses of the USSR" (Tsvelev, 1976) and the Broad Unified Classifier for *Trifolium* L. (Uzhik et al., 1983). The collected accessions represented various ecological groups, including mesophytic forage species such as alfalfa (*Medicago* spp.; 30 accessions), melilot (*Melilotus* spp.; 3), awnless brome (*Bromus inermis*; 2), prostrate summer cypress (*Kochia prostrata*; 3), winterfat (*Eurotia ceratoides*; 3), and bluegrass (*Poa* spp.; 4). Additional xerophytic and salt-tolerant species were collected from drier steppe and semi-desert zones, including four accessions of *Agropyron*, two of *Camphorosma*, three of *Festuca valesiaca*, and two of *Calligonum*. Each accession was assigned a passport entry containing coordinates, habitat description, collector details, and seed quantity.

Morphological and ecological descriptions of each accession were carried out using standardized descriptors of the V.R. Williams All-Russian Research Institute of Feed (Shamsutdinov et al., 1993). Traits recorded included bush architecture, regrowth form, stem height, number of internodes, branching intensity, foliage density, leaf pubescence and waxiness, stipule structure, inflorescence shape and color, pod type (size, whorl number, coloration), seed characteristics (size, shape, hard-seed percentage), and root morphology including nodulation. Foliage abundance was scored on a three-point scale: 1.0 = sparse foliage (<40% stem coverage), 2.0 = moderate foliage (40–70%), and 3.0 = dense foliage (>70%). Ecological suitability of each species was assessed based on optimal habitat conditions, including moisture level, soil characteristics, terrain type, and plant community structure.

A controlled greenhouse experiment was performed to evaluate germination and early phenological development of *Medicago* accessions. Seeds from each accession were sown in a Completely Randomized Design (CRD), with three replications of 30 seeds each (90 seeds per accession). The greenhouse was maintained at day/night temperatures of 22 ± 2°C and 18 ± 2°C, respectively, with a 14-h light/10-h dark photoperiod, relative humidity of 55–65%, and a light intensity of 250–300 μmol m^{-2} s^{-1}. Irrigation was applied to maintain near field capacity. Plants were grown in 2.5-L pots containing a substrate composed of 50% loamy soil, 30% peat, and 20% washed river sand, adjusted to pH 6.5–7.0. Phenological observations—including cotyledon emergence, appearance of simple leaves, and the appearance of the first three true leaves—were recorded every 48 hours. Germination percentage was assessed according to GOST 12038-84 ("Crop seeds"), where a seed was considered germinated once the radicle reached at least 2 mm. All accessions were dried, cleaned, and placed into medium-term seed storage at the Department of the Gene Pool of Field Crops, Kazakh Scientific Research Institute of Agriculture and Plant Growing (KazNIIZiR), under controlled humidity and temperature conditions to maintain viability.

Statistical Analysis

Statistical analyses were performed using Statistica 7.0. For each accession, descriptive statistics (mean, standard deviation, and range) were calculated for germination percentage and for phenological traits. Germination data were arcsine-transformed prior to analysis to meet normality assumptions. Differences among accessions for germination rate and key phenological intervals (days to cotyledon, simple leaf, and true leaf stages) were evaluated using one-way ANOVA. When significant differences were detected ($P<0.05$), post-hoc comparisons were conducted using Tukey's Honest Significant Difference (HSD) test. In addition, Pearson correlation coefficients (r) were calculated to quantify relationships between germination percentage, phenological timing, and selected morphological traits (e.g., stem height, foliage score, branching). The strength of correlation was interpreted as weak (0.2–0.39), moderate (0.40–0.69), or strong (0.70–1.0). All analyses were carried out at a significance level of $P<0.05$.

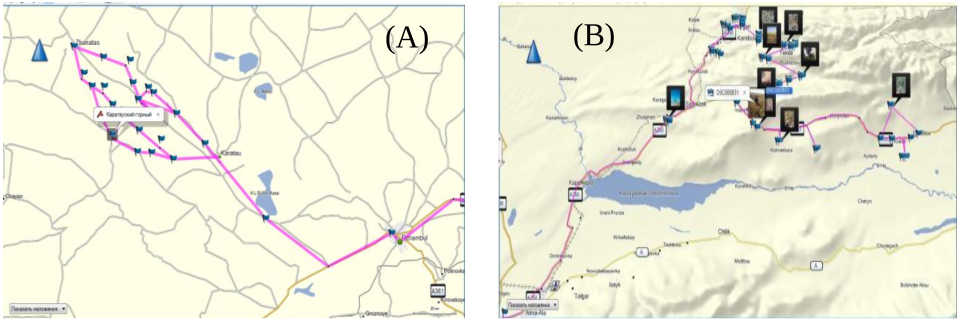

Fig. 1: Routes of expeditions to collect wild species of forage crops: A) Jambyl region; B) Turekestan region.

Description of the Studied Territories

The Jambyl region is situated in the southeastern part of Kazakhstan. It is bordered on the west and east by the Turkestan and Almaty regions, on the north by the Karaganda region, and on the south by the Republic of Kyrgyzstan. Despite the predominance of flat terrain, the Jambyl region boasts a variety of natural areas. The northern part of the Chu River is located in the clay or rocky Betpak–Dala Desert. To the south of the Chu River lies the sandy Moyinkum Desert, characterized by bumpy, hilly sands. The Karatau ridge occupies the southwest of the region (altitude up to 1600 m). The Kendyktas Mountains (reaching up to 1,503 m) extend to the east and southeast, gradually merging with the Ili Alatau range. The region's climate is continental, resulting in a significant temperature difference and low precipitation amounts. The average January temperature in the lowlands is -15°C, in the foothills -6°C, -8°C; in July +16°C and +24°C, +25°C, respectively (Fig. 2). The Turkestan region is situated in the extreme south of Kazakhstan, in the desert zone, and encompasses part of the Turan lowland and the foothills of the Tien Shan. The region is mainly located on a plain. In the north lies the clay Betpak-Dala desert, and to the south and west are the Moyinkum desert, the Shardara steppe, and the sands of Kyzylkum. The Ugam Range, Karzhantau, and Talas Alatau stretch in the southeast, while the Karatau range occupies the central part. The climate of the region is sharply continental. The area experiences dry and hot summers (with an average summer temperature of +31°C and a maximum of +49°C), as well as cold winters (with a lowest temperature of -38°C and an average of 1.5°C). The average annual temperature is +12°C. The hottest month is July (with an average temperature of +32°C), and the coldest month is January (with an average temperature of 2.7°C). The average number of sunny days is 320. The average rainfall is 360 mm. The maximum amount of precipitation (70%) falls in the spring and winter periods. The wettest month is April, while August is the driest. The prevailing winds are from the northeast and west, often quite intense. There is a shortage of moisture in the soil during the summer months, when plants grow intensively. Relatively low air humidity and elevated temperature lead to evaporation of moisture from the soil, several times higher than the amount of precipitation (Fig. 3).

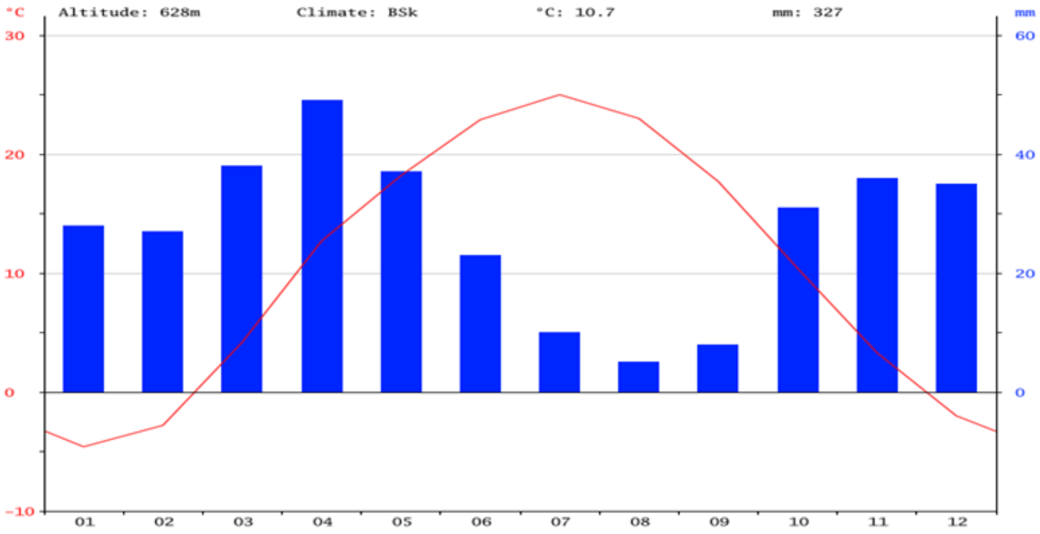

Fig. 2: The weather and climatic conditions of the Jambyl region.

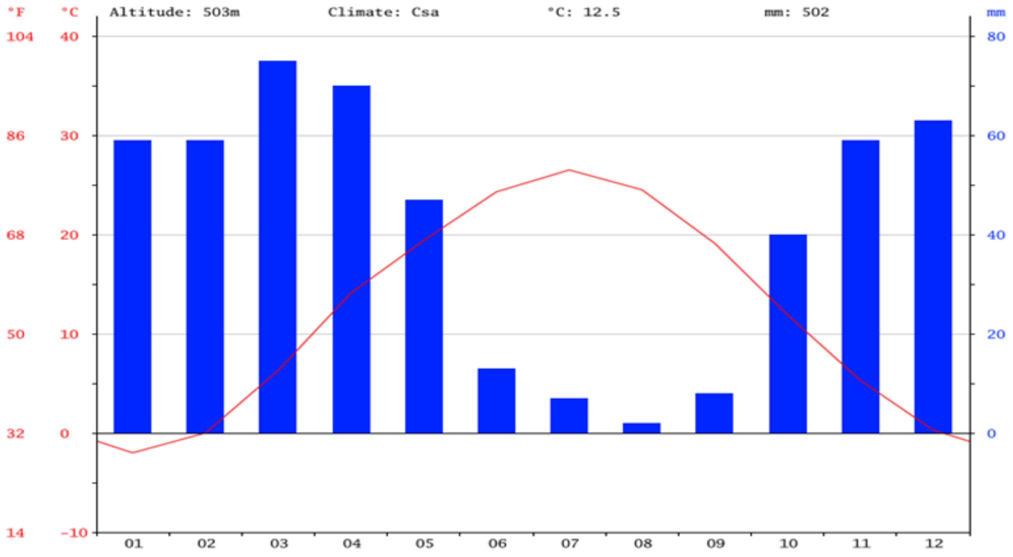

Fig. 3: The weather and climatic conditions of the Turkestan region.

RESULTS & DISCUSSION

In the open steppe and arid conditions, wild alfalfa species cannot compete with grasses and other steppe plant communities. Wild alfalfa species were most frequently encountered in natural lowlands, roadside slopes, and small depressions, environments that generally reflect mesophytic conditions and suggest a preference for moderate soil moisture within many Medicago taxa. However, this interpretation requires nuance. Naydenova et al. (2022) reported that the occurrence of Medicago spp. across a wide range of habitats—including dry, shallow, and nutrient-poor soils—demonstrates considerable ecological plasticity rather than a strict association with mesophily. Thus, while certain local populations may display mesophytic tendencies, the genus as a whole encompasses species and ecotypes well adapted to drought-prone and heterogeneous environments. In our survey, the flora of the East Kazakhstan region exhibited substantial taxonomic and morphological diversity, with multiple wild alfalfa species represented. Yellow-flowered forms were markedly more common in natural habitats than blue-flowered forms, as evidenced by expedition collections from 2023 and phenological observations recorded in 2024 (Fig. 4 and 5).

According to the ploidy classification, wild perennial alfalfa species are divided into three groups: diploid (2n=16), tetraploid (2n=32), and hexaploid (2n=48). They differ not only in ploidy, but also in color and size of flowers, bean shape, leaves, bush shape, rosette of autumn growth, productivity, etc. (Aknazarov & Salimshoeva, 2006; Lubenets, 1972). In special experiments, we studied five species of wild perennial alfalfa collected from East Kazakhstan and the Abai regions. Their field germination ability was very low (2-3%); however, it was possible to obtain single plants (3-6) for each species. The primary data on phenology and some morphological features are presented in Table 1. Zhang et al. (2021) in their study explains that the low field germination of wild alfalfa may be due to certain factors such as hard seeds which are can be caused by environmental issues such as high temperature and low precipitation. Other causative agents could include: fungal infections, autotoxicity from compounds in the soil, drought stress, and unsuitable soil conditions like low pH. We recommend that future research should consider screening test samples for possible fungal infections before applying.

The collected samples exhibit considerable intra-population diversity, particularly in bush architecture, which ranges from prostrate to fully erect growth forms. Floral coloration is also highly variable, encompassing blue, variegated, yellow, purple, and white phenotypes. Inflorescence morphology varies from round-headed to cylindrical trusses, while pod whorl number ranges from 0.5 to 4 per node (Meiirman & Masonichich-Shotunova, 2013). The expansion of the collection fund is guided by two principal directions: (1) collecting local forms displaying high levels of resistance to biotic and abiotic stresses and possessing valuable biochemical traits; and (2) incorporating elite germplasm generated through advances in global breeding programs. Seed preservation under conventional indoor conditions has been widely practiced and validated through long-term studies at KazNIIZiR. Despite the inherent hard-seededness characteristic of many leguminous forage species, periodic regeneration is essential, as seeds remain susceptible to loss of viability over time. Research has demonstrated that physiological and genetic changes continue to occur during storage, including the accumulation of point mutations and potential chromosomal rearrangements. Consequently, collection samples may undergo gradual genetic shifts, and there remains a persistent risk of mechanical or hybridogenic contamination (Zheleznov et al., 2008).

In addition to ex situ storage of legume germplasm as seed, field-based living collections or field genebanks are also utilized. However, this approach has a critical limitation: maintaining genetic purity is challenging in cross-pollinated species such as legumes. To safeguard the integrity of germplasm, isolated plots are established specifically for the regeneration and maintenance of collection samples, thereby minimizing unintended cross-pollination and preserving the genetic identity of each accession. As a result of the long-term and productive work of the feed crop breeding laboratory at KazNIIZiR, varieties combining early ripening and winter hardiness have already been created (Shamsutdinov & Novoselova, 2015). However, issues of increasing productive longevity, seed productivity, and

Fig. 4: Yellow alfalfa.

Fig. 5: Trautvetter's alfalfa.

Table 1: Phenology and morphological features of wild alfalfa species collected by the expedition in the East Kazakhstan and Abai regions (sowing in 2023, recording in 2024)

Ecological origin (district)	Spring regrowth	The beginning of budding	Blossom		Maturation of the seeds	Height (length) the plant	Foliage of score	Bush shape	Color of the flowers	Truss shape	Bean whorl
			beginning	full							
1	2	3	4	5	6	7	8	9	10	11	12
Trautvetter's alfalfa											
Kokpekti district	27.03	25.05	5.06	15.06	12.08	90	3.0	prostrate	blue, yellow	cylindrical	0.5-1.0
Kokpekti district	27.03	21.05	30.05	20.06	12.08	70	3.0	prostrate	blue, multicolored	round-headed, cylindrical	0.5; 0.5-0.8
Yellow alfalfa											
Ayagoz district	27.03	22.05	30.05	20.06	12.08	85	3.0	prostrate	blue, yellow	round-headed, cylindrical	0.5-1.0 1.0-1.5 1.0-2.5
Ayagoz district	27.03	22.05	29.05	20.06	12.08	80	2.5	prostrate	blue, yellow, multicolored	cylindrical	0.5-0.8 0.5-1.5 0.5-1.0
Ayagoz district	27.03	27.05	09.06	25.06	13.08	70	2.0	prostrate	blue	round-headed	0.5 0.5-1.0
Bolshenarymsky district	27.03	21.05	21.06	30.06	13.08	65	3.0	prostrate	blue, yellow	round-headed	0.5 0.5-1.0
Blue alfalfa											
Bolshenarymsky district	27.03	26.05	07.06	15.06	13.08	80	2.0	prostrate	blue	cylindrical	1.0-1.5 1.5-2.5
Along highway 70 km from Ayaguz to Zaisan, M. Falcata, East Kazakhstan region, at the foot of the Tarbagatai Mountains up to 10-12 km	27.03	26.05	06.06	18.06	13.08	78	2.0	prostrate	blue	round-headed, cylindrical	0.5 1.5-2.5 1.5-3.0
Glubokoye district	27.03	25.05	04.06	15.06	13.08	85	2.0	prostrate, erect	blue	round-headed, cylindrical	0.5-1.5 1.0-1.5
Ayteke Bi district	27.03	25.05	05.06	15.06	13.08	81	1.5	prostrate, erect	blue, multicolored	round-headed	0.5-1.0 1.0-3.5
Denisov district	27.03	25.05	06.06	12.06	13.08	75	2	prostrate	blue	round-headed	0.5 0.5-1.2
Variegated alfalfa											
Zharma district	27.03	22.05	28.05	10.06	13.08	71	2	prostrate	blue, multicolored	round-headed	0.5 1.0-1.5 1.5-2.0
Tarbagatay district	27.03	22.05	26.05	10.06	13.08	74	2	prostrate	blue, multicolored	cylindrical	1.0-1.5 0.5-1.5 1.0-2.0
Medicago caerulea											
Zharma district	27.03	22.05	26.05	10.06	13.08	26	2	prostrate	light blue	round-headed	1.0-2.5

resistance to diseases and pests remain on the agenda. Therefore, when examining alfalfa wild plants, special attention was paid to these very features. Experiments were conducted using wild alfalfa samples collected during the 2024 expedition in greenhouse conditions (Fig. 6). Table 2 shows their characteristics based on phenological observations. The appearance of the embryonic cotyledon leaf (shoots) in alfalfa samples was prolonged and lasted from December 29 to February 29. The emergence of simple leaves in alfalfa occurred over a broad temporal window, extending from 5 January to 11 March. The appearance of the third true leaf was recorded between 1 February and 27 March across the evaluated samples. Analysis of the progression through early developmental stages—from cotyledon emergence to the appearance of the third true leaf—revealed substantial phenological variation among alfalfa accessions. The total inter-accession difference in development time reached 55 days, while intra-accession variability ranged from 23 to 38 days. This wide range of developmental timing indicates considerable genetic and physiological diversity that may be advantageous for breeding programs targeting stress escape, persistence, and adaptation.

Wild relatives of cultivated species constitute a critical genetic reservoir for the improvement of forage crops. Wild forage species and ecotypes possess complex adaptive traits shaped by long-term natural selection within heterogeneous environments. These taxa can serve as valuable gene donors, as they often carry resistance to pests and diseases, as well as traits that enhance yield stability and forage quality. Owing to their broad ecological adaptability, such traits can be introgressed into cultivated forage crops to address both environmental challenges and evolving market demands. The identification and deployment of novel resistance genes are particularly urgent in the context of global climate change, where reducing pesticide dependence and enhancing resilience to abiotic stresses are key priorities for sustainable agriculture (FAO, 2014). For the full use and preservation of the genetic diversity of the region's plant resources, considerable work was done to establish a collection fund of economically valuable legume genera.At KazNIIZiR, conditions were established for a rapid search for the source genetic material required for breeding work, as well as control over the maintenance of samples in either a live form or as a seed bank. Although several limitations constrain the wider applicability of the findings, this research underscores the critical importance of mobilizing and characterizing wild forage grass genetic resources in Kazakhstan. Firstly, the expeditions' geographic reach was restricted to three southern regions:

Table 2: Phenological observations of wild alfalfa samples in greenhouse conditions (expedition collection in 2024, recording in 2025)

Sample number	Germination ability, %	Date of appearance				
		of the cotyledon leaf	of the simple leaf	of the ternate leaf		
				first	second	third
Samples of yellow-flowered alfalfa						
1	80	15.01-22.02	25.01-26.02	27.01-04.03	01.02-16.03	08.02-18.03
2	100	29.12-05.02	05.01-08.02	11.01-11.02	15.01-18.02	18.01-26.02
3	100	29.12-20.01	05.01-27.01	06.01-29.01	11.01-01.02	15.01-08.02
4	90	15.01-01.02	15.01-01.02	18.01-18.02	22.01-11.02	25.01-26.02
5	100	29.12-01.02	05.01-05.02	11.01-11.02	15.01-11.02	20.01-18.02
6	50	29.12-11.02	05.01-18.02	11.01-27.01	15.01-26.02	20.01-29.02
7	100	29.12-18.01	05.01-05.02	11.01-18.02	15.01-22.02	18.01-26.02
8	100	29.12-01.02	05.01-08.02	06.01-08.02	11.01-22.02	15.01-26.02
9	90	29.12-18.01	11.01-05.02	18.01-08.02	27.01-05.02	05.02-22.02
10	70	29.12-11.02	05.01-18.02	06.01-22.02	15.01-26.02	20.01-29.02
11	100	29.12-18.01	05.01-25.01	06.01-05.02	01.02-11.02	15.01-11.02
12	100	29.12-22.02	05.01-26.01	06.01-29.02	11.01-04.03	15.01-11.03
13	100	29.12-01.02	05.01-27.01	11.01-11.02	15.01-29.02	20.01-26.02
14	100	29.12-11.02	05.01-18.02	06.01-05.02	11.01-11.02	15.01-18.02
16	80	20.01-11.02	08.02-18.02	11.02-26.02	15.02-29.02	26.02-04.03
17	60	15.01-01.02	20.01-05.02	25.01-08.02	01.02-15.02	05.02-18.02
18	100	29.01-11.02	05.01-22.02	11.01-26.02	15.01-04.03	27.01-11.03
19	100	22.12-22.12	29.12-20.01	05.01-22.02	15.01-08.02	20.01-18.02
20	100	15.01-08.02	22.01-18.02	27.01-26.02	01.02-22.02	22.01-04.03
Samples of blue-flowered alfalfa						
21	90	29.12-25.01	05.01-05.02	06.01-11.02	11.01-18.02	15.01-26.02
22	90	18.01-05.02	22.01-08.02	27.01-11.02	01.02-18.02	08.02-26.02
23	90	15.01-01.02	22.01-05.02	25.01-08.02	27.01-18.02	05.02-26.02
24	80	29.12-22.02	05.01-26.01	06.01-29.02	11.01-04.03	15.01-05.02
25	80	05.01-04.03	06.01-11.03	11.01-16.03	20.01-18.03	01.02-27.03
26	100	29.12-08.02	05.01-26.02	06.01-18.02	11.01-04.03	15.01-11.03
27	100	29.12-20.01	22.01-11.02	27.01-18.02	01.02-22.02	08.02-29.02
28	90	05.01-01.02	11.01-08.02	15.01-18.02	20.01-22.02	27.01-26.02
29	100	29.12-18.01	11.01-08.02	18.01-11.02	22.01-18.02	27.01-22.02
30	100	29.12-18.01	05.01-11.02	11.01-18.02	20.01-26.02	27.01-29.02

Fig. 6: The general appearance of plants in a greenhouse.

Almaty, Jambyl, and Turkestan. These areas are rich in species diversity, but they do not fully capture the ecological gradient of Kazakhstan's enormous steppe, semi-desert, and mountainous systems. Consequently, the study may have overlooked potential genetic diversity in the unexplored northern and western regions (Tynykulov et al., 2024; Meldebekova et al., 2024). Secondly, although morphological and phenological characteristics were used in the study to describe the collected accessions, they don't reveal much about the underlying genetic variation. The identification of allelic diversity, phylogenetic relationships, and potential adaptive genes that are essential for breeding programs aimed at climate resilience is limited by the lack of molecular or genomic analyses. Thirdly, there may have been bias in the assessment of traits due to environmental influences during field collection and greenhouse cultivation that affected phenotypic expressions, specifically germination rates and growth dynamics (Moore et al., 2020; Rognli et al., 2021; Sustek-Sánchez et al., 2023). Standardized viability testing under various environmental and soil conditions is necessary, as evidenced by the reported low field germination (2–3%) among certain wild alfalfa species. Furthermore, because the study only looks at the genus Medicago, it is not possible to compare other important forage taxa like Bromus, Trifolium, or Poa, which might also have important adaptive traits for Kazakhstan's semi-arid and arid agroecosystems (Muzbay et al., 2023; Irmulatov et al., 2025).

Given the known risks of viability loss and hybridogenic contamination in cross-pollinated species, the use of medium-term seed storage without concurrent evaluation of long-term ex situ or in situ strategies poses a vulnerability to genetic erosion over time from a conservation perspective (Pathirana & Carimi, 2022). To better understand genetic diversity both within and between wild populations, future research should try to overcome these limitations by utilizing molecular marker-assisted selection and genome-wide association studies (GWAS). The representation of Kazakhstan's forage gene pool would be increased by extending expeditionary surveys to underrepresented ecological zones, especially the northern steppe and Caspian lowland habitats (Sitpayeva et al., 2020). Future collection routes could be optimized, and climate-resilient hotspots could be identified by combining remote sensing and GIS-based habitat modeling. Additionally, cooperation with international programs like the Global Crop Diversity Trust, FAO, and ICARDA may make it easier to align Kazakhstan's germplasm management with global conservation frameworks. To assess genetic drift, adaptive stability, and germination longevity, longitudinal studies contrasting ex situ seed banks within situ populations are advised. The domestication and field evaluation of wild accessions could be accelerated by participatory breeding programs that involve local farmers (Perić et al., 2024). This would guarantee that future cultivars are not only genetically diverse but also agronomically viable and regionally adapted. A key novelty of this study is the combination of expedition-based sampling, ecological profiling, and controlled greenhouse evaluation within a single framework. Previous work in Kazakhstan has often focused exclusively on botanical surveys or cultivar evaluation, but rarely both. The integration of field and greenhouse phenology provides a clearer understanding of how wild accessions may perform in early establishment phases—an important trait for pasture renovation, where poor seedling vigor often limits success. The wide phenological window documented here suggests that breeding efforts could strategically select for either early or delayed emergence depending on the target production zone (Bouton, 2023).

Despite its strengths, the study has several limitations. The geographic range—while substantial—was restricted to southern Kazakhstan, and many ecologically important northern, eastern, and western regions remained unexplored. The study relied on morphological and phenological markers, which, although informative, provide limited insight into underlying genetic structure. Incorporating molecular profiling (e.g., SSRs, SNP arrays, GBS) would allow a more precise assessment of genetic diversity, population differentiation, and potential redundancy among accessions. Additionally, multi-year field evaluations are needed to verify whether greenhouse phenology and vigor correspond to long-term field performance under variable climatic conditions (Fu et al., 2024).

Overall, the collected accessions represent a strategically important expansion of Kazakhstan's forage germplasm base and contribute new material to the global pool of *Medicago* diversity. These materials present immediate opportunities for incorporation into pre-breeding pipelines aimed at improving persistence, stress tolerance, and productivity of alfalfa and other forage crops under continental climate conditions. Strengthening collaborations with ICARDA, USDA-GRIN, and other international genebanks would further enhance the conservation and utilization of this unique genetic heritage while positioning Kazakhstan as a key contributor to global forage crop improvement.

Conclusions

This study mobilized a diverse gene pool of wild Medicago from steppe–foothill ecotones of southern Kazakhstan and documented substantial variation in phenology, morphology (e.g., epicuticular wax, branching, raceme compactness), and controlled-environment germination. Taken together, these accessions constitute a practical donor set for climate resilience—targeting heat and drought escape (earlier flowering), drought avoidance/persistence (waxy leaves, thicker stems), and establishment under challenging soils (vigorous seedlings under osmotic stress). The greenhouse–field contrast in emergence, consistent with physical (hardseed) dormancy, signals that seed-technology solutions must accompany genetic gains to ensure on-farm impact.

Conservation and data stewardship are integral to breeding success. We recommend safety duplication of all priority accessions, full georeferenced passport data, and FAIR release of characterization/phenotyping datasets to catalyze reuse by national programs and international pre-breeding partners. Multi-environment trials across steppe, foothill, and irrigated zones should be embedded early to capture genotype-by-environment responses and refine ideotype definitions.

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Author's Contribution: S. Abayev: conceptualization, project administration; Sh. Bastaubayeva, S. Yerzhanova: methodology, investigation; M. Yesimbekova, G. Meiirman: visualization, validation, writing; S. Toktarbekova, N. Kaskabayev: data curation, writing, review & editing; Zh. Kabden, D. Medeubekov: writing original draft, visualization. All authors have read and approved the final manuscript and agree to be accountable for all aspects of the work.

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