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Mobilization and Conservation of the Genetic Diversity of Wild Relatives of Forage Legumes in Kazakhstan

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ABSTRACT

Conserving the genetic diversity of forage legumes is crucial for ensuring agricultural resilience, particularly in regions with varied climatic conditions like Kazakhstan. The paper discusses the results of studies on the collection, evaluation, development, and conservation of forage plant genetic resources. It presents the results of an expedition in Kazakhstan, where variable climatic conditions necessitated a survey to identify valuable populations of forage grasses and collect seeds for studying and reproducing economically valuable plant samples. A total of 177 samples of wild forage legumes were collected, including 144 samples of alfalfa, 24 samples of melilot, and 9 samples of sainfoin. Laboratory analyses and cytological studies showed that the number of chromosomes in the metaphases of mitosis of the collected samples remained genetically stable. Some collected samples were placed in collection nurseries, where their phenological, morphological, and other valuable traits, as well as their reproductive characteristics and disease resistance, were studied. Based on the annual evaluation of collection samples, sources of valuable plant breeding traits were identified, and targeted trait collections were established for use in the breeding process. Thus, 15 samples of alfalfa, 3 samples of melilot, and 1 sample of sainfoin were selected based on a combination of traits. The results of the study demonstrated significant potential for utilizing wild legume species in the development of sustainable agricultural practices, especially in addressing the challenges posed by global warming and drought. Findings on genetic resource collection support the development of breeding programs to enhance agricultural sustainability and food security in the region.

1. INTRODUCTION

Due to global warming and the increasing aridification of a significant portion of the planet's land, interest in drought-resistant and salt-tolerant forage plants, especially those growing on rainfed farmland (without irrigation), is increasing [1]. These plants are essential for agricultural production in Kazakhstan, where increasing aridity necessitates more resilient crops to ensure the country's food security under varying climatic conditions. Plant genetic resources are key components of plant biodiversity and have actual or potential value in producing animal forage. They also play a crucial role in the development of environmentally sustainable agriculture. Therefore, collecting, creating, studying, and effectively using plant genetic resources are the most important areas for breeding and introduction in Kazakhstan and the world [2, 3].

Materials from collections of plant genetic resources are primarily used in breeding programs to develop high-yield, high-quality crop varieties, as well as in research and education [4]. In recent years, the collection of wild ecotypes has been conducted through expeditions to various regions of Kazakhstan, including remote ones [5].

The need to establish independent genetic resources in Kazakhstan arose following the decentralization of efforts to manage plant genetic resources after the dissolution of the former Soviet Union. At that time, the problems of agricultural biodiversity and ways to solve them took their rightful place among the priorities of the agro-industrial complex and science. This is evidenced by the strategic approaches and activities of such scientific centers as the Food and Agriculture Organization (FAO), the International Maize and Wheat Improvement Center (CIMMYT), the International Center for Agricultural Research in Dry Areas (ICARDA), Bioversity International, etc. [6]. To develop the collection of genetic resources, Kazakhstan signed the Convention on the Conservation of Biodiversity, which assumes international obligations [7]. It is designed to solve the modern problems of genetic erosion of many wild relatives of cultivated plants and the disappearance of old folk-bred varieties, exceptionally interesting as genetic sources of high resistance to diseases, pests, cold, and drought [8].

To provide biological diversity and mobilize genetic resources, gene banks have been established in many countries, including Kazakhstan, where plant collections are preserved in ex-situ conditions. This type of conservation is the most significant and widespread method of preserving plant genetic resources today [9].

Collections of ex-situ plant genetic resources are preserved as dry seeds at low temperatures. Storing collection samples of the national gene pool in medium- and long-term storage allows for a significant extension of seed viability, thereby enhancing the reliability of gene pool preservation and reducing the costs associated with sample regeneration [10, 11]. Work with the collection includes preserving, describing, and evaluating the collected materials and managing data and information related to plant genetic resources. In collection nurseries, researchers continuously study and reproduce crop collection samples by their phenological, morphological, and economically valuable traits, record the diseases, and identify species. Based on the annual study of collection samples, sources of plant breeding valuable traits are identified, and target trait collections are formed for their use in further breeding [12-15].

Apart from collecting plant genetic resources, their introduction is also a factor in increasing the sustainability of agriculture in the face of climate change. Within this framework, the urgent task is to create early-ripening forage varieties of southern drought-resistant plants and introduce them into Kazakhstan's biome.

Forage production is focused not only on the production of cheap high-protein and energy-saturated forage for highly productive animal husbandry but also on expanded reproduction and preservation of soil fertility using the environmental functions of forage grasses and their systemic formations (forage agrobiocenoses). Arid territories, including most of Kazakhstan, are entirely represented as forage lands, where agriculture is impossible without irrigation. Creating high-yield varieties of forage crops and introducing wild plants from local flora and little-known crops from other regions remain important reserves in forage production, allowing adaptation to new conditions in animal husbandry and crop production [16].

Forage production on arable land in field crop rotations, forage crop rotations, and irrigated cultural pastures is a promising way of cultivating stable crops. In Kazakhstan, field forage production is based on the cultivation and harvesting of forage made of perennial legumes (melilot, alfalfa, sainfoin, clover) and grasses (wheatgrass, cocksfoot, awnless brome, Kentucky bluegrass, ryegrass, common timothy, meadow fescue) and some annual species (Sudan grass, sunflower, forage millet, triticale, sweet sorghum, sorghum, soybeans, barley, peas, oats, forage root crops and melons/gourds).

Some of the crops are versatile. For example, the herbages of alfalfa, sainfoin, melilot, and intensive grasses can be used to produce green mass, harvest hay, and vitamin-rich grass meal, and create long-term cultivated pastures. This makes forage crops an important research object in the framework of the current climatic situation [17, 18].

Despite the abundance of wild forage legumes in Kazakhstan, their genetic potential remains underutilized in breeding and conservation efforts.

Thus, this study aimed to identify forage grasslands in Kazakhstan and evaluate their economically and agronomically valuable traits following seed collection and propagation.

2. METHODS

The study was conducted in 2021-2024 at the Kazakh Research Institute of Agriculture and Crop Production (KazNIIZiR).

The main objective was achieved by consistently completing three interrelated stages (Figure 1).

Stage 1 (2021-2023)	Collection of a population of wild legume ecotypes with a description of samples in natural habitats, soil conditions, and plant composition for their transfer to the gene storage and laboratory and field study
Stage 2 (2022-2024)	Introduction of wild legume species obtained as a result of the collection in the expedition
Stage 3 (2023-2024)	Identification of sources of heat resistance, drought resistance, and disease resistance and their involvement in hybridization with cultivated varieties by recurrent breeding

Figure 1. A general scheme for the implementation of research on the collection, study, and use of wild legume species

The first-stage tasks relate to collecting samples for further introduction in the framework of an organized expedition. The expedition routes were based on geobotanical scientific data on species distribution in Kazakhstan. The expedition targeted diverse ecological zones across the Almaty, Jetisu, Abai, and East Kazakhstan regions. They covered mountainous, foothills, and steppe zones in the Almaty, Jetisu, Abai, and East Kazakhstan regions (Figures 2-5).

During the collection, legumes were a priority. Sample collection was conducted mainly with seeds, and in some cases, when there were no ripe seeds, with live plants for further transplantation in introduction nurseries. At each site, plants were sampled based on ecological variation, with 10-15 plants sampled per site. Wild species of forage grasses from the legume family were collected at the population level (ecotypes) and, in some cases, as individual plants (Figure 6).

Figure 2. Fragments of the expedition route through the East Kazakhstan region, from the border of the PRC (Druzhba), the Dzungarian Gate to the city of Ayaguz

Figure 3. Fragments of the expedition route in the Ayaguz district of the Abai region

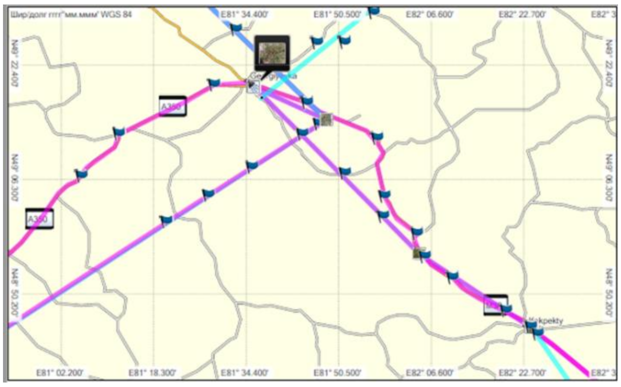


Figure 4. Fragments of the expedition route through the Kokpekti and Zharma districts of the Abai region

Figure 5. Fragments of the expedition route through the Kokpekti district to Lake Zaisan in the East Kazakhstan region

Figure 6. Collection of samples in the form of seeds and root-digging

It is worth noting that environmental conditions at collection sites, such as soil pH, temperature, and moisture levels, significantly influence the traits of the collected forage legume samples. For instance, soil pH affects nutrient availability, shaping traits like growth and resilience, as seen in the salt-tolerant *M. coerulea* found in alkaline soils. Samples collected from arid steppe zones, such as *M. falcata*, displayed strong drought resistance due to adaptation to low soil moisture. It was also found that populations from mountainous regions exhibited greater cold tolerance compared to those from lowland areas. Such environmental factors could greatly contribute to the genetic and phenotypic diversity of the samples, shaping traits like plant height, foliage density, and flowering time. Understanding these influences provides valuable insights for breeding programs, enabling the development of resilient crops tailored to specific climatic and ecological conditions.

To prepare the collected material for the second stage, laboratory analysis was conducted. It involved the description of fruit elements and seeds by morphological characteristics, which is important to clarify the identification of the species. The following morphological features were described: the color and shape of the bean, the size of the seeds, the shape of the leaf, etc.

After laboratory analysis and sample preparation procedures, the seeds were divided into three groups: one part was transferred to gene storage, the second part was used for cytological analysis, and the third part was used for sowing in a greenhouse during seedling preparation or for direct sowing in a field (nursery) following the methodological guidelines of the All-Russian Research Institute for Plant Breeding (VIR) [19] and the Williams Fodder Research Institute (VNIIK) [20] for the study of the world collection of forage crops. Seedlings were propagated in greenhouse conditions to overcome the hardness of the seeds and ensure controlled germination. Once established, the seedlings were transplanted into field nurseries with a planting density of 10 plants per row, spaced 10 cm apart, and a row spacing of 30 cm.

The second group of seeds underwent karyotyping, or the study of mitotic chromosomes. Samples of wild legumes collected by the expedition in Almaty and East Kazakhstan regions were taken for karyological studies.

Further cytological studies were conducted on the expedition samples according to the VIR method developed by N.I. Vavilov used squashed preparations. For its implementation, we harvested young growing roots that give the best results during studies.

Preparation for cytological studies included the following stages:

1) Germination. The seeds were germinated in Petri dishes on wet, filtered paper. The Petri dishes with seeds were placed in a thermostat with an air temperature of 23-25°C optimal for active mitosis, after which roots appeared in 4-5 days. They were then extracted with tweezers and placed on a slide.

2) Fixation of the material. Before fixation, the roots were treated with special reagents (8-oxynoline, chloral hydrate, and paradichlorobenzene) and cold temperatures.

Fixation of legume materials was conducted on explants 2-3 mm in size with a colchicine concentration of 0.01-0.03%. A solution of paradichlorobenzene was used on some of the samples. To prepare it, 5-10 g of crystalline paradichlorobenzene was dissolved in 500 ml of distilled water and left in a closed container in a thermostat at a temperature of 60°C for 10-12 hours. To avoid it becoming unusable, the resulting fixative was prepared immediately before use. Small tools (scissors, tweezers, scalpel) were prepared in advance for fixation. With their help, the material was treated with the solution for 2-3 hours at a temperature of 12-16°C. After using the fixator, the material was washed in water to completely remove its residues, and then partial dehydration with ethyl alcohol (75% and 96%) was conducted. The fixed roots were crushed into small pieces.

3) Staining. The sections were stained with acetocarmine, used in the production of temporary preparations, and heated on an alcohol lamp.

To prepare acetocarmine, 1-2 g of carmine was dissolved in 45 ml of glacial acetic acid and 55 ml of distilled water. The dissolution was conducted in a flask with a reverse refrigerator and then in a heated water bath for 30-60 minutes. After cooling, the dark red carmine solution was filtered and placed in a dish with the lid lifted. The staining itself was performed after the stainer was poured into a drip.

The root contents were viewed on a slide with a drop of 45% acetic acid.

To count the chromosomes, the metaphase of the first division of mitosis is used when the chromosomes are clearly visible. The acid was pulled off with filtered paper, after which the roots were placed in a new drop of acid, covered with a covered glass, and squashed with light pressure with a bent needle.

As part of the second stage, the sowing of the collected wild legume species was conducted on December 22-25 after the preliminary preparation of the seed material.

Due to the hardness of the collected seeds and the limitation in their quantity, the seedling method was chosen for sowing. Seedlings previously prepared in greenhouse conditions were used for planting in the nursery, which allowed the formation of a full-fledged introduction nursery for primary study and further seed collection.

During cultivation, the phases of seeding emergence, the first, second, and third true leaves were noted. In the phase of the third true leaf, the plants were transferred to field conditions to study the population of wild legume species in cultivation conditions with an assessment of selectively valuable traits, such as drought resistance, disease resistance, forage yield, seed productivity, and cold tolerance and properties and to obtain seed material.

Drought resistance was assessed by observing plant performance in arid conditions, while disease resistance was evaluated through field observations for susceptibility to common legume diseases. Forage yield was determined by measuring plant height, foliage density on a 3-point scale, and green mass productivity per plant. Seed productivity was assessed by counting seeds per plant and recording their weight, and cold tolerance was inferred from the survival and growth of plants in low-temperature nursery environments.

For each sample, 10 plants were planted in single-row plots 10 cm apart, with a row spacing of 30 cm. After sowing, phenological observations continued with recording the budding phase, the beginning of flowering and mass flowering, and the maturation of beans and seeds. During the flowering period, the height of the plant was measured, foliage and green mass were recorded, and seeds were collected according to the VNIIK method [20]. The foliage of plants was evaluated according to a 3-point system: 1: slight; 2: good; and 3: excellent.

3. RESULTS AND DISCUSSION

3.1 Sample collection and phenological observations

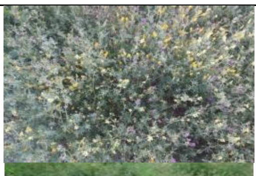
Based on the results, 177 samples of ecotypes and species of forage grasses were collected, including 144 samples of alfalfa, 9 of sainfoin, and 24 of melilot.

Alfalfa (*Medicago falcago* (Reichb.) Grosch.). The most valuable alfalfa species in terms of food belong to the perennial subgenus Falcago, which includes 21 species [21].

The flora of Kazakhstan is rich in wild alfalfa species with two levels of ploidy: tetraploids (2n = 32) and diploids (2n = 16), which are the progenitors of cultivated species and the genetic center of origin of alfalfa in the Central Asian center and the center of species such as *M. varia*. Mart. and *M. falcata*. The diploid species *M. coerulea* Less. is predominantly found in West Kazakhstan, and in the foothills of southern Kazakhstan, *M. difalcata* Sinsk. and *M. trautvetteri* Sumn are found. Of the tetraploid species, *M. varia*. Mart. and *M. falcata* L. are found in North Kazakhstan and *M. tianschanica* Vass. is found in the south. The natural tetraploid species *M. varia* Mart. is a product of hybridization between *M. sativa* and *M. falcata* and is found everywhere.

These wild species are characterized by increased drought resistance, but *M. falcata* stands out most favorably among them as the most common wild species found in the form of both drought-resistant and mesophilic ecotypes. In the open steppe, *M. difalcata* is characterized by the greatest drought resistance. *M. coerulea* is the most salt-tolerant species. Along with the traits of drought resistance and salt tolerance, wild species are noticeably distinguished by their resistance to major diseases and longevity. The characteristics of alfalfa species found in Kazakhstan are given in Table 1.

Table 1. Characteristics of alfalfa species found in Kazakhstan

Picture	Species	Characteristics
	*M. varia. Mart.*	Variegated alfalfa. It is characterized by intermediate traits between blue and yellow alfalfa in the color of the corolla, the bean shape, leaves, root system, and stability.
	*M. falcata L.*	Yellow alfalfa. Crescent-shaped, characterized by bright yellow and light-yellow corolla color; the beans are straight or crescent-shaped on straight stems, the leaves are small, narrow, linear, or wide and lanceolate, pubescent on the underside. The root system is powerful, branched with a weakly expressed main root. The tillering zone is immersed in the soil by 3-8 cm. Soboliferous forms are found. Many ecotypes are characterized by high winter hardiness and drought resistance. The growth is slow, one cutting is formed.

	*M. tianschanica Vass.*	Tian-Shan alfalfa. Not demanding to soils and powerfully developed. It is promising for breeding work in many areas. It is widespread in the mountains of the Western Tian Shan, especially in the Kyrgyz and Talas Alatau and Syrdarya Karatau. Vertically, its distribution reaches 2,500 m above sea level in the subalpine belt.
	*M. sativa subsp. Transaxona*	It grows wild in the southern part of the Tian Shan foothills. It goes far in the most fertile, well-moistened lands, on the borders of crop rotation areas. This species is the ancestor of the cultural species *M. sativa L.* with numerous varieties.
	*M. coerulea Less.*	Blue alfalfa. It is widespread in West Kazakhstan. It is a very salt-tolerant species. Its main range is in the Caspian lowland to the Mugodjar Mountains. The populations of blue alfalfa growing in West Kazakhstan belong to two types: floodplain and sandy. The first type is characterized by greater height (from 120-150 to 200 cm), a semi-sprawling bush shape, salt tolerance, high tilling capacity, medium foliage, slight hairiness, disease resistance, high seed productivity, small but well-twisted (3-5 coils) beans. The second type is more xeromorphic, with small recumbent bushes, weak foliage, and narrow lanceolate or linear pubescent leaves. The root system is powerful, going deep into the ground. The stems are thin, coarse, slightly leafy, glabrous, or slightly pubescent, 50-100 cm high. The leaves are small, almost linear, with few teeth in the upper part, almost glabrous from above, with pressed hairs from below. The truss is quite thick, with many flowers. The corolla is blue or bluish-violet. The beans are small, spirally twisted into two or three closely joined coils, with prominent reticulated veins.
	*M. trautvetteri Sumn.*	It is localized in the Mugodzhary mountains, on the Ustyurt plateau, in the southern part of the foothills of the Zhongar Alatau, Western Tian Shan in the floodplain of the Ili River. It is characterized by high salt resistance, drought resistance, winter hardiness, and disease resistance. In habitats with good water supply, wild populations have an erect bush shape, less often a sprawling one. The foliage is slight, the leaves are small, elongated, and pubescent. The most productive populations of this species grow in the Aktobe region, in the Mugodzhary Mountains, on the Ustyurt plateau, in the basin of the Bolshaya Chobda River, and in the south of Kazakhstan in the floodplain of the Ili River.
	*M. difalcata L.*	It occupies extensive dry-steppe, semi-desert, and desert zones. A variety of ecotypes are concentrated north of Lake Balkhash. It is the most drought-resistant species of the subgenus *Falcago* among others. Large foci of the cenosis areal of this type of alfalfa are located in the Jezkazgan, Karaganda, south of Kostanay, Abai, Pavlodar, East Kazakhstan, east of Akmola, and north of Jambyl regions.

All alfalfa species are found in the natural flora with localization of genetic diversity in certain ecological growing conditions. Sometimes, alfalfa species occupy large distribution centers, where it is even possible to use them for harvesting dry forage.

As a result of the expedition, 144 samples of alfalfa were collected. The characteristics of some of the collected ecotypes of wild species are presented below.

Expedition number 02: collected in the Almaty region, Kerbulak district, near the village of Sarybastau, coordinates N=45039I663, E=080023I416. The altitude was 1,184 m. The terrain is a roadside lowland, the soil is gray with average moisture content, and the plant grows in a plant community consisting of yellow melilot, Aristida, and wheatgrass. The height of the plants is 70 cm, the tilling capacity is slight, the foliage is good, the flower color is yellow, the bush shape is prostrate, and the bean shape is straight and slightly curved.

Expedition number 38: collected in the East Kazakhstan region, Bogas bekety, on the border of Aksuat-Zaisan at the Tarbagatai crossing point, coordinates N=480081012, E=0820551959, altitude: 418 m. The plant community contains grasses, prostrate summer cypress, etc. The terrain is flat, the soil is moist, and it is next to the artificial planting of poplars.

Expedition number 43: collected at the turn to Aulie Yrgyzbai kesheni, in the East Kazakhstan region, coordinates N=470571805, E=0820181382, altitude: 650 m. The plant community contains grasses, needlegrass, chamomile, licorice, etc. The soil is gravelly and sandy. The tilling capacity is strong, and the foliage is good. The leaf shape is oval and large (medium), the flower color is blue hybrid, the stems are thick and creeping, the bush is 4-5 years old, and the roots are powerful with strongly developed lateral roots. The bean shape is crescent-shaped with 1 coil.

Expedition number 48: collected on the border of the Tarbagatai and Ayaguz districts of East Kazakhstan region, N=470481676, E=0810261488, altitude: 990 m. The plant community is a sheep fescue and needlegrass steppe, the terrain is a roadside depression, the height of the plants is 30 cm, the tilling capacity is slight, the leaf shape is small and narrow, and the flower color is yellow.

Expedition number 52: collected on the coast of Zhalanashkol island on the border of the Alakul district with China (the Druzhba station), N=450351122, E=0820071119, altitude: 381 m. The plant community consists of bulrush, wormwood, and licorice, the terrain is represented by dam slopes, and the soil is rocky, with average moisture content. The tilling capacity is very good, the stems are sprawling and erect, the foliage is good, the leaves are small, and the flower color is blue and purple, growing in small trusses. The bean shape is closer to 1 coil, and the bean formation is abundant.

Sainfoin (Onobiychis L.). The global flora includes 164 species of the genus Onobiychis Mill. The flora of the Commonwealth of Independent States (CIS) countries includes 62 species belonging to the Leguminosae family (legumes), genus Onobrychis Mill., 2 subgenera, 6 sections, and 10 subsections. Taxonomic analysis based on literary material and our herbarium collections in various regions of Kazakhstan allowed us to identify 10 species of the sainfoin genus, which belong to 2 subgenera, 5 sections, and 4 subsections, with one of the species, Onobrychis megaloptera Kovalevsk, identified as endemic. This species occurs within Kazakhstan in the form of isolated populations (Chatkal and Ferghana ranges in the basin of the Yassy River).

3 of the 10 species belong to the subgenus Euonobrychis DC., of which 2 species belong to the section Dendrobrychis DC., 2 species belong to the section Lophobrychis Hand.–Mazz., subsection Orientales Sirj., and 1 species belongs to the section Eubrychis DC., subsection Vulgatae Eland.-Mazz. There are 5 species in the subgenus Sisyrosema Bunge, of which 1 species belongs to the section Anthyllium Nabelek, subsection Lipskyanae Sirj., and 4 species belong to the section Hymenobrychis DC., subsection Pulcherrimae Sirj.

The expedition collected samples in the following places: 64(12) in the Sarkand district, Almaly village, 66(26) in the Sarkand district, Abai village, 67(43) in the Sarkand district, Karaboget village, 69(65) in the Alakol district, Karabulak village, 70(86) in the Zhambyl district, Koktobe village, 71(88) in the Eskeldi district, 72(55) in the Kerbulak district, Altyn Emel pass, 51(6) in the Sarkand district, Cherkassk village, 52(7) in the Alakol district, Usharal village, and 53(32) in on the border of the Ayaguz and Tarbagatai districts.

Melilot (Melilotus Desr.). The genus consists of 16 species. The most famous cultivated species are white and yellow melilot. They grow in the north in areas with severe winters, and in the south in arid steppe zones. The following melilot species are of great practical value: Russian melilot, Melilotus dentatus, Polish melilot, Melilotus tauricus, and Melilotus suaveolens. However, these species have not yet become widespread in culture.

Samples with the following origin were collected for the white melilot:

Expedition number 48(11): collected in the Aktogay village of the Jetisu region, N=46035I391, E=080035I462.

Expedition number 49(39): collected in the Balapanov settlement of the Jetisu region, N=45056I247, E=080037I027. Altitude: 584 m.

24 samples were collected for yellow melilot, some of them are described below.

Expedition number 01: collected in the Jetisu region, Kerbulak district, near the village of Arkharly, coordinates N=44016I820, E=077048I565. Altitude: 1184 m. The terrain is plain, with virgin land on the edge of arable land, the soil is gray, with average moisture content, the plant grows in a plant community consisting of individual plants like yellow alfalfa, Aristida, wheatgrass, and field weeds. The height of the plants is 80-90 cm, with a slight tilling capacity, slight foliage, and yellow flowers.

Expedition number 18: collected in the village of Aktogay, along the Almaty – Ust-Kamenogorsk highway, coordinates N=460351391, E=0800351462, roadside lowland. The plant community consists of bayal, wormwood, small groups of couch grass, etc. The terrain is a lowland where meltwater accumulates, the soil is gray clay, the height of the plants is 70 cm, the tilling capacity is average, the foliage is slight, the leaf shape is elliptical, and the flowers are white.

Expedition number 23: collected in the East Kazakhstan region, at the turn to Taskesken opposite the cell tower, N=470141420, E=0800411245, altitude: 657 m. The plant community is a heavy sod of grass herbage, the soil is meadow, with sufficient moisture. The height of the plants is 50 cm, the tilling capacity is average, the foliage is good, and the leaf shape is small and lanceolate.

3.2 Karyological analysis

Cytological control and further study of karyotype stability for inclusion in the breeding process are important in breeding perennial forms of wild alfalfa. However, there is no literature information on the methodological foundations of the study of mitosis, especially the process of division in maternal roots. Thus, it is important to conduct genetic and cytological studies to create new alfalfa varieties with a balanced number of chromosomes (between wild and cultivated ones).

By the ploidy classification, wild perennial alfalfa species are divided into three groups: diploid (2n=16), tetraploid (2n-32), and hexaploid (2n=48). They differ in ploidy, flower color, size, bean shape, leaves, bush shape, autumn regrowth rosette, productivity, etc.

In most studied wild alfalfa samples, cytological data on the number of chromosomes in somatic cells correspond to the species. Wild tetraploid alfalfa species showed the greatest stability in the number of chromosomes. Therefore, they can be directly included in hybridization, that is, inbreeding to enhance the adaptive properties of cultivated varieties.

We present the results of the sample 02 study. The stage of prophase and metaphase in the primary roots of sample 02 of yellow alfalfa proceeded very slowly. Thus, the counting of chromosomes lasted 12-15 days. Cytological analysis showed that the number of chromosomes in the metaphases of mitosis was genetically constant, and the somatic set of this number was 32 chromosomes (Figure 7).

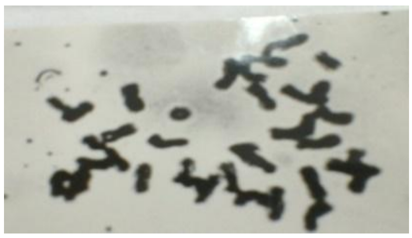
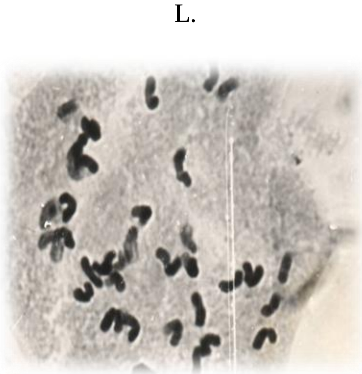
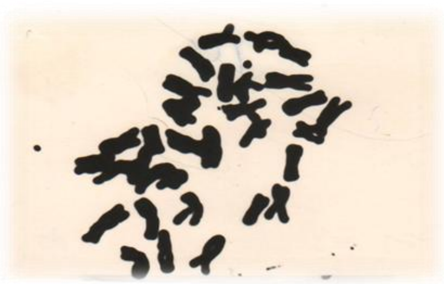


Figure 7. Metaphase plates in a wild specimen 02, *M. falcate* L.



a) No. 05



b) No. 07

Figure 8. Metaphase plates in wild alfalfa specimens *M. falcate* L.

In samples 05 and 07, the cytological splitting had a wider spectrum. The number of chromosomes here is 30 and 32. However, among them there are often cells with the number of chromosomes equaling 32, approaching the cultivated form (Figure 8).

Cytological studies of samples from the group of tetraploid species *M. sativa* (blue alfalfa) also showed the predominance of normal somatic cells with the number of chromosomes 2n-32. Moreover, many of these cells occur with almost the same frequency, except for individual cells. It is interesting to note that 30 or 31 chromosome cells had not been found in these samples. Various rearrangements of the chromosomal apparatus (translocations, divisions, inversions, or non-squashed chromosome phenomena) were not observed. Thus, it was possible to determine the specific number of chromosomes in the squash preparations.

In a wild sample of tetraploid forms 49, the set of chromosomes in somatic cells varies insignificantly. There are mainly cells with 31 and 32 chromosomes, and there are more 32-chromosome cells than other preparations (Figure 9).

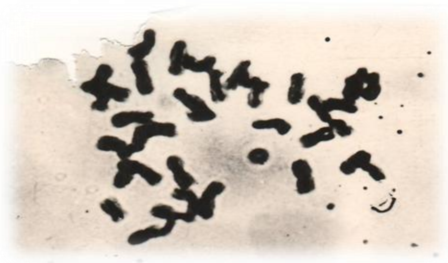


Figure 9. Metaphase plates in a wild alfalfa specimen 49, *M. sativa L.*

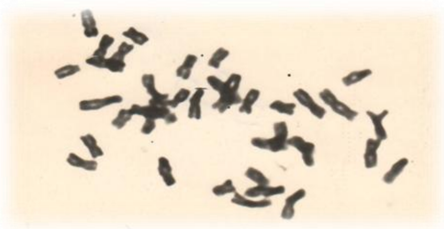


Figure 10. Metaphase plates in wild alfalfa specimens 52, *M. sativa L.*

Sample 52 has slight variations in the number of chromosomes from 31 to 33, but normal metaphase plates with 32 chromosomes are more common (Figure 10).

After the laboratory analysis and sample preparation procedures, 117 seed samples were transferred to the gene storage department of the field crops gene pool for medium-term storage, which was documented in a report.

Introduction of wild legumes: Based on the remaining samples, an introduction nursery was established in 2022, consisting of 67 alfalfa samples, including 13 samples planted over an area of 5-10 m^2. The remaining samples were individually planted in an area of 70×20 cm, with each sample containing 15 to 23 plants.

After the samples were planted using the seedling method, the emergence of cotyledon leaves in alfalfa was prolonged, lasting from December 29 to February 29. The emergence of the first simple leaf in alfalfa occurred between January 5 and March 11. The appearance of the third true leaf was recorded from February 1 to March 27 (Figure 11).

Figure 11. General view of seedlings of wild legumes in a greenhouse

a) sainfoin

b) alfalfa

c) melilot

Figure 12. General view of the plants transplanted into the KazNIIZiR field

An analysis of the passage of the initial phases, starting from the appearance of an embryonic cotyledon leaf to three true leaves, showed the lengthiness of development between alfalfa samples that equaled 55 days. The variation among the samples ranged from 23 to 38 days. All alfalfa samples were grown in a greenhouse, except for sample 15(47), which was collected in the village of Koktobe, Jambyl District, Almaty Region.

Alfalfa, sainfoin, and melilot exhibit notable differences in their phenological development due to both environmental and genetic factors. Alfalfa showed a longer germination period, particularly in greenhouse conditions, with variations across ecotypes reflecting differences in seed hardness and adaptability to moisture. Sainfoin demonstrated quicker flowering but limited seed production, possibly due to its lower adaptability to the nursery's environmental conditions. Melilot showed robust growth in terms of plant height and green mass productivity but delayed flowering or absence of it, which may be linked to its genetic predisposition to longer photoperiods.

This study involved the reproduction of a population of wild legumes under controlled conditions, along with the assessment of breeding-valuable traits and the selection of genetic sources. After being transferred from greenhouse conditions to a field nursery, 44 out of 45 transplanted alfalfa samples, 6 out of 8 melilot samples, and 4 out of 8 sainfoin samples successfully took root (Figure 12).

The phase of plant development after the plants' transplantation was long. In each sample, flowering was delayed due to insufficient pollination and limited bean formation. Ultimately, a limited number of seeds were collected from samples 10(45), 16(21), 20(59), 21(61), 23(23), 25(87), 27(90), 41(54), 42(68), 44(15), 45(51), 46(35), 47(37), and 48(95).

The melilot samples accumulated a harvest of green mass and did not have flowering phases. In the sainfoin sample 68(46), the flowering phase lasted from June 12 to June 22, and in the alfalfa sample 10(45), flowering occurred from June 20 to July 7, and seeds were collected from all samples in the range of 8.6-26.8 g.

The dates of the phenological phases of the expedition samples collected in 2022-2023 are shown in Table 2.

Table 2. Phenological observations of transplanted expedition collection samples (planting and data for 2021)

Sample Expedition Number	Budding	Flowering		Seed Maturation
		Beginning	Mass	
Yellow-flowered alfalfa samples				
1(1)	01.06	11.06	20.06	
2(2)	01.06	11.06	21.06	
3(3)	03.06	14.06	25.06	
4(4)	02.06	13.06	23.06	
5(11)	01.06	11.06	20.06	
6(19)	30.05	12.06	22.06	
7(22)	29.05	10.06	21.06	
8(27)	03.06	13.06	26.06	
9(28)	03.06	14.06	27.06	
10(45)	02.06	11.06	22.06	26.09 (10.10)
11(38)	01.06	11.06	20.06	
12(39)	04.06	15.06	30.06	
13(40)	01.06	12.06	20.06	
14(44)	02.06	13.06	01.07	
16(21)	28.05	10.06	24.06	26.09
17(17)	28.05	09.06	22.06	
18 (53)	01.06	13.06	02.07	
19(58)	01.06	11.06	04.07	
20(59)	01.06	10.06	05.07	26.09
21(61)	29.05	11.06	07.07	26.09
22(63)	29.05	12.06	04.07	
23(23)	01.06	12.06	05.07	26.09
24(66)	30.05	13.06	05.07	
25(87)	01.06	10.06	06.07	26.09
26(89)	02.06	10.06	04.07	
27(90)	02.06	11.06	01.07	26.09
Blue-flowered alfalfa samples				
28(6)	28.05	11.06	03.07	
29(13)	30.05	12.06	28.06	
30(14)	01.06	12.06	27.06	
31(15)	01.06	13.06	30.06	
32(32)	01.06	10.06	02.07	
33(49)	02.06	14.06	02.07	
34(48)	02.06	15.06	05.07	
38(64)	30.05	15.06	04.07	
39(50)	30.05	14.06	30.06	
40(52)	01.06	12.06	29.06	
41(54)	01.06	12.06	20.06	26.09
42(68)	30.05	11.06	19.06	26.09
43(5)	30.05	10.06	21.06	

44(25)	30.05	12.06	23.06	26.09
45(51)	01.06	11.06	22.06	26.09
46(35)	30.05	12.06	22.06	26.09
47(37)	29.05	10.06	20.06	26.09
48(95)	02.06	10.06	21.06	26.09
49(57)	27.05	11.06	20.06	
Melilot samples				
50(8)	30.05	12.06	23.06	
51(16)	01.06	11.06	25.06	
52(18)	29.05	08.06	22.06	
50(8)	30.05	12.06	23.06	
51(16)	01.06	11.06	25.06	
52(18)	29.05	08.06	22.06	
53(20)	01.06	10.06	20.06	
54(24)	02.06	10.06	21.06	
55(42)	03.06	12.06	21.06	
56(56)	02.06	11.06	18.06	
57(31)	02.06	09.06	24.06	
Sainfoin samples				
64(12)	26.05	05.06	14.06	
65(30)	27.05	02.06	12.06	26.09
66(25)	29.05	09.06	18.06	
67(43)	30.05	10.06	22.06	26.09
68(46)	30.05	07.06	16.06	26.09
69(65)	01.06	11.06	23.06	
70(86)	01.06	10.06	21.06	
71(88)	29.05	09.06	21.06	
72(55)	29.05	08.06	19.06	26.09

3.3 Economic traits assessment

Figures 13-16 show the results of the assessment of grown samples in terms of height, plant foliage, green mass productivity, and seed material collection.

The height of plants in yellow-flowered alfalfa samples varied from 61 to 125 cm and in blue-flowered ones from 60 to 160 cm. In this indicator, one can distinguish the following yellow alfalfa expedition samples: 1(1), 10(45), 9(28), 16(21), 17(17), 20(59), 22(63), 25(87), 27(90) and the following blue alfalfa samples: 30(14), 32(32), 33(49), 38(64), 39(50), 41(54), 42(68), 43(5), 46(35), 47(37). Some of them (22(63), 38(64), 33(49), 22(63)) were unusually tall, with a height of more than 100 cm. In the melilot samples, the height of the plants varied from 97 to 145 cm. All melilot samples, except 53(20), reached a height of more than 100 cm. In the sainfoin samples, the height of the plants varied from 90 to 130 cm.

The foliage level is an important integrated indicator that determines the forage quality since the leaf mass contains 1.5-2.0 times more protein than other parts. The following samples received the maximum score on the assessment scale for foliage from the yellow-flowered samples: 2(2), 5(11), 11(38), 12(39), 17(17), 20(59), 22(63), 23(23), 24(66), 26(89); from the blue-flowered samples: 29(13), 31(15), 33(49), 39(50), 44(25), 45(51), 48(95), 49(57); from the melilot samples: 51(16), 56(56), 57(31); and from the sainfoin samples: 65(30), 72(55).

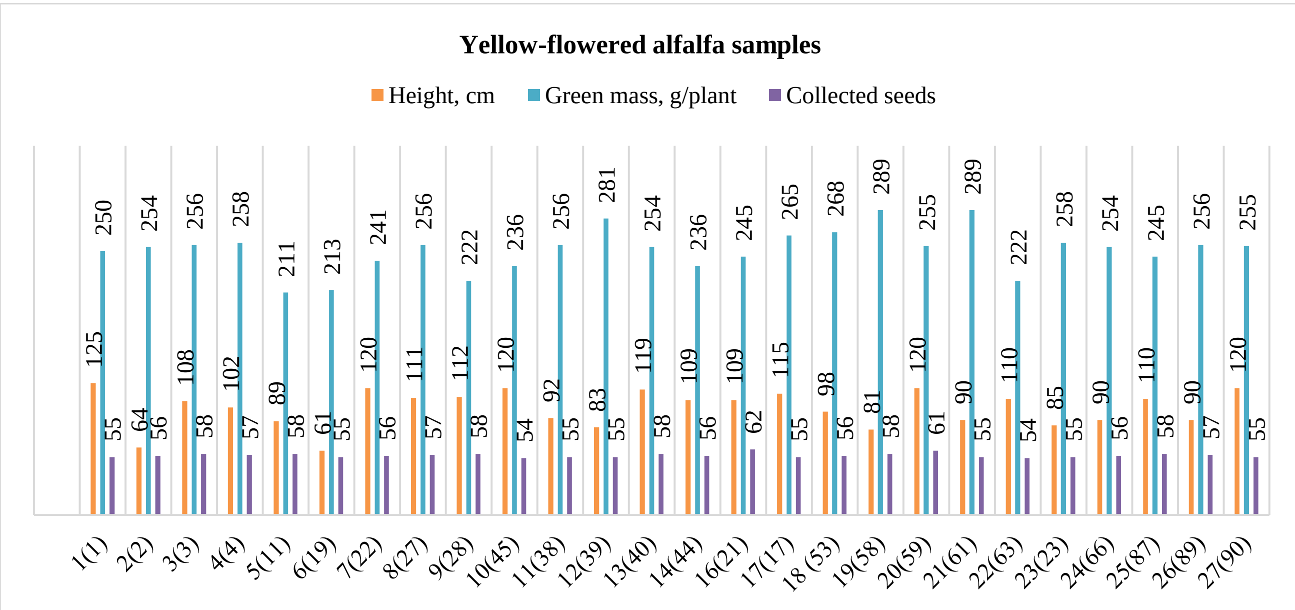


Figure 13. Indicators of economically valuable traits in yellow-flowered alfalfa

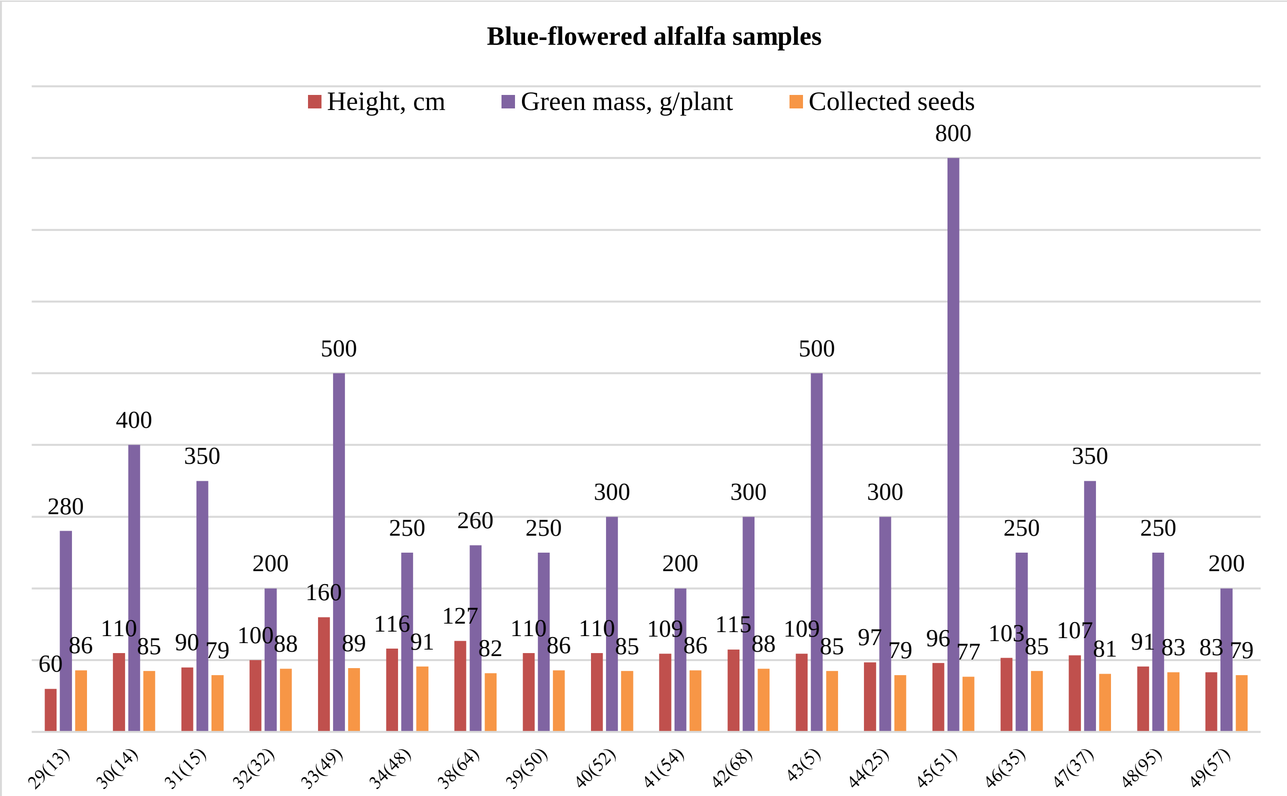


Figure 14. Indicators of economically valuable traits in blue-flowered alfalfa

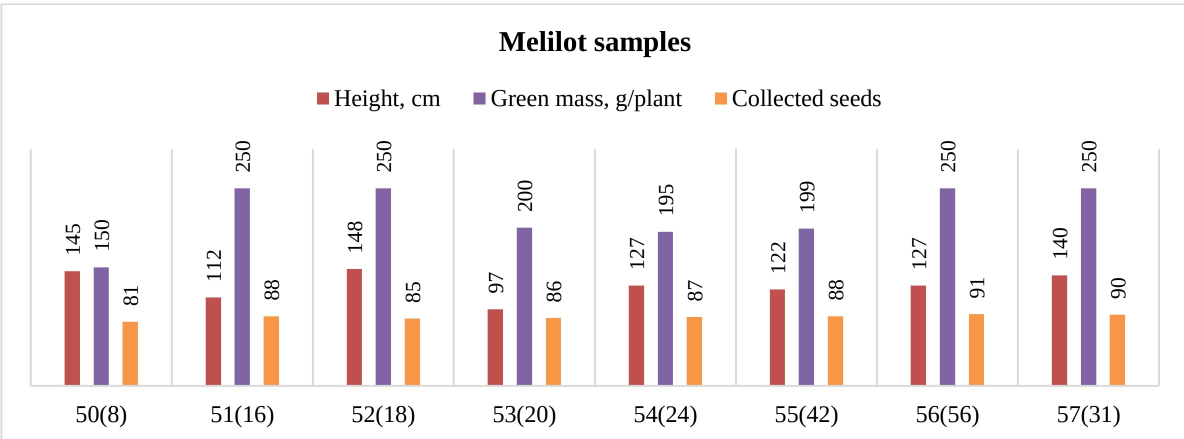


Figure 15. Indicators of economically valuable traits in melilot

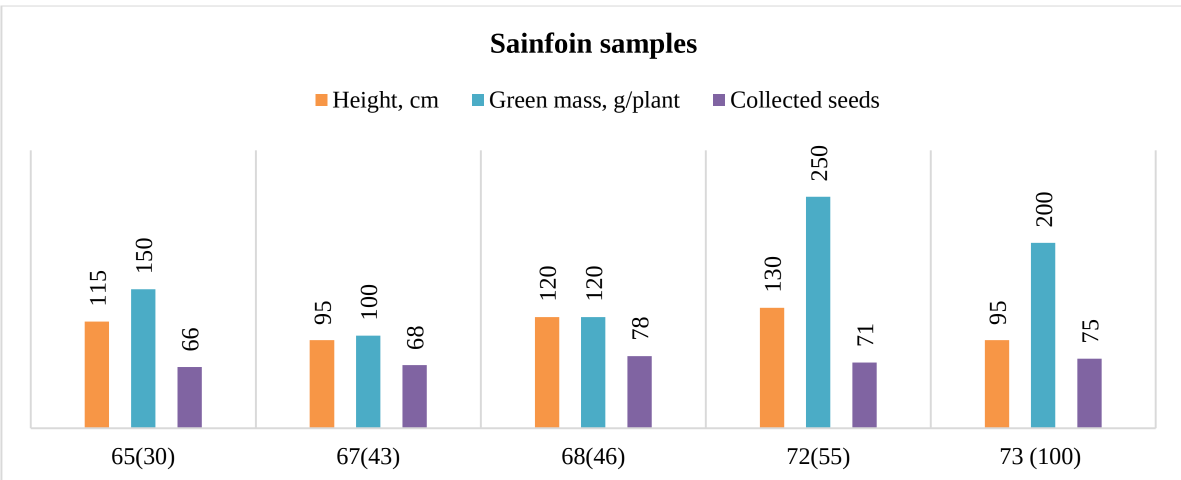


Figure 16. Indicators of economically valuable traits in sainfoin

The productivity of the green mass yield for each plant in the composition of the samples reached a high value of 500 g per plant in the yellow-flowered alfalfa samples 10(45), 13(40), 14(44), 18(53), 25(89), 27(90); and in the blue-flowered alfalfa samples 33(49), 40(52), 43(5), 45(51). The blue-flowered alfalfa sample 45(51) had the highest productivity, equaling 800 g of green mass per plant.

The melilot samples gave a green mass yield of up to 250 g per plant, except for 50(8), and the sainfoin samples were the least productive. Individual accounting per plant allows the selection of highly productive elite genotypes in the future. The population composition of the samples is very diverse, which affects the inclusion of low-yielding genotypes with a green mass of less than 100 g per plant in the samples.

Seed collection for 2023 was also conducted for each plant. The number of seeds in the yellow-flowered alfalfa sample 20(59) was 4.0 g, in 16(21) 0.4 g, in 23(23) 0.2 g, in 25 (87) 0.1 g, in 10(45) 54 pcs., in 21 (61) 65 pcs., and in 27(90) 15 pcs. In blue-flowered alfalfa samples, the indicators were as follows: in 41(54) 2.0 g, in 44(25) 0.1 g, in 45(51) 0.7 g, in 47(37) 0.1 g, in 48(95) 0.2 g, in 42(68) 160 pcs., and in 46(35) 35 pcs. The sainfoin samples had the following indicators: 65(30): 26.8 g, 67(43): 12.5 g, 68(46): 15.6 g, 72(55): 8.6 g, and 73(100): 0.3 g.

Except for some samples (6 samples), seeds were collected from each plant within the range of 0.5-15.0 g in the year of planting (September 29). In total, seeds from 1,359 plants were collected in 61 samples.

In the second year of life (planted in 2022), by the combination of economically valuable traits and properties, 6 alfalfa samples were identified as a source of valuable qualities from breeding: numbers 20(59), 16(21), 23(23), 25(87), 10(45), 21 (61). In the first year of life (planted in 2023), 9 samples were selected: 58(46), 59(47), 67(55), 72(60), 73(61), 87(75), 90(75), 69(57), 74(62). For the final assessment of the sample value, the tests in the 1st and 2nd year of life are not enough, and research will continue on all samples of the introduction nursery.

The melilot and sainfoin plants developed up to the flowering phase. By a visual assessment, the melilot samples 50(42) 45(12), and 44(11) and sainfoin sample 53(32) were considered valuable.

The results of this study highlight the significant genetic potential of wild forage legumes in Kazakhstan, providing a critical resource for enhancing drought and disease resistance in cultivated varieties. It underscores the relationship between Kazakhstan's diverse environments and the genetic diversity of legumes. It follows current research trends that study adapted traits of leguminous forage crops. It agrees with Humphries et al. [21], who investigated developed adaptive mechanisms of alfalfa for surviving dry soil in low rainfall environments, high temperatures, and short growing seasons with winter freezing, such as broad and well-branched root systems or lower stomatal conductance. It also aligns with Calleja-Satrustegui et al. [22], who investigated drought resilience in wild Medicago species in arid and semi-arid regions. Li et al. [23] and Zhang et al. [24] highlighted the superior salt tolerance and drought resistance of *M. falcata*, which aligns with the results obtained in this study.

The cytological stability observed in tetraploid species such as M. varia and M. sativa aligns with the findings of Innes et al. [25], who highlighted the effect of tetraploidy or ploidy size on vigor, productivity, and potential adaptation to climate change. The karyological analyses present Kazakhstan's wild legumes as prime candidates for hybridization with cultivated varieties to enhance resilience traits. The study's novelty rests on the identification of region-specific traits, such as the high drought tolerance of M. difalcata in arid steppes and the salt tolerance of *M. coerulea* in alkaline soils, providing guidelines for targeted breeding programs and valuable information for optimizing forage production in arid environments, aligning with global efforts to ensure food security under changing climatic conditions. Future research should focus on the molecular characterization of the collected samples to identify specific genes associated with drought and disease resistance, especially those with region-specific traits [26]. Long-term studies on the performance of selected ecotypes under various climatic conditions would further support the use of resistant varieties in breeding programs.

4. CONCLUSIONS

As a result of an expedition to East Kazakhstan and Abai regions, 177 wild forage grass ecotypes were collected, including 144 alfalfa samples, 9 sainfoin samples, and 24 melilot samples. An ecological assessment of their growth conditions was conducted for all species, considering factors such as habitat, surrounding vegetation, soil composition, and terrain.

The samples underwent laboratory analysis and cytological analysis of mitotic chromosomes. Cytological analysis showed that the number of chromosomes in the metaphases of mitosis was genetically constant, and the somatic set of most numbers included 32 chromosomes. In collection nurseries, the study and reproduction of 172 legume collection samples continue based on phenological, morphological, and economically valuable traits, with attention to disease resistance and the identification of specific characteristics.

Future research should focus on the molecular characterization of the collected samples to identify specific genes associated with drought and disease resistance. Therefore, a part of the gathered collection was deposited in the KazNIIZiR gene pool for modern centralized storage. This provides comprehensive information on the legumes of Kazakhstan and supports research programs focused on crop production development based on this data. The identified alfalfa samples with stable tetraploid chromosome counts and high green mass productivity should be prioritized for hybridization with cultivated varieties. The emphasis on preserving crop sustainability enabled Kazakhstan to deposit 522 forage grass samples in the global seed repository for the first time, with support from Crop Trust in the Svalbard Global Seed Vault (SGSV). The project guarantees Kazakhstan's rights to germplasm in the global gene bank system in the framework of current international law.

This study helps enhance the resilience of the agricultural sector to climate and environmental changes. Further research is recommended in the established nursery, along with the expansion of the geographical and biological scope for studying legumes in Kazakhstan.

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19676041 "Conservation of genetic resources of forage plants based on expedition collections from the wild flora of Kazakhstan").

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