Genetic Diversity of Perennial Wild Species of Alfalfa Subgenus Falcago (Reichb) Grossh. in Kazakhstan and Their Involvement in the Breeding

Bauyrzhan Baktyzhanovich Kalibayev¹, Galiolla Tulendinovich Meiirman², Sakysh Tanyrbergenovna Yerzhanova², Serik Sarybaevich Aбаev² and Amankeldi Turgambekovich Kenebaev²

¹) Kazakh National Agrarian University, Abai Avenue, 8, Almaty, Republic of Kazakhstan
²) Kazakh Scientific Research Institute of Agriculture and Plant Growing, Erlepesov Street, 1, Almalybak, Karasai district, Almaty region, Republic of Kazakhstan

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* Corresponding author:
E-mail: bauyrzhan.kalibayev@gmail.com

ABSTRACT

The field expedition has collected 144 samples at the ecotype level of seven wild alfalfa species, which are the source of adaptive properties. One part of the original seeds has been placed for medium-term storage as the gene pool, and the other part has been sown in the culture to use wild-growing species in recurrent breeding. It has been found that the productivity of wild species is inferior to that of M. sativa L., but they are valuable as a source of adaptation traits: drought tolerance, salt tolerance, and winter hardiness, which are important with the development of recurrent breeding to adapt the crops to the global climate change. The yield of the hybrid plants in the offspring from crossing seven ecotypes of M. falcata L. (yellow-flowered) with M. sativa L. (blue-flowered) using a marker-trait has been established: the hybrid nature of the plants obtained from free entomophilic cross-pollination has been established, and the yield of the hybrid plants has amounted to 61–91%. In the hybrid population obtained from M. sativa sybsp. transoxona, M. falcata L., and M. tianschanica Vass. upon crossing them, 50 best phenotypes have been selected to continue the backcrossing with M. sativa L.

INTRODUCTION

Kazakhstan is considered to be a traditional alfalfa-growing area. Alfalfa is cultivated in dry farming conditions in the foothill zone in the southern and southeastern regions, and with irrigation — everywhere. The temperature resources in irrigation in the south are sufficient for obtaining up to five-six mows with a total hay yield of 25 t/ha, in the southeast — four mows (15 t/ha), and in the northern part — two mows (6 t/ha). Compared to other legumes, alfalfa has a wide cultivation area. The value of alfalfa is not limited to its nutritional value alone. Alfalfa is an excellent crop precursor. After two- or three-year cultivation, it accumulates 10.0–12.0 t/ha of crop residues in the soil, which, in terms of the content of nitrogen and other nutrients, is equivalent to the introduction of 4.0–7.0 t/ha of manure. In the case of a good harvest, alfalfa fixes up to 300 kg/ha of nitrogen, which improves soil fertility and increases the yield of subsequent crops.

All alfalfa varieties cultivated in Kazakhstan belong to two tetraploid species of M. sativa L. and M. varia Mart. Varieties of M. sativa L. are cultivated in the southern, southeastern, and western parts of Kazakhstan, and varieties of M. varia Mart. — in the northern part of the republic due to their increased winter hardiness. In the natural flora of Kazakhstan, there are seven species of perennial alfalfa of the Falcago (Reichb) Grossh. subgenus of 21 species as per the latest classification compiled by P. A. Lubenets. The diploid species are M. difalcata Sinsk., M. traufetteri Sum., and M. coerulea Less., and tetraploid species are M. sativa sybsp. transoxona, M. varia Mart., M. falcata L., and M. tianschanica Vass., which are endemic for Kazakhstan (del Pozo et al., 2017). Their productivity in the cultivation...
conditions is low, but they stand out significantly in terms of the degree of drought resistance, winter hardiness, salt tolerance, disease resistance, and leafiness (quality).

However, due to their low productivity and several negative properties, it is impossible to cultivate them on a production-significant scale in the conditions of intensive farming. They are characterized by the slow growth rate, seed hardness (up to 90%), poor regrowth, weak reaction to humidification, and the lying tuft shape. In this regard, they remain a valuable component of natural pastures and hayfields, as they grow in various plant communities, and sometimes there are areas with 60–80 % of wild alfalfa.

Of the wild species, it is more likely to use the best ecotypes of *M. falcata* L. for a direct introduction. First of all, the *M. falcata* L. species is more productive in the conditions of a floodplain meadow and withstands long-term flooding (up to 20 days). The most important thing is to isolate and genetically fix the semi-wide type of tuft for removing the difficulties of combined harvesting for obtaining seeds. In flooded meadows, the plant community is mainly represented by grasses, and the share of legumes is insignificant. To increase the forage value of meadow grasses, the introduction of *M. falcata* L. along with *Lotus corniculatus* L. seems an essential technique in meadow growing.

The wild alfalfa species are used to create source material for alfalfa breeding for adaptability to drought, resistance to diseases, saline soils, and winter conditions using their germplasm based on their hybridization with the cultivated *M. sativa* L. species (Humphries et al., 2021; Krasteva, Uzundzhalieva, & Ruseva, 2012; Toktarbekova, Meirman, Yerzhanova, Abayev, & Umbetov, 2020). Their use is associated with studies in the following areas and stages: (1) collecting wild-growing alfalfa species at the level of their ecotypes and studying them in the cultivation conditions in the introduction nurseries for isolating the initial forms; (2) surveying the proposed territory through a local expedition for identifying introgressive (hybrid) forms in the form-building process arising from free hybridization in the foci of their joint growth. These forms are searched for in the following distribution areas: Tarbagatai and Kalbatau mountains, foothills of the Ili Alatau, Karatau, and the Mugadzhar mountains; (3) polyploidization of the diploid wild alfalfa species (2n = 16) to the tetraploid level (2n = 32) for overcoming their inability for hybridization with *M. sativa* (2n = 32); (4) hybridization of *M. sativa* (the recipient) with wild tetraploid species: *M. falcate, M. tianchanica, M. varia* (adaptability donors), using the method of backcrossing; and (5) studying the hybrids obtained from the BC$_1$ – BC$_3$ backcrossing with the selection of the starting material for the breeding of synthetic varieties.

The problems of studying the wild species and their use in breeding are closely related to alfalfa adaptation to global climate change. Aridization of the climate stands out in this problem; therefore, alfalfa breeding for drought resistance is considered to be a priority (Humphries et al., 2021; Liatukiene, Skuodiene, Tomchuk, & Danyte, 2020; Shi, Nan, & Smith, 2017). The sources and donars for this trait are its wild relatives that grow in the natural flora of Kazakhstan. The crop wild relatives (CWR) are wild species that are more or less genetically related to their cultivated counterparts that may be used for introducing useful genes to improve productivity, biotic and abiotic stress resistance, and quality. They play an important role in improving the yield for achieving food security for the growing population and for overcoming the problems posed by climate change and the new threats from diseases and pests. These genetic resources are increasingly threatened by the excessive anthropogenic load in their natural habitat (Lala, Amri, & Maxted, 2018).

The experiment aimed at isolating valuable starting materials and using them in breeding to create highly productive, drought-resistant varieties of alfalfa.

**MATERIALS AND METHODS**

The studies were performed as part of the breeding process with the use of the germplasm of the wild-growing alfalfa species, starting from their collection from the natural flora to including them in hybridization testing in the cultivation conditions, studying the hybrid offspring in F$_1$, with the selection of elite plants for reuse in the backcrossing scheme in the dryland foothill zone of the Almaty region in the Kazakh Research Institute of Agriculture and Plant Production LLP, the village of Almalybak in the period 2017-2019.

The wild samples (ecotypes) were collected by organizing a field expedition to the territories of the Almaty, the Zhambyl, the Turkestan, East Kazakhstan, the Pavlodar, the Karaganda, and the Akmola regions of Kazakhstan in 2016-2019.
The sampling units were seeds from typical plants in the natural species habitats. In certain cases, valuable samples were transferred to the introductory nurseries of living plants. The samples were sown or planted in these nurseries in the 60 × 60 cm pattern for individual observation and care. In these nurseries, wild species were tested in the cultivation conditions: cultivated soil, high soil fertility, and management system. The nurseries consisted of samples of tetraploid specimens (M. sativa sybsp. transoxona — 12, M. falcata L. — 59, M. varia Mart. — 25, M. tianschanica Vass. — 16) and those of diploid species (M. difalcata Sinsk. — 20, and M. trautvetteri Symn. — 10 samples). The study covered all species with a significant set of ecotypes, except for M. coerulaca Less. The growing area of M. coerulaca is Western Kazakhstan. It is a very valuable species that is distinguished by its resistance to drought and salt.

The introduction nurseries were specially placed among the crops of the Semirechenskaya local variety (M. Sativa L.) so that it would serve as a paternal form during the free cross-pollination of wild tetraploid species. The interspecific hybridization was performed by the method of free cross-pollination in the variants of crossing tetraploid species of M. sativa sybsp. transoxona, M. tianschanica Vass., M. varia Mart., M. falcata L. with M. sativa L. Here, the variants of cross-pollination of diploid species with the Semirechenskaya local variety were not considered due to their heterogeneity.

To determine the yield of the hybrid plants, an analysis was performed for some experimental variants of crossing M. falcata L. with M. sativa L., where seven ecotypes of M. falcata L. were used. The plant hybridity in the crosses of M. falcata L. was recognized by yellow flowers, and in the crosses of M. sativa L. — by blue flowers. The dominating color of the flower corolla in F₁ was used for determining the plants’ hybridity. The blue color of the corolla dominated.

The affinity of M. sativa and other blue-flowered alfalfa species (M. tianschanica Vass., M. varia Mart.) of the Central Asian region, which have many common morphological traits, does not allow establishing the level of hybridization when they are freely pollinated. All the main species systematic traits in M. sativa and M. tianschanica Vass., M. varia Mart. either coincide or are within the range of the variability of the trait.

The other combinations of crossing M. sativa sybsp. transoxona, M. varia Mart., and M. tianschanica Vass. with M. sativa L. were practically used to create the hybrid background to select the best genotypes from the numerous combinations of genes in the structure of the newly synthesized populations. The hybrid population F₁ of wild species with cultivated sown alfalfa (M. sativa) was placed in a 60 × 60 pattern, 50 plants in each, with 112 combinations for hybridization.

To characterize the wild species and their hybrid offspring, the green mass productivity was determined per single plant. For each combination, a sample consisting of at least 25 specimens was analyzed.

The Semirechenskaya local variety (M. sativa L.), which is widely cultivated in Kazakhstan, was taken as reference. The elite plants were selected from the interspecific hybrid population based on a set of economically valuable traits of the plants with blue-flowered corolla and erect tuft shape to be used in repeated backcrossing with M. sativa L. to displace the unwanted traits from the wild species.

RESULTS AND DISCUSSION

The wild species of alfalfa of the Central Asian gene center, including the territory of Kazakhstan, represent the world’s potential for drought tolerance. Populations with the following strong manifestations of xerophytism grow here: lowering of the aboveground organs, pigmentation of the stem with a very wide, and sometimes recumbent or rising tuft shape, lanceolate leaves, weak leafiness, and thin and deep-penetrating rod-shaped root system.

The diploid species are M. coerulaca Less., which is localized in the Caspian lowland of Western Kazakhstan, and M. trautvetteri Symn., which is localized in the Near-Ustyurt zone to the west of the Mugadzhir hillocks on the Ustyurt plateau, and in the Emba sands. It is a rare species that has been spontaneously selected towards the blue flower color and has other morphological features: the densely rising tuft shape, low foliage, strong stem pigmentation, pubescence, thin stems, and prolonged flowering period (May to September). It has originated from the spontaneous crossing of diploid species — M. coerulaca Less, and M. difalcata Sinsk. M. difalcata Sinsk. is found in the Central Kazakhstan desert — hillock belt, and occupies the southern half of the East Kazakhstan region.
The tetraploid species are as follows: *M. sativa* sybsp. *transoxona*, the natural localization range of the species in the East is limited by the Tien Shan mountain ranges and occupies the foothill belt of the south of Kazakhstan and further the neighboring Central Asian states; *M. falcata* L., this species is found in the Eastern Tien Shan, Zhungar Alatau, throughout the desert-steppe territory of Kazakhstan and further to Altai, Siberia, and the Far East; *M. tianschanica* Vass., this species is common on the foothills of the Western Tien Shan, it is found only in the mountain valleys of the extreme South of Kazakhstan, on the passes and slopes of the mountain ranges. At the junction of *M. sativa* sybsp. *transoxona* and *M. falcata* L., there is the natural habitat of *M. varia* Mart., which is a polymorphic hybrid species that stably keeps its range not only in the natural but also in the cultivation conditions, and occupies a large territory of Kazakhstan, up to the Tien Shan foothills.

The tetraploid species *M. sativa* sybsp. *transoxona* and *M. falcata* L. in the nature and in the cultivated conditions form a group of the ecotypes that are morphologically different and adapted to certain conditions. For instance, within Kazakhstan, *M. sativa* L. has formed varieties of cultivated alfalfa known in the breeding that have valuable biological properties: the Lowland Turkestan and Semirechensky, from which alfalfa introduction originated, and which are also the ancestors of many commercial varieties in the American continent.

Modern varieties of the *M. sativa* L. and *M. varia* Mart. species are characterized by the erect (sometimes semierect) tuft type, which gives them an advantage over the other species in terms of regrowth and multi mowing, forages productivity, and seed mass. The other species, especially the diploid ones, having wide or rising tuft shapes, are unsuitable for haymaking and industrial seed production in the cultivation conditions. For these reasons, wild species remain in nature as components of the natural phytocenosis that can be used for grazing animals.

The Tien Shan mountain system within the territory of Kazakhstan and Central Asian states is one of the ancient centers of forming the *M. sativa* L. cultivated species. The phylogenesis of this species proceeded from *M. tianschanica* Vass. to *M. sativa* sybsp. *transoxona*, which are close to the most ancient ecological groups: the Khiva, the Turkestan, and the Semireche ones. Thus, the Semirechye population has been introduced on the territory of Kazakhstan for a millennium, and according to the scientists, the territory of Central Asia is the primary center for the introduction of alfalfa to cultivation. However, there is information that the Semirechensk ecological group of *M. sativa* L. was improved by folk breeding in the southern part of Kazakhstan. In 1934, when switching to the varietal zoning, it got an official status as the Semirechenskaya local variety. The Semirechenskaya local variety is a product of introduction, and it has been included with some breaks as important source material in scientific breeding.

The Semirechenskaya alfalfa has been sufficiently studied in terms of general growth biology and the development of cultivation technology and the use in breeding (del Pozo et al., 2017). For the breeding use, its various populations have been decomposed into biotypes based on the study of their trait variability, the inbred lines have been created and bred in $J_1$ – $J_3$ generations, and in some methodological studies – up to $J_7$, based on the study of the self-fertility and the overall combining ability. Inbred lines called Kokzhazyk-1 and Kokzhazyk-2, which are protected by patents and are widely used in the breeding of synthetic varieties, have been derived from the Semirechenskaya local variety (Nuraliyev, Meirman, Abayev, Bulatova, & Yerzhebayeva, 2018).

Many varieties of the *M. sativa* L. alfalfa species have been created with the participation of the Semirechenskaya alfalfa species. For instance, the Kapchagayskaya 80 variety has been bred based on the biotypic selection, and the genetic structure of the multiclonal synthetic varieties has included the inbred lines selected from the local population (del Pozo et al., 2017) of the Semirechenskaya variety, such as Darkhan 80, Kokorai, Kokbalausa, and Osimtal. All these varieties surpass the original Semirechenskaya local variety by 15–21% with a yield of 120–140 cwt/ha of dry mass in the irrigation conditions in the South and Southeast of Kazakhstan, and they have statutory protection in the form of patents and copyright certificates.

One of the directions in creating the source material for breeding is the attraction of the wild-growing species. In terms of evolution, many forage crops, including alfalfa, have retained their wild counterparts and these can become the valuable
source and the donors of crop improvement in terms of certain economically valuable traits and properties, in particular, longevity, drought, and salt tolerance (Humphries et al., 2021; Kumar et al., 2018; Lapina, Grauda, & Rashal, 2011; Prosperi et al., 2014).

The authors examined the territory of Kazakhstan and collected samples of various ecotypes and species of forage grasses from the natural flora in a total of 272 specimens, including 144 alfalfa samples (Meirman, Kenenbayev, Yerzhanova, Abayev, & Toktarbekova, 2017). They were distributed by the following species: *M. sativa* sybsp. *transoxona* — 12 samples, *M. falcata* L. — 59 samples, *M. varia* Mart. — 25 samples, *M. tianschanica* Vass. — 16 samples, *M. difalcata* Sinsk. — 20 samples, *M. coerulea* Less. — two samples, and *M. trautvetteri* Symn. — 10 samples.

Ecotypes of *M. falcata* L. are ubiquitous in natural landscapes. This species is distinguished by its longevity and high competitiveness. It is found more often than the other species in dense grass stands. Depending on the growth location, the specific marker-trait of alfalfa — the shape of the beans — changes from straight and half-curled to completely curled in one and sometimes 1.5 curls. Regularity is observed that straight beans are fixed due to natural selection in the offspring of the *M. falcata* L. species that is spread in the mountains.

In the valley belt and the foothills, the formation of 1.0 – 1.5 curls was characterized. The beans of the *M. trautvetteri* Symn. variety may be almost straight or have up to 2.0 curls, with the blue corolla of the flower; the beans of *M. difalcata* Sinsk. are relatively large, sickle-shaped, and do not form curls. *M. sativa* sybsp. *transoxona* is widely spread mainly in the valley belt, in the foothills, and the lower tier of the mountains. The plants always form beans with one to 3.5 curls. The beans of the *M. coerulea* Less. variety is very small with 1.5–3.5 curls. The shape of the beans of the *M. tianschanica* Vass. and *M. varia* Mart. varies from slightly straight to four curls. In general, blue-flowered species are characterized by the formation of curled beans, and yellow-flowered species are characterized by straight, slightly straight, and sickle-shaped beans without curls. The wild populations of alfalfa species are in general characterized by lanceolate narrow leaves and lying tuft type (Fig. 1). It should be noted that the *M. sativa* L. and *M. varia* Mart. varieties of cultivated species usually have erect and sometimes semierect tuft. The ecotypes of wild species in the conditions of high soil fertility, but without irrigation, in the introductory nurseries created directly with the seeds from the expeditionary harvests, showed the following productivity results. All specimens of wild species were inferior to *M. sativa* L. (Fig. 2).

**Fig. 1.** Wild ecotypes of *M. falcata* L. with lying tuft: a) The second-year offspring of alfalfa in an introductory nursery, collected in the Tarbagatai Mountains in the midst of motley-grass association; b) the second-year offspring of alfalfa in the introductory nursery, collected in the area of intersection of the Kyrgyz Alatau and Karatau mountains (Turkestan region); c) the second-year offspring of alfalfa in an introductory nursery, collected in the valley of the Katon-Karagai district of the East Kazakhstan region
The productivity of the tetraploid species was closer to the indicators of \( M. \text{sativa} \) L.. The green mass productivity of wild \( M. \text{varia} \) Mart. was 93.2 % with the variation of 81–104%, of \( M. \text{sativa} \) sybsp. transoxona was 86 % with the variation of 74–87% for individual ecotypes, of \( M. \text{falcata} \) L. was 80.3% with the variation of 75–83.6% from the level of \( M. \text{sativa} \) L., of \( M. \text{tianschanica} \) Vass. was 75.6% with the variation of 70–83.1%. The relative productivity of the diploid species of \( M. \text{difalcata} \) Sinsk. was only 49.3%, of \( M. \text{trautvetteri} \) Symn. was 40.6%, and of \( M. \text{coerulea} \) Less. was 38.1% from the level of \( M. \text{sativa} \) L..

Their direct introduction into the crops for intensifying forage production is not promising due to their low productivity, though wild species are more adapted to the unfavorable climatic conditions, resistant to diseases, feature longevity, and drought and salt resistance. They are of interest for breeding as sources and donors of their adaptive properties (Chernyavskikh, Dumacheva, & Borodaeva, 2019; Dzyubenko, Bukhteeva, & Kochegina, 2017; Humphries et al., 2018; Meiirman & Yerzhanova, 2015; Meiirman, Kenenbayev, Yerzhanova, Abayev, & Toktarbekova, 2017; Meyrman, Yerzhanova, Abayev, Toktarbekova, & Kenebaev, 2016; Nuraliyev, Meiirman, Abayev, Bulatova, & Yerzhebayeva, 2018; Xu, Zhou, & Shimizu, 2010; Zubair, Pratley, Sandral, & Humphries, 2017).

Alfalfa is a plant with entomophilous pollination, the pollen is mainly transferred from flower to flower by wild bees, bumblebees, and young broods of domestic bees, which ensure cross-pollination. Self-pollination occurs based on the manifestations of auto tripping, but its share in the populations does not exceed 1–6%. Two methods of hybridization are known: 1) artificial — isolation of the inflorescences, collection of pollen from the staminate parent, and applying the pollen to the snouts. Alfalfa flowers are very small, emasculation is a laborious process, it is not always possible to obtain hybrid seeds; 2) controlled free pollination with the selection of pairs for crossing with the help of pollinators to increase the volume of the resulting hybrid populations, from which strict selection can be made; it is advisable to use this inbreeding method.
All alfalfa species freely interbreed with each other within the tetraploid set of chromosomes. In the foci of introgressive hybridization, where tetraploid and diploid species grow together, the morphogenetic process is observed. Spontaneous hybridization between heteroploid species is extremely rare in the natural conditions and the ploidy of the genotypes in the offspring is aligned at the tetraploid level, which in the case of crossing diploids with tetraploids in the artificial conditions usually produces 5 to 15 hybrid offsprings per 1,000 crossings. To remove the genetic barrier of incapacity for hybridization, the diploid species are first transferred to the tetraploid level by polyploidization and then crossed with tetraploids (Humphries et al., 2021).

To determine the yield of the hybrid plants, an analysis has been performed for some experimental variants of crossing M. falcata L. with M. sativa L., where seven ecotypes of M. falcata L. are used:

1) the steppe ecotype of M. falcata L. (expeditionary No. 56); tuft type is wide, the leaves are lanceolate, and the beans are almost straight or slightly curved. The flower corolla is yellow. It is very drought-resistant. It was collected on the territory of the Almaty region in the Alakul district, 5 km away from the settlement of Balapanov, coordinates N = 45° 56' 247'', E = 080° 27' 070'', the altitude of 584 m.

2) the steppe ecotype of M. falcata L., tuft type is slightly erect, the leaves are lanceolate and pubescent, the racemes are small. The flower corolla is yellow, the beans are spiral. It is drought-resistant. The material was obtained from K. E. Kanapyanov (Pavlodar Agricultural Research Institute LLP). It had been collected in the territory of the Pavlodar region.

3) the steppe ecotype of M. falcata L. (expeditionary No. 5 (11)), tuft type is lying, the leaves are small, narrow, and lanceolate, and the beans are sickle-shaped. The flower corolla is bright yellow. It is drought- and salt-resistant. It was collected on the territory of the Almaty region in the Raiymbek district near the Tuzdykol Lake, the coordinates: N = 44° 16' 820'', E = 077° 48' 565'', the altitude of 884 m.

4) the floodplain ecotype of M. falcata L. (expeditionary No. 98) tuft type is semirecumbent, the leaves are large, broad, and lanceolate, and the beans are sickle-shaped; it can withstand flooding for up to 20–30 days. It was collected in the territory of the East Kazakhstan region on the floodplain of the Shar River near the settlement of Kalbatau.

5) the foothill ecotype of M. falcata L. (expeditionary No. 25 (87)), tuft type is semi-leafy, the leaves are relatively large, the beans are almost straight and small, and the flower corolla is bright yellow. It is cold-resistant. It was collected on the territory of the Almaty region near the settlement of Kyzylagash, coordinates: N = 45° 39' 887'', E = 080° 23' 070'', the altitude of 742 m.

6) the foothill ecotype of M. falcata L. (expeditionary No. 24 (66)), tuft type is lying, the stems are long, the leaves are large, the beans are straight, and the flower corolla is pale yellow. It is cold-resistant. It was collected on the territory of the Almaty region near the settlement of Kabanbai, coordinates N = 45° 40' 562, E = 080° 22' 984'', the altitude of 770 m.

7) the mountain ecotype of M. falcata L. (expeditionary No. 7 (22)), tuft type is pressed to the ground and short, the racemes are small, the leaves are lanceolate, the beans are strongly bent, and the flower corolla is yellow. It was collected on the territory of the Zhambyl region near the settlement of Sulutor, coordinates: N = 45° 34' 887'', E = 080° 27' 070'', the altitude of 934 m.

The yield of the hybrid plants from free cross-pollination is shown in the example of M. falcata L. x M. sativa L. (Fig. 3). The F1 hybrids are dominated by blue-colored flowers (violet, blue, etc.), while yellow flowers do not appear in the hybrids of M. falcata L. In the case of free cross-pollination with the use of insect pollinators of wild-growing tetraploid species with the cultivated M. sativa L. (the Semirechenskaya local variety of this species), as shown in the example with the ecotypes of M. falcata L., the level of hybridization in the offspring was quite high (61–91 %). This high value of hybridization was because single plants of the wild species involved in cross-pollination were surrounded by a dense stand of their staminate parent, the Semirechenskaya local variety, which produced a huge mass of pollen transferred by the pollinating insects for effective fertilization. It can be assumed that in the variants of crossing with other
tetraploids, such as *M. sativa* sybsp. *transoxona* x *M. sativa* L., *M. varia* Mart. x *M. sativa* L., and *M. tianschanica* Vass. with *M. sativa* L., the level of hybridization was not less than that in the variant with *M. falcata* L. x *M. sativa* L.

During an expedition for collecting the ecotypes of various alfalfa species, it was found that in the centers of joint growth of the tetraploid species of *M. sativa* L. and *M. falcata* L., specimens of hybrid origin with polychroic flower corollas were often found among them: bluish-yellow, greenish-yellow, purple, white, creamy, and multicolored with various shades and color saturation. As a rule, these specimens had the semierect tuft type and spiral beans with 1–1.5 clearly expressed curls. The appearance of such morphological traits indicates the morphogenetic process in the *M. varia* Mart. species. Transgressive forms can also serve as the starting material for breeding natural hybrids. Their inclusion in breeding for adaptability, firstly, can accelerate the breeding process, and secondly, as the material that has passed natural selection in the natural conditions, they can be more valuable.

The authors collected seeds from transgressive forms and set up the introduction nurseries in the amount of 196 offsprings for studying them in the cultivation conditions and using them in breeding along with the artificially synthesized interspecific hybrids. Thus, breeding for adaptability at the first stage was made with interspecific hybrids with the use of the tetraploid species (*2n = 32*). The many years’ experience shows that the tetraploid level is the optimal set of chromosomes for alfalfa if the problem is considered from the standpoint of productivity; the other levels of ploidy in both diploid (*2n = 16*) species and hexoploid species (*M. cancellata* Bieb. and *M. saxatilis* Bieb.) are considered manifestations of the longitudinal variants in phylogeny, ensuring survival and reproduction in adverse environmental conditions. The parallel existence of the alfalfa species with various levels of ploidy both in the cultivation conditions and in nature creates a unique opportunity for breeding in terms of combining the productivity traits with the adaptability to the unfavorable environmental conditions.

In analyzing the productivity of the *F*₁ hybrids from free interspecific cross-pollination, the average productivity in 59 hybrid combinations with *M. falcata* was 83%, in 16 combinations with *M. tianschanica* was 75%, in 25 combinations with *M. varia* was 70%, in 12 combinations with *M. sativa* sybsp. *transoxona* was 89% of the indicator of the Semirechenskaya local reference variety (*M. sativa* L.). From the standpoint of the green mass yield after the first mow in the second year of life, the best elite plants were identified in the composition of productive combinations that exceeded the reference (Table 1).

**Fig. 3.** The yield of the hybrid plants in % from free cross-pollination of the ecotypes of *M. falcata* L. with the Semirechenskaya local variety (*M. sativa* L.)
The selection had been performed that left 10% of the hybrid plants for re-pollination with the Semirechenskaya local variety (M. sativa L.) for obtaining seeds from re-pollination with the recipient. The rejected plants were mowed before flowering to exclude their participation in hybridization. In addition to productivity, the selection criteria were the following: erect tuft type, semi-wide, high leafiness, resistance to diseases, and regrowth rate after the first mow.

From the tetraploid species freely cross-pollinated with the staminate M. sativa L., the F₁ offspring was obtained in 112 combinations with the participation of M. sativa sybsp. transoxona — 600 plants, of M. falcata L. — 2,350 plants, of M. varia Mart. — 1,250 plants, of M. tianschanica Vass. — 800 plants. This set of five thousand hybrid plants served as the genetic background for the selection of 50 best phenotypes to continue hybridization in backcrossing with M. sativa L.. A sufficient number of hybrid seeds were collected for the establishment of the next breeding nursery for hybridization and selection.

**CONCLUSION**

The flora of Kazakhstan is rich in the gene pool of perennial alfalfa of the Falcago (Reichb) Grossh subgenus. 144 samples have been collected, and they are used in the breeding. From free entolophilic cross-pollination of the tetraploid species, such as M. falcata L., M. varia Mart., and M. tianschanica Vass. with M. sativa L., 112 hybrid combinations have been obtained (five thousand hybrid plants), of which 50 elite plants have been selected by their phenotype for recrossing to displace the unwanted traits from the wild species.

| Combination                  | The number of analyzed crossbreeding combinations | Green mass | Variability limit (min. and max.) (g) | The best selected phenotypes | Obtained hybrid seeds from a single plant (min. and max.) (g) |
|------------------------------|---------------------------------------------------|------------|--------------------------------------|------------------------------|---------------------------------------------------------------|
| M. falcata                   | 59                                                | 374        | 83                                   | 225 – 504                    | 15 5 – 8                                                      |
| M. tianschanica              | 16                                                | 337        | 75                                   | 270 – 540                    | 10 9 – 12                                                     |
| M. varia                     | 25                                                | 314        | 70                                   | 279 – 518                    | 12 7 – 13                                                     |
| M. sativa sybsp. transox.    | 12                                                | 401        | 89                                   | 315 – 545                    | 13 6 – 11                                                     |
| M. sativa (reference Semirechenskaya local) | -                                             | 450        | 100                                  | 405 – 495                    |                                                              |

| REFERENCES | | |
|-------------|---|---|
| Chernyavskikh, V. I., Dumacheva, E. V., & Borodaeva, Z. A. (2019). Osobennosti adaptatsii ekotipov medicago varia m. k razlichnym usloviam ekotop [Peculiarities of the medicago varia m. ecotypes adaptation to various conditions of the ecotopes]. Proceedings of the V International Scientific and Methodological Conference “Role of Physiology and Biochemistry in Introduction and Breeding of Agricultural Plants” (pp. 104–107). Retrieved from https://vstisp.org/vstisp/images/Sbornik_T-1-2.pdf |
| del Pozo, A., Ovalle, C., Espinoza, S., Barahona, V., Gerding, M., & Humphries, A. (2017). Water relations and use-efficiency, plant survival and productivity of nine alfalfa (Medicago sativa L.) cultivars in dryland Mediterranean conditions. European Journal of Agronomy, 84, 16–22. https://doi.org/10.1016/j.eja.2016.12.002 |
| Dzyubenko, N. I., Bukhteeva, A. V., & Kochegina, A. A. (2017). Perennial and annual drought- and salt-resistant forage plants in the vavilov collection. Proceedings on Applied Botany, Genetics and Breeding, 178(1), 5–23. https://doi.org/10.30901/2227-8834-2017-1-5-23 |
| Humphries, A. W., Ovalle, C., Hughes, S., del Pozo, A., Inostroza, L., Barahona, V., ... Kilian, B. (2021). Characterization and pre-breeding of diverse alfalfa wild relatives originating from drought-stressed environments. Crop Science, 61(1), 69–88. https://doi.org/10.1002/csc2.20274 |
| Humphries, A., Ovalle, C., del Pozo, A., Inostroza, L., Barahona, V., Ivelic-Saez, J., ... Kilian, B. (2018). Introgression of alfalfa crop wild relatives for climate change adaptation. In D. Basigalup, M. Spada, A. Odorizzi, & V. Arolfo (Eds.), Proceedings of the Second World Alfalfa Congress, Global |
Interaction for Alfalfa Innovation, 11-14 November, Cordoba (pp. 72–76). Argentina: Instituto Nacional de Tecnología Agropecuaria (INTA). Retrieved from https://alfalfa.ucdavis.edu/+symposium/proceedings/2018/Oral%20Presentations/Introgression-For-Climate-Change-Adaptation%20by%20Hymphries,%20A._WAC_Argentina_Nov2018.pdf

Krasteva, L., Uzundzhalieva, K., & Ruseva, R. (2012). Plant genetic resources as a part of the biodiversity. Агрознање, 13(1), 5–14. https://doi.org/10.7251/agren1201005k

Kumar, T., Bao, A. K., Bao, Z., Wang, F., Gao, L., & Wang, S. M. (2018). The progress of genetic improvement in alfalfa (Medicago sativa L.). Czech Journal of Genetics and Plant Breeding, 54(2), 41–51. https://doi.org/10.17221/46/2017-CJGPB

Lala, S., Amri, A., & Maxted, N. (2018). Towards the conservation of crop wild relative diversity in North Africa: checklist, prioritisation and inventory. Genetic Resources and Crop Evolution, 65(1), 113–124. https://doi.org/10.1007/s10722-017-0513-5

Lapiņa, L., Grauda, D., & Rashal, I. (2011). Characterization of latvian alfalfa Medicago sativa genetic resources. Acta Biologica Universitatis Daugavpiliensis, 11(2), 134–140. Retrieved from http://sciences.lv/wp-content/uploads/ACTA/2011/11-2/4_Rashal.pdf

Liatukiene, A., Skuodiene, R., Tomchuk, D., & Danyte, V. (2020). Evaluation of agro-biological traits of Medicago sativa and M. varia in a Cambisol and Retisol. Zemdirbyste-Agriculture, 107(1), 41–48. https://doi.org/10.13080/z-a.2020.107.006

Meirman, G. T., & Yerzhanova, S. T. (2015). The formation and study in the culture of genetic resources of forage crops by the expeditionary collection of wild forms from the natural landscapes of Kazakhstan. Ekin Journal of Crop Breeding and Genetics, 1(2), 70–77. Retrieved from https://dergipark.org.tr/tr/download/article-file/211579

Meirman, G. T., Kenenbayev, S., Yerzhanova, S., Abayev, S. S., & Toktarbekova, S. (2017). Results of selection studies of alfalfa based on inbred lines. Journal of Agricultural Science and Technology A, 7(5), 309–316. https://doi.org/10.17265/2161-6256/2017.05.003

Meyrman, G. T., Yerzhanova, S. T., Abayev, S. S., Toktarbekova, S. T., & Kenebaev, A. T. (2016). Creation of highly productive polycomponent agrocoenosis of fodder crops to improve the quality of fodder. Science and World, 5(33), 69–75. Retrieved from http://scienceph.ru/fs/Science_and_world_no_5_33_may_vol_ii.pdf

Nuraliyev, S. K., Meirman, G. T., Abayev, S. S., Bulatova, K. M., & Yerzhebayeva, R. S. (2018). Selection of inbred lines of alfalfa for creating synthetic varieties. OnLine Journal of Biological Sciences, 18(1), 7–16. https://doi.org/10.3844/objsci.2018.7.16

Prosperi, J.-M., Jenczewski, E., Muller, M.-H., Fourtier, S., Sampoux, J.-P., & Ronfort, J. (2014). Alfalfa domestication history, genetic diversity and genetic resources. Legume Perspectives, 4, 13–14. Retrieved from https://hal.archives-ouvertes.fr/hal-01216251/document

Shi, S., Nan, L., & Smith, K. F. (2017). The current status, problems, and prospects of alfalfa (Medicago sativa L.) breeding in China. Agronomy, 7(1), 1. https://doi.org/10.3390/agronomy7010001

Toktarbekova, S. T. K., Meirman, G. T., Yerzhanova, S. T., Abayev, S. S., & Umbetov, A. K. (2020). Productivity of the green mass of new alfalfa cultivars depending on the effect of macro- and microfertilizers on various phosphorous backgrounds. Journal of Ecological Engineering, 21(2), 57–62. https://doi.org/10.12911/22998993/116347

Xu, Z., Zhou, G., & Shimizu, H. (2010). Plant responses to drought and rewatering. Plant Signaling & Behavior, 5(6), 649–654. https://doi.org/10.4161/psb.5.6.11398

Zubair, H. M., Pratley, J. E., Sandral, G. A., & Humphries, A. (2017). Allelopathic interference of alfalfa (Medicago sativa L.) genotypes to annual ryegrass (Lolium rigidum). Journal of Plant Research, 130(4), 647–658. https://doi.org/10.1007/s10265-017-0921-9