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RESEARCH ARTICLE
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Innovative Breeding Methods for Alfalfa Adaptation to North Kazakhstan's Agroecosystem

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ABSTRACT

The paper presents the results of laboratory and field experiments on the source material of alfalfa characterized by low seed productivity in North Kazakhstan. The study aimed to assess the breeding value of alfalfa variety populations and identify promising source material for developing high-yielding seed varieties. Field trials were conducted in 2021-2023 at the Kokshetau Experimental and Production Facility located in the coniform hill and plain zone of North Kazakhstan. An effective method of alfalfa breeding was developed and validated, considering for the biological characteristics of plant growth and development traits as well as the influence of environmental factors on seed productivity. The promising alfalfa line- (SGP-04-09-3) obtained using this method is characterized by high feed and seed weight yields, significantly exceeding the standard. The variety has winter hardiness, drought resistance, a relatively high percentage of self-pollination, intensive regenerative ability, and a high bean-setting rate. Moreover, seven lines with the potential for further selective hybridization aimed at increasing the seed productivity of alfalfa were selected using the polycross method. The experimentally verified source material is recommended for practical breeding in North Kazakhstan to breed varieties resistant to adverse weather conditions.

Keywords: Alfalfa, Gene pool, Assessment, Selection, Seed productivity, Polycross method, Complex hybrid population, Bush shape, Bean setting rate, Self-pollination.

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INTRODUCTION

Alfalfa (*Medicago sativa*), which belongs to the *Fabaceae* family, is a perennial plant species, a high-quality forage crop widely grown throughout the world. Its significance lies in its remarkable adaptability to extreme climate and salinity, its ability to enrich soil through nitrogen fixation, absorbency of minerals and high source of nutrients for cattle (Hadidi et al., 2023). Alfalfa is one of the most widespread crops in the world and is cultivated in more than 30 million ha. The wide geographical distribution of the crop is explained by its ease of adaptation to various climatic and soil conditions (Omelko & Fomichev, 2022; Yue-gao & Cash, 2009; Riday, 2011; Shpaar, 2011). Such perennial crops reduce the annual destruction of the soil structure, which affects many biochemical processes, and are key to ensuring the sustainability and stability of agricultural ecosystems (Islam & Ashilenje, 2018). Alfalfa reduces the level of nitrate leaching from soils, which lowers water pollution, increases biodiversity and carbon fixation in the soil, and improves critical habitat for wildlife (Syswerda & Robertson, 2014; Picasso et al., 2019). The powerful root system of alfalfa is deeply located. It contributes to the enrichment of the soil with humus, improving its structure and pore volume, increasing water and air permeability, and creating water-resistant aggregates and fertility. In three years of cultivation, alfalfa leaves an amount of organic matter per ha equal to the introduction of 60 t of manure, while the humus content increases by 8-10%. Alfalfa crops are widely appreciated as a preceding crop, since the volumes of symbiotic nitrogen fixation after its sowing reach 180-250 kg/ha, which contributes to the accumulation of 80-195 kg/ha of biological nitrogen in the soil and is equivalent to the

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introduction of 270-291kg of nitrogen fertilizers. 180-250kg/ha of nitrogen accumulates on alfalfa roots and crop residues, equivalent to 4-5kg of nitrogen fertilizers or 30-40tons of manure per ha into the soil (Humphries et al., 2020; Zhang et al., 2021). Alfalfa is actively cultivated in some regions of Kazakhstan. In North Kazakhstan, which has a large share of plowed agricultural land, the Kokshe variety of local selection has become widespread. The following are also cultivated: Karabalykskaya raduga, Shortandinskaya 2, Karagandinskaya 1, Lutsia 14, Raykhan, Lazurnaya, and others. The features of the varieties approved for sowing in North Kazakhstan include the high productivity of their vegetative mass, a prolonged flowering period, and a low indicator of seed productivity, which is one of the most problematic issues of alfalfa cultivation (Bulakhtina et al., 2024). Breeding and production practice revealed that alfalfa productivity of feed yield and seeds is inversely correlated. Practically, it is impossible to obtain an economically suitable seed harvest from the third year of life of alfalfa plants, since from this period, the formation of reproductive organs in the plant structure decreases. A significant increase in alfalfa seed productivity is challenging and requires accelerated and mandatory action (Shayakhmetova et al., 2024). Alfalfa varieties have not become more widespread in Kazakhstan due to unstable winter hardiness, insufficient drought resistance, and susceptibility to pests and diseases. This necessitates the development of new intensive varieties with consistently high seed productivity (Nazarova et al., 2025). The correct selection of varieties and their use in breeding is paramount, especially using the effective method of polycross with forming experimental hybrid synthetic populations (Leskudov, 1993).

In various parts of the world, research has been committed to improving the breeding methods of the Alfalfa species. According to a study by (Zadorozhna, 2022), the research on alfalfa in Ukraine has gone through various developmental stages. Highly productive synthetic varieties have been created to optimize the nutrition system and nitrogen fixation. Through this developmental stage that has spanned over fifty years, competitive alfalfa varieties and hybrids such as alfalfa Vinnichanka, Lyubava, hop alfalfa Bereginya, alfalfa Regina, Sinyukha, Rosana, Radoslava, Rodin, Amag and Ramin with increased productivity and longevity has been developed (Belyakov & Nazarova, 2022). Notably, in Kazakhstan, according to a study by Kalibayev et al. (2021), the genetic diversity that exists among wild alfalfa species was implemented as an innovative tool in breeding. Desirable phenotypical traits such as drought tolerance, salt tolerance, and winter hardiness were achieved in the hybrid species after crossing seven ecotypes of *M. falcata L.* (yellow-flowered) with *M. sativa L.* (blue-flowered). This study highlighted the importance of genetic diversity in innovative breeding and how it can be used to achieve desirable traits needed for productivity. Another significant advancement in this area is the development of a model that studies the influence of timing on the yield and quality of alfalfa. Koptileuov et al. (2023) concluded that in addition to using high-yielding alfalfa varieties, the implementation of resource-saving cultivation and harvesting technologies, the optimal organization of cultivation production processes from tillage to harvesting, time of harvest and the efficient use of agrotechnical means contribute to the production of high-quality alfalfa feed. Studies such as Kuznetsov et al. (2022) aimed to identify promising alfalfa species with desirable biological and economical traits such as high seed productivity, a high yield of forage mass, good quality, and stress resistance. They concluded in their five-year study that varieties such as P-88044, U-73+149 and S-302 had stable seed/green mass yields regardless of climatic conditions; hence, they may be used for alfalfa selection. In conclusion, recent research and initiatives in Kazakhstan and globally have focused on innovative breeding methods to develop alfalfa varieties with high seed productivity and resilience to climatic challenges. By combining traditional selection techniques with the incorporation of crop wild relatives, genetic crossing, creation of synthetic populations and resource-efficient farming practices, these efforts aim to enhance the sustainability and stability of agricultural ecosystems. The study aims to assess the breeding value of alfalfa variety populations and isolate experimental starting material to create alfalfa varieties with high seed yields.

MATERIALS & METHODS

The study was conducted in 2021-2023 at the Department of Experimental Crop Production of the Kokshetau Experimental and Production Facility in the village of Shagalaly, Zerendi district, Akmola region (Fig. 1).

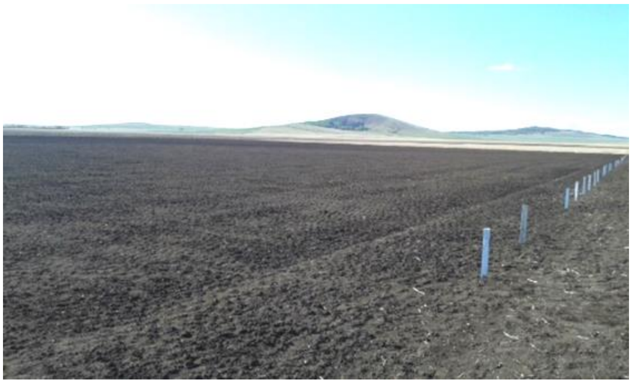

Fig. 1: Experimental field of Kokshetau Experimental and Production Facility.

The soil of the experimental plot was ordinary chernozem, moderately deep, with medium humus content. The zonal soils make up most of the soil cover of the region. The arable horizon reached 34 cm; below, there was a transitional horizon B (14-20 cm) dark gray, with a brown tinge of dense build, further passing into the horizon BC. By the mechanical composition, it was slightly stony heavy loam. By chemical composition: humus content: 4.71% (determined according to Tyurin), pH of the medium: 7.1-7.5, mobile forms of phosphorus: 2.16, potassium: 40.9 (according to Machigin), nitrogen: 3.21 (Grandval-Lajoux) mg/100 g of soil. The nitrogen content was average, the phosphorus content was low, and the potassium content was high. The soil had significant reserves of total forms of nitrogen and phosphorus. The preceding crop was black fallow, tillage was conducted according to zonal technology. All nurseries were established on black fallow without cover in the spring (in the first decade of May) manually. Sowing was conducted in a square-cluster (70x70 cm) method, in wide-row crops with a manual RS-1 seeder. The method of sowing in the collection nursery, in progeny evaluation nurseries (PEN), polycross nursery (PN), and complex hybrid populations nursery (CHP, SGP in the variety name) was square cluster (70x70cm) (Fig. 2). In the control nursery (CN) and the nursery of competitive variety testing (CVT) for seeds, the sowing was performed in wide rows (row spacing: 70cm). Each number in the collection nursery and the CHP occupied 5m^2 in six repetitions. In the CN, all numbers were established in six-fold repetition, and CVT in eight-fold repetition. The area of plots in the CN was 10m^2, and in CVT 25m^2. The zoned alfalfa variety of the local Kokshe selection was adopted as the standard. The standard was seeded after every 10 numbers. The plants were treated manually and mechanically. The side protective strips were 0.7m, the end ones were 10m. The total area of breeding crops was 1 ha, and the preliminary reproduction of promising numbers was 0.2ha. The main method of alfalfa breeding, along with hybridization, was the polycross method based on the complete cross-pollination of selected populations, varieties, hybrids, biotypes, or plants. After cross-pollination, the seeds from the best plants were combined, giving rise to a new synthetic variety. The design of the breeding process, the establishment of nurseries, evaluation, hybridization, selection, and variety testing were conducted according to the methodological guidelines for the study of the perennial grass collection developed by the All-Russian Institute for Plant Breeding (VIR) (Stephanovich et al., 2021), the guidelines for the selection of perennial grasses by the researchers of Ural Federal University (Stephanovich et al., 2021), the guidelines of the Siberian Feed Research Institute (Monsen et al., 2004), the methodology of the state crop variety testing (Suhara, 2017) and the methodological foundations and techniques of breeding perennial grasses in North Kazakhstan (Ministry of Natural Resources and Protection of Environment of the Republic of Kazakhstan, 1999).

Fig. 2: Sowing on experimental plots.

In the CHP nursery, forming populations into a biomechanical mixture took place, which became the final stage of selection before variety testing. The main selection methods in hybrid populations were mass and family group selection. Annually, 600-800 numbers according to two schemes of the breeding process were evaluated according to a set of traits. The harvesting of the selected numbers and the accounting of the harvest in the nurseries were conducted manually. The threshing of the selected sheaves was conducted on stationary laboratory threshing machines. During the growing season, two field and one laboratory screenings were conducted. Phenological observations. When evaluating the samples for seed productivity, the dates of full flowering, the beginning of fruit formation and full fruit formation, maturation, and cutting of the seed herbage were additionally noted. Observations were conducted every other day, and at the onset of the main phases (budding, flowering) daily. The phase of plant development was noted in two non-adjacent repetitions, after which the average date was calculated. The beginning of the phase was its onset in 10% of the total number of plants in the plot, and the full phase in 75%.

Herbage Density

To determine the herbage density, the number of plants and stems per unit area was calculated. For this purpose, permanently fixed platforms with an area of at least 0.25-0.5m^2 of square or elongated shape were used. Linear measures were also used, 0.5-1m^2 of the seeding row. The counting by variety was repeated at least 3-4 times.

Herbage Height

The height of the herbage served as an indirect indicator of yield (Fig. 3). Height measurement was performed by calculating the distance from the soil surface to the end of the inflorescences of plants, without pulling the shoots. In legumes, the length of the plant was determined from the base to the tip of the elongated stem. 10 measurements were conducted on each plot. During lodging, the height of the lodged herbage was additionally determined without stretching the stems.

Green Mass Productivity

The productivity index of green and dry mass was determined in collection and breeding nurseries with individual plant accounting. Productivity is expressed in terms of one or more plants (g, kg) or a limited area (1-3 m2) with further conversion of yield in c/ha. When evaluating the number, the green mass of all plants from the plot was weighed, and their number was determined for subsequent recalculation per plant. From the total mass of cut plants, a sample was taken from each variety to determine the dry matter content and structural analysis.

Yield Accounting

The yield of the feed mass served as one of the main indicators of the value of the varieties. The yield during haymaking was considered in the phase of the beginning of flowering of legumes. The accounting was conducted by direct weighing of the green mass from the plot, immediately after cutting and taking a test sheaf to determine the yield of hay, the foliage of the herbage, and the crop structure. A test sheaf to determine the hay yield was collected in a gauze bag and dried to an air-dry state. The sample was weighed until its mass became constant during the control weighing.

Fig. 3: General view of the herbage and measurement of the height of alfalfa plants: a) measurement of the height of regrowth on the 20th day after cutting; b) alfalfa in the budding/beginning of flowering phase.

Foliage

To determine the foliage, a sample weighing 1 kg was taken in different parts of the plot and divided into two fractions: leaves and stems. In leguminous grasses, inflorescences were also attributed to the leaf fraction. The leaves in this fraction were isolated together with the petioles.

Crop Structure

To determine the crop structure, a trial sheaf weighing 1kg was selected. When analyzing the sample, the shoots were divided into the following categories.

1. Generative: bearing inflorescences (in leguminous grasses, the stem with all lateral branches was considered a shoot);
2. Elongated vegetative: stems with clearly elongated internodes, but not bearing inflorescences;
3. Shortened vegetative: without elongated internodes and inflorescences (rosettes in legumes).

After analyzing the sample, the shoots of each category were counted, and the crop structure was investigated. The shoot categories were divided into fractions. Generative shoots were divided into inflorescences (in legumes: a stem with all lateral branches) and leaves (in legumes: with petioles). The elongated vegetative shoots were divided into straw (stem) and leaves. The shortened vegetative shoots were not separated. Each fraction is weighed on a technical scale with an accuracy of 0.1g. To analyze the structure of the seed herbage, we took samples before harvesting seeds on plots from sites of 0.25m^2 in four-fold repetition. When analyzing the samples, the number of generative shoots per unit area, the number of inflorescences (on average on 25 shoots), the number of inflorescences (10 inflorescences were selected randomly), the number of seeds, the weight of 1,000 seeds (2 samples of 500 seeds), and the yield of full seeds (%) were calculated after threshing and cleaning the sample. Chemical analysis of the nutritional value of the dry mass was conducted using a Kjeldahl micrometer, indicating the content of crude protein in the plant sample. The mathematical processing of the results was performed using a personal computer and standard software. Statistical processing of the results, in particular, variance and correlation analysis, was conducted according to Dospekhov (1985).

RESULTS & DISCUSSION

The development of innovative breeding methods for alfalfa in North Kazakhstan's Agroecosystems demonstrates promising advancements in adaptive crop improvement. This study has significant implications for the region's agricultural productivity and environmental resilience. Based on the results, valuable numbers were selected according to traits, primarily seed productivity. Of the 46 samples, 18 were considered promising (Table 1). We selected 905 biotypes out of 1,711 for winter hardiness (100% overwintering), 401 biotypes out of 912 for drought resistance, 125 biotypes out of 414 for self-pollination (22-27%) and 19 biotypes out of 43 for regenerative ability (67-75%). On their basis, a synthetic CHP was formed. The PN for limited free cross-pollination included promising biotypes from the varieties Kokshe, Omskaya 7, Nurilya, Pamyati Khasenova, Rambler, Raykhan, Flora 4, Tulunskaya gibridnaya, Zheltogibridnaya 55, Oranzhevaya 115, Kapchagayskaya 80, Zhainak 96, Raduga, and Aisulu, which formed 19 CHPs. As a result of cross-pollination, 124 polycross hybrids were isolated based on the complex of selected traits according to the model of future varieties. In 2022, a nursery cycle of 12 numbers was established for these purposes. In the PEN, 124 polycross progeny varieties of 2021-2023 sowing and 65 progeny varieties of 2022 sowing were evaluated using the polycross method

Table 1: Main parameters of the assessment of the alfalfa model with increased seed productivity (2021-2023)

No.	Parameter	Standard variety	Model variety
1	Yield of green mass, c/ha	80-110	120-150
2	Seed yield, kg/ha	0.6-1.0	1.6-3.3
3	Shape of the bush	sprawling	erect
4	% of overwintering	70-80	95-100
5	Winter hardiness, drought resistance, points	2-3	5
6	Self-pollination, %	1-6	10-13
7	Bean setting rate, %	10-18	25-39
8	Regenerative capacity, % of biotypes with high seed productivity	12-15	66-74
9	Tilling capacity, stems (pcs.)	14-26	34-44
10	Shedding of flowers and beans, points	4-5	0-2
11	Duration of flowering, days	44-56	30-36
12	Susceptibility to powdery mildew and leaf spot, points (a complex of spot diseases), points	3-5	1-2
13	Damage by pests of seeds (clover-seed chalcid, Tychius), %	25-39	5-10
14	Plant height, cm	61-70	77-84

with an alternate arrangement of parental forms and hybrids. The new PEN cycle of 2023 included 65 polycross hybrids. 12 CHPs from each sowing cycle (2021-2023) passed the preliminary test. The characteristics of the CHPs based on the main traits are presented in Table 2 and 3. SGP-9-21, SGP-11-21, and SGP-12-21 were identified as the most promising populations for the nursery in 2021. Based on the results of testing and evaluation in the nursery in 2022, the following populations became the most promising for inclusion in the variety testing: SGP-3-22 for drought resistance, SGP-7-22 for disease resistance, SGP-10-22 for green mass productivity, SGP-5-22 for overwintering (Table 3). Recent studies have highlighted the importance of the polycross breeding technique and its importance in sustainable agriculture (Ijaz et al., 2021).

Table 2: Composition of populations by bush shape in various alfalfa varieties (2021-2023)

Species	Shape of the bush, %		
	Erect (x_2+m)	Sprawling (x_2+m)	Spreading (x_2+m)
Erect bush	55.1-90.4	90.6-44.9	-
Chaglinskaya 14	70.1±0.7	10.9±1.7	-
Chaglinskaya 17	78.7±1.9	10.7±1.1	-
Khanshaim	74.5±1.8	15.5±1.8	-
Nurilya	65.1±1.3	44.9±1.3	-
Sprawling bush	11.6-30.4	4.9-82.1	6.3-22.7
Kokshe	14.7±1.1	71.9±1.1	13.4±1.1
Rambler	20.6±1.9	50.4±1.9	17.0±1.9
Sarga	20.4±1.7	50.8±1.8	16.9±1.3
Uralochka	20.1±1.9	50.3±1.9	18.3±1.2
Karabalykskaya raduga	19.7±1.8	49.8±1.5	17.0±1.7
Viola	20.3±1.4	44.4±1.2	15.5±1.4
Karabalykskaya zhemchuzhina	19.6±1.7	43.4±1.1	16.0±1.4
Raykhan	18.5±1.2	41.4±1.9	16.0±1.0
Lazurnaya	18.6±1.0	48.4±0.8	17.0±1.3
Starbak	17.6±1.1	45.4±1.3	16.0±1.4
Bokkara	20.6±1.7	49.4±1.0	16.0±1.9
Kokorai	20.2±1.8	48.6±1.7	15.0±1.2
Baralfa	19.5±1.1	47.4±1.2	15.4±1.0
Lutsiya	19.3±1.2	49.3±1.4	17.0±1.1
Spreading bush	-	29.8-47.7	52.5-1.2
Flora 6	-	26.5±1.6	42.5±1.6
Omskaya 7	-	28.1±1.8	41.9±1.7

Table 3: Average duration of alfalfa flowering (2021-2023)

Variety, sample, number	Average duration of flowering, day		
	flowers	Raceme	plant
Kokshe	3.4	7.1	14.2
Omskaya 7	3.9±0.3	6.7±0.7	15.6±1.6
Flora 6	3.5±0.2	6.8±0.7	15.7±1.6
K-45589	4.1±0.4	7.8±1.4	17.8±1.7
Sarga	4.2±0.5	7.7±1.3	15.8±1.6
Uralochka	4.1±0.4	7.6±1.2	15.1±1.6
K-3793	4.5±0.6	7.4±1.2	17.9±1.7
K-2192	4.8±0.7	7.6±1.0	17.6±1.6
Nurilya	3.5±0.2	6.9±0.8	14.8±1.5
Khanshaim	3.6±0.3	7.0±0.9	14.1±1.4
Chaglinskaya 14	3.5±0.2	6.9±0.8	14.4±1.6
Chaglinskaya 17	3.6±0.3	7.0±0.9	14.3±1.5
Raykhan	3.9±0.4	6.7±0.7	15.1±1.6
K-930	4.0±0.6	7.3±1.4	17.8±1.6
Lazurnaya	4.0±0.6	7.0±0.9	15.1±1.6
Karabalykskaya zhemchuzhina	3.9±0.6	6.8±0.7	14.8±1.5
Karabalykskaya raduga	3.9±0.4	6.9±0.8	14.6±1.4
K-41422	4.6±0.9	7.6±1.0	17.9±1.6

Yun et al. (2022) reported in their study that polycrossed perennial grasses displayed superior advantages such as superior stem thickness, fresh yield and improved plant heights. These traits correlate with our results from SGP-10-22. Based on the two cycles of CHP evaluation, the following numbers were recognized as the most promising and having breeding value for further inclusion and variety testing: SGP-4-11-15-2, SGP-10-11-14-5, SGP-9-11-6-6, SGP-5-11-11-1, SGP-4-11-10-8, SGP-9-11-17-2, and SGP-5-11-12-7.

Based on the promising variety samples with valuable biotypes, CHPs were created. They were further evaluated according to biological properties and economically valuable traits in the variety tests of the CN. By the yield of green mass, hay, and seeds, seven varieties of the 2022 sowing year and seven varieties of the 2023 sowing year were distinguished in the CN (Table 4). Our evaluation of the biological and economic characters of alfalfa aligns with the outcomes of Marinova and Ivanova-Kovacheva (2022), who conducted comparable experiments on six alfalfa species. Their study measured dry and green mass production over three years and identified the most productive and economically viable alfalfa variety based on their results.

Table 4: The degree of damage of various alfalfa populations by powdery mildew (2021-2023)

Species	Degree of damage, points			
	in the first year of life	in the second year: 1st cutting	in the second year: 2nd cutting	limit per cycle
Resistant	0-2	1-4	1-3	0-4
Khanshaim	1.5	1.3	1.3	1.4
Chaglinskaya 14	1.2	1.2	1.1	1.2
Chaglinskaya 17	1.1	1.0	1.1	1.2
Rambler	1.0	1.2	1.5	1.2
Flora 6	1.5	1.7	1.7	1.6
Omskaya 7	1.7	3.5	3.0	2.7
Moderately resistant	2-6	3-7	1-7	2-7
Kokshe	3.2	6.0	4.0	4.7
Raykhan	3.6	5.7	4.7	5.4
Sarga	3.0	3.9	4.5	5.2
Uralochka	3.1	3.4	4.3	5.0
Karabalykskaya raduga	3.1	3.4	4.0	4.1
Viola	4.4	3.0	4.2	5.2
Karabalykskaya zhemchuzhina	2.3	3.2	4.1	5.0
Lazurnaya	3.1	3.1	4.0	4.1
Starbak	4.2	4.8	4.1	5.1
Bokkara	3.5	4.5	4.2	5.2
Kokorai	3.6	4.2	4.0	4.2
Lutsiya	3.4	3.4	4.7	5.2
Baralfa	2.3	3.4	4.3	5.0

The characterization of promising CN numbers according to traits conducted for inclusion in the CVT showed that they were distinguished by high winter hardiness (5 points), drought resistance (5 points), and disease resistance (4 points), were taller (by 11-20 cm), and had a higher foliage by 2-3%. According to the length of the vegetation, the varieties were at the standard level. Almost all 12 numbers exceeded the control variety in seed productivity (Table 5). The seed yield of the standard grade of alfalfa (Kokshe) was 0.9 c/ha, while the new number SGP-04-09-3 had 2.1 c/ha, with an excess of 133%. The variety formed a highly productive seed herbage due to high winter hardiness (overwintering: 100%), drought resistance (green leaves during the drought period amounted to 63.5%), and tilling capacity (44.0 stems/bush). In the new population, 67.7% of biotypes had a high regenerative capacity with a bean setting rate of 33.0% and self-pollination of over 28.6%. SGP-04-09-3 was characterized by a relatively vigorous flowering period (53 days for standard, 45 days for the new promising number) and resistance to diseases and pests (Table 6). Renewing genetic material by attracting new source forms is the basis for crop breeding (Nazzicari et al., 2024; Zafar et al., 2024). To effectively create competitive varieties, it is necessary to have a genetically diverse and comprehensively studied source material, which forms the basis for plant improvement (Zafar et al., 2023). In the breeding of cross-pollinating perennial grasses, the formation of complex hybrid synthetic populations by polycross is becoming increasingly used and important (Zafar et al., 2021). This method allows for crossing populations with a heterogeneous genetic nature, causing a high and stable effect of heterosis (Zafar et al., 2022).

Table 5: The degree of damage to the alfalfa population by leaf spot (2021-2023)

Type, variety sample	Degree of damage, points			
	in the first year of life	in the second year		limit per cycle
		1st cutting	2nd cutting	
Resistant	0-2	1-4	1-3	0-4
Khanshaim	1.5	1.3	1.3	1.4
Chaglinskaya 14	1.2	1.2	1.1	1.2
Chaglinskaya 17	1.1	1.0	1.1	1.2
Rambler	1.0	1.2	1.5	1.2
Flora 6	1.5	1.7	1.7	1.6
Omskaya 7	1.7	3.5	3.0	1.7
Moderately resistant	1-3	2-4	2-5	0-5
Kokshe	1.1	2.0	2.0	1.7
Raykhan	1.5	2.7	2.7	1.4
Sarga	1.0	1.9	1.2	1.9
Uralochka	1.1	1.4	1.0	1.8
Karabalykskaya raduga	1.1	1.4	1.0	1.6
Viola	1.3	2.0	2.0	1.7
Karabalykskaya zhemchuzhina	1.3	2.2	2.1	1.7
Lazurnaya	1.1	1.1	1.0	1.8
Starbak	1.2	2.8	2.0	1.9
Bokkara	1.5	2.5	2.1	1.9
Kokorai	1.7	2.2	2.2	1.6
Lutsiya	2.4	2.4	2.2	1.5
Baralfa	1.4	2.4	2.4	1.4

Table 6: Variability of the number of stems in the bush depending on the shape of the bush of various alfalfa varieties (2021-2023), pcs.

Shape, variety samples	x±m
Erect bush	3-9
Khanshaim	3.1±1.1
Chaglinskaya 14	6.5±1.7
Chaglinskaya 17	6.9±1.1
Nurilya	6.7±0.1
Sprawling bush	10-14
Kokshe	10.1±1.7
K-39112	12.0±1.9
Sarga	13.4±1.1
Uralochka	9.1±1.7
Karabalykskaya raduga	10.0±1.9
Viola	11.4±1.1
Karabalykskaya zhemchuzhina	9.1±1.7
Raykhan	11.0±1.9
K-45589	12.4±1.1
K-930	10.1±1.7
K-41422	9.0±1.9
Spreading bush	29-46
Rambler	29.3±3.1
Omskaya 7	45.5±2.7

Based on the study of the biological characteristics of plant growth and development and the influence of environmental factors that reduce potential seed productivity, an effective method of breeding alfalfa with increased seed productivity was developed and tested (Table 7; Table 8). The new method includes several stages: ecological variety testing, development of trait parameters, selection of biotypes, and assessment against a provocative background for overwintering, drought resistance, self-pollination, regenerative ability, and bean setting rate. Our results on the influence of environmental factors agrees with the study of (Feng et al., 2022) who concluded on the relationship between yield and ecological factors. Feng et al. (2022) highlighted that high soil nutrient availability positively correlated with yield and high nitrogen and potassium content increased protein content in the alfalfa species. This correlation was also affected by the maturity and age of the alfalfa. We recommend that further studies be carried out to identify the correlation between our selected species and environmental factors not limited to drought and climatic condition. Wang et al. (2021) also highlighted the positive effect of practices such as combined tilling and ploughing to the yield of alfalfa especially in semi-arid regions like North Kazakhstan. The method is based on the complete cross-pollination of selected populations, varieties, hybrids, biotypes, or plants.

Table 7: Bean setting rate of prospective alfalfa populations (2021-2023)

Variety, number	Bean setting rate, %
Kokshe (standard)	5.7±0.4
Omskaya 7	6.4±0.1
Nurilya	15.5±0.2
Viola	6.1±0.3
Karabalykskaya zhemchuzhina	5.5±0.2
Bokkara	7.3±0.2
Serpovidnaya	4.4±0.1
Baralfa	8.3±0.1
Lazurnaya	9.2±0.2
Rambler	9.5±0.3
Raykhan	7.5±0.4
Yaroslavna	10.4±0.3
Starbak	7.6±0.2
Flora 6	9.1±0.2
Uralochka	8.1±0.2
Sarga	7.2±0.3
Lutsiya	7.5±0.3
Khanshaim	7.4±0.3
SGP-02-21-9	24.4±0.4
SGP-04-09-3	28.1±0.5
SGP-09-10-7	29.4±0.3

After cross-pollination, the best plants' seeds are combined, which gives rise to a new synthetic variety (Table 9; Table 10). A necessary condition for the work is the evaluation of plants by their combinational ability. Methods for evaluating combinational ability include diallelic crosses, topcross, and polycross (Yun et al., 2022). The polycross method is the most effective in perennial grass breeding. To assess the combinational ability of this method, after cross-pollination, seeds from each component are collected separately and evaluated in comparison with the parent, the average values of polycross hybrids, and the zoned variety. Only plants with the best combinational ability are combined into a biomechanical mixture (Popa et al., 2024). Our application of the polycross method in perennial grass and our selected desired traits (adaptability, green mass yield, and seed productivity) agrees with the methodology of (Bekuzarova et al., 2021), who employed the same technique in the formation of complex hybrid populations involving clovers, alfalfas and salonins. The synthetic variety obtained as a result of crossing, according to the definition of (Monirifar et al, 2023), "represents a complex hybrid population obtained as a result of cross-pollination of several populations, mixtures, clones, or hybrid lines with high combinational ability".

Table 8: Composition of alfalfa variety populations by self-pollination (2021-2023), plants (pcs.)

Variety, population	Self-pollination, %					
	5	10	15	20	25	30
Kokshe	36	2	-	-	-	-
Omskaya 7	32	11	2	-	-	-
Lazurnaya	34	4	-	-	-	-
Karabalykskaya zhemchuzhina	31	-	-	-	-	-
Raykhan	38	7	1	-	-	-
Raduga	32	10	3	-	-	-
Yaroslavna	41	12	7	-	-	-
K-3793	31	11	4	-	-	-
Nurilya	43	24	11	3	-	-
Lutsiya	34	5	6	2	-	-
Flora 6	48	26	12	5	-	-
Sarga	44	23	11	3	-	-
K-45589	28	12	1	3	-	-
Severo-Kazakhstanskaya 8	31	30	27	7	5	-
Rambler	24	20	19	8	5	-
Khanshaim	23	27	29	9	7	4
SGP-02-21-9	21	22	23	14	12	8
SGP-04-09-3	20	21	22	17	14	6
SGP-09-10-7	20	11	20	15	13	7

Table 9: Promising alfalfa variety samples by vegetative mass structure (2021-2023)

Variety, sample	Foliage, %	Plant height, cm	Tilling capacity, pcs/stems
Kokshe (standard)	48±0.39	56±0.25	11±0.03
Flora 6	49±0.32	59±0.32	16±0.02
Nurilya	49±0.31	58±0.22	16±0.02
Raykhan	49±0.29	58±0.36	15±0.1
Karabalykskaya zhemchuzhina	50±0.22	59±0.20	15±0.1
Omskaya 7	51±0.26	59±0.21	14±0.02
K-45589	52±0.28	55±0.27	13±0.02
K-41121	50±0.31	57±0.34	15±0.01
K-43833	51±0.31	55±0.34	14±0.02
Viola	49±0.32	59±0.35	16±0.02
Lutsiya	50±0.38	59±0.28	16±0.02
Uralochka	48±0.38	56±0.28	14±0.02
K-2192	47±0.34	57±0.26	14±0.02
Sarga	47±0.31	59±0.37	16±0.02
Khanshaim	48±0.28	55±0.34	14±0.02
Chaglinskaya 17	47±0.29	57±0.33	15±0.01
Rambler	46±0.29	56±0.32	15±0.01
Raduga	47±0.27	57±0.29	15±0.01

Several methods are used to create synthetic populations: ecological-geographical, evolutionary, and periodic selection. Their application is determined by breeding tasks and the crop's biological features. The method of periodic selection, the method of halves, or the method of reserves, as it is called by breeders, is used when the focus of breeding is quality. The evolutionary method is based on the fact that if breeding is conducted for traits controlled by natural selection, then it should be based on widespread cross-pollination of diverse genetic material (Annicchiarico et al., 2025).

Using ecological-geographical method, not isolated clones, lines, and biotypes are used as the starting material for cross-pollination, but entire populations that differ in origin. There is no consensus among breeders on the minimum and maximum number of components of synthetic populations to avoid noticeable depression from inbreeding and ensure uniformity of composition. According to A. Ivanova (Ivanova et al., 2023), their optimal number ranges from 3 to 15. Thus, a high-yielding synthetic variety of alfalfa Sinalfa consisting of 15 clones was created in Hungary. Other promising synthetic varieties of alfalfa were obtained: Sinbeta (seven biotypes), Singamma (eight biotypes) and Nematol (eight biotypes). Our methodology agrees with (Annicchiarico & Pecetti, 2021; Ruta et al., 2022) whose synthetic alfalfa variety consisted of clones within the established range of 3-15. Breeding practice shows that a synthetic population is usually created based on specially selected 6-16 biotypes. A sufficient degree of genetic integration of the resulting population is achieved by free cross-pollination of previously obtained biotypes. In our study based on the polycross method, we started work in various breeding areas. The evolutionary method is mainly used when breeding for traits controlled by natural selection (productivity, resistance to adverse environmental factors, diseases, and pests). The essence of the method is a wide and complete cross-pollination on a different genetic basis, ensured by attracting various source materials. Replanting such a complex population under certain conditions creates an opportunity for the action of natural selection and cross-pollination. As a result, a balanced genetically synthetic population adapted to a set of conditions is formed. Selection operates based on artificially created genetic diversity, enhanced by polycross, recombination of genes, and their segregation (Table 11; Table 12). For future research, integrating genomic selection as highlighted by Annicchiarico et al. (2022) that can help in identifying superior traits especially in stress prone areas. Genomic selection has been shown to be efficient in determining desirable traits, cost effective and requires less time (Annicchiarico & Pecetti 2021). This study's innovative breeding methods for alfalfa adaptation in North Kazakhstan are a testament to the power of combining traditional and modern approaches in crop improvement. Moving forward, we recommend integrating genomic tools and leveraging cross-disciplinary insights to further enhance the resilience and productivity of alfalfa varieties, ensuring sustainable agricultural systems for years to come.

Table 10: Promising alfalfa samples in terms of feed quality, % (on average for 2021-2023)

Variety, sample	Dry matter	Protein	Fat	Ash	Fiber	Humidity
Kokshe (st)	92.12	15.1	3.10	6.70	18.9	7.8
Uralochka	91.60	19.0	3.30	7.0	18.7	8.2
K-41422	91.53	18.2	3.30	6.80	19.5	8.8
K-930	92.17	17.5	3.10	6.70	19.0	8.8
SGP-02-21-9	91.40	18.7	3.40	6.70	26.8	7.7
Flora 6	91.37	18.0	3.40	6.60	20.1	8.7
Sarga	91.96	15.6	3.30	6.40	20.4	8.7
K-2192	91.87	16.8	3.30	6.80	17.6	8.9
Yaroslavna	91.21	17.8	3.40	6.80	18.8	9.3
SGP-5-11-12-7	91.76	19.3	3.40	6.50	23.0	8.2
Raykhan	91.81	18.2	3.40	6.60	17.3	9.8
SGP-04-09-3	91.42	18.6	3.30	7.00	21.4	7.7
Khanshaim	91.28	18.1	3.40	6.90	14.6	9.4
Chaglinskaya 17	90.85	17.8	3.20	6.90	13.9	8.6
K-45589	92.08	17.8	3.0	6.50	16.3	9.1
Karabalykskaya zhemchuzhina	91.39	17.0	3.10	7.20	20.0	9.1
SGP-09-10-7	92.20	21.5	3.40	6.90	19.5	8.0

Table 11: Promising varieties by seed yield and the main elements of its formation (2021-2023)

Population	Flowering vigor, days	Resistance to prolification, points	Bean setting rate, %	Regenerative ability, %	Seed yield, kg/ha
Kokshe	45	3	5.7	6.5	1.6
Flora 6	42	4	9.1	8.2	1.8
Karabalykskaya zhemchuzhina	42	4	6.6	7.2	1.7
Nurilya	40	5	5.1	8.1	1.9
Uralochka	42	4	9.4	6.3	1.8
Lazurnaya	40	5	9.2	16.0	1.8
Sarga	42	4	7.2	9.4	1.7
Baralfa	44	4	5.7	8.0	1.6
Raykhan	41	4	6.5	6.1	1.9
Raduga	40	4	7.2	7.4	1.7
Rambler	43	4	6.1	9.6	1.7
Viola	40	4	5.4	8.0	1.5
Khanshaim	42	4	6.1	14.1	1.6
Starbak	40	4	7.6	7.1	1.9
Omskaya 7	41	4	7.1	5.1	1.7
Bokkara	40	3	5.9	7.7	1.6
K-3793	41	3	5.1	8.4	1.3
K-2192	40	3	5.0	8.1	1.2
Least Significant Difference $(LSD)_{0.5}$					0.2

Table 12: Structure of seed productivity of alfalfa varieties (on average for 2021-2023)

Population	Quantity, pcs.			Weight of 1,000 seeds, g	Seed productivity, g/m^2
	racemes per shoot	beans in the inflorescence	seeds per bean		
Kokshe St	19.1	8.8	2.1	1.9	5.1
Viola	16.4	7.1	1.6	1.6	5.2
Lutsiya	17.5	7.9	1.9	1.7	5.3
SGP-02-21-9	16.2	6.7	1.7	1.9	5.6
SGP-09-10-7	18.9	8.2	1.8	1.9	5.8
Baralfa	16.6	7.9	1.7	1.7	5.2
SGP-04-09-3	19.6	8.2	1.8	1.9	5.7
Bokkara	17.3	7.7	1.6	1.7	5.2
Starbak	16.9	7.4	1.6	1.8	5.3
Omskaya 7	19.1	8.9	1.9	1.8	5.2
Sarga	19.7	8.7	1.8	2.0	5.7
Rambler	18.1	7.9	1.6	1.7	5.4
Karabalykskaya zhemchuzhina	17.6	7.9	1.6	1.7	5.0
Khanshaim	19.8	8.7	1.7	1.9	5.2
SGP-5-11-12-7	18.6	7.7	1.8	2.0	5.8
Uralochka	19.8	8.2	1.9	2.1	5.6
Raykhan	19.2	8.1	1.7	2.1	5.5
Raduga	18.9	7.9	1.7	1.8	5.3
$LSD_{0.5}$					0.5

Conclusion

The samples derived in the study are the basis for hybridization conducted to increase the seed productivity of alfalfa and overcome the instability of varieties facing adverse environmental factors. According to the two cycles of the CHP assessment, the following numbers were the most promising: SGP-4-11-15-2, SGP-10-11-14-5, SGP-9-11-6-6, SGP-5-11-11-1, SGP-4-11-10-8, SGP-9-11-17-2, and SGP-5-11-12-7. Using the developed method of breeding alfalfa with increased seed productivity, we created the promising alfalfa number SGP-04-09-3 characterized by high feed and seed weight. The excess over the standard in hay yield was 28% and in seeds, 133%. The variety showed winter hardiness, drought resistance, and a relatively high percentage of self-pollination, regenerative ability, and bean-setting rate. This confirms the high efficiency of the polycross method. The period of creation of a new variety is reduced from 18-20 years with classical breeding to 12-14 years. The experimentally verified source material in the form of complex hybrid populations may later be used in practical breeding in North Kazakhstan.

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