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RESEARCH ARTICLE
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Substantiation of the Basic Biological Properties and Economically Valuable Features of the Alfalfa Population Model with High Seed Yield

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ABSTRACT

One of the main indicators of the value of an alfalfa variety is high seed productivity. At its low rates, it is impossible to expand the acreage of the plant further. The varieties zoned in North Kazakhstan with a sufficiently high yield of feed mass are characterized by relatively low seed productivity. Therefore, the study aimed to select, evaluate, and create a new experimental starting material for breeding alfalfa plants with increased seed productivity. The experiments were conducted in 2021-2023 at the Kokshetau Experimental and Production Facility located in the steppe zone of North Kazakhstan. 18 of the most valuable samples in terms of a complex biological and economic characteristics were identified for further breeding work. Their assessment helped identify promising alfalfa populations, combining high seed productivity and resistance to adverse environmental factors. Thus, among the studied variety samples, the variety samples Rambler, Nurilya, Sarga, Uralochka, Flora 6, Lazurnaya, Starbak, Karabalykskaya zhemchuzhina, Raduga, Raykhan, K-3793, and K-2192 stood out with the structure of seed yield, bean setting rate, resistance to prolification, and flowering vigor. In further breeding work on the formation of complex hybrid populations, it is recommended to use the selected alfalfa variety model with optimal values of the listed characteristics, providing an increased yield of green mass and seed productivity of the plant.

Keywords: Alfalfa, Source material, Selection, Seed productivity, Polycross method, Complex hybrid population, Assessment, Model, Sample, Bush shape, Bean setting rate, Self-pollination, Resistance to prolification, Regenerative ability

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INTRODUCTION

Animal husbandry is a priority branch of agriculture in Kazakhstan. Increasing the number of livestock requires creating a solid forage based on high-yielding varieties and using advanced agricultural technologies.

An analysis of the state of feed production development in recent years shows that the low productivity of forage lands, especially in years unfavorable in terms of moisture conditions, does not allow for providing a sufficient supply of the available farm animal livestock with complete feed. Perennial grasses with valuable biological and economic properties play an important role in increasing the efficiency of the production of high-protein and energy-saturated feeds.

Perennial leguminous grasses in different areas of Kazakhstan are cultivated in field rotations and forage crop rotations on long-term cultural pastures and hayfields. The system of measures to increase feed production includes the introduction of new varieties of perennial grasses that meet the current level of agricultural development.

Therefore, strengthening the livestock forage base in Kazakhstan is inextricably linked with the cultivation of alfalfa, the most valuable long-term crop globally. Alfalfa is a high-protein forage crop that has great practical value in field and meadow grass cultivation. Over the years, different cultures and peoples have shown interest in alfalfa, and the area under this crop is increasing annually.

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Today, alfalfa is cultivated on an area of more than 30.0 million ha on the scale of world agriculture. The largest areas are concentrated in North and South America, making up more than 18.9 million ha, or 64% of the global area. In Europe, this number is more than 7.12 million ha, in Asia 2.23 million ha, in Africa 170.0 thousand ha, and in Australia and Oceania 125.0 thousand ha.

The wide geographical distribution of the crop is explained by its adaptability to various climatic and soil conditions. Its unique biological properties namely perenniality, multifocality, the ability to fix biological nitrogen, responsiveness to irrigation, high productivity of feed mass, and the protein content with a balanced amino acid composition, allowed alfalfa to be called the "queen" of forage crops in many countries. It can produce protein up to 3 t/ha and is a source for many feeds, namely, green mass, hay, haylage and vitamin and grass meal (Yuegao & Cash, 2009; Riday, 2011; Shpaar, 2011). In Kazakhstan, alfalfa is the main forage crop and the area of its cultivation at various times reached up to 1.2 million ha (Yuegao & Cash, 2009). However, in the structure of the acreage of perennial grasses in the arable land of the hill-plain zone of North Kazakhstan, alfalfa occupies insignificant volumes. Its wider spread is hindered by the low and unstable seed productivity of the crop and the associated acute shortage of seeds. This factor is due to the lack of alfalfa varieties that fully meet the requirements of intensive farming in the region.

In North Kazakhstan, the locally selected variety Kokshe has become widespread. The following are also cultivated: Karabalykskaya raduga, Shortandinskaya 2, Karagandinskaya 1, Lutsia 14, Raykhan, Lazurnaya and others. However, the varieties have poor winter hardiness and drought resistance and are affected by pest and disease infestation, which is why they have not become widespread in the region. Therefore, there is a need to develop new intensive alfalfa varieties with consistently high seed productivity.

New promising alfalfa varieties in the regions of North Kazakhstan should have the following indicators and properties: high yield of feed mass (40-50kg/ha of hay without irrigation and up to 100kg/ha with irrigation) combined with high protein content (17-19%) and sufficiently high (2-3kg/ha) and stable level of seed productivity. The varieties should also have high winter hardiness and drought resistance, longevity in use, intensive post-harvest regrowth, and resistance to major diseases and pests.

The study aims to identify new experimental material for the creation of alfalfa varieties with high seed yield and to analyze its breeding value for use in the agricultural industry of North Kazakhstan.

MATERIALS & METHODS

The study was conducted in 2021-2023 at the Department of Experimental Crop Production of the Kokshetau Experimental and Production Facility located in the village of Shagalaly, Zerendinsky district, the Akmola region, Kazakhstan (Fig. 1) (54.178132/ 69.523074).

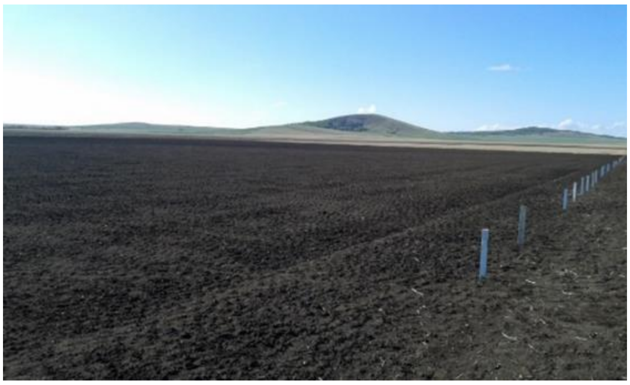

Fig. 1: Experimental field of Kokshetau Experimental and Production Facility.

The soil of the experimental site was ordinary chernozem, moderately deep, with medium humus content; such soils make up most of the soil cover of the region. The arable horizon reached 34cm; below, there was a transitional horizon B (14-20cm) of dark gray color, 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 equaled 4.71% (determined according to Tyurin), pH of the medium: 7.1-7.5, mobile forms of phosphorus: 2.16, potassium: 40.9 (determined according to Machigin), nitrogen: 3.21 (Grandval-Lajoux) mg per 100g of soil. The nitrogen content was average, phosphorus content was low, and potassium content was high. The soil had significant reserves of total forms of nitrogen and phosphorus.

The preceding crop was black fallow. Tillage was carried out according to zonal technology. All nurseries were established on black fallow without coverage in the spring (in the first decade of May) manually. Sowing was carried out in a square-cluster (70x70cm) method in wide-row crops with a manual RS-1 seeder (Fig. 2).

Fig. 2: Sowing on experimental plots.

The nursery of the source material was sown periodically as new crop materials were accumulated from other research institutes and expeditions to collect wild forms. From 2021 to 2023, the collection nursery was established three times, with 46 samples each year.

The sowing in breeding nurseries was performed in spring (May). The nurseries were established on black fallow without coverage in spring manually. The soil was represented by ordinary chernozem with a medium humus content, the depth of humus horizon equaling 25-27cm, and an average humus content of 4.01%.

The method of sowing in a collection nursery, in a nursery of complex hybrid populations (CHP), was performed using the square-cluster technique (70x70cm). In the control nursery (CN) and the nursery of competitive variety testing (CVT) for seeds, the sowing was wide-row (with a row spacing of 70cm). Each number in the collection nursery and the CHP occupied $5m^2$ in six repetitions. In the CN, all numbers were established in 6-fold repetition, and in the CVT, in 8-fold repetition. The area of plots in the CN was $10m^2$ and in the CVT nursery 25m2.

The zoned alfalfa variety of the local Kokshe selection was adopted as the standard. Standards in breeding nurseries were placed after 10 numbers. The plants were treated manually and mechanically. The side protective strips equaled 0.7m; the end ones were 10m. The total area of breeding crops was 1ha, and the preliminary reproduction of promising numbers was 0.2ha.

The design of the breeding process, the establishment of nurseries, and variety evaluation, hybridization, selection, and testing were carried out according to the methodological guidelines for the study of the perennial grass collection developed by the All-Russian Institute for Plant Breeding (Feoktistova, 2023), the guidelines for the selection of perennial grasses by the All-Russian Feed Institute (Kosolapov et al., 2021), the guidelines of the Siberian Feed Research Institute (Bekuzarova et al., 2021) and the methodology of the state crop variety testing (Liveseed, 2020).

To evaluate the studied forms, records, observations, and analyses were carried out in nurseries according to generally accepted methods of working with perennial grasses, including the methodology "State crop variety tests" issued by the State Commission for Crop Variety Testing under the Ministry of Agriculture, and "Methodological guidelines for the selection of perennial grasses" developed by the All-Union W.R. Williams Feed Research Institute.

Mass and family group selection have become the main selection methods in hybrid populations. Every year, 600-800 numbers of two breeding process schemes were evaluated according to a set of characteristics.

The harvesting of the selected numbers and the accounting of the yield in the nurseries were carried out manually. The threshing of the selected sheaves was carried out on stationary laboratory threshing machines. During the growing season, two field and one laboratory screenings were carried out.

Phenological Observations

In the second and subsequent years of the plant's life, we noted the dates of the beginning and full spring regrowth; the nature of regrowth (vigorous, slow); the intensity of regrowth on a five-point scale (1: very weak, 2: weak, 3: average, 4: good, 5: excellent); budding of legumes; the beginning of flowering; the date of cutting; the beginning and full regrowth after cutting; termination of vegetation; the phase of plant development before winter; the state of the herbage. Additionally, the dates of full flowering, the beginning and full fruit formation, maturation, and cutting of the seed herbage were noted.

Observations were made every other day, and at the onset of the main phases (budding, flowering) they were made daily. The phase of plant development was noted in two non-adjacent repetitions, after which the average date of its onset was derived. The beginning of the phase was its onset in 10% of the samples, and the full phase was the onset in 75% of the total number of plants in the plot.

During flowering, its duration was determined, and the number of flowers was calculated. The duration of flowering of the raceme depends on the location of the racemes on the plant, therefore, racemes from the lower, middle, and upper parts were selected for observation. The study was carried out three times on the same plants where the flowers were counted.

Accounting for the Density of Herbage

To determine the herbage density, the number of plants and stems per area unit was calculated. For this purpose, permanently fixed platforms were used, the size of which in various nurseries was at least $0.25\text{-}0.5m^2$. The shape of the platforms was square or elongated. Linear measures were also used, $0.5\text{-}1m^2$ of the seeding row. The counting by varieties was repeated at least 3-4 times.

Determination of the Height of the Herbage

In field studies, the height of the herbage served as an indirect yield indicator. Heights were defined as the distance from the soil surface to the end of the inflorescences of plants, without stretching the shoots. The length of the plant from the base to the tip of the elongated stem was determined for legumes. 10 measurements were carried out on each plot. During lodging, the height of the fallen herbage was additionally determined without stretching the stems. Moreover, the shape of the bush was recorded as the main constitutional feature in determining the variability of morphobiological traits of alfalfa.

Productivity of the Green Mass

The productivity index of green and dry mass was determined in collection and breeding nurseries at the time of individual plant counting. Productivity was expressed in terms of one or more plants (g, kg) or a limited area ($1\text{-}3m^2$) with further conversion of yield to the value ofc/ha.

When evaluating the variety, the green mass of all plants from the plot was weighed, and their total number was determined for subsequent recalculation per plant. From the total mass of cut plants, a sample was taken from each variety to determine dry matter content and structural analysis.

Yield Recording

The main indicator of the value of the varieties was indicated by the yield of the feed mass, calculated in the phase of the beginning of flowering of legumes during cutting. The yield was recorded by direct weighing of the entire green mass from the plot obtained immediately after cutting and taking a test sheaf to determine the yield of hay, the foliage of the herbage, and the structure of the crop. The test sheaf was collected in a gauze bag and dried to an air-dry state. The sample was then weighed until its mass became constant during the control weighings.

Determination of Foliage

To determine the foliage, a sample weighing 1 kg was taken in different parts of the plot. The resulting samples were then divided into two fractions: leaves and inflorescences/stems. The leaves were isolated along with the petioles.

Crop Structure

To determine the crop structure, a test sheaf weighing 1 kg was selected. The shoots found in the sample were divided into the following categories:
1. Generative, bearing inflorescences (in leguminous grasses, the stem with all lateral branches was taken as shoots);
2. Elongated vegetative stems with clearly elongated internodes, but not bearing inflorescences;
3. Shortened vegetative stems without elongated internodes and inflorescences (rosettes).

After analyzing the entire sample, the shoots of each category were counted, after which an analysis of the crop structure was carried out. The individual shoot categories were also divided into fractions. Thus, generative shoots were divided into inflorescences (the stem with all lateral branches) and leaves (with petioles). The elongated vegetative shoots were divided into the straw (stem) and leaves. The shortened vegetative shoots were not separated. Each fraction was then weighed on a technical scale with an accuracy of 0.1g.

To analyze the structure of the seed stand, samples were taken from 0.25m^2 plots in 4-fold repetition before harvesting seeds on plots. When analyzing the samples, the number of generative shoots per unit area, the number of inflorescences (on average on 25 shoots), the number of seeds on inflorescences (for this 10 inflorescences were taken without selection), the number of seeds, and the weight of 1,000 seeds (2 samples of 500 seeds each) were calculated. After threshing and cleaning the entire sample, the yield of full seeds was also determined as a percentage. Chemical analysis to determine the nutritional value of dry weight for the content of crude protein was carried out using the Kjeldahl micrometer.

The mathematical processing of the results was carried out using a personal computer and standard software. Statistical processing, in particular, variance and correlation analysis, was carried out using the methods developed by B.A. Dospekhov (Jha et al., 2021).

RESULTS

As a result of research on alfalfa, its morphological and economically valuable characteristics, and trends in the development of breeding and production requests, we compiled a model of a new type of alfalfa variety populations with high seed yield (Table 1).

The main parameters of the characteristics of the model alfalfa variety with increased seed productivity are as follows: yield of green mass: 120-150c/ha, seeds: 1.6-3.3 c/ha; bush shape: erect; winter hardiness: 95-100%, drought resistance: 5 points; self-pollination: 10-13%; bean setting rate: 25-39%; the regenerative ability of biotypes with high seed productivity: 66-74%; tilling capacity: 34-44 stems; shedding (flowers, beans): 0-2 points; flowering duration: 30-36 days; susceptibility to diseases (a complex of spot diseases): 1-2 points; plant height: 77-84cm. The correlation coefficient between seed productivity and trait parameters is within the range of r=0.5-0.9. Further description and characterization of the samples according to morphobiological characteristics are given for promising populations selected for further breeding work.

The Shape of the Bush

Based on the shape of the bush, three eco-elements are distinguished in different populations: 1: erect, 2: spreading, 3: sprawling. In plants with an erect bush shape, several identical stems extend from the root neck almost at an acute angle, parallel to each other. This bush shape prevailed in 54.9-90.4% of plants in alfalfa populations. In 44.8 to 71.7% of plants with a sprawling bush shape, in addition to a well-defined main stem, less developed stems of different heights also branch off in different directions. The spreading shape of the bush is characterized by the absence of a clearly defined main stem, the presence of several stems of the same height, extending in different directions and strongly inclined. The proportion of plants with this form of bush was 51.3-61.4% (Table 2).

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

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

The erect shape of the bush was noted in the varieties Chaglinskaya 14, Chaglinskaya 17, Khanshaim, and Nurilya, which, along with high feed productivity had increased seed productivity. The sprawling shape of the bush was noted in the varieties Kokshe, Rambler, Sarga, Uralochka, Karabalykskaya raduga, Viola, Karabalykskaya zhemchuzhina, Raykhan, Lazurnaya, Starbak, Bokkara, Kokorai, Baralfa, and Lutsia. They had a high vegetative mass and a relatively average seed yield.

The spreading shape of the bush was characteristic of varieties of West Siberian origin, which had high winter hardiness, but average seed yield due to their lodging and difficulties for harvesting with a combine harvester.

The Duration of the Flowering Period

According to the results, the flowering time of one alfalfa flower averaged 4.8 days, one raceme 7.8, and one plant 17.9 days (Table 3).

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

Omskaya 7, Nurilya, Flora 6, Khanshaim, Chaglinskaya 14, Chaglinskaya 17, Karabalykskaya zhemchuzhina, and Karabalykskaya raduga were characterized by a relatively short flowering period (3.5-3.9 days). The longest flowering period was observed in K-41422, Lazurnaya, K-930, K-2192, K-3793, Sarga, Uralochka, and K-45589 (4.0-4.8 days).

Disease Resistance

The susceptibility to powdery mildew in alfalfa varieties was different (Table 4). Most samples were included in the moderately resistant group with a damage score ranging from 4 to 6. Among them were the varieties Kokshe and Raykhan, zoned in the study area.

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		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	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

In different years, leaf spots also appeared on alfalfa crops. the maximum number of alfalfa variety sample plants affected by leaf spot was noted in 2021, when favorable conditions were developed: morning dew, rains, and long periods with high temperatures. The assessment of the alfalfa collection nursery showed that the majority of populations had a leaf spot damage value similar to the Kokshe standard (Table 5). The smallest number of affected plants was observed in the populations of Lazurnaya, Karabalykskaya raduga, Uralochka, and Sarga.

Alfalfa plants were also affected by root rot. Plants were most often affected in the fall of the first year of life, which is why they did not grow back in the spring of the following year, making it difficult to evaluate the samples by overwintering. Diseased plants were visible among healthy bushes: they easily detached from the root neck, which rotted and became crumbly.

Tilling Capacity

According to the results, yellow alfalfa was characterized by low tilling capacity. The average tilling capacity was mainly noted in local populations of variegated alfalfa. Relatively few specimens had high tilling capacity. According to the number of stems on the bush, alfalfa samples were distributed as follows:

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

The first structural group with an erect bush shape was characterized by relatively weak tilling capacity (no more than 3-9 stems); Populations with a sprawling bush shape had an average tilling capacity (10-14 stems); Alfalfa samples with a spreading bush shape had an increased number of stems in the bush. Here, they exceeded the representatives of the first group by 7-10 times (Table 6). The number of plant stems is the most important distinctive morphobiological feature and is decisive in the crop structure of forage mass and seeds.

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

The Setting Rate of the Beans

In the 2021-2023 studies, the following samples showed the highest results under conditions of free cross-pollination: Nurilya, Yaroslavna, SGP-02-21-9, SGP-04-09-3, SGP-09-10-7, and Rambler. The varieties had a bean setting rate of 10.4-29.4% (Table 7). The bean setting rate of the Kokshe standard was 5.7%; for other known varieties, it ranged from 7 to 9%. The results showed a direct correlation (r=0.40-0.95) between the bean setting rate and the seed yield of alfalfa samples under conditions of free cross-pollination. Samples with a high percentage of bean setting rate usually produced more seeds.

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

Self-pollination

The study of the population composition of 100 plants of each variety allowed us to identify and select the necessary biotypes with a high level of self-pollination. The most promising biotypes from the variety samples concerning self-pollination include the Severo-Kazakhstanskaya 8 (5 plants) and new complex hybrid synthetic populations of SGP-09-10-7 (13 plants), SGP-02-21-9 (12 plants) and SGP-04-09-3 (14 plants). Thus, 25% of the biotypes from the selected populations were self-pollinating (Table 8).

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

These elite biotypes, which show seed productivity even under unfavorable conditions of flowering and fruit formation, are a promising basis for the future of a new variety of alfalfa with high seed yield.

The Structure and Quality of the Vegetative Mass

The results showed that, by the structure of the vegetative mass, the varieties Karabalykskaya zhemchuzhina, Omskaya 7, K-45589, K-41121, K-43833, Viola, Flora 6, Nurilya, Raykhan, and Lutsia had the greatest foliage (Table 9).

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

The maximum plant height was shown by the samples of Flora 6, Nurilya, Raykhan, Karabalykskaya zhemchuzhina, Omskaya 7, Viola, Lutsia, and Sarga. The samples of Sarg, Viola, Lucia, Flora 6, and Nurilya showed a high tilling capacity. The general view of the alfalfa herbage is shown in Fig. 3.

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.

The Quality of the Green Mass

Analysis of the chemical composition of alfalfa varieties and populations from the collection nursery indicates a different nutrient content in the plants. A comprehensive analysis of quality indicators allowed us to identify 17 alfalfa samples that exceeded the Kokshe standard (Table 10).

The largest amount of dry matter was observed in samples of K-930, K-45589, and SGP-09-10-7. The maximum protein content was shown by the samples of Uralochka, K-41422, SGP-02-21-9, SGP-5-11-12-7, SGP-04-09-3, and SGP-09-10-7. The samples of Khanshaiym, Chaglinskaya 17, SGP-09-10-7, Karabalykskaya zhemchuzhina, K-2192, Yaroslavna, and K-41422 showed a high ash content. The smallest amount of fiber was found in the samples of Khanshaim, Chaglinskaya 17, K-45589, K-2192, and Uralochka.

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

Yield and Structure of the Seed Herbage

As a result of the study, promising crop samples were identified for the main elements and seed yield in an average of three years. Among the studied crop samples in terms of seed yield, namely, flowering vigor, resistance to prolification, bean setting rate, and regenerative ability, biotypes from the population of Rambler, Nurilya, Sarga, Uralochka, Flora 6, Lazurnaya, Starbak, Karabalykskaya zhemchuzhina, Raduga, Raykhan, K-3793, and K-2192 showed the best results (Table 11).

As a result, 18 varieties and samples were selected for further breeding work.

The quality of the seed material was determined by measuring the weight of 1,000 seeds. Accordingly, the larger the seeds were, the higher the yield of alfalfa seeds. According to the study data, the weight of 1,000 seeds of the studied varieties ranged from 1.6 to 2.1 g.

The number of racemes per shoot, beans in the inflorescence, seeds per bean, and the weight of 1,000 seeds are important indicators in assessing seed productivity. During the years of the study, the number of racemes per shoot ranged from 16.2 to 19.8, the number of beans in the inflorescence was from 6.7 to 8.9, and the number of seeds per bean was 1.7 pcs. (Table 12).

The correlation between morphobiological characteristics and alfalfa yield varied depending on meteorological conditions during the years of the study. The correlation between the weight of 1,000 seeds and yield was characterized by a weak positive dependence r=0.21±0.10.

The morphological features of alfalfa and its requirements for environmental conditions during fruit formation result from significant differences in the biology of its flowering and pollination and depend on meteorological factors. There is an opinion that alfalfa is a "whimsical" crop, and the productivity of its seeds depends more on the weather conditions of the year than on agricultural techniques. This opinion largely corresponds to the actual state of things. Therefore, during breeding activities, it is necessary to consider the conditions and factors contributing to the abundant flowering and fruiting of alfalfa.

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

Among such factors, it is worth mentioning diseases caused by fungi and viruses that affect variegated alfalfa (*M. varia* Mart.). For example, vegetative organ disease significantly affects the yield of seed alfalfa. Alfalfa, in comparison with other forage crops, is characterized by general resistance to diseases and pests. Despite this, the reason for the decrease in yields and feed qualities of alfalfa varieties is often the susceptibility to damage from diseases and pests.

The yield of alfalfa seeds decreases by 41-52% with moderate to severe leaf spot damage and by 42-61% with leaf rust damage. Numerous studies have shown that alfalfa culture affected by viral and fungal diseases accumulates less organic substances than healthy plants. This phenomenon is explained by a decrease in photosynthetic energy and an increase in the respiration energy of the plant.

One of the most common diseases of alfalfa is powdery mildew. It manifests as a whitish cobweb coating on the leaves of affected plants in the flowering phase. The powdery mildew is dangerous because it weakens the growth processes in alfalfa plants, makes leaves and ovaries fall prematurely, and results in severe deterioration of feed qualities and seed productivity. Furthermore, plants affected by powdery mildew are not eaten by animals in their green form and cannot be used to prepare high-quality hay.

The alfalfa yield conditions include the structure of its varieties. Unlike other legumes, in the first year of life, alfalfa after emergence, does not form a rosette of leaves but a single slowly growing stem. The aboveground part dies off in late fall, and only the roots overwinter. In the upper part of the roots, the buds for renewal are established.

In Sagalbekov's studies (1994), most of the samples, particularly wild samples of variegated alfalfa, had a low tilling capacity of about 3-5 stems. Wild species were also characterized by low tilling capacity, amounting to about 2-3 stems per bush.

According to the results of a more detailed analysis, low seed yield and bean setting rate (under 5-7%)are among the main limiting yield traits that depend on soil and climatic conditions. The combination of air,temperature, humidity, and the sum of active temperatures significantly affect the intensity of pollination, the completeness of the bean setting, and the number of set seeds in the beans. Therefore, the yield of alfalfa seeds at a significant level depends on the seed setting in the flower.

According to the model of future varieties among the studied variety samples, the following were distinguished: by the shape of the bush: Chaglinskaya 14, Chaglinskaya 17, Khanshaiym, Nurilya; by the duration of the flowering period: Omskaya 7, Nurilya, Flora 6, Khanshaiym, Chaglinskaya 14, Chaglinskaya 17, Karabalykskaya zhemchuzhina, and Karabalykskaya raduga – varieties with a short flowering (3.5-3.9 days) and K-41422, Lazurnaya, K-930, K-2192, K-3793, Sarga, Uralochka, and K-45589 – varieties with the longest flowering period (4.0-4.8 days).

In terms of resistance to pests and diseases, K-502, K-930, K-42340, K-39112, Rambler, Bokkara, Baralfa, Lutsia, Chaglinskaya 14, Chaglinskaya 17, Khanshaiym, and Raykhan showed the best results. In terms of tilling capacity, Khanshaiym, Chaglinskaya 14, Chaglinskaya 17, Nurilya, K-39112, Sarga, Uralochka, Karabalykskaya raduga, Viola, Karabalykskaya zhemchuzhina, Raykhan, K-45589, K-930, and K-41422 showed the best results. Flora 6, Karabalykskaya zhemchuzhina, Omskaya 7, Viola, Lutsia, Sarga, and Nurilya were distinguished by yield and quality of vegetative mass. A comprehensive analysis of quality indicators allowed us to identify 17 alfalfa samples that exceeded the standard of the North Kazakhstan Kokshe variety.

DISCUSSION

Our results from this study on substantiating the basic biological properties and economically valuable features of the alfalfa population model with high seed yield align with recent research trends on sustainable and high-yielding forage crops. Our objectives agree with the work of these authors (Tucak et al., 2023), who concluded a multi-year trial to identify top-performing seeds in the alfalfa population in various climatic conditions. We also agree with studies (Lin et al., 2023) identifying yield-related and resistance traits as important targets when breeding alfalfa plants, especially in regions with fluctuating climatic conditions.

Our model enhanced alfalfa with increased productivity agrees with the work of these authors (Wang & Zhang, 2023), who highlighted the importance of developing a new model of alfalfa planting by mixing several alfalfa varieties with contrasting phenotypes and genetic backgrounds.

Our results highlight the shape of the bush as one of the most essential morphological characteristics for a promising population selected for further breeding. This finding agrees with the work of (Abdushaeva, 2024), who concluded that the basis for selection for elements of seed productivity was the shape of the bush since it affected the formation of generative shoots, resistance to lodging, and the possibility of regenerating the herbage by seed under natural growing conditions.

Our study's focus on winter hardiness and drought resistance is aligned with the current research trend, which identifies these traits as essential for alfalfa varieties in the harsh climates of Kazakhstan and similar regions. We agree with (Xu et al., 2021), who employed a multi-site evaluation to highlight the importance of winter hardiness as a criterion when selecting seeds for alfalfa breeding. We also agree with (Vozhehova, 2021), who highlighted drought resistance and the ability to survive drought with the lowest yield reduction as another vital factor to consider during selection.

In line with recent research on disease resistance of forage crops, our results show that the alfalfa populations under study showed low susceptibility to powdery mildew and leaf spot. We agree with the authors (Yang et al., 2022), who reviewed the theoretical basis of plant immunity and the genes responsible for disease resistance of alfalfa plants, thereby supporting the breeding of resistant varieties and exploring the gaps in genetic editing of alfalfa breeds. This also supports our conclusion that incorporating varieties with these specific traits could increase seed productivity in low-pollination environments.

In this study, we observed flowering duration of 30-36 days and bean-setting rates of 25-39%. This is consistent with research exploring how these traits contribute to higher seed yields, such as (Putnam, 2021; Qin et al., 2022), who concluded that delayed flowering stages are necessary when selecting model alfalfa varieties.

The study’s observation of tilling capacity and its correlation with seed yield aligns with recent research identifying tilling as a vital morphological characteristic influencing forage quality. It also agrees with the works of (Magomedova et al., 2021; B. Wang et al., 2024), who highlighted the importance of tilling capacity and practices to improve the growth of alfalfa in semi-arid regions.

This study proposes that the introduction of alfalfa varieties exhibiting high seed productivity and disease resistance could address Kazakhstan's forage crop limitations and aid in building a stable livestock forage base (Bastaubayeva et al., 2023; Ahmed et al., 2023).

Future research could focus on further integrating gene-editing techniques to refine these desirable traits, as recent works suggest this could facilitate even more significant improvements in seed productivity and stress resistance (Zheng et al., 2022; Chen et al., 2024). Adapting our model to other regions with similar climates could yield insights that further optimize alfalfa as a globally essential crop.

Conclusion

As a result of the study on the set of biological properties and economically valuable traits, 18 samples of alfalfa plants were selected for further breeding work. Based on the study of the gene pool based on a complex of traits and factors of reducing seed productivity, a model of a variety with increased seed yield was created.

Pollination and the formation of alfalfa seeds are greatly facilitated by the temperature and relative humidity of the air, the amount of precipitation, the number of sunny clear days during the flowering period, and the fruit formation. These factors contribute to productivity not separately but in a comprehensive way.

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