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REGULATION OF INTRAPOPULATION HETEROSIS WITH INCLUSION OF INBRED LINES FOR DEVELOPING SYNTHETIC CULTIVARS IN ALFALFA (*MEDICAGO SATIVA* L.)

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SUMMARY

The conducted experiments sought to create inbred alfalfa (*Medicago sativa* L.) lines based on the study of self-fertility, autotripping, inbreeding depression, and the general combining ability of lines in inbreeding generations from the first to the seventh generation. The self-fertility trait showed manifestations in all samples with a high value of up to 100% during artificial flower opening. Given the level of "autotripping" is insignificant (up to 5%), it limited self-fertility in freely propagated populations. The efficiency of inbreeding was successful in their establishment up to the second and third generations, where the lines achieved the highest value of the general combining ability effect. The developed promising lines served as a genetic basis for the formation of high-yielding cultivars (Kokbalausa, Kokoray, Osimtal, Kokshalgyn, and Shabyt 80) with the optimum effect of intrapopulation heterosis.

Keywords: Alfalfa (*M. sativa* L.), heterosis, self-fertility, autotripping, inbred lines, intra-populations, general combining ability, synthetic cultivars

Key findings: In alfalfa (*M. sativa* L.), the inbred lines have succeeded in developing with their optimal level of inbreeding. For developing synthetic cultivars, the use of inbred lines continued through GCA and intrapopulation hybridization and heterosis. Five cultivars of alfalfa have reached formation and inclusion in the State Register of Cultivars Approved for Cultivation.

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INTRODUCTION

Alfalfa (*Medicago sativa* L.) is a perennial herbaceous legume, widely cultivated as a forage crop for livestock due to its high protein content and adaptability. Alfalfa provides a high-quality forage as hay or silage and, to a lesser extent, as a grazing crop. It also served as a cover crop and green manure, improving soil fertility by fixing nitrogen. Alfalfa is popular for its deep roots, which help enhance drought resistance and prevent soil erosion. In the farming community of Kazakhstan, alfalfa has earned recognition as the high-protein forage crop. In crop rotation effects, it promotes the accumulation of biological nitrogen in the soil, suppresses the growth of weeds and pests development, and improves soil structure and fertility. Alfalfa has an important soil-improving and environmental significance, contributing to the sustainable development of agriculture and forage production (Lancefield *et al.*, 2009; Yue-Gao, 2009; Riday, 2011; Adhikari, 2017).

Alfalfa is an entomophilous pollinated plant, with its pollens transferred by bees of the genus *Andrena*, which facilitates effective cross-pollination. This, in turn, maintains the intra-population heterosis, which serves as the considerable genetic basis for heterotic effects in open-pollinated populations (Hu *et al.*, 2016; El-Rouby *et al.*, 2017). Self-pollination is a very rare phenomenon in alfalfa, which can refer to the flower's anatomical structure, self-incompatibility, and the absence of autotripping—automatic opening of the flower without the mechanical action of bees (Meirman and Masonich-Shotunova, 2012).

The flower structure and genetic mechanisms in the phylogenetic development sought to maintain the heterozygosity in alfalfa. The same was also evident in the fact that the individual selection did not lead to the desired and fruitful results in alfalfa. Alfalfa cultivars function as complex hybrid populations consisting of different biotypes, individuals, and their mutual cross-pollination, providing the highest viability based on the manifestation of 'multiple heterosis.' The concept of multiple heterosis originated from Palilov (1976) to characterize the synthetic cultivars of cross-pollinated crops, with a

change of generations from the original under conditions of panmictic pollination. This definition is quite applicable to alfalfa, since it is descriptive of a high degree of panmixia—free crossing, occurring as a result of polycross cross-pollination among the structural individuals forming a population. Such genetic diversity promotes the manifestation of stable heterotic effects and ensures the adaptability, productivity, and stability of cultivars under various environmental conditions.

The idea of developing simple F_1 hybrids in alfalfa at the interlinear and inter-varietal levels with open cross-pollination, while using the CMS form for this purpose as in maize crops, emerged as impractical due to the crop's low multiplication coefficient (Bouton, 2012). A promising direction in alfalfa breeding remains the development of synthetic cultivars (Brummer, 1999). The methodological features of synthetic cultivars are that original forms undergo evaluation and selection according to the general combining ability (GCA) before including them in the composition of newly developed alfalfa cultivars. The average indicators of parental lines for combining also indicate the action of adaptive genes (Sprague and Tatum, 1942). Therefore, this is more important in the selection of alfalfa synthetics than the specific combining ability; however, the latter one is more crucial in developing paired hybrids.

Individual genotypes, biotypes, and inbred lines that have undergone evaluation and selection for economically valuable traits served as initial forms of synthetics. From the genetic viewpoint, it was more effective to use inbred lines that have passed through evaluation in generations of inbreeding and selection for general combining ability (Riday and Brummer, 2002). Inbred lines reached development through artificial self-pollination based on the principles using the trait of self-fertility. Selection of inbred lines and their use in the population structure of synthetic cultivars entailed a) study of self-fertility and autotripping (the ability of flowers to spontaneously open and pollinate without the participation of pollinating insects); b) analysis of inbreeding depression and multi-stage selection for economically valuable traits; and

c) evaluation of the inbred lines through GCA, and then by crossing with other lines to obtain the productive offspring. Formation of synthetics relied on selected inbred lines with high GCA to obtain stable and high-yielding alfalfa cultivars.

MATERIALS AND METHODS

The development of inbred lines in alfalfa (*M. sativa* L.) utilized plants with the self-fertility trait through artificial self-pollination in several inbreeding generations (J_0 – J_7). The study used a population of the alfalfa local cultivar Semirechenskaya to conduct the experiment. For practical and effective selection, the experiment chose plants of the population from 155 best samples by productivity and other valuable traits. The inbreeding depression levels of the traits in succeeding generations entailed determining inbreeding in a comparative experiment with the original forms. For this, the lines from different generations involved studies under the conditions of the same sowing year, which made it possible to objectively assess the degree of inbreeding depression under identical growing conditions.

The manifestation of autotripping incurred observation using an 'odor background' that repels pollinators. Observations continued on flowers with a sample size of at least 100 plants. The assessment of the overall combining ability engaged the top cross-crossing method with testers, with plants isolated from the alfalfa cultivars Semirechenskaya Mestnaya, Iolatskaya 1763, and Mestnaya from China (k-32860). By starting the crossing, adult buds

attained preliminary isolation with a gauze isolator to increase the number of flowers ready to open. At the same time, before the flowers opened, the unsealed inflorescences of the paternal parent entailed placing them under the sail (corolla) of the maternal parent. Alfalfa flowers open with great force, and the pistillate-stamen apparatus, hitting the sail, creates conditions for the germination of foreign pollen. In alfalfa, foreign pollen germinates faster than its own, and on this biological basis, the hybridity of the resulting seeds occurs. After crossing, removing isolators followed, since bees do not visit already opened flowers. The volume of crossing for each combination was at least 100 flowers.

Using dispersion analysis, the total phenotypic variability of the traits in top cross hybrids reached a breakdown into environmental and genotypic. The latter, in turn, subdivides into variations arising due to differences in inbred lines according to general combining ability (GCA) and specific combining ability (SCA) (Table 1) using the methodology developed by Savchenko (1973). The mean squares, which served as a measure for assessing the variances due to GCA and SCA, appeared significant ($p < 0.01$) for all studied traits. Variances due to GCA were significantly higher than the SCA variances, indicating the predominant importance of the additive type of action genes in the genetic system for controlling the productivity of green mass, plant height, and bushiness of alfalfa lines. For alfalfa breeding, it is important to have data characterizing the GCA, since the value of this indicator in original forms determines the value of synthetic cultivars.

Table 1. Analysis of variance of the combining ability of alfalfa lines in generations J_0 – J_7 .

Traits	Average square		
	General combining ability (GCA)	Specific combining ability (SCA)	GCA - SCA
Green mass productivity	33853.7	10829.0	3.1
Plant height	26.58	6.92	3.8
Bushiness of the plant	18.72	12.47	1.5

The dispersion analysis of the indicators of top cross F_1 hybrids made it possible to establish the presence of significant differences between $F_\phi < F_r$. It provided the base for determining the effects of the GCA of inbred lines depending on the level of inbreeding. For the phenotypic characterization of the alfalfa's newly developed synthetic cultivars and their original inbred lines, key agronomic traits assessed comprised green mass yield, plant height, foliage, and regrowth rates after mowing. All the conducted tests were under field conditions in four-fold repetition, using a standard evaluation system (ARIF, 1985).

RESULTS AND DISCUSSION

Self-fertility and autotripping

In alfalfa (*M. sativa* L.), the self-fertility with various aspects has achieved quite extensive studies. During artificial self-pollination, the variations in self-fertility vary in populations from self-incompatible to almost 100%. Thus, Kvasova *et al.* (1971) studied the structure of three populations by the self-fertility trait and established the proportion of completely self-sterile plants (ranging from 14% to 18%). About half of the plants got placed into the class with self-fertility (5%–30%), and the proportion of completely self-fertile plants was 1% of the total number of plants studied. Numerous studies established the relationship between self- and cross-fertility, with the seed productivity in alfalfa depending upon the weather conditions (Tysdal, 1946; Wilsie, 1951; Melton *et al.*, 1976). Under better conditions for insect pollinators on self-fertile plants, the cross-fertility index revealed a positive association with seed productivity, while under poor conditions, the self-fertility index showed closer correlations with seed yield.

Analysis of self-fertility by artificial self-pollination of the original samples showed these populations comprised a mixture of genotypes of absolutely self-incompatible and partially self-fertile individuals. In populations, the self-incompatible genotypes emerged to be

17%, and self-fertile forms ranged from 5% to 100%. Under free pollination by bees, the self-fertile plants exhibited cross-fertility in a significant expression and almost up to 100%. Self-fertility manifests itself when alfalfa flowers open spontaneously without mechanical action (autotripping). This fact revealed that alfalfa has alternative reproductive properties and the ability to self-pollinate, self-fertilize, and cross-fertilize with the participation of insect pollinators, particularly bees. This makes possible the isolated reproduction and genetic enrichment during cross-pollination, which is essential for selection and seed production. No mutual negation existed between self- and cross-fertility. However, the self-fertile plants simultaneously exhibited cross-fertility. In these experiments, their relationship received the regression equation description, i.e., $y = a + bx$ (Meirman, 1988).

In alfalfa, the flower structure facilitates the strict cross-pollination, and the flowers that open under natural conditions occur advantageously due to the activity of wild bees. Pollination and fertilization in alfalfa do not occur in closed flowers, and these processes are possible only after the so-called tripping—when a stamen column hits the velum of the flower, causing it to open. The description of the phenomenon of spontaneous flower opening in alfalfa first came from Piper (1914). The absence of autotripping is a limiting factor for the manifestation of self-fertility in alfalfa; therefore, inbreeding continues exclusively manually—by pressing a needle on the base of the flower to artificially open it and then self-pollinate.

In the presented experiment using a 'scented background' that repels pollinators, it was evident that autotripping (automatic opening of the flower) occurs with the high temperature and low relative humidity. Observations carried out at the population level showed individual flowers can open without mechanical action in 2%–5% of the sample. Autotripping does not cover all the flowers of the whole plant. Therefore, one can conclude the entire evolution of alfalfa focused on ensuring cross-pollination for maintaining heterozygosity of the population. The presence

of self-fertility opens up new possibilities in breeding work. Particularly, it seeks the development of inbred lines to enhance the genetic homogeneity of material with increased OCA and the formation of synthetic cultivars with stable characteristics based on intra-population heterotic effects with wide panmixia.

Nevertheless, geneticists set strategic goals for developing the absolutely self-pollinating populations so as not to be dependent on pollinating bees. The said problem also proved to be associated with the catastrophic reduction in the number of pollinating bees due to the mass use of chemicals in agriculture. The prospects of this direction, in genetic terms, incur dictates in the presence of self-fertile individuals in the population structure of alfalfa. Shumny (2004) reported that to obtain highly self-fertile forms and complete reconstruction of the flower—obtaining forms with self-opening flowers and with self-pollination in a closed flower (autogamy)—deeper inbreeding up to 50 generations took place. Similarly, it was also possible to considerably enhance the level of self-fertility; however, its potential manifestation was due to the low level of autotripping.

In alfalfa, self-fertility has become a trait that increases seed productivity as well as sets the goal of developing partially self-pollinating cultivars (Shumny and Kvasova, 1971; Bober *et al.*, 1984; Bober and Kharba, 1986). Past studies believe self-fertility will lead to significant inbreeding depression in almost all important traits (Ivanov *et al.*, 1982; Tereshchenko and Biletsky, 1984). However, in freely propagated alfalfa populations, the self-fertility remains only a slight manifestation of autotripping of flowers. In the populations, about 1%–6% of plants are prone to autotripping (Torsell, 1940; Zykov, 1964; Kvasova, 1975). In alfalfa, self-pollination does not occur in a closed flower, i.e., 'cleistogamous pollination.' For populations, the self-fertility trait was seemingly a condition for the development of inbred lines with increased OCA for inclusion in the genetic structure of newly developed populations (Meirman and Masonich-Shotunova, 2012).

Inbreeding depression and selection for valuable traits

Based on the genetic reproduction system, cross-pollinating crops appeared to be very sensitive to self-pollination. Specifically, the morphological features of flower structure facilitate cross-pollination and protect against self-pollination. From an evolutionary perspective, cross-pollination is considerably more advanced than self-pollination. In breeding, attempts have repeatedly made use of inbreeding to preserve and stabilize the desired traits in cross-pollinators, accompanied by undesirable phenomena, and forced breeders to either completely abandon this method or use it with great caution. Fascination with the theoretical aspects of the problem of linear selection has given rise to an incorrect idea about the possibility of breeding highly productive cultivars homozygous for dominant genes using inbreeding. The inbreeding results in differentiation from the original form, allowing the breeders to identify and remove the recessive genes that control undesirable traits. Genetic splitting also occurs as a result of which aroused inbred lines receive different sets of genes.

Under normal conditions, the alfalfa seedlings' characterization resulted in the presence of one cotyledon and one first true leaflet. However, during inbreeding (intra-family crossing), especially at the early stages of seedling development and during transition from a heterozygous to a homozygous state, the abnormal types were also emergent. Such types of seedlings exhibited deviation from typical morphology, with 3–4 cotyledonary leaves and several rounded leaflets and other atypical features. Such mutations probably had associations with disturbances in the process of embryogenesis and genetic destabilization at the highest level of inbreeding. However, the frequency of such abnormal seedlings was low and ranged from 0.05% to 0.09%. Under present field conditions, 67 abnormal seedlings were visible. It was also evident all these abnormal seedlings lagged after normal plants in development; 14 of them also died, and some did not even lay down the generative organs.

During seedlings' emergence, chlorophyll deficiency occurred in the seedlings of the 2nd generation inbred lines, occasionally recording true albinos (without chlorophyll). In adult plants with inbred origins, dwarfism, chlorophyll deficiency, leaf shape and pubescence, small leaves, shorter internodes at the stem, and various defects were also noticeable in the flower structure. During inbreeding, plants with white corolla flowers were often apart, and this recessive trait was the characteristic of all the alfalfa inbred families. However, in the offspring, the frequency of their appearance varies. When the population was successful in reproducing freely, the abnormalities were notable, and usually in the heterozygote, it remains unnoticed. Only with self-pollination did the conditions develop for elimination from the original population. Numerous studies expressed inbreeding depression ranging from 30% to 40% in alfalfa inbred lines (Torsell, 1940; Wilsie, 1958; Downey, 1962; Panella and Lorenzetti, 1966; Briggs and Knowles, 1972; Pozdiyakov, 1975; Dzyubenko, 1982; Espanov, 2008).

The obtained results revealed the sensitivity of alfalfa to inbreeding depression. Maximum inbreeding depression was noteworthy in the first and second generations, and the green mass productivity of the local line Semirechenskaya was 68.6%–62.6%, in Cardinal (79.7%–73.4%), in Dawson (87.4%–80.5%), and in Local from China (82.9%–78.6%) compared with their initial levels of production. However, from the third generation of inbreeding, the productivity became particularly stable in all lines. The difference between generations J₃ and J₇ was unreliable. In scientific literature, inbred lines reached common designations with the symbol J with an indication of generations. Theoretically, with homozygosis, the productivity has been considerably reduced from generation to generation due to inbreeding. According to the hypothesis of dominance, intervarietal difference in inbreeding attained an explanation by the complexes of recessive genes found in the alfalfa populations, which exhibited a negative effect in homozygotes.

However, the selection effect turned out to be stronger, which softened the effect of inbreeding and affected the productivity of alfalfa lines in J₃ to J₇.

With inbreeding depression, plant types with new properties of practical value also appear, such as high foliage, large inflorescences resistant to diseases, upright bushes, and reduction in the intercutting period. In the methodical experiments (J₀–J₇), as well as in the mass selection (J₂–J₃), the selection of breeding elites continued for plant vigor (plant height, bushiness, foliage, and upright bush type) in inbred populations with subsequent assessment of GCA for inclusion in the newly synthesized cultivars. In the inbreeding process, 200 promising and rapidly growing genotypes entailed selection from the splitting generations of inbred lines with a constant assessment of the GCA using the top cross method. Some valuable lines attained patenting as initial forms for selection, such as Kokzhazyk-1 (with patent No. 896, dated 09.04.2019) and Kokzhazyk-2 (with patent No. 897, dated 09.04.2019).

General combining ability and formation of synthetic cultivars

The development of alfalfa inbred lines for utilization in breeding populations involves long-term inbreeding of the best initial samples of the collection. Additionally, it is also important to determine the inbreeding frequency to achieve the optimal level of homozygosity for better expression of the heterotic effects. The genetic composition of synthetic cultivars should consist of the combination of valuable lines. Experience with cross-pollinated crops showed, based on using inbred lines, it is possible to sharply enhance the value of such an essential factor as the GCA. In this regard, several methodological issues arise related to the establishment of the inbreeding generation, due to which it is advisable to select inbred clones with the highest desirable GCA effects.

In the experiment, obtaining the inbred lines J₁–J₇ came from clones of the local genotype Semirechenskaya. Through a large-

Table 2. Effect of general combining ability (GCA) and specific combining ability (SCA) on breeding-valuable traits in the second-year alfalfa inbred lines selected in the alfalfa local cultivar Semirechenskaya.

Generation inbreeding	1-slope	2-slope	3-slope	4-slope	Average
Green mass					
J ₀	-49.28	-49.00	-32.17	-26.43	-213.53
J ₁	20.72	-6.33	13.94	1.54	35.58
J ₂	31.28	22.11	15.94	20.68	100.03
J ₃	26.39	49.44	21.94	6.13	113.92
J ₄	2.06	26.33	-8.28	5.79	35.92
J ₅	-12.94	-13.89	10.50	5.90	-11.53
J ₆	2.50	-3.78	-4.94	17.68	19.47
J ₇	-20.72	-24.89	-16.94	-13.32	-83.86
Error	6.89	5.64	4.38	6.21	22.53
Plant height					
J ₀	-5.14	-3.17	-3.88	-3.30	-3.66
J ₁	10.01	6.80	3.12	-1.05	4.89
J ₂	5.13	4.97	2.27	3.43	4.14
J ₃	-1.85	-3.67	-4.75	-3.76	-1.54
J ₄	-3.42	-1.95	-0.68	2.33	-0.76
J ₅	-3.49	0.30	2.67	-2.62	-0.68
J ₆	-1.14	-1.57	0.80	-1.59	-1.98
J ₇	-0.12	-1.72	0.47	-0.95	-0.41
Error	1.23	1.17	0.81	0.85	0.65
Bushiness of plants					
J ₀	0.49	0.31	4.98	-1.56	1.06
J ₁	3.41	-2.29	1.66	1.49	1.07
J ₂	2.32	4.36	12.63	-0.31	4.74
J ₃	0.24	-0.56	-6.47	9.78	0.74
J ₄	-1.93	1.96	-4.79	-2.54	-1.81
J ₅	-1.26	2.59	-0.89	-3.46	-0.78
J ₆	-2.18	-2.49	-1.67	0.01	-1.58
J ₇	-1.09	-3.89	-5.44	-3.39	-3.46
Error	0.79	2.01	1.72	0.66	0.79

scale application of inbreeding, using the possibilities of selection for acceleration of generation turnover, it is possible to significantly enhance the efficiency of selection in alfalfa. Genetic differences between lines J₀–J₇ were noticeable, as observed through the average squares of standard deviations of the GCA in comparison with the SCA (Table 1). The mean squares used to assess general combining ability and specific combining ability were significant for all studied traits at $P < 0.01$. The results of the mowing assessment with GCA effects in inbred lines J₁–J₇ and the original form (J₀) are available in Table 2. The analysis of the GCA in each mowing revealed the formation of mowing occurs under different

environmental conditions. These circumstances will certainly be reflected in the manifestation of heterosis and variability in GCA effects. Through the mowing analysis, it was possible to obtain fairly accurate results of the GCA and determine the prospects of the lines. An increase in the GCA was notable in lines of the second (J₂) and third (J₃) inbreeding generations, which showed this property in all four mowings.

The fluctuation in the GCA effects in J₂ on the productivity of green mass depending on the mowing was 15.94–31.28, and in J₃, it was 6.13–49.44. The studies showed the most optimal level of inbreeding for the selection of synthetic cultivars based on the subsequent

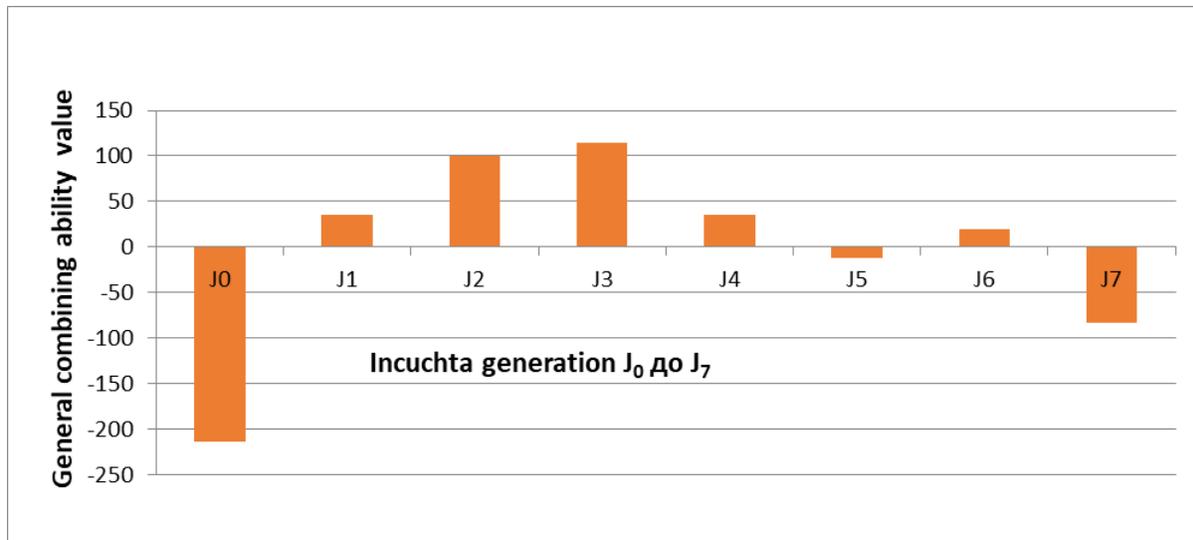


Figure 1. Effect of general combining ability (GCA) on the green mass productivity J_0 – J_7 generations inbred lines selected in the alfalfa local cultivar Semirechenskaya.

hybridization in polycross among the inbred lines of J_2 and J_3 generations (Figure 1). Using inbred lines in generations J_2 – J_3 , multi-clonal synthetic cultivars of alfalfa succeeded in their development. Based on state varietal evaluation results, these synthetic cultivars of alfalfa (Kokbalausa, Kokorai, Osimtal, Kokshalgyn, and Shabyt 80) were varieties included in the State Register of Plant Cultivars and approved for cultivation in the Republic of Kazakhstan.

Some of the alfalfa cultivars, as discussed

Cultivar Kokorai: Developed by selecting the best plants for a complex of economically valuable traits and a combination of valuable inbred lines and polycross crossing. The said cultivar consists of four inbred lines and one heterozygous plant: I2-132 from sample K-6940 (India), I2-101 from cultivar Kapchagayskaya 80, I3-53 from cultivar Semirechenskaya local, I2-212 from sample K-41340 (Italy), and plant 23-4 from cultivar Omskaya 8893.

Cultivar Kokbalausa: Developed through selection and combination of valuable inbred lines and polycross crossing. The cultivar consists of seven inbred lines: K14/27 from the

cultivars Kapchagayskaya 80 and S-6/33 from the local cultivar Semirechenskaya–Kazakhstan; D17/09 from the Dawson cultivar (USA); I17/54 and I20/12 from the cultivar Iolotanskaya-1763–Turkmenistan; and P-12/02 and P-14/08 from the cultivar Poiton–France.

Cultivar Shabyt 80: Developed through breeding, selection, and combination of inbred lines and polycross crossing. The synthetic cultivar comprised six inbred lines: C-21/14, C-5/08, L-8/33, L-13/04, K-7/3, and I-6/33 of the second and third generations of inbreeding.

Cultivar Kokshalgyn: Developed through inbreeding and selection in combination with inbred lines and polycross crossing. The synthetic cultivar consists of six inbred lines: K-24/41, S-25/12, I-45/067, K-16/75, S-21/32, and I-43/779.

Cultivar Osimtal: Synthesized from five inbred lines: D-19/24 from the cultivar Dawson–USA, D-8/65 from the cultivar Dawson–USA, S-21/14 from the local cultivar Semirechenskaya–Kazakhstan, K-8/41 from the local cultivar Kapchagayskaya-80–Kazakhstan, and I-7/3 from the cultivar Iolotanskaya-1763–Turkmenistan and mill.

Efficient selection of the lines for OKS and their utilization in the developing synthetic cultivars of alfalfa gained expression from a significant increase in the productivity of the population due to the highest level of heterosis (Meirman and Masonich-Shotunova, 2012). The restoration of plant vigor as a result of free hybridization of inbred lines depends upon their genetic characteristics. In various cases, free pollination of inbred lines also revealed an advantage due to the sharp manifestation of heterosis. This was in relation to the development and selection of the combination of valuable inbred lines. If these newly developed genotypes meet the selection requirements (homogeneity by phenotype, the least inbred depression, and high OKS), it is possible to sharply increase the efficiency of the research work. In self-pollination, numerous genes pass into a homozygous state, and as a result, it becomes possible to quickly eliminate forms with negative traits and isolate valuable lines for heterosis selection. By studying, it is possible to identify OKS lines that carry additive genes in the development of any trait, which is important for synthetics.

CONCLUSIONS

In alfalfa (*M. sativa* L.) populations, a wide range of variability was evident, from complete self-incompatibility to high self-fertility. Self-fertility does not exclude the possibility of cross-fertility. In the South and Southeast conditions of Kazakhstan, the manifestation of self-fertility during free reproduction of the population was scarce due to the absence of autotripping. The manifestation of self-fertility makes it possible to conduct selection of inbred lines. Inbreeding depression occurs quite sharply in the lines of the first and second generations. The greater GCA effect was the characteristic of inbreeding generations J_2 - J_3 , and deeper inbreeding does not have a significant impact on the GCA indicators. The effectiveness of using inbred lines has reached validation with the development of several high-yielding cultivars of alfalfa.

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