Name of the project: Num.14870651 «Production early maturing soybean material with high productive and adaptive potential using molecular breeding methods for conditions of Northern Kazakhstan»

Relevance: Soybean is a new, previously uncultivated and promising crop for Northern Kazakhstan. The culture is difficult to cultivate in Kazakhstan due to the climatic features of our country, however, it is in demand and promises farmers high profitability. However, due to climatic conditions, the choice of soybean varieties or hybrids in Kazakhstan, unlike other regions, is small, it is necessary to use precocious and ultra-ripe varieties. In dynamics, there is an increase in the area under soybeans from 24.9 thousand hectares to 227.8 thousand hectares in 2021. On average, the yield of soybeans in Kazakhstan is 20-21 kg /ha, but the highest indicators are characteristic of the Almaty region (irrigation farming zone). In other regions of Kazakhstan, the yield of soybeans does not exceed 10 kg/ha. In Kazakhstan, the main growing regions are Almaty and East Kazakhstan regions.

When creating soybean varieties for the conditions of Northern Kazakhstan, it is necessary to take into account many factors, in particular, an insufficient amount of temperatures during the growth period and a long daylight day, since soybean is a short-day plant. Soybean varieties with weak photoperiodic sensitivity can bloom relatively early and form seeds in long daylight conditions.

For the northern region, 1-2 varieties of non-district selection are allowed, for which there is no seed production. This is the problem of cultivating a highly profitable crop in the conditions of the north of Kazakhstan: the lack of varieties and, accordingly, the lack of seed production by culture. For the full implementation of this culture, it is necessary to create varieties adapted to the conditions of the north of Kazakhstan, taking into account the terms of vegetation of plants and the photoperiodic reaction of the plant to the length of daylight.

Precocity is the most important indicator for agricultural plants in the conditions of Northern Kazakhstan. Using the methods of molecular biology, it will be possible to obtain the raw material of soybeans in order to create new highly productive and precocious varieties for specific conditions. The project provides for the use of traditional and molecular breeding methods, including DNA marker methods, which makes it possible to simplify, accelerate and reduce the cost of the breeding process when creating new varieties for 3-5 years. Also, molecular markers in plant breeding make it possible to assess the genetic diversity of the soybean source material and classify breeding forms and traits.

Goal: study and create new precocious forms of soybeans using traditional methods of breeding and molecular analysis for the conditions of Northern Kazakhstan.

Project objectives:

1. Study of the source material of soybeans according to the main economically valuable characteristics and determination of their breeding significance, identification of valuable initial forms of soybeans with high values of morphobiological, morphophysiological and economically valuable characteristics, to include them in the breeding process to create varieties with high adaptive ability. Identification of soybean genotypes based on molecular genetic polymorphism in the conditions of Northern Kazakhstan;
2. To create a new soybean source material by classical selection and evaluation based on molecular analysis using DNA markers associated with precocity. Selection of sources and donors of precocity and productivity based on field research and molecular analysis.

1. Generation and accelerated reproduction of the obtained soybean hybrids in greenhouse conditions;

2. Hybridological analysis of soybeans according to the main economic and valuable characteristics in the conditions of Northern Kazakhstan;

5. Molecular analysis for precocity and field assessment for the productivity of generations F3 F4 and the selection of precocious forms for further passage of the links of the breeding process.

Expected results

1. A comprehensive assessment of the source material of soybeans of various origins according to the main economic and valuable characteristics. Selection of genotypes for further use in practical breeding. Documentation of soybean samples;

2. Optimization of protocols for the development of DNA markers associated with precocity and molecular analysis of the soybean source material with subsequent selection of highly productive and adaptive lines will be carried out;

3. Selection of precocious soybean samples to include them in the breeding process. Hybridization of selected parental soybean forms, obtaining F1 generation hybrids;

4. Generation and production of hybrid grains of F2 generations. Hybridological analysis of A3 and A4 generation hybrids according to the main economically valuable characteristics and field assessment on the productivity of A3 A4 generations and selection of precocious forms.

5. Publication of at least 2 (two) articles and (or) reviews in peer-reviewed scientific publications indexed in the Science Citation Index Expanded of the Web of Science database and (or) having a CiteScore percentile in the Scopus database of at least 35 (thirty-five).

6. Publication of 2 articles in a peer-reviewed foreign or domestic publication recommended by COXON, indicating the identification number and name of the project, as part of its financing.

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Information for potential users: the direction of breeding and seed production of agricultural crops

The results of the study 2022 year: The study of a new source material of soybeans according to the main economically valuable characteristics was carried out, valuable initial forms of soybeans were identified. Ивушка, Suiyang 1, СК Элана, Beidou 26, Heihe 43, Heihe 58, Beidou 56 varieties were the best in terms of the complex of elements of productivity, yield and grain quality, breeding lines 7-21-2, 28-21-1, 31-21-1, 46-21-1, 92-21-1, 100-21-1,122-21-1 etc., varietals were marked by precocity and yield. According to the height of the attachment of the lower bean, the degree of correlation was r = 0.51, the number of beans on the plant r = 0.74, the size of the seeds and their number were characterized by a high positive relationship. The conducted analysis of variance showed a high confidence score and a lower deviation rate in the experiment. A molecular analysis of the soy source material using DNA markers, as well as the

selection of genes controlling precocity, was carried out. Primers for the soybean SEP3 gene have been selected (Genbank number XM_006579370). Work has been carried out on the amplification of full-size sequences of SEP3 genes of soybeans of different varieties using selected primers. On primers 22GmSEP3aNachS1 and 2GmSEP3aKonA1 with DNA of different varieties, PCR products of the expected size (about 735 bp) were obtained, which were then sequenced on ThermoFisherScientific. Work has been carried out on the creation of a new source material, reproduction and evaluation of breeding material in breeding nurseries. According to the results of the hybridological analysis, 2 combinations of crosses were identified.

The results of the study 2023 year: The study of soybean varieties of various origin according to the main economically valuable characteristics and properties and the determination of their breeding significance for practical breeding was carried out. There were more than 180 varieties of soybeans in the study. The conditions of the growing season of 2023 in terms of air temperature values exceeded the average annual values during the growing season of the crop. The onset of hot and dry weather was noted during budding-flowering, which negatively affected the formation of fruits and the yield of soybean varieties. According to the average monthly data, May was the coolest: the air temperature for this month on average varied from 7.6 to 24.1 ° C, while the average monthly temperature was 15.3 °C. The hottest month was July: the air temperature varied from 19.2 to 28.0 ° C, the sum of the effective temperatures during the period of growth and development of culture – 756.4 °C. Precipitation varied by month. The amount of precipitation was significantly lower than the average annual values in May, June, July and August. The greatest amount of precipitation (13.2 mm) fell in June. May was the driest (2.5 mm). The air temperature indicators were slightly higher compared to the long-term average data, and precipitation indicators were significantly lower. The HTC varied from 0.1 to 0.31. The lowest value of the HTC was noted during the sowing- shoots period. All interphase periods of soybean crop growth and development took place in drought conditions, which led to a reduction in the growing season of soybean varieties. The sum of active temperatures during the growing season was 2266.4 °C, and the value of the hydrothermal coefficient was 0.27. An increase in precipitation during the ripening period (late august-September) led to a significant lengthening of the harvest time, which is primarily due to high grain humidity.

According to the goals and objectives of the project, the polymorphism of photosynthetic activity of soybean varieties was evaluated. How to determine the amount of pigments and chlorophyll, the index of "greenery", the area of leaves, the dynamics of accumulation of dry matter, the dynamics of linear growth, the length of the stem and internodes of plants, the intensity of photosynthesis, etc. According to the studied signs of photosynthetic activity, the following were isolated: Alta 1245 I, Kendou 60, Artika, Heihe 33, Cheremshanka, Beidou 53, Chera 1-3, Nadezhda, Nur +, Otan +, and at line №.92, Bara (Russia), Ivushka, LongKen 336 (China), selected varieties can be used in practical breeding.

The duration of the growing season of soybean varieties was: in the ultra-early groups of ripeness from 75 to 88 days, in the early groups from 92 to 110 days. The most precocious according to the results of the study (less than 85 days) were varieties Suiyang 1, Heihe 33, Kendou 69, Chera-1-3, Osmon, Bara 1247 I, Artika, Kasatka, Dina, Eldarado, Altom. At the level of the Ivushka standard, there were the following varieties: Svetlyachek, SK Doka, Beidou 43, Beidou 26, Heihe 58, Beidou 36, Kendou 60, Chera 1-1, Chera 1-2, №92, № 73, SIBNIISHOZ, Zaryanitsa, Cheremshanka. All varieties of the early group matured significantly earlier than the standard variety Bara.

The size of seeds is one of the important elements of the crop structure. Accounting for this feature was carried out by the weight of 1000 seeds in grams. The average seed size of the ultra-early groups standard was 133.3 g. The average seed size of the entire set of studied samples was 137.7 g. The minimum value was 95.8 g for the Bara 1247 I sample, and the

maximum was 233.3 g for the Beidou 26 sample from China. The entire set of samples under study by the weight of 1000 seeds can be divided into variants in accordance with the "International Classification of CMEA of the genus Glycine Willd: small (71-130 g) – 16 sample., medium (131-190 g) – 29 sample., large (191-250 g) – 4 sample. The samples of Chinese and Russian breeding with large seeds may be of the greatest interest on this basis: Beidou 26, Kendou 60, Beidou 41, Suinong 10, Beidou 51, Heihe 49, Huajiong 2,Heihe 35, LongKen 336, Heike 59, Kenfeng 6, Lider 1, Veselitsa, SIBNIIK 315, TSLS-15-14.21 1411 I.

The results of the quality assessment (fat and protein content) showed its varietal specificity. The varieties of the ultra-early ripeness group were characterized by high values of fat content, in comparison with the early-ripening group. The best varieties of this group presented in Table 7 were characterized by a fat content of 40....47.25%. At the same time, some soybean varieties were at the standard level, while a high protein content was noted. These include the varieties Zusha, Artika, Alta, Lancetnaya, Larisa and Eldorado. The varieties Osmon, Lider 10, Cheremshanka, Altom, and Mezenka were characterized by a high fat content.

Productivity is one of the fundamental signs in breeding. In soybeans, as in other leguminous crops, yield instability is noted, the influence of growing conditions affects productivity more strongly compared to the influence of the genotype. In the ultra-early group of the Mezenka variety, TSLS -15-23 1399 I, Beidou 43, Chera 1-2 and perspective line №92, exceeded the standard grade by 0.1-1.4 c/ha. In the early ripe group, varieties of Chinese selection exceeded the standard grade by 0.1-7.5 c/ha, varieties of Russian selection by 0.5-5.1 c/ha, varieties of domestic selection by 0.2-6.8 c/ha. In the ultra-early group, the average yield was 10.1 c/ha, the maximum yield of the variety Chera 1-2 from the Russian selection. The minimum yield indicator was for the Svetlaya sample, the yield of which was 3.6 c/ha.

In the early group, the average indicator of this trait was 9.9 c/ha. The maximum yield in this group was shown by the LongKen 333 variety from China – 16.1 c/ha. The minimum yield was the sample Beidou 52 – 3.3 c/ha. According to the average yield indicator, it can be concluded that early-ripening varieties are inferior to the ultra-early group.

There was a positive and strong relationship between the yield and the weight of seeds from one bean (r = 0.98); between the sign of the weight of seeds from the plant and the amount of grain in the bean (r = 0.95); between the yield and the amount of grain in the bean (r = 0.93); between the sign of the weight of seeds from the plant and side branches (r = 0.80); between yield and lateral branches (r = 0.76); between the amount of grain in a bean and lateral branches (r = 0.74).

The coefficients of variation according to the characteristics of the number of seeds in the bean and the growing season had low values of 3.78 and 6.02%, respectively, which indicates the stability of the formation of the studied characteristics by varieties. The variability of plant height had an average value (CV = 18.3%).

In the early groups of ripeness, the variability of the sign of the growing season was insignificant, as evidenced by the low values of the coefficients of variation by varieties (CV=5.4%). It was found that the average cultivating (CV=11.16-17.29%) economically useful signs include plant height, the number of seeds in the bean, the high-cultivating (CV = 21.78–25.55%) - the height of attachment of the lower bean, the number of productive nodes, the weight of seeds from 1 bean, the weight of 1000 seeds, yield.

As the main abiotic factor, cold stress limits the area of cultivation of crops and reduces yields. Crop loss due to exposure to low positive temperatures reduces the yield of soybean seeds by an average of 24%. The results obtained in assessing the cold resistance of soybean samples clearly revealed statistically significant differences between the samples. Seed germination (GP) in the studied samples at 25 ± 1 ° C (control) temperature ranged on average from 10 to 96%; germination rate (SE) from 2.4 to 9.0 days; emergence rate index (SEI) from 2.3 to 17.4 days; the coefficient of occurrence rate (SEC) from 11.1 to 40.8 days; at a low positive temperature of 10 ± 1 °C GP from 2% to 96%; SE from 4.8 to 15.0 days; SEI from SEC 1.5 to 6.5 days; SEI from 6.7 to 72.7 days. The samples were characterized by the least number of days according to

SE: 127/1; 36/3; 5/2; 139/3; 94/2; 95/3; 124/2; 105/3; 151/2; 19/1; 17/1/1; 23/1/1; 34/1 and 102/1, on average, this indicator varied 3-7 days at a temperature of 10 0C, which allows us to continue breeding work with them to create cold-resistant starting materials. Samples 8/3; 24/2; 25/3; 46/3; 86/1; 103/2; 118/2; 140/3; 143/2; 3/3/1; 4/3/1; 6/3/1; 9/2/2; 15/3/1 and 125/3 were cold-sensitive to low temperatures, this indicator was zero. Grade 62/1 SE was the highest - 15 days, which indicates a high sensitivity to cold of this sample. The rate of occurrence index - SEC in the control variant ranged on average from 2 to 14.6, then under the action of cold stress from 0 to 6.3. Also, under the action of a low positive temperature, there was a strong variation in SEC from 6 to 72.7, which indicates different reactions of the collection to cold.

As a result of cluster analysis, a clear division into two groups was revealed. The first group included 2 genotypes of Chinese (14/3 and 62/1) and 11 genotypes of domestic (89/3, 90/2, 91/2, 94/2, 95/3, 102/1, 105/3, 107/3, 108/3, 125/3 and 130/3) of the collection, while the second group included the remaining 55 samples of the Russian, Chinese and Kazakh collections. The second group, in turn, was divided into 2 subclusters: 1 subcluster consisted of 19 samples, the second of 16. The genotypes included in the first group comparatively had the most cold resistance according to the screening results.

Thus, the most cold-resistant genotypes were selected based on seed germination (GP), germination rate (SE), emergence rate index (SEI) and emergence rate coefficient (SEC).: 14/3, 62/1, 89/3, 94/2, 95/3, 102/1, 105/3, 125/3 and 130/3 for the breeding process.

A molecular analysis of the initial soybean material was carried out using DNA markers associated with precocity, 100 soybean samples were studied. Soybean - Glycine max (L.) Merr. a typical plant of a short day and demanding of heat. The yield of soybean plants largely depends on the time of flowering and ripening in various ecological regions. Currently, more than 8 loci are known in soybeans that control the duration of the growing season and the reaction to the photoperiod and are designated as *E* loci. In conditions of a long day, dominant alleles of E genes lengthen the growing season, and recessive alleles of *e*, on the contrary, shorten. Genotyping of soybean cultivars to *E1-E4* genes was carried out using DNA markers. For the analysis of the E1 gene, the markers E1_*HinfI* and *e*1-re_STS, flanking the site of dominant and recessive alleles, were used. The allelic variation of the *E*1 gene includes a single nucleotide polymorphism (SNP) in nucleotide 44, leading to a missense mutation (arginine to threonine) (*e1-as*), deletion of one base (adenine) in nucleotide 49, leading to a premature stop codon in nucleotide 124 (*e1-fs*) and a null allele (*e1-nl*), in which the entire *E*1 gene was removed. As a result of PCR analysis of 7 soybean samples, the presence of the *e1-fs* allele was detected, 17 samples had the e1as allele and the remaining 66 genotypes had the dominant *E*1 allele. In the *E*1 gene, the main product was found to be 235 bp, 117 bp and 80 bp in size, and in the e1-asallel was characterized by the presence of PCR products of 235 bp, 117 bp, 46 bp and 33 bp in size.

According to the *e*1-re_STS marker, the dominant *E*1 gene had a PCR product with a size of 840 bp, whereas the recessive *e*1 and *e1-fs* alleles had a PCR product with a size of 841 bp.

Thus, as a result of the research, molecular markers of various alleles of the *E*1–*E*4 genes responsible for sensitivity to photoperiod and maturity were tested. As a result of PCR analysis, a high frequency of dominant alleles of *E*1, *E*2 and *E*4 loci was established in this soybean collection, according to the *E*2 locus, most of the studied samples contain recessive *e*2-*ns*, which, apparently, make the main contribution to the reduction of maturation time. The tested set of molecular markers can be used for the selection of domestic soybean varieties based on sensitivity to photoperiod and maturation periods, on which the productivity of soybeans largely depends, especially in temperate climates, atypical for its cultivation.

In the conditions of 2023, 12 combinations of crosses were carried out in order to create a new soybean source material characterized by precocity. The parent forms were selected using the molecular analysis method and high yield (phenotypic effect). The combination of hybrids Bara♀× Milyausha ♂, Svetlyachek ♀× Beidou 26, Ivushka ♀×Heihe 58 ♂, SK Doka ♀×Cheremshanka, SK Doka ♀×Beidou26 ♂, Ivushka ♀× Beidou 52♂, Bara♀× Beidou 19 ♂, Ivushka ♀×JInYAAn55 ♂, SK Doka ♀×Beidou 40 ♂, SK Doka ♀× Heihe 33♂ etc.

In the conditions of the phytotron, accelerated reproduction of F_1 generation hybrids obtained in 2023 is laid in order to obtain the F_2 generation. For optimal growth and development, LED lamps EG-240C 1750 A are used, all necessary measures for the growth and development of culture are carried out. Bookmark conducted in the following combinations of crossing the Bara♀× Milyausha ♂, Svetlyachek ♀× Beidou 26, Ivushka ♀×Heihe 58 ♂, SK Doka ♀×Cheremshanka, SK Doka ♀×Beidou26 ♂, Ivushka ♀× Beidou 52♂, Bara♀× Beidou 19 ♂, Ivushka ♀×JInYAAn55 ♂, SK Doka ♀×Beidou 40 ♂, SK Doka ♀× Heihe 33♂.

For generations F_3 and F_4, a hybridological analysis was carried out on the main economically valuable characteristics for the conditions of the north of Kazakhstan. As a result of the conducted studies, it was revealed that by the weight of seeds from one plant, the hybrids exceeded the parent forms, the level of heterosis varied from. Intermediate inheritance was observed in all combinations.

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