**Name of the project theme:** AP14870014 “Applications of DNA technologies in breeding and genetic studies of proso millet culture at creating new local drought tolerant varieties”.

**Scientific novelty:**

The plant millet (*Panicum miliaceum* L.) is a valuable cereal and fodder crop in the world. Currently, millet is grown mainly in East and Central Asia and to a lesser extent in Eastern Europe and from West Asia to Pakistan and India (Kate S.M.et.al, 2018). According to FAO agriculture organization in the world, the sown area of millet ranks 6th in sown area (34.7 million ha) and gross grain harvest (31.6 million tons) among grain crops, second only to wheat, rice, barley, corn and sorghum (Zotikov V.I. et.al, 2012).

There are 21 varieties of pearl millet for grain and 13 varieties for fodder in the State Register of Breeding Achievements of the Ministry of Agriculture of the Republic of Kazakhstan. Much of the new varieties belong to the breeding of Aktobe Agricultural Experimental Station, three varieties of food and fodder direction were created in "A. I. Barayev Research and Production Center for Grain Farming " LLP, 2 varieties were created in Pavlodar Agricultural Experimental Station and 1 variety in West Kazakhstan Scientific Research Institute. Millet varieties began to enter the Gossortoset since 1930. Beginning in 1937, the selection of millet was resumed in the major sowing regions, including Aktobe region. An example of the use of folk breeding, based on careful repeated individual selection, are the works of the world famous Aktobe millet breeder Shyganak Bersiev, who established during 1937-1944, several world achievements in growing millet in irrigation. An outstanding achievement of Bersiev is the record yield of millet of 201 cwt/ha in 1943 (Tsygankov I.G. et al., 2004). Most millet varieties have been developed using classical breeding methods. It is practically impossible to achieve a combination of many desirable valuable traits in one genotype using only classical breeding methods because of negative genetic correlations (Sokurova L.Kh., 2014). Selection with molecular markers, marker-assisted selection, MAS, is a comprehensive and innovative approach that includes both traditional genetic and molecular. Traditional methods of variety identification are based on morphological traits. The number of such traits is limited, while the number of varieties is in the tens of thousands. Due to the progress in the study of molecular organization and variability of the genome, marker technologies have been developed, contributing to a significant increase in efficiency and acceleration of the process of creating new varieties (Suvolap J.M., 2013). Taking into account the fact that in the state variety testing system and in production there is a limited assortment of pearl millet crops, creation of new drought tolerant varieties to the conditions of dry-steppe zones of Kazakhstan is an urgent task for breeders.

**Project Objective:**

Comprehensive study of the proso millet gerplasm by using DNA marker analysis, selection of initial material in the breeding process and the creation of a new local drought tolerance variety for the steppe and dry steppe zones of Kazakhstan.

**Expected results of the project:**

-The efficiency of using microsatellite SSR and ISSR markers for genetic polymorphism analysis will be evaluated;

-The calculation of genetic distance and clustering of the collection to identify heterogeneity of genotypes will be carried out;

-Perspective samples containing highly efficient and economically valuable genes will be identified;

-Collection and breeding nurseries will be established in conditions of dry-steppe zone of Kazakhstan and highly productive and drought-resistant genotypes will be selected;

-will be transferred to a new local competitive drought tolerant proso millet variety in the State variety trials in the regions of Kazakhstan joint breeding S. Seifullin Kazakh agrotechnical university and Aktobe Agricultural Experimental Station and filed an application to NIIS MJ RK to obtain a patent of the RK on the new breeding achievement.

During the implementation, the establishment of demonstration sites (plots) will be carried out on the breeding plots of the research originator Aktobe Agricultural Plant LLP, as well as a number of beneficiaries of the project "Di Land LLP" (Kargaly district, Aktobe region). On these sites (sites) it is planned to place a number of promising varieties and constant lines of millet of domestic selection in order to demonstrate the existing varietal potential and elements of their varietal agricultural technology in the dry steppe zones of the Republic of Kazakhstan.

Based on the results of the scientific project, 3 (three) articles and (or) reviews will be published 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 50 (fifty).

Currently, two PhD doctoral students of the department are actively conducting research in this area. A sufficient amount of source material has been accumulated for breeding and genetic work. To date, the collection includes about 200 samples of various ecological and geographical origins: Afghanistan, Belgium, Hungary, China, Canada, India, Iran, Mexico, Pakistan, Russian Federation, USA, Turkey, Ukraine, France. The gene pool of foreign and domestic collections was tested in the field conditions of Northern and Western Kazakhstan. Research on this culture took place in different directions, biochemical, physiological and valuable-economic characteristics were studied, which made it possible to evaluate the source material comprehensively; the use of different geographical latitudes allows the results obtained to be used not locally, but throughout Kazakhstan as a whole. New hybrid materials of different generations have been obtained using traditional selection and mutagenesis. Based on the research results, articles with a high percentile were published in foreign journals. The proposed project will allow us to continue breeding and genetic research using innovative methods at the molecular level and replenish the local collection with new varieties that meet the requirements of the cereal industry.

**Research team members:**
**Project Manager – Rysbekova Aiman Bokenovna**
**Research Group:**

| # | Name, surname, education, degree, academic rank | Main place of work, position | Hirsch index, Researcher ID, ORCID, Scopus Author ID (if available) |
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**List of publications and patents published within the framework of this project: (with links to them):**

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**Information for potential users:**

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**Additional Information:**

A collection nursery and breeding nursery for millet lines was established in the Akmola and West Kazakhstan regions. The collection nursery consisted of 120 specimens of various ecological and geographical origins and a breeding nursery of 4 lines obtained as a result of individual selection. The beginning of the germination phase of millet plants in the conditions of the West Kazakhstan region was noted on the 5-6th day, full germination - on the 8-9th day. Field germination of the majority of millet assortment in nurseries was 70-80%; for a number of samples it was in the range of 55-65%. In the conditions of the Akmola region, due to the lack of precipitation during the sowing period, seedlings were obtained on the 12-14th day, full seedlings on the 16-18th day, field germination was at the level of 70-75%, in some samples it was at the level of 50 -55%. Evaluation of the lines for all economically valuable traits showed that the most promising line, in comparison with the standard variety of the given region, Pamyati Bersiev, was Line R-1553; in all indicators, this line surpassed other lines, including the phenotype score assessment. This line stood out in both regions. Drought resistance of varieties was assessed according to a scoring system in both regions. Line R-1553 showed high results of 4.4 and 4.2 points, Line S-8/82 and Line S-12/82, respectively, scored 3.5 and 3.8 points. The yield of the line P-1553 in the conditions of the West Kazakhstan region was 318 g/m2, which is 38 g/m2 higher than the standard variety; a study of the line in the Akmola region also showed an increase in yield in comparison with the standard of this region Saratovskoe 6, so the yield of the line was 302 g/m2, which is 64 g/m2 higher than the standard grade.

To assess the intravarietal polymorphism of the world and domestic millet gene pools, represented by 120 samples of various origins, DNA extraction was carried out using a modified CTAB method. Selected 20 SSR markers: SSR-67; SSR-70; SSR-71; SSR-82; SSR-85; SSR-86; SSR-92; SSR-100; SSR-109; SSR-120; SSR-121; SSR-127; SSR-128; SSR-129; SSR-131; SSR-142; SSR-143; SSR-144; SSR-146; SSR-182. PCR conditions for the listed DNA markers were optimized. PCR analysis using the SSR-131 marker did not detect intravarietal polymorphism; a

PCR product of 349 nucleotide pairs (bp) in size was amplified in all samples. Polymorphism was identified for markers SSR-142 and SSR-143. When using SSR-142, the majority of samples (~90%) amplified a band of 124 bp, with the exception of some samples that had a PCR product of 118 bp. Using the SSR-143 marker made it possible to visualize the presence of a PCR product of size 144 p.n. and 160 bp Using markers SSR-144 and SSR-146, 2 amplicons, approximately 450 bp, were identified in the studied samples. and 200 bp for SSR-144 marker, 200 bp. and 95 bp for SSR-146 marker.