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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2	V.I. Tsygankov, Candidate of Agricultural Sciences	Aktobe ARS, Leading Scientific Associate	Hirsch index -4, ORCID 0000-0002-3652-3888; Scopus Author ID 572 212 898-46
3	I.A. Zhirnova, Master of Agricultural Sciences	S. Seifullin Kazakh agrotechnical university, PhD student in the Department of Agriculture and Crop Production	Hirsch index -1, ORCID 0000-0003-1716-8793; Scopus Author ID 571203111547;
4	E. N. Dyusibayeva, PhD	S. Seifullin Kazakh agrotechnical university,, Associate Professor in the Department of Agriculture and Crop Production	Hirsch index -1, ORCID 0000-0002-5960-6328, Scopus Author ID 57195609258;
5	A.E. Zeinullina, Master of Agricultural Sciences	S. Seifullin Kazakh agrotechnical university, PhD student in the Department of Farming and Crop Production	ORCID 0000-0001-6880-0969; Scopus Author ID 57208920657;
6	L.A. Dzhikiya, Master of Agricultural Sciences	S. Seifullin Kazakh agrotechnical university, Senior lab assistant at	ORCID 0000-0002-5197-6359;

		the Department of Agriculture and Crop Production	
7	Yessenbekova G.T., PhD	S. Seifullin Kazakh agrotechnical university, Senior lecturer of the Department of Plant Protection and Quarantine	Hirsch index -1 ORCID 0000-0002-5747-8860; Scopus Author ID: 57210697811
8	L.P. Zotova, PhD.	S. Seifullin Kazakh agrotechnical university, Senior Lecturer, Department of Agriculture and Crop Production	Hirsch index -5 ORCID 0000-0001-8610-2689; Scopus Author ID: 57197867176; Researcher ID Web of Science AAE-9553-2022
9	A.V. Tsygankov, Bachelor of Agriculture	Aktobe ARS, Researcher	ORCID-ID 0000-0002-1782-962X

Main results for 2022:

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 8-9 th 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/m^2 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/m^2, which is 64 g/m^2 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.

Main results for 2023:

To detect intra-varietal polymorphism of DNA of proso millet collection, the informative ISSR-primers were selected according to the primer efficiency scale. PCR conditions optimization

was performed for each marker. Performance of each ISSR marker was individually analyzed by ISSR method with genomic DNA in PCR reaction. A total of 16 ISSR primers were tested: ISSR 807, ISSR 808, ISSR 809, ISSR 810, ISSR 811, ISSR 816, ISSR 817, ISSR 819, ISSR 820, ISSR 822, ISSR 823, SSR 824, ISSR 826, ISSR 834, ISSR 835 and ISSR 840. The analyzed 16 ISSR primers contained sequences of di-, tri- and polynucleotide microsatellite motifs. 5 of the 16 ISSR markers showed high efficiency as they detected the highest number of well amplifiable DNA fragments, the remaining markers showed medium or low efficiency.

To assess genetic differentiation of the gene pool based on SSR scoring data, the number of different alleles (Na), number of effective alleles (Ne), Shannon index (I), expected heterozygosity (He), unbiased expected heterozygosity (uHe), polymorphism information content (PIC value) were calculated with GenAlEx6.5 software package for MS-Excel. 9 out of 20 SSR markers, 9 had polymorphism: SSR 67, SSR 82, SSR 85, SSR 86, SSR 92, SSR 100, SSR 109, SSR 142 and SSR 146, while the remaining 11 were monomorphic. Among the proso millet cultivars divided into 6 origin groups, 20 SSR primer pairs managed to identify a total of 47 alleles, with an average of 2.35 alleles per marker. Genetic diversity analysis showed that the average number of different alleles (Na) per SSR locus ranged from 1.77 to 3.66 for which no unique alleles were obtained. Of the 9 SSR markers, four showed high PIC values, SSR 67 (0.536), SSR-82 (0.756), SSR-85 (0.795) and SSR-109 (0.758), indicating exceeding the critical value of 0.5. Markers SSR 85 and SSR 86 showed significant association with variance of productive bushiness trait, p-value was 0.013 and 0.008, respectively. SSR-85 marker was also associated with average grain yield trait, at $p<0.05$.

In the current year, field experiments of the nursery collection were laid in the conditions of Akmola and Aktobe regions. During the study of the world collection of proso millet, which contained 61 foreign varieties and 29 local varieties, phenotypic variability depending on the region was recorded including the most important seven agronomic traits. As a result, it was revealed that in soil-climatic conditions of A.I. Baraev SPC GF (Akmola region) the average value of plant height ranged from 63.1 to 95.6 cm. In the conditions of Aktobe Agronomical Experimentation Station (Aktobe region), this trait varied from 67.4 to 99.2 cm. The number and the weight of grains from the main panicle had the most significant association to growing conditions. Observations in the experimental areas showed that there was a fluctuation in the number of panicle grains in the conditions of A.I. Baraev SPC GF: 460±10.9 pieces in average, 706±16.6 pieces for the western region, the weight of panicle seeds: 2.6±0.09 and 2.55±0.08, respectively. Productive bushiness was 1.0-1.5 piece per plant in average in the conditions of Akmola region, on the contrary, in the conditions of Aktobe region there was a significant variation for this trait: 0.8-3.8 piece per plant plant, respectively.

The obtained field data show that plants grown in different regions of Kazakhstan and formed in contrasting soil and climatic conditions, do not differ in their yield traits, such as seed weight per panicle and weight of 1000 seeds. Average indicators affecting productivity of proso millet genotypes in the terms of the mass of 1000 seeds amounted to 5.7 g in the conditions of A.I. Baraev SPC GF, to 6.45 g in Aktobe Agronomical Experimentation Station, respectively. Evaluation of the grain yield average value of proso millet varieties revealed that the yield of the northern region (225-1248 g/m^2) was almost twice higher than the one of the western region (49.0-668.0 g/m^2). The difference in productivity between the two regions was 209 g/m^2. The study of seed productivity of proso millet plants per square meter allowed to identify the most productive genotypes, such as: Saratovskoye 3 (608.5 g/m^2), PI 209790 (635.3 g/m2), K - 2241 (636.0 g/m^2), Shortanda 7 (713 g/m^2), PI 177481(720.3 g/m^2), PI 211058 (738.5 g/m2), K-2468 (1206.2 g/m^2), which managed to give a stable yield regardless of climatic conditions prevailing in the year of research. According to the vegetation period, the collection can be divided into three groups: faster-ripening (67-70 days); intermediate (71-99); late-ripening forms (100 and more). In the current year, the vegetation period of three samples was shorter by 3.3% compared to the standard. In Aktobe region, 18 varieties of domestic collection had a vegetative period shorter by 20% than the «Pamyati Bersiyeva» local standard variety. The studies conducted in this region showed that the vegetation periods of foreign 27 genotypes were shorter than the local standard «Pamyati

Bersiyeva» local standard variety. According to the results of phenological observations the vegetation period averaged 90.4±2.1 days in the conditions of A.I. Baraev SPC GF. In the conditions of Aktobe Agronomical Experimentation Station it was shorter by 13.8 days and averaged 75.8±2.3 days.

Thus, the field trials of proso millet collection were conducted in the conditions of Aktobe and Akmola regions. As a result of research, the evaluation of initial material of proso millet on valuable-economic traits was carried out and the sources presenting high indices of growth, development and resistance to stress factors were selected. Promising constant lines were selected for the conditions of dry-steppe zones of Kazakhstan according to structural analysis. PCR mode for ISSR markers was optimized and DNA polymorphism of proso millet germoplasm was detected. Statistical t-test showed that SSR 85 and SSR 86 markers were associated with agronomic traits such as productive bushiness (pieces) and grain yield (g/m^2). The obtained results can be used in breeding process to improve proso millet productivity.

List of publications and patents published within the framework of this project: (with links to them):

Within the framework of this project, 3 articles were published in the national journals recommended by Committee for Quality Assurance in the Sphere of Education of the Ministry of Education of the Republic of Kazakhstan and 5 articles were published as part of the international conferences:

1 Зейнуллина А.Е., Рысбекова А.Б., Дюсибаева Э.Н., Жирнова И.А., Цыганков В.И., Цыганков А.В. Активность фотосинтетических пигментов растений проса под воздействием азида натрия // Вестник Кызылординского университета имени Коркыт Ата №1 (64), 2023. С.144-154. https://doi.org/10.52081/bkaku.2023.v64.i1.014.

2 Зейнуллина А.Е., Рысбекова А.Б., Дюсибаева Э.Н., Жирнова И.А., Цыганков В.И., Цыганков А.В. Натрий азидтің мутаген ретінде тары (*Panicum miliaceum* L.) генотиптерінің шаруашылық құнды белгілеріне әсері // Вестник Кызылординского университета имени Коркыт Ата, №3-1 (66), 2023. https://doi.org/10.52081/bkaku.2023.v66.i3.065.

3 Зейнуллина А.Е., Рысбекова А.Б., Дюсибаева Э.Н., Жирнова И.А., Есенбекова Г.Т, Мухина Ж.М. Эффект колхицина на структурные показатели растений проса посевного (*Panicum miliaceum* L.) в поколении M_1 // Вестник науки Казахского агротехнического исследовательского университета им. С. Сейфуллина (междисциплинарный), 2023. -№ 3(118). - С.235-249. – ISSN 2710-3757, ISSN 2079-939X. doi.org/ 10.51452/kazatu.2023.3 (118).1447

-в международных конференциях:

1 Zeinullina Aiym, Rysbekova Aiman Mutagenic effect of colchicine on photosynthetic pigments of two proso millet genotypes / International Conference “Scientific research of the SCO countries: synergy and integration” July 12, 2023. Beijing, PRC. P.144-150.

2 Zeinullina A.Y., Rysbekova A.B., Dyusibaeva E.N., Zhirnova I.A. Mutagenic effect of colchicine on photosynthetic pigments of proso millet M2 generation / Proceedings of the XXIX International Scientific and Practical Conference. Warsaw, Poland. 2023. P. 9-13.

3 Zhirnova I., Rysbekova A., Kurishbayev A., Dyusibaeva E., Zeinullina A. Evaluation of the initial millet material for breeding / Proceedings of the XXIX International Scientific and Practical Conference. Warsaw, Poland. 2023. P. 14-17.

4 Zeinullina Aiym, Rysbekova Aiman, Dyusibaeva Elmira, Zhirnova Irina Influence of colchicine on seeds germination and coleoptile length of proso millet genotypes / V International Scientific and Practical Conference “World science priorities”, August 10 – 11, 2023, Vienna. Austria. P.5-10.