

The project title: IRN AP13067944 "Molecular SNP-marking of bread wheat by genes *TaGW*, *TaGS* and *Rht* for grain weight and lodging resistance"

Significance: The grain loss during wheat lodging can reach 50%; such plants tend to form shrunken, poor-quality seeds. Coarse-grain ones have a higher market value of seeds, flour yield and products quality. Most wheat varieties in Kazakhstan are small-grain, even when grown in favorable years with minimal abiotic stresses. And the process of selecting the best genotypes from splitting hybrid populations is very long, accompanied by lengthy observations, and significant expenditures of human and financial resources. The creation of lodging-resistant, large-grain forms of wheat is possible when obtaining hybrids using samples and methods of foreign breeding. The method of molecular-assisted selection (MAS) is based on modern molecular technologies that make it possible to change the principle of selection of productive plants based on their genotypes, which will make it possible to more accurately determine the donors of the trait under study. Despite the knowledge, the method of SNP-marking of soft wheat is practically not used in Kazakhstan today. And the study of the trait of coarse-grain size and resistance to lodging of varieties of Kazakhstan selection at the level of genes *Rht*, *TaGW* and *TaGS* is an urgent task, the solution of which will allow breeders to speed up the selection process for the desired trait and carry it out at the level of the genotype of wheat plants.

Project goal: Development of promising molecular SNP markers to study the genetic diversity of hybrid populations of soft wheat and increase its productivity by enlarging the 1000-grains mass and resistance to lodging.

Expected and achieved results: As a result of the research, molecular SNP markers will be developed for the analysis of genetic polymorphism for genes controlling plant height (*Rht*) and a sign of 1000-grains mass (*TaGW2*, *TaGW6* and *TaGW8*), as well as a sign of grain size (*tags5*). The work will be carried out on varieties of bread wheat of Kazakh and foreign origin, as well as on hybrids obtained using varieties of domestic and foreign breeding. The work is the first molecular analysis using our own developed SNP markers and the application of the MAS method on soft wheat in Kazakhstan based on coarse grain and lodging resistance.

Based on the results of the 2022 study, 111 collection samples of spring soft wheat were selected and studied in field and laboratory conditions in two geographical points of Northern and Central Kazakhstan. According to the results of field tests of 21 hybrids in the conditions of the dry steppe zone of Akmoла and Karaganda regions, the most promising combinations were selected in terms of coarse grain and short stem, with a weight of 1000 seeds above 40 g and a height of up to 75 cm: Xn-10 x Astana, Xn-08 x Shortandinskaya 2017 (F2); Xn-10 x Karaganda 22 and Xn-08 x Karaganda 29 (F4).

Based on the results of bioinformatics analysis, the *Rht-B1a*, *Rht-D1*, *TaGW2-A1*, *TaGW8-B1a*, *TaGW6*, and *TaGS5-3A* genes were identified as target genes, for which specific primers for the coding regions of the genes were selected.

Based on the results of sequencing, single nucleotide polymorphisms were found for the genes that control plant height *Rht* (Reduced Height), coarse grain (*TaGS5*) among parental forms (Karaganda 29, Karaganda 30, Karaganda 31, Karabalykskaya 90) and varieties of foreign breeding (Xn-02, Xn-08, Xn-10).

In the results of the 2023 study, collections and hybrid samples of wheat were analyzed as prospective breeding materials based on the traits of lodging resistance and large grain size. Field trials of the wheat collection and hybrid material were conducted in two geographical locations, Northern and Central Kazakhstan. Sequencing of gene fragments *Rht*, *TaGW*, *TaGS* of the parental forms of hybrids (Karagandinskaya 29, Karagandinskaya 30, Karagandinskaya 31, Karagandinskaya 22, Karabalykskaya 90, Xn-02, Xn-08, Xn-10) was performed.

Alignment of the nucleotide sequence for the *Rht_D1* gene revealed a polymorphism (G/T) in the Chinese selection variety Xn-10. Multiple polymorphisms were detected in the *TaGW6* gene in the combinations Xn-08 x Karabalykskaya 90 and Xn-08 x Karagandinskaya 29, as well as substitutions C/T in the variety Xn-10. Polymorphisms (A/C) were identified in the *TaGS5* gene between the group of domestically bred varieties (Karabalykskaya 90,

Karagandinskaya31, Karagandinskaya22, Karagandinskaya30, Karagandinskaya29) and the group of Chinese selection (Xn-02, Xn-08, Xn-10).

Based on the identified polymorphisms, SNP markers were developed. Genotyping of 250 wheat lines was conducted according to the optimized protocol, depending on the primer structure. Clear differentiation of parental forms (Xn-10 x Karagandinskaya22, Xn-10 x Karagandinskaya30, Xn-08 x Karagandinskaya29) and segregating hybrid populations into homo- and heterozygotes was achieved for genes controlling plant height (Rht), 1000-grain weight (TaGW), and grain size trait (TaGS).

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Information for potential users: The expected results will represent a significant contribution to the field of genetics and wheat breeding, and a significant scientific and socio-economic effect is expected from the implementation of the project in the field of applied research and agriculture of the Republic of Kazakhstan. The target consumers of the project results can be scientists from universities and research institutes, breeders from research centers and breeding stations for the selection of grain crops and wheat, specialists from State-of-the-art testing stations, as well as farmers who are interested in the application of modern methods in real field conditions.