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MOLECULAR GENETIC ANALYSIS IN SOFT WHEAT BIOFORTIFICATION

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Annotation. *Since grain, which is more common in wild wheat relatives such as Aegilops tauschii, contains high concentrations of iron and zinc, ancient varieties are studied to increase the content of these nutrients in modern elite varieties. This paper presents data on molecular differences between ancient varieties using DNA microsatellite markers.*

Keywords: *biofortification, selection-genetic, grain, minerals, vitamins, Aegilops tauschii, DNA microsatellite markers.*

МОЛЕКУЛЯРНО-ГЕНЕТИЧЕСКИЙ АНАЛИЗ В БИОФОРТИФИКАЦИИ МЯГКОЙ ПШЕНИЦЫ

Аннотация. *Поскольку зерно, которое чаще встречается у диких сородичей пшеницы, таких как Aegilops tauschii, содержит высокие концентрации железа и цинка, изучаются древние сорта с целью повышения содержания этих питательных веществ в современных элитных сортах. В статье представлены данные о молекулярных различиях между древними сортами с использованием ДНК-микросателлитных маркеров.*

Ключевые слова: *биофортификация, селекция и генетика, зерно, минералы, витамины, Aegilops tauschii, микросателлитные ДНК-маркеры.*

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Introduction. Cereals are one of the most widely consumed products of the world's population. Therefore, for a population whose consumption is mainly based on grain products, the content of micro and macroelements and vitamins in grains is of great importance. Today, the role of molecular genetic methods in scientific research is unparalleled in accelerating the selection process and creating high-quality and targeted yielding varieties.

Marker-based breeding is important for implementing biofortification of wheat based on Fe and Zn elements. Therefore, it is crucial for breeders to understand the genetic basis of Fe and Zn element concentrations and successfully implement MAS methods.

The hexaploid T. aestivum common wheat (2n = 6x = 42; genome BBAADD) consists of A, B, and D genomes, with the genome donors being the A-subgenome of T. urartu, the B-subgenome of A. speltoides (or a closely related species), and the D-subgenomes of A. tauschii. [1].

Therefore, since high concentrations of iron and zinc in the grain are common in wild relatives of wheat, such as Aegilops tauschii, this species is increasingly being used to increase these substances in modern elite varieties [2].

Genetic biofortification is a long-term, cost-effective and sustainable solution. Next-generation sequencing technology is proving to be a very useful method for obtaining accurate information about crops. In addition, developments in next-generation sequencing technology and statistical methods are helping to identify and exploit regions in the wheat genome that are responsible for its high mineral and other biofortification properties [3].

Materials and methods. It is important to conduct biochemical analysis of valuable elements in the grains of ancient local varieties of soft wheat, which have been cultivated since ancient times, as well as molecular genetic analysis of research samples using DNA markers genetically linked to iron and other elements.

Research samples. The ancient local wheat varieties "Kizil Sharq" and "Shalola" in the Kashkadarya region, as well as the varieties "Krasnodar-99", "Yaksart", "Gozgon", and "Grom" currently grown in the region, were selected.

Genomic DNA isolation from samples. Genomic DNA from the studied samples was isolated using the STAV (cetyltrimethylammonium bromide) method [4]. The DNA concentration was determined visually by comparing it with lambda (λ) phage DNA at a concentration of 25 ng/ml.

Primers, polymerase chain reaction (PCR), gel electrophoresis, and genotyping. A total of 26 microsatellite (SSR - simple sequence repeat) primer pairs were selected from the Wheat Microsatellite Marker Collection (https://wheat.pw.usda.gov) for molecular studies.

A working mixture was prepared for the PCR reaction based on the following components (Table 1), and the amplification process was carried out using a Hot-start program consisting of 45 cycles (Table 1).

DNA analysis. The study samples were subjected to PCR screening using primer pairs of 26 wheat genome microsatellite markers, including 5 BARC, 9 GPW and 6 CDM and WMC SSR sets (Table 1). According to the results of gel electrophoresis analysis, 1 of the 26 markers included in the molecular screening was found to be PCR-negative.

Table 1.**PCR analysis results**

№	SSR marker collection set	Number of primer pairs used for PCR	from that		
			PCR fragments not detected	**Polimorf**	**Monomorphic**
	BARC	5	-	4	1
	CDM	6	1	5	-
	GPW	9	-	6	3
	WMC	6	-	2	4

	Total:	26	1	**17**	**8**

Of the remaining 25 markers, 17 (68%) were polymorphic and 8 (32%) were monomorphic (Figure 1). Among the SSR markers used, the GPW set showed the highest polymorphism (24%), while the WMC SSR set showed the lowest polymorphism (8%). It was also observed that all CDM microsatellite markers used in the molecular analysis were polymorphic. Polymorphic markers were selected for further studies.

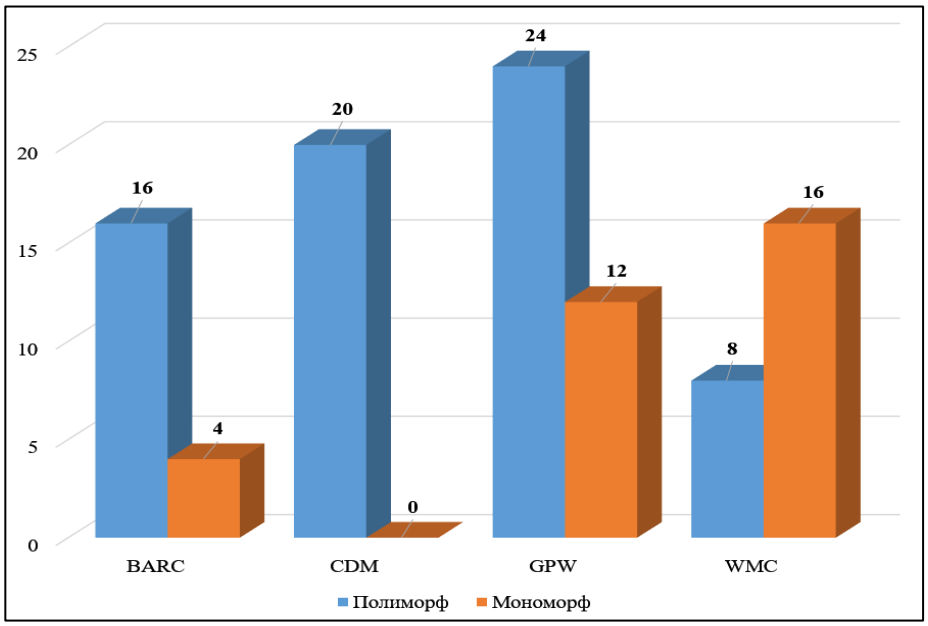

Figure 1. Polymorphism levels of SSR markers among study samples.

Thus, molecular differences between important microelements and DNA microsatellite markers in the study samples were investigated. Further studies will investigate the genetic relationship of the identified polymorphic markers with these microelements.

Conclusion. The results of the studies showed that 17 out of 26 SSR markers involved in molecular studies showed polymorphism. When analyzing the level of polymorphism of primers by sets, it was observed that the group of primers showing the highest polymorphism belonged to the GPW set. It was found that polymorphism was very high between the old local variety “Kizil Sharq” and the remaining varieties.

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