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Selection of wheat-emmer lines with naked colored grains using molecular markers

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Motivation and aim: Wild relatives of modern wheat, such as emmer, carry many beneficial traits related to nutritional value and grain quality. These genetic resources can be used as invaluable assets for wheat improvement. The advent of sequencing technology and the development of molecular breeding methods based on the decoding of the primary wheat genome sequence allow these genomic resources to be effectively used for the development of new varieties. At the same time, there are no any hard tetraploid cultivated wheat (*Triticum turgidum* L., $2n = 4x = 28$, BBAA), which grains can be produced anthocyanin's in Russia.

Materials and methods: The modern white-grained and naked spring emmer variety Gremme with good yield was selected as a recurrent parent. Purple-grained hulled wheat-emmer hybrids, inherited of purple color genes from the tetraploid wheat *Triticum aethiopicum* Jakubz., coming from the Abyssinian region in Ethiopia were obtained by us earlier. Plants were selected visually based on the nakedness of grains during threshing.

In wheat, these important for human health compounds can accumulate in the pericarp, giving the grains a purple color. Their biosynthesis is controlled by complementarily interacting *Pp3* and *Pp-1* genes on chromosome 2A and on chromosome 7A or 7B or 7D, respectively. By decoding the primary sequence of the regulatory genes *Pp*, the intragenic allele-specific diagnostic markers for the dominant alleles of *Pp3* / *TaMYC1* (Patent No. 2774444, Russian Federation State Register of Inventions.) and *Pp-1* / *TaMYB* to hexaploid bread wheat were designed using the PrimerQuest™Tool. Using the NCBI database (https://www.ncbi.nlm.nih.gov/), the MultAlin software (http://multalin.toulouse.inra.fr/ multalin), the primary DNA sequence of the *Pp3* gene in bread wheat, six 261-nucleotide tandem repeats were identified in the promoter of the dominant allele, tissue-specifically activating the expression of structural genes for the synthesis of anthocyanins in the pericarp of the grain, while only one such repeat was found in the recessive allele of white-grained wheat varieties. After decoding the primary DNA sequence of the *Pp1* gene in bread wheat, differences within the first exon and first intron of the gene between the dominant and recessive alleles were revealed. These allele-specific diagnostic markers amplified PCR products of different lengths and, together with microsatellites linked to genes *Pp,* were used for selection of the tetraploid wheat plants with dominant alleles. During the work, the use of SSR markers in the selection did not provide reliable linkage with the target genes, which was especially evident in the selection of the *Pp-1* gene on chromosome 7B. On the contrary, the use of molecular intragenic markers made it possible to accurately select samples with target genes. In the F_2 and F_3 progenies, after crossing white naked emmer variety Gremme with the purple-grained wheat-emmer hybrids, containing the dominant alleles of the *Pp-1*and *Pp3*, naked purple-grained plants accumulating high anthocyanins content in grains were selected.

Conclusions: As a result, after molecular genetic selection, the collection of spring purple-grained naked wheat-emmer lines were created. The obtained lines contain functional working genes that trigger the biosynthesis of anthocyanins in the pericarp of the grain, and were easily threshed. This collection will serve as the basis for the selection of spring hard wheats of the Siberian region with high anthocyanin content.

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