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Improvement of nutritional value of sorghum grain using site-directed mutagenesis of kafirin genes

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Sorghum (Sorghum bicolor (L.) Moench) is one of the most important crops of world agricultural production, a reliable source of fodder and food grains in the arid regions. However, unlike other cereals, sorghum grain has a lower nutritional value, the main reason for which is the resistance of its storage proteins (kafirins) to protease digestion. Modern biotechnology has an arsenal of methods for solving this problem, in particular, RNA interference and genome editing technologies. Using RNA interference technology, we previously obtained mutants with improved digestibility of kafirins and increased lysine content in two varieties of grain sorghum. Currently, to create sorghum lines with improved digestibility of kafirins we are using an approach based on genome editing technology. For this purpose, we have created a series of binary vectors for site-directed mutagenesis of the k1C5 and gKAF1 genes encoding 22 kDa α- and γ-kafirins, respectively.

Nucleotide sequences encoding the signal polypeptides of these proteins responsible for their packaging into the protein bodies of endosperm cells were chosen as targets. In total, four vectors were created: p1C and p2C – for editing k1C5 gene, and p3C and p4C – for editing the gKAF1 gene; each of these vectors contained the cas9 endonuclease gene and genomic target motifs (23 bp sequences). Using Agrobacterium-mediated genetic transformation these vectors were introduced into genome of the grain sorghum variety Avans. In total, 22 transgenic plants carrying cas9 gene were obtained. Among the regenerants obtained from experiments with the p2C, the plants were identified in which the kernels had a modified endosperm texture with a disturbed development of the vitreous endosperm, as well as the plants with a reduced grain size. SDS-electrophoresis of kernel proteins revealed a number of mutants with a significantly higher level of kafirin digestibility (up to 87%) compared to the original cv. Avans (60%). DNA sequencing of the k1C5 gene sequence, encoding the signal polypeptide of 22 kDa α-kafirin, in one of the mutants with improved digestibility of kafirins from T1 generation revealed the presence of a deletion of the third nucleotide of the target, which may have led to a frameshift and a change in the amino acid composition of the polypeptide. Among the plants obtained from experiment with the p3C, two mutants with a deletion and a substitution in the nucleotide sequence of the gKAF1 gene, encoding the γ-kafirin signal polypeptide, were identified. Thus, based on the genome editing technology, we obtained the mutants with significantly improved digestibility of kafirins, which can be used in sorghum breeding.

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Biography

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