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RNA-interference and genome editing as an efficient tool for improving kafirin digestibility in grain sorghum

Lev A. Elkonin

Federal Agrarian Research Centre of South-East Region, Saratov, 410010, Russia

Sorghum (*Sorghum bicolor* (L.) Moench) is one of the world's most important drought-tolerant crops. However, unlike other cereals, sorghum grain has a lower nutritional value, the main reason for which is the resistance of its seed storage proteins (kafirins) to proteolytic digestion. Suppressing the synthesis of individual kafirin subclasses is an effective approach to solve this problem, since distortion of the prolamine synthesis in cereals leads to a rebalancing of the kernel proteome, and to the synthesis of other proteins with a higher content of essential amino acids. This goal can be achieved by RNAi silencing of kafirin genes or genome editing by introducing mutations into the nucleotide sequences of these genes. Using *Agrobacterium*-mediated genetic transformation, in two cultivars of grain sorghum, we obtained the RNAi-mutants bearing the genetic construct for RNA-silencing of the γ-kafirin gene (gKAF1). The mutants are characterized by complete or partial reduction of the vitreous endosperm, increased digestibility of endosperm proteins, and increased content of essential amino acids. The genetic construct for silencing inherited under self-pollination for several generations. In order to use genome editing we have created two series of vectors, which contain genomic target motifs (23 bp sequences) of the α-kafirin (p1C, p2C, p05, p06) and γ-kafirin (p3C, p4C, p07, p08) genes, Cas9 gene, and the marker gene bar. Using these vectors, we have obtained transgenic sorghum plants, with modified endosperm texture and increased in vitro protein digestibility. The work was funded in part by the Russian Foundation for Fundamental Sciences, grant 19-016-00117.