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# SITE-directed mutagenesis for producing grain sorgum mutants with improved kafirine digestibility

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The use of genome editing technologies opens wide opportunities for the targeted mutagenesis in important agricultural crops. In the context of global warming, sorghum, an important drought- and heat-tolerant crop is of particular importance. However, compared to other cereals, sorghum grain has a lower nutritional value, due to the resistance of its storage proteins (kafirins) to proteolytic digestion. A decrease in the synthesis of kafirins as a result of mutations or the expression of the RNAi genetic constructs modifies the ultrastructure of protein bodies and improves their digestibility by proteases. To obtain mutants with improved protein digestibility, we created four binary vectors for site-directed mutagenesis of the *k1C5* and *gKAF1* genes encoding α- and γ-kafirin, respectively. Each of these vectors contained the *cas9* endonuclease gene and a guide RNA targeted the nucleotide sequences encoding the kafirin signal polypeptides. By means of agrobacterial transformation, the created vectors were introduced into the genome of the grain sorghum cv. Avans. 14 transgenic plants were regenerated. Sequencing of 5 regenerants obtained using a vector for the *k1C5* mutagenesis revealed 3 plants with mutations. The offspring of these mutants had a higher digestibility of grain proteins *in vitro* (86–92%) compared to the initial cv. Avans (63–67%). Notably, the $T_1$ plants lacked the *cas9* gene and the *bar* marker gene, which indicates the production of mutants with the edited *k1C5* gene sequence, which lack the genetic construct that induced this mutation. Two mutants with mutations in the *gKAF1* sequence were obtained. Thus, using the CRISPR/*Cas* technology, we have obtained mutants with improved digestibility of kafirins, which can be used in practical sorghum breeding.

**Keywords:** SITE-directed mutagenesis; sorgum; kafirine digestibility.

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