

Improving the digestibility of seed storage proteins in sorghum by RNA silencing of the gamma-kafrin gene: inheritance and expression of the RNAi genetic construct in mutants of cv. Avance and their hybrids

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Key words: RNAi; γ -kafrin; endosperm; *in vitro* protein digestibility; sorghum

Motivation and Aim: RNA interference technology is an effective tool for functional genomics and plant breeding down-regulating expression of various genes encoding biological and agronomically important traits. Sorghum is a high-yielding and drought-tolerant crop. However, sorghum seed storage proteins, kafirins, are resistant to protease digestion that makes sorghum grain less nutritious. Suppression of the synthesis of kafirins improves protein digestibility of sorghum grain [1]. Previously, by *Agrobacterium*-mediated genetic transformation, in the grain sorghum cv. Avance, we have obtained a mutant carrying a genetic construct for RNA silencing of the γ -kafrin gene [1]. Herein, we report on inheritance and expression of this genetic construct.

Methods and Algorithms: PCR, qPCR and RT-PCR methods as well as *in vitro* protein digestibility (IVPD) assay and protein SDS-PAGE were used. To study manifestation of agronomically important traits the T₃–T₅ progenies of the initial mutant carrying RNAi genetic construct were grown in experimental field in 3 replications for 3 seasons.

Results: It was found that the construct is stably inherited during self-pollination up to the T₅ generation. qPCR showed that in different plants from T₄ generation, the genetic construct is present in 1 or 2 copies. RT-qPCR revealed that the expression level of the γ -kafrin gene in developing kernels of T₄ plants was reduced by 3.5–84.1 times. Homozygous lines with different numbers of construct copies were identified, the copy number does not affect IVPD level. All transgenic lines with the RNAi construct differed from the original cultivar by high IVPD, floury endosperm, reduced height, 1000-grain weight, and grain yield per panicle. RNAi construct is inherited during hybridization, improving the digestibility of grain proteins in the F₁ hybrids.

Conclusion: The mutant lines reported herein may be used in functional genomics and for obtaining F₁ heterotic sorghum hybrids with high digestibility of grain proteins.

Funding: The research was supported by the grant of the Russian Science Foundation No. 24-16-00063.

References

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