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## Nutritional and biophysical characteristics of CRISPR-generated low kafirin, waxy sorghum

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Grain sorghum (*Sorghum bicolor* (L.) Monech) is a crop of tremendous significance. Grain sorghum is cultivated for consumption by humans and livestock alike worldwide and is valued for its resistance to biotic and abiotic stresses. However, sorghum grain protein is deficient in essential amino acids and has low digestibility. Furthermore, sorghum does not yield flour with desirable bread making properties. These nutritional shortcomings can be attributed to the structure and amino acid content of the kafirin storage proteins that constitute >70% of proteins expressed in the endosperm that form low-digestibility protein bodies. This study was conducted to evaluate nutritional and biochemical characteristics of reduced kafirin, low-amylose sorghum grain. Previously, a single-guide RNA (sgRNA) Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR/Cas9) construct was used to target members of the alpha-kafirin gene family, *k1C*, which reduced kafirin expression in endosperm cells and elicited a proteome re-balance wherein the increase in nonkafirin expression and reduction in protein body morphology would increase lysine and improve digestibility of the grain. Additionally, introgression of the waxy mutant into k1C-edited F1 sorghum was performed to confer the low-amylose starch trait to improve the dough-making potential of sorghum grain. Here, we report on results from a highly-inbred edited line with ~400kb of the *k1C* family (12 active genes) deleted. These results include transmission electron microscopy (TEM) images of protein bodies, dissecting microscope imagery of endosperm texture, protein-bound and free amino acid profile, and kafirin and non-kafirin sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS PAGE). The results of these tests will provide insight into the nutritional and biophysical properties of sorghum grain produced from this novel genotypic combination.

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## Phenotyping transgenic events for drought resistance

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Drought and heat stress limit maize growth and development, decreasing annual yield in many regions worldwide. As temperatures continue to rise, tropical regions will be disproportionately affected, adversely impacting the quality and production of agricultural products, requiring integrated management practices and the adoption of stress-resistant varieties to mitigate losses. Maize plants evolved different morphological and physiological mechanisms to grow under water-limited conditions, affecting several agronomic traits, but only a few major genes were reported associated with yield. At the Genomics for Climate Change Research Center (GCCRC), we study both genes of unknown function involved in drought response in extremophile plants and genes of the alternative respiratory pathway from cultivated species. After a significant increase in shoot and root biomass under water restriction in a greenhouse experiment, different events overexpressing the UCPI (Uncoupling protein 1) from *Arabidopsis thaliana* or an uncharacterized gene (gccrc candidate gene 20) from a halophyte species were tested in the field under well water and drought conditions in two maize genetic backgrounds (B104xCML488 and B104xCML360) during the Brazilian second season in 2023. We observed variation among events in agronomic and yield components for each gene. Some events demonstrated superior performance compared to the controls. In the B104xCML360 background under drought conditions, both genes contributed to an increase in yield components, whereas in the B104xCML488 background, they did not have the same effect. While CML488 is a drought-tolerant line adapted to the Africa CYMMIT program, CML360 is adapted to acid soils from South America. Field phenotyping of additional genes is in progress in the GCCRC pipeline.

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