Genome editing for precision breeding of cassava and other clonally propagated crops

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Abstract

Root, tuber, and banana (RTB) crops are important staples for people in low and middle income countries around the world. Able to grow in marginal conditions, and high in calories per hectare, these food security crops hold great potential in the context of climate change, and great possibility for genetic improvement. RTB crops are clonally propagated, which allows for reliable preservation of phenotype from generation to generation, but goes hand in hand with heterozygosity and arduous conventional breeding. Thus, genome editing is particularly alluring for these species, as a method for altering specific genes and/or traits in a single generation without disrupting complements of preferred traits, or homozygosing deleterious mutations.

The starchy root crop cassava naturally produces the neurotoxin cyanide. Dietary exposure to cyanide is associated with neurodevelopmental deficits, the paralytic disease Konzo, and other diseases. Post-harvest processing of cassava to remove cyanide, however, can be laborious and reduce nutrient content in the resulting products. We have applied CRISPR-Cas9 genome editing to block the cyanide pathway in three cassava cultivars. By generating disabling mutations in *CYP79D1* and *CYP79D2*, the genes that encode the first enzyme in the cyanogenesis pathway, we engineered cassava lines that do not make cyanide. Single-gene knockouts suggest that *CYP79D1* and *CYP79D2* carry different burdens in cassava cyanogenesis; *CYP79D2* knockout lines with very low (but detectable) cyanide levels may be useful where some cyanide is desired. The mutant lines enable well-controlled field trials to test hypotheses regarding the role of cyanogenesis in cassava. This work demonstrates cassava genome editing for improved food safety and reduced labor burden.

Especially in clonally propagated crops, genome editing is useful for testing candidate genes from association studies, and for uncoupling alleles that recombination has not. Going forward, transgene-free genome editing may obviate GMO concerns and drawn-out regulatory processes, easing adoption of engineered improved varieties.

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Key words: genome editing, clonally propagated crops, cassava, cyanide, food safety