

Generation of semi-dwarf tropical maize by multiplex genome editing

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Motivation and Aim: Maize is the most important modern agricultural crop. A significant portion of global maize production comes from tropical regions. Tropical maize is better adapted to heat and drought than varieties from temperate regions, but it is characterized by lower productivity. The height of plants in many tropical genotypes is significantly greater than that of elite material from temperate climates, which is associated with lower yield and less resistance to lodging. Reducing the height of maize plants is one of the most pressing tasks in breeding this crop. Until recently, all methods of improving tropical corn varieties were limited to classical breeding. However, we have recently developed a genome editing method for tropical maize [1], which allows the application of targeted gene modification methods to enhance the most important traits. Gibberellin (GA) 20-oxidase (GA20ox) genes are known to control plant height. In this study, genome editing approach was applied to the GA20ox gene family in few tropical maize lines with the aim of reducing plant height.

Methods and Algorithms: Few guide RNAs (gRNAs) were designed for directing Cas9 simultaneously to multiple genes of the maize Ga20ox gene family. Cas9/gRNA expressing cassette together with *Wus/Bbm* morphogenic genes was coupled with ternary vector system for increasing the efficiency of *Agrobacterium*-mediated transformation. T0 and T1 plant generations were genotyped by Sanger sequencing. Combination of digital and manual phenotyping was performed to assess morphological characteristics of individual plants in T1. Homozygous mutant lines were established by self-pollination of selected individual mutants.

Results: Modification of multiple genes of the Ga20ox gene family was performed on three tropical maize lines from the CIMMYT collection and temperate model line B104. In all lines, a wide range of phenotypes in the T0 generation associated with mutations in the target genes was obtained. Some T0 regenerant plants from two tropical lines were used to produce the next generation through self-pollination. In the T1 generation, phenotypes obtained in the T0 generation were reproduced, and several new phenotypes associated with changes in plant height and shape were also identified.

Conclusion: The use of the most advanced genome editing methods enables overcoming genotype-specific transformation barriers and obtaining modifications in exotic genotypes of crop plants. Modification of genes in the gibberellic acid regulatory pathway allows obtaining a spectrum of morphological changes potentially valuable for breeding.

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References

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