

## UTILIZAÇÃO DE GENES MORFOGÊNICOS PARA POSSIBILITAR A EDIÇÃO GENÔMICA DE LINHAGENS TROPICAIS DE MILHO

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### Abstract:

Genome editing (GE) is a powerful tool to accelerate plant breeding, but its large-scale application still faces relevant obstacles. Prominently among these is the recalcitrance to traditional genetic transformation protocols exhibited by many genotypes of important crop species. Despite the widespread utilization of a few maize genotypes amenable to genetic transformation, these are unfit for high-confidence phenotyping in field trials or commercial ends. This hindrance is somewhat aggravated by the fact that most of the known transformable maize lines are adapted to temperate geographies, whereas a considerable proportion of maize production occurs in the tropics. Different strategies have been recently developed and are constantly being improved to overcome the low efficiency and genotype dependency of genetic transformation. Ectopic expression of morphogenic regulators (MRs) is among the most promising approaches to achieve "universal" GE capabilities in maize. Here, we report the successful GE of tropical maize lines using a MRs-based protocol. To this end, we used a CRISPR/Cas-based construct aiming at the knockout of the *VIRESCENT YELLOW-LIKE (VYL)* gene, which results in an easily recognizable phenotype. Three out of eight tropical lines were amenable to transformation, with efficiencies reaching up to 5%. Remarkably, most of the events showed the *vy1* loss-of-function phenotype, with 96.4% of the recovered events presenting indels at the target site. A zygosity analysis showed that approximately 40% of the T<sub>0</sub> regenerants are heterozygous for the mutations (i.e. only one allele edited). Further investigation revealed a SNP present in heterozygosity at the target site of some lines, explaining the high rate of heterozygous T<sub>0</sub> and highlighting the importance of investigating the target sequence in new genotypes for specific sgRNA design. Finally, these results demonstrate the efficient GE of relevant tropical maize lines, expanding the current availability of GE-amenable genotypes of this important crop.

**Key-words:** CRISPR/Cas; morphogenic regulators; maize transformation; ;