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CRISPR/Cas9-targeted Mutagenesis of *Flowering Promoting Factor 1* Gene to Induce Brachytic Dwarfing trait in Tomato

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[Introduction]

Brachytic dwarfing (also known as brachytic or brachytic dwarf) is characterized by a shortening of only the internodes with little or no effect on other tissues and organs. Short plant height is especially beneficial for management practices in fresh-market tomatoes in the USA. Fruit size is an important standard in fresh-market tomatoes in the USA. Fruits greater than 5.715 cm in diameter are screened as marketable fruit. CRISPR/Cas9 system has been broadly used for CRISPR/Cas9-mediated genome editing in plants including tomato. To develop tomato with short plant height and no reduction in fruit size, brachytic dwarfing tomatoes were induced by CRISPR/Cas9-targeted mutagenesis.

[Materials and Methods]

Two gRNAs specifically targeting *flowering promoting factor 1* gene (*FPF1*, Solyc01g066980) were designed and two *Agrobacterium* strains containing gRNA1 or gRNA2 were prepared and used for *Agrobacterium*-mediated transformation. Mutants were identified by Sanger sequencing. Transgene-free mutants were screened by PCR using primers targeting *Cas9* gene or *hph* gene. Homozygous mutants were screened by analyzing chromatograms. Potential off-targets were searched using whole genome re-sequencing data. Homozygous transgene-free mutant lines with no off-target were grown in a greenhouse and tomato field at the University of Florida. Phenotypes, including internode length and fruit size, were investigated. In addition, two paralogues (Solyc01g066950 and Solyc01g066970) of *FPF1* were used for CRISPR/Cas9-targeted mutagenesis.

[Results and Discussion]

The mutation efficiency of gRNA1 and gRNA2 was 50% and 53.8%, respectively. We obtained 2 homozygous transgene-free mutant lines with no off-targets. On *FPF1*, *br.8.1^{CR}* has a 7 bp deletion induced by gRNA1 and *br.8.2^{CR}* has a 2 bp deletion induced by gRNA2. The mutant lines showed internode length reduction by 35.07% and 29.1% in *br.8.1^{CR}* and *br.8.2^{CR}*, respectively. The *br.8.1^{CR}* and *br.8.2^{CR}* lines showed no remarkable reduction in fruit size, which is a typical characteristic of brachytic dwarfing. However, yield reduction was observed in both mutant lines (by 34.36% and 46.83%). In addition, we obtained Solyc01g066950 and/or Solyc01g066970 mutant lines. On Solyc01g066970, *br.7^{CR}* has a mutation. On both Solyc01g066950 and Solyc01g066970, *br.57.1^{CR}* and *br.57.2^{CR}* have mutations. All the three mutants showed internode length reduction, and dosage effect was observed in *br.57.1^{CR}* and *br.57.2^{CR}*. We developed a new plant material that contains an agriculturally favorable and an unfavorable trait simultaneously through CRISPR/Cas9-targeted mutagenesis.

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