**Brief Communication**

**Using CRISPR/Cas9 genome editing system to create MaGA20ox2 gene-modified semi-dwarf banana**

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Banana is an important staple crop and widely grown in more than 130 countries around the world. Plant height is one of the major characteristics of crops (Wang et al., 2018); however, the height of commercial banana varieties is more than 2 m, and higher varieties are frequently challenged by the weak lodging and severe damages caused by typhoons and storms. Dwarf varieties are suitable for mechanized plant maintenance and fruit harvesting (Dash and Rai, 2016); thus, development of semi-dwarf and dwarf banana is important to current farming systems.

Gibberellin (GA) is one of the most important determinants of plant height (Sasaki et al., 2002), and mutations in genes for biosynthesis or signal transduction of GAs generally result in dwarf phenotypes. For example, a 383 bp deletion in the OsGA20ox2 caused a premature stop codon and lower bioactive GA20ox2 activity, resulting in the semi-dwarf phenotype in IR8 sd1 mutant (Sasaki et al., 2002; Spielmeyer et al., 2002). Similarly, Chen et al. (2016) found that the dwarf phenotype of banana mutant ‘8818-1’ was mainly derived from differential expression in 6 genes regulating GA production compared with its wild-type Williams 8818. In this study, we found five GA20ox2 homologous genes specifically expressed in certain tissues, such as Ma11g17210 and Ma06g27710 which exhibited higher mRNA levels in leaves, while Ma11g10500 was highly expressed in fruit peels. By contrast, Ma04g15900 exhibited higher transcript level in fruit axis, peel and pulp, and Ma08g32850 was much lower in spite of its similar expression pattern to Ma04g15900 (Figure 1a). To date, the CRISPR/Cas9 system has been extensively used for generating targeted mutations in crop genomes for functional analysis and plant precision breeding (Feng et al., 2018; Hu et al., 2017). To examine the functions of five MaGA20ox2 genes in ‘Gros Michel’, we selected two sgRNAs that specifically target the second exon of each gene (Figure 1b), and these two sgRNAs were separately driven by an U6a or U3 promoter. In addition, a GFP gene was fused with HPT of the pYLCRISPR/Cas9P vector (Ma et al., 2015) to form a pCRISPR/Cas9-GA2T expression plasmid (Figure 1c) which was transformed into *Agrobacterium* strain EHA105.

To confirm the mutations induced by the CRISPR/Cas9 system, we amplified the target regions of transformed and wild-type (WT) plants and performed high-throughput sequencing. The results showed that 7 mutant lines with semi-dwarf phenotype contained different types of mutations (Figure 1k). In the T1 site of Ma04g15900 and Ma08g32850, the short insertions (+1) were the much common mutation in seven mutants, and short deletions (~1) appeared in #7, #12, #31 and #45, while the 19-bp deletion was only found in #7. In the T2 site of Ma11g10500, short deletion (~3) appeared in #7, #31, #45, #51 and #60, and short insertions (+1) were only found in #7 or #60 (Figure 1k). Furthermore, we found that mutations in Ma04g15900 and/or Ma08g32850 genes may play important roles for semi-dwarf phenotype in ‘Gros Michel’. These results indicated that the CRISPR/Cas9-mediated system is efficient for precise genome editing in banana.

To investigate the changes in plant height, we measured the height of six mutant lines and found that it was significantly decreased compared with the WT at three developmental stages (Figure 1l). To understand how these mutations affect the MaGA20ox2 genes, the transcript level of these five genes in banana pseudostems was performed. Compared with WT, the transcript level of Ma04g15900 and Ma08g32850 was significantly down-regulated, while Ma11g10500 and Ma06g27710 were significantly up-regulated.
was significantly up-regulated in #12, and that Ma04g15900 was significantly down-regulated in #31 (Figure 1m). To confirm whether GA biosynthesis was impaired in mutants, endogenous GAs of different tissues were quantified. The results showed a higher content of GA33 and lower content of GA40, GA19, GA20 in #31 compared with WT. Also, the amounts of active GA3, GA3 and GA4 were decreased in roots and pseudostems of #31, while the content of inactive GA8 and GA20 was much higher (Figure 1n).

To our surprise, mutant lines had thicker and dark greener leaves than WT and that was similar to Arabidopsis (Peng and Harberd, 1997). We analysed the differences in middle leaves and petioles of #31 and WT at 100 days after transplanting. The results showed that corneum and epidermal cells of WT were regular and smooth (Figure 1o), while these cells of #31 were irregular (Figure 1p). The cell numbers of upper corneum (UC), lower epidermis and lower corneum of #31 were significantly reduced compared with WT (Figure 1q). In addition, the alterations in cell length were different from changes in cell number. The cell length of UC and upper epidermis was longer and that of #31 was similar to that of WT. The upper epidermis was shorter than WT (Figure 1r).

These results may explain why the leaves of mutants were thicker than the controls. Furthermore, longitudinal sections of the thinner parts of petioles showed that cells of WT were regular, while inner cells of #31 were partially irregular (Figure 1s). In addition, the cell length of #31 was shorter than WT (Figure 1t).

These results of plant height and leaf cell structures were consistent with Carrizo citrange (Fagoaga et al., 2007) and provide a new insight into understanding the roles of MaGA20ox2 genes in plant architecture.

In summary, we have successfully applied the CRISPR/Cas9 system to edit the MaGA20ox2 genes in ‘Gros Michel’ and obtained semi-dwarf mutants. Manipulation of Ma04g15900 and/or Ma08g32850 genes is likely to be an efficient strategy to develop semi-dwarf or dwarf banana germplasm resources. Future efforts will be given to evaluate the characteristics of growth and yield of these mutants at multiple locations in different years to minimize the environmental influences. Results from these current and future studies will be of significant impact on banana dwarf breeding.

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Conflict of interest

The authors declare no conflict of interest.

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