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Dear Editor,

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The CRISPR (Clustered Regularly Interspaced Short Palindromic Repeat)/Cas9 provides straight-forward ways for genome editing in many plants (Yin et al., 2017), but has never been applied in bamboo. Here, we reported the generation of bamboo mutants with CRISPR/Cas9 technology by targeting one specific copy or all homoeologous genes.

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was simultaneously co-transformed with CRISPR/Cas9 plasmids (Figure 1B). Around 1.8% of the protoplasts transformed with the UBI-Cas9/OsU6b-sgRNA construct showed strong signals within 72 h, indicating that the mGFP function was restored by the CRISPR/Cas9 system through deleting the additional “guanine” (Figure 1C). The OsU6a and OsU6c promoters work as well, however, with lower efficiency than the OsU6b promoter, as positive signals were only occasionally observed with more than 10 replicates. Taking together, the UBI-Cas9/OsU6b-sgRNA construct effectively works in bamboo protoplast, and were used for the following endogenous gene editing in Ma bamboo.

The putative phytoene synthase (PSY1) in bamboo, whose homolog in maize functions in carotenoid biosynthesis (Zhu et al., 2016), was selected for the initial test. Three bamboo PSY1 alleles (DlmPSY1-A, DlmPSY1-B, DlmPSY1-C) were identified and cloned by a homology cloning strategy (Figure 1D). To mutate all copies of DlmPSY1, sgRNA1 targeting a conserved site among all DlmPSY1 loci was designed (Figure 1D). In addition, the sgRNA2 target site containing 2–3 single-nucleotide polymorphisms (SNPs) in the spacer region among three DlmPSY1 homoeoalleles and was selected to test the tolerance of sgRNA mismatches (Figure 1D).

1,600 bamboo calluses induced from stem were transformed as described previously (Ye et al., 2017). In total, 34 independent transgenic lines were confirmed positive (2.1%) by PCR. Based on Sanger sequencing results, 22 (100%) and 10 (83.3%) independent T0 lines were edited in the sgRNA1 and sgRNA2 region, respectively (Figure 1E), indicating that both constructs effectively induce endogenous gene editing.

The editing profiles were further analyzed by sequencing. Eighteen lines (81.8%) contained putative homozygote/biallelic mutations in all subgenomes at the sgRNA1 target site. In some lines, putative homozygote/biallelic mutations exist in one
subgenome while heterozygote or chimeric mutations appear in other subgenomes (T0-10 and T0-26) (Figure 1F). Eight mutation types were identified from 590 independent clones (Figure 1G). The most frequent mutation type was deletion (75%), of which 59.1% are small deletions (<2bp). The ratios of large fragment deletions (≥14bp), insertions, and combined indels were 15.9%, 2.21%, and 7.82%, respectively (Figure 1G). Since bamboo propagates through asexual budding, those homozygote/biallelic mutations will remain in the genome of their offspring clones during breeding.

sgRNA2 that perfectly targets DlmPSY1-A1, but not DlmPSY1-B1 or DlmPSY1-C1 was designed to study the recognition specificity (Figure 1D). Sequencing results confirmed that 10 transgenic lines contain mutations in DlmPSY1-A1, but none in DlmPSY1-B1 and DlmPSY1-C1 (Figure 1E). Two lines (20%) were putative homozygous or biallelic mutations (T0-12 and T0-14), while 7 lines (70%) were heterozygous/chimeric (T0-30 to T0-32 as representative examples, Figure 1H). The ratios of deletions, insertions, and combined mutations were 86%, 9%, and 5%, respectively (Figure 1I). The mutations were predominantly short nucleotide changes (1–26bp), and 22.7% were 1bp nucleotide deletions (Figure 1I). Those data demonstrated the successful application of the CRISPR/Cas9 system in mutating a specific DlmPSY1 allele.

Eighteen lines (81.8%) with homozygote/biallelic mutations in all subgenomes at the sgRNA1 site exhibited albino phenotypes (Figure 1J), which appeared at an early stage during tissue culture, and persisted at the plantlets stage (Figure 1J). Those results suggest that genome editing takes place at an early stage in embryonic cells, and led to the loss-of-function of all DlmPSY1 alleles. Similar results were reported in rice, wheat or cotton (Wang et al., 2018; Wang et al., 2014; Zhang et al., 2014). In case of sgRNA2, although DlmPSY1-A was mutated, no visible phenotypic change was observed due to the existence of the wild-type DlmPSY1-B and DlmPSY1-C.
Next, we applied this technology in bamboo molecular research. Bamboo is the tallest grass in the world, while the underlying mechanism is unknown. Previously, we identified several Gibberellin-responsive genes including GRG1 (GA-responsive gene 1, PH01004823G0070) that potentially acts in controlling bamboo height (Zhang et al., 2018). Here two homozygote grg1 mutants (efficiency 40%) in Ma bamboo were produced using our optimized CRISPR/Cas9 technology. Mutation in GRG1 increased plant height (Figure 1K), mostly due to elongated internodes (Figure 1L–N). Sequencing results confirmed that the grg1 mutant has the putative homozygous mutation in A1-subgenome, biallelic mutation in B1-subgenome, and homozygous mutation in C1-subgenome (Figure 1O), indicating the loss-of-function of GRG1 in transgenic bamboo. To our knowledge, this is the first example on controlling bamboo height through gene manipulation, which will contribute to subsequent studies on the molecular mechanisms behind the fast growth of bamboo.

In summary, for the first time we engineered the hexaploid Ma bamboo through CRISPR/Cas9 technology. The homozygote mutations were obtained in the first generation of transgenic lines, which is extremely important for bamboo species due to its long vegetative growth periods. We also confirmed the albino phenotype of dlmpsy1 mutant in bamboo and generated a bamboo mutant with altered plant height. This demonstrates the applicability of CRISPR/Cas9 in bamboo and thereby boosts future bamboo research and breeding.

References


Conflict of interests

The authors declare that they have no conflict of interests.

Fundings

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Authors’ contributions

Q.Z. conceived this project; L.F.G., Y.S.Z., X.Q.M. C.T.L. and Q.Z. designed experiments, and interpreted the results; S.W.Y., G.C. and M.V.K. performed the experiments and analyzed the data, W.J.W., C.Y.C., C.W., and D.W.S. helped to perform the experiments and collect the data. All authors read and approved the submission of this manuscript.

Figure Legend

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A. Bamboo protoplast isolation and transformation. a. Microscopic image of isolated bamboo protoplast transformed with 35S:tdTomato. b-d. Images of bamboo protoplasts co-expressing the fluorescence proteins tdTomato (b) and GFP (c) driven by the 35S promoter, and their overlay (d).

B. CRISPR/Cas9 plasmids for bamboo protoplast. Top: CRISPR/Cas9 constructs expressing the sgRNA directed against mGFP and driven by OsU6a/OsU6b/OsU6c respectively; Middle: mGFP-expression construct, mGFP contains one additional guanine (lower-green case) downstream of the translational start site (red); bottom: GFP-expression construct. The sgRNA was designed to produce the presumptive cleavage site at the third nucleotide upstream of the PAM sequence (blue).

C. Representative bamboo protoplasts co-transfected with mGFP and OsU6b-sgRNA/UBI-Cas9 reproducibly emitting fluorescence signals (red
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D. *DlmPSY1* gene structure and sequences of the target sites. Gray boxes: exons; black lines: introns; number in brackets: positions of start codon, stop codon and sgRNA target sites (red and orange rectangles). The PAM regions (blue), SNPs (red), and nucleotide sequences of the *sgRNAs* and *DlmPSY1* genes were given.

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Authors’ contributions

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