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LETTER

CRISPR/Cas9-mediated *SiYABBY1* mutagenesis enhances cleistogamy and seed number and size in foxtail millet (*Setaria italica*)

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Highlights

- CRISPR/Cas9-mediated *SiYABBY1* (*Seita.2G037900*) mutagenesis enhances cleistogamy and increases seed number and size in foxtail millet.
- Overexpression of *SiYABBY1* (*Seita.2G037900*) can significantly inhibit the normal growth and development of foxtail millet.
- Understanding the molecular mechanism of *SiYABBY1* will contribute to achieving the breeding goal of high yield.

Due to the continuous growth of the global population, the intensification of climate change and the on-going reduction of agricultural land area, increasing crop yields has become an urgent challenge (Bailey-Serres *et al.* 2019). Foxtail millet is an ancient crop that originated in China, but its yield still has great potential for further improvement compared to staple crops (Diao and Jia 2016). However, current research on the genes regulating inflorescence development in foxtail millet remains limited (Zhi *et al.* 2021; Zhang *et al.* 2024).

The *YABBY* gene family plays a significant role in regulating various biological processes in plants (Han *et al.* 2025). Previous studies have shown that the *YABBY* gene is involved in the regulation of plant inflorescence development (Siegfried *et al.* 1999; Cong *et al.* 2008; Zhang *et al.* 2023; Zuo *et al.* 2024; Cai *et al.* 2025). In foxtail millet, recent studies have found that heterologous expression of the *YABBY* gene in *Arabidopsis thaliana* leads to changes such as delayed flowering, leaf curling, shorter siliques, and reduced seed yield of the plants (Guo *et al.* 2022; Ren *et al.* 2025). However, there are few reports on the *YABBY* gene regulating inflorescence development in foxtail millet. The findings of this study indicate that *SiYABBY1*-overexpression significantly inhibits the development of foxtail millet. On the contrary, knocking out this gene affects the size of lodicules, promoting 72.8% of the spikelets to exhibit cleistogamy, while it also causes changes in panicle morphology, ultimately increasing grain length by 11.27–14.02% and the number of spikelet grains by 31.16–33.67%. This study not only provides new insights into the role of *SiYABBY1* in foxtail millet inflorescence development but also offers important theoretical support for strategies aimed at increasing foxtail millet yield.

In this study, based on our previous RNA-seq data (Zhang *et al*. 2025), we conducted an RT-qPCR analysis on nine *SiYABBY* family genes and chose the one highly expressed in the green anther (GrA) period for subsequent experiments (Appendices A and B). To investigate its function, we heterologously expressed the *SiYABBY1* gene in *Arabidopsis thaliana* (Appendix C). The resulting seedlings displayed a purple phenotype, and the overexpression of *SiYABBY1* caused varying degrees of dwarfing (Appendix D), with significant differences observed in traits such as leaf area, root length, and seed size (Appendix E). In addition, overexpression of *SiYABBY1* in foxtail millet (*SiYABBY1-OE*) led to notable phenotypic changes (Appendix F). Most transgenic plants lacked roots (Appendix G), demonstrated significantly inhibited growth and failed to progress normally to the heading stage (Fig. 1-A and E). Furthermore, the leaves of *SiYABBY1-OE* plants were shorter and narrower (Fig. 1-B). Observations of the epidermal cells of these leaves revealed significant differences in cell length, width, number, and area (Fig. 1-C, D, and F). To further explore its molecular mechanisms, we conducted RNA-seq analysis on *SiYABBY1-OE*. Differentially expressed genes (DEGs) were screened with Fold change>2 as the threshold. The cluster analysis and volcano plot results revealed that *SiYABBY1-OE* (*Seita.2G037900*) was significantly up-regulated

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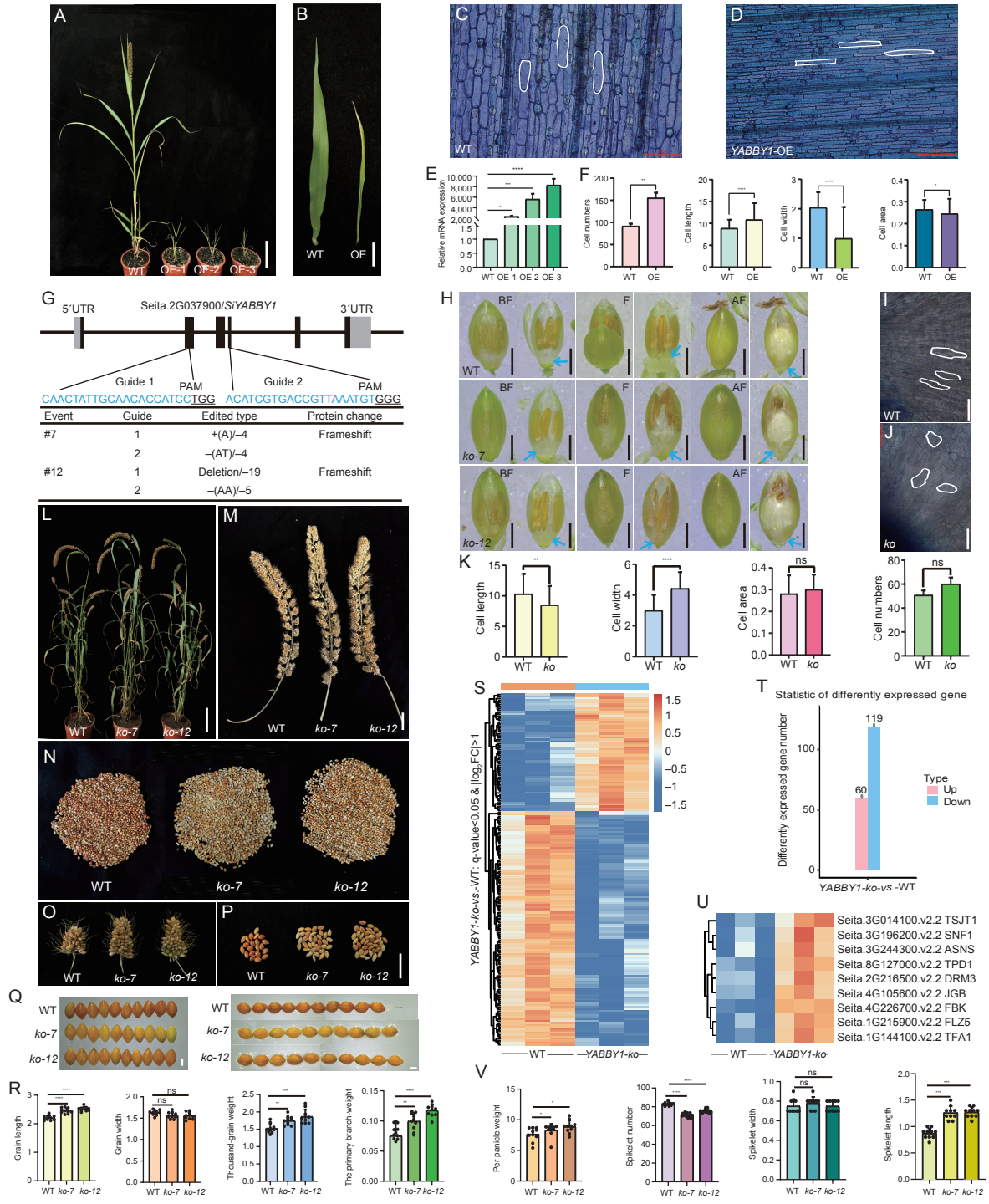


Fig. 1 *SiYABBY1* affects the panicle development and yield of foxtail millet. A and B, phenotypes of *SiYABBY1*-OE lines. Scale bar in A=10 cm; scale bar in B=1 cm. C and D, leaf epidermal cell phenotypes of *SiYABBY1*-OE lines. Scale bar=10 μm. E and F, expression levels of *SiYABBY1*-OE lines (E); and cell length, cell width, cell area and cell number (F). Bars mean SD (n=3). G, sequencing analysis of the genomic structure of the *SiYABBY1* gene and the targeted sites of the CRISPR/Cas9 system. H, analysis of the panicle cleistogamy phenotypes of the edited lines. The blue arrows indicate the lodicules. Scale bar=1 mm. I and J, cell morphology analysis of wild type (WT) (I) and *SiYABBY1-ko* (J) lodicules. Scale bar=10 μm. K, statistical analysis of cell length, cell width, cell area, and cell number of the *SiYABBY1-ko* lodicules. Error bars indicate SD (n=3). L, overall plant phenotypes of the WT and *SiYABBY1-ko* lines. Scale bar=10 cm. M, panicle morphology of the WT and *SiYABBY1-ko* lines. Scale bar=2 cm. N, yield per plant in the WT and *SiYABBY1-ko* lines. Scale bar=1 cm. O and P, primary branch and grain morphology analysis of WT and edited plants. Scale bar=1 cm. Q, comparison of grain width and length between the WT and edited lines. Scale bar=1 mm. R and V, statistical analysis of grain length and width, thousand-grain weight, primary branch weight, per panicle weight, spikelet number, spikelet width and spikelet length. Error bars indicate SD (n=10). S, cluster analysis of genes expressed in WT and *SiYABBY1-ko*. T, numbers of up- and down-regulated genes in WT and *SiYABBY1-ko*. U, genes affecting the inflorescence and grain development of *SiYABBY1-ko*. BF, before flowering stage. F, flowering stage. AF, after flowering stage. Statistically significant differences between samples were determined by Student's t-test. *, P<0.05; **, P<0.01; ***, P<0.001; ****, P<0.0001; ns, not significant.

in the transgenic plants. KEGG enrichment analysis indicated that the DEGs were predominantly enriched in pathways associated with plant hormone signal transduction, amino acid metabolism, and glucose metabolism. Meanwhile, significantly different expression levels of genes related to cell wall synthesis, cell cycle and defense were also found (Appendix H).

Subsequently, CRISPR/Cas9 technology was used to edit *SiYABBY1* (*SiYABBY1-ko*) (Fig. 1-G; Appendices I–L). Compared with the control, 72.8% of the glumes exhibited the cleistogamy feature, and the glumes were mostly purple (Appendix M). The morphology of lodicules in edited plants was similar to the WT during the before flowering (BF) stage, but the lodicules became slender during the flowering (F) stage (Fig. 1-H–K). Furthermore, NBT staining of the lodicules at various developmental stages revealed that the accumulation of $O_2^{-}\cdot$ in the edited plants was significantly higher than in WT (Appendix N). In summary, the loss of function of *SiYABBY1* may lead to ROS accumulation, which probably results in malformations in lodicule development and the cleistogamous phenotype in foxtail millet.

The phenotypic observations and analysis results from the *SiYABBY1* gene-edited plants indicated no significant differences in plant height or panicle length between the edited lines and WT (Fig. 1-L and M). However, the spikelet length of the *SiYABBY1-ko* lines increased compared to the WT, and the number of spikelet grains also increased, ultimately leading to a significant rise in the number of grains per panicle (Fig. 1-O and P). Further statistical analysis indicated that editing the *SiYABBY1* gene could significantly enhance most yield traits (Fig. 1-N, V, Q, and R; Appendix O). Consequently, spikelets from *SiYABBY1* edited plants were collected for transcriptome analysis to explore the regulatory mechanisms underlying foxtail millet inflorescence and grain development. Screening based on Fold change>2 (Fig. 1-S) revealed a total of 22,454 DEGs between WT and *SiYABBY1-ko*, including 60 significantly up-regulated genes and 119 significantly down-regulated genes (Fig. 1-T; Appendix P). Further analysis indicated that in *SiYABBY1* edited plants, the expression levels of genes associated with inflorescence and grain development were relatively high (Fig. 1-U), suggesting that the up-regulation of these genes might have contributed to the 8.69–16.06% increase in per panicle weight of foxtail millet.

In conclusion, this study successfully identified the key gene *SiYABBY1* that regulates the development of foxtail millet. The results show that *SiYABBY1* may affect lodicule development by modulating the accumulation of ROS, thereby regulating the process of cleistogamy in foxtail millet. In addition, the knockout of this gene positively effects multiple yield traits, but the underlying molecular mechanism has not yet been fully elucidated. Future studies can identify and confirm the direct downstream target genes of *SiYABBY1* through molecular biological and genetic strategies, and verify the functions of its downstream target genes by means such as gene editing. This study lays an important theoretical foundation for the in-depth analysis of the developmental mechanism of foxtail millet panicles.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that they did not use AI in the preparation and writing of this manuscript.

Appendices associated with this paper are available at https://doi.org/10.1016/j.jia.2026.03.007

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