AcMYB61 improves drought tolerance in transgenic kiwifruit by reducing water loss

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Introduction

Kiwifruit is more sensitive to drought than many fruit trees because of its larger leaves and shallow root system. Drought has caused a large reduction in kiwifruit production every year, so improving its drought resistance has become an urgent problem to be solved.

In our preliminary work, *AcMYB61* was screened out from transcriptome data, which significantly induced by drought, but its mechanism of action is still unclear. Here, we verified the function of *AcMYB61* in drought resistance by overexpressing *AcMYB61* in kiwifruit. The results will enrich the resistance mechanism of kiwifruit.

Material and Method

Gene clone; Subcellular localization; transactivation activity analysis; Stable transformation in kiwifruit; Gene expression profile assayed by qRT-PCR; Autofluorescence observation of lignin; Y1H, etc. .

Results

◆ *AcMYB61* was identified in kiwifruit and it was expressed preferentially in stem and leaves.

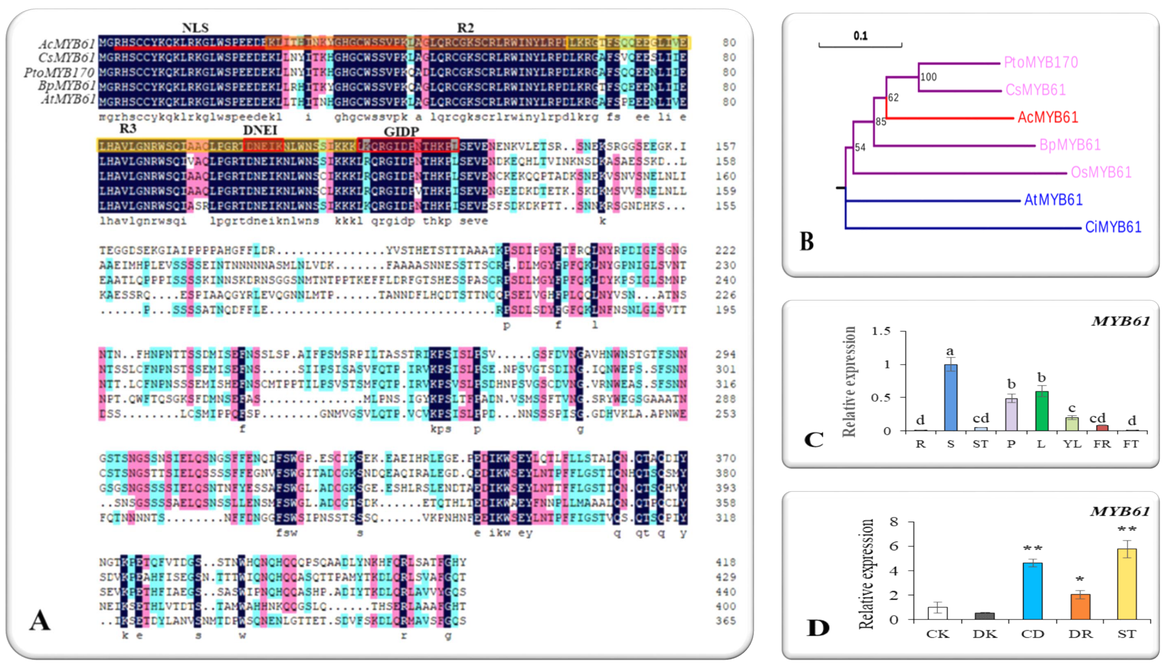


Fig. 1 Alignment (A) and phylogenetic tree (B) of amino acid of *AcMYB61*, and other *MYBs* and expression profile of *AcMYB61* in different tissues (C) and under different treatments (D). NLS: Nuclear localization signal; DNEI: Negative regulation of flavonoid; GIDP: activator activity. Pto. *Populus tomentosa*; Cs. *Citrus sinensis*; Ac. *Actinidia chinensis*; Bp. *Betula platyphylla* Suk. ; Os. *Oryza sativa*; At. *Arabidopsis thaliana*; Ci. *Chrysanthemum indicum* var.aromaticum. Bar = 0.1 substitutions per site. R. Root; S. stem; ST. stem tip; P. petiole; L. leaf; YL. young leaf; FR. Flower; FT. fruit. CK, Control; DK, Dark; CD, Cold; DR, Drought; ST, Salt. Different letters: significant difference at $p < 0.05$ level. *, $P < 0.05$; **， $P < 0.01$.

◆ *AcMY61* is localized to the nucleus and has no transcriptional activation activity.

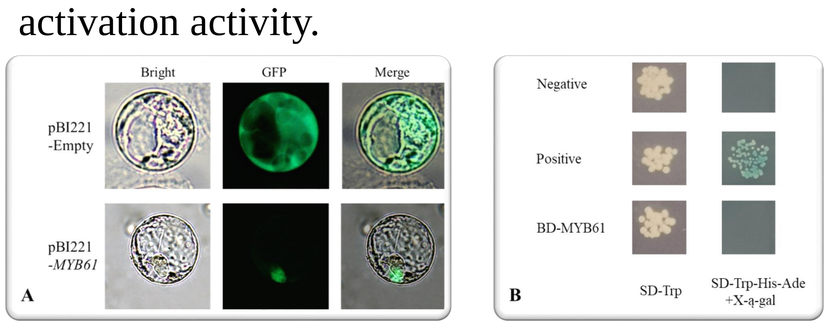
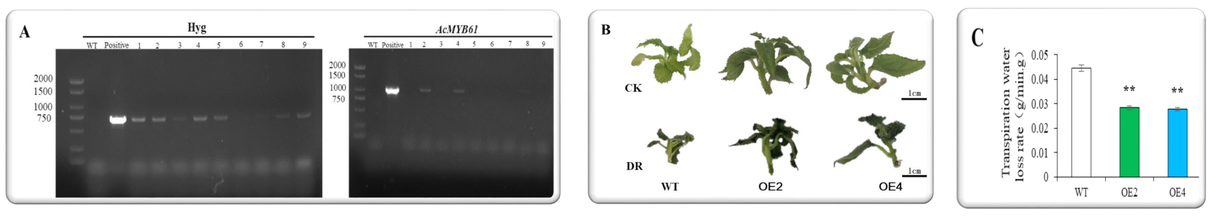


Fig. 2 Subcellular localization (A) and transcriptional activity analysis (B) of *AcMYB61*.

◆ The transgenic lines reduced leaf water loss rate under drought stress.



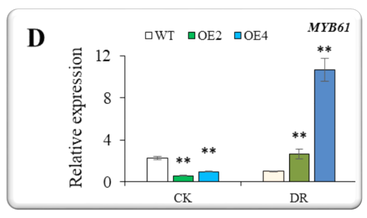


Fig. 3 Validation of transgenic lines by PCR (A) and morphological phenotype (B) and water loss rate (C) and expression profile of *AcMYB61* (D) of transgenic lines under drought stress.
WT: wild type; OE2 and OE4: 2 transgenic lines. CK, Control; DR, Drought. *, $P < 0.05$; **， $P < 0.01$.

◆ The transgenic lines decreased stomatal opening area.

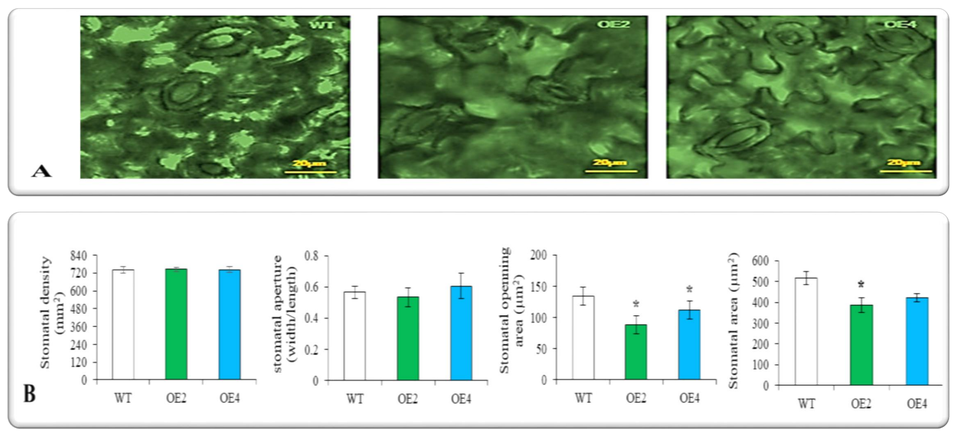


Fig. 4 Stomatal state (A) and index (B) of wild type and two transgenic lines. WT: wild type; OE2 and OE4: 2 transgenic lines.

◆ The transgenic lines reduced the autofluorescence intensity of lignin in the vein and the number of root system.

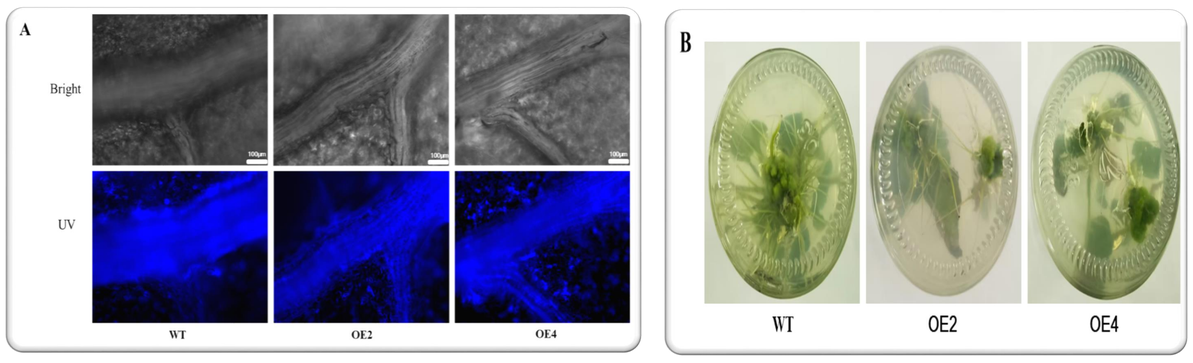


Fig. 5 Fluorescence observation of lignin in vein (A) and the root state (B) of transgenic lines. WT: wild type; OE2 and OE4: 2 transgenic lines.

◆ Overexpression of *AcMYB61* down-regulated the expression of lignin-biosynthesis-related gene.

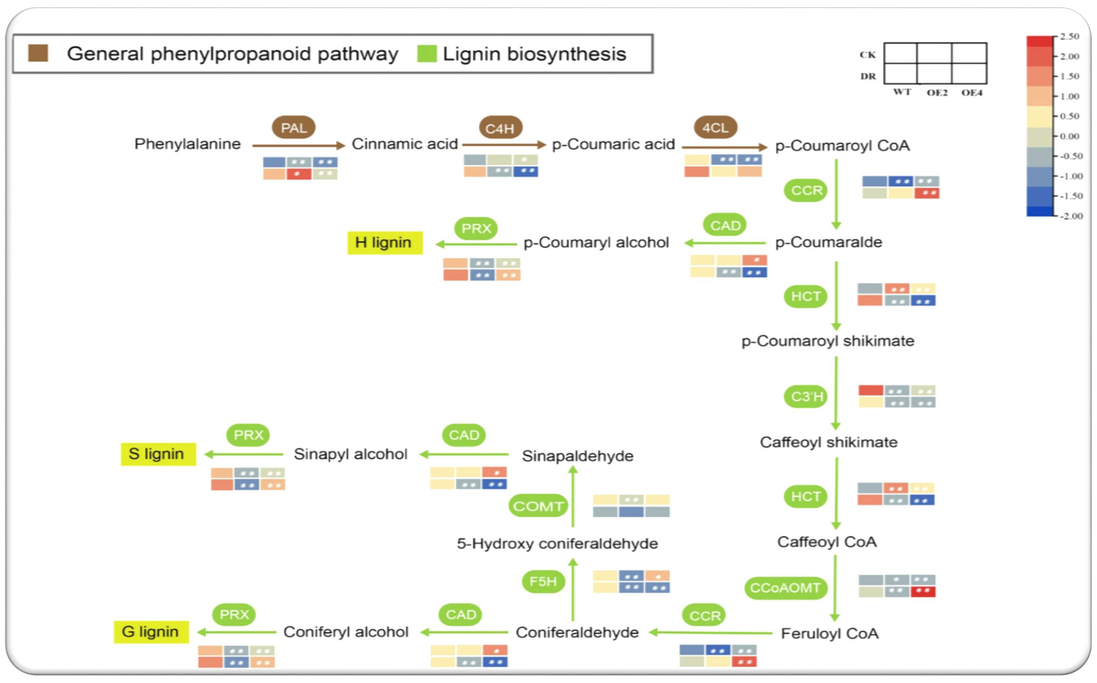


Fig. 6 Expression profile of genes involved in lignin-biosynthesis pathway.
WT: wild type; OE2 and OE4: 2 transgenic lines. CK, Control; DR, Drought.

◆ *AcMYB61* bind the promoter of *AcPRX* to active its expression.

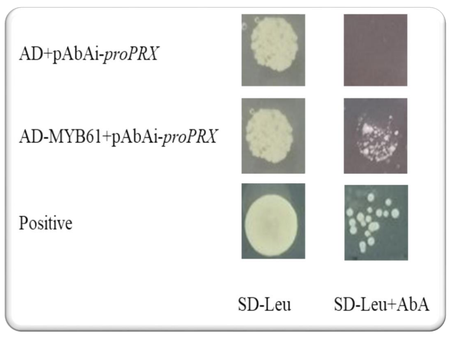


Fig. 7 Interaction between *AcMYB61* and *AcPRX* assayed by Y1H.

Conclusion

Overexpression of *AcMYB61* significantly reduced leaf water loss and stomatal opening area, thus improving drought tolerance. Moreover, the transgenic lines decreased the autofluorescence intensity of lignin in the veins and the number of root systemby *AcMYB61* binding to *AcPRX* promoter to inhibit its expression.

Acknowledgements

The work was supported by the fund received from the Sichuan Science and Technology Program (2022YFH0049) .