

## The apple bHLH transcription factor MdbHLH3 functions in determining the fruit carbohydrates and malate

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### Summary:

Changes in carbohydrates and organic acids largely determine the palatability of edible tissues of horticulture crops. Elucidating the potential molecular mechanisms involved in the change of carbohydrates and organic acids, as well as their temporal and spatial crosstalk are key steps in understanding fruit developmental processes. Here, we used apple (*Malus domestica*, Borkh.) as research materials, and found that MdbHLH3, a basic helix-loop-helix transcription factor (bHLH TF), modulates the accumulation of malate and carbohydrates. Biochemical analyses demonstrated that MdbHLH3 directly binds to the promoter of MdcyMDH that encodes an apple cytosolic NAD-dependent malate dehydrogenase, activating its transcriptional expression, thereby promoting malate accumulation in apple fruits. Additionally, MdbHLH3 over-expression increased the photosynthetic capacity and carbohydrate levels in apple leaves, and also enhanced the carbohydrate accumulation in fruits by adjusting carbohydrate allocation from sources to sinks. Overall, our findings provide new insights into the mechanism of how the bHLH TF MdbHLH3 modulates the fruit quality. It directly regulates the expression of cytosolic malate dehydrogenase MdcyMDH to coordinate carbohydrate allocation and malate accumulation in apple.

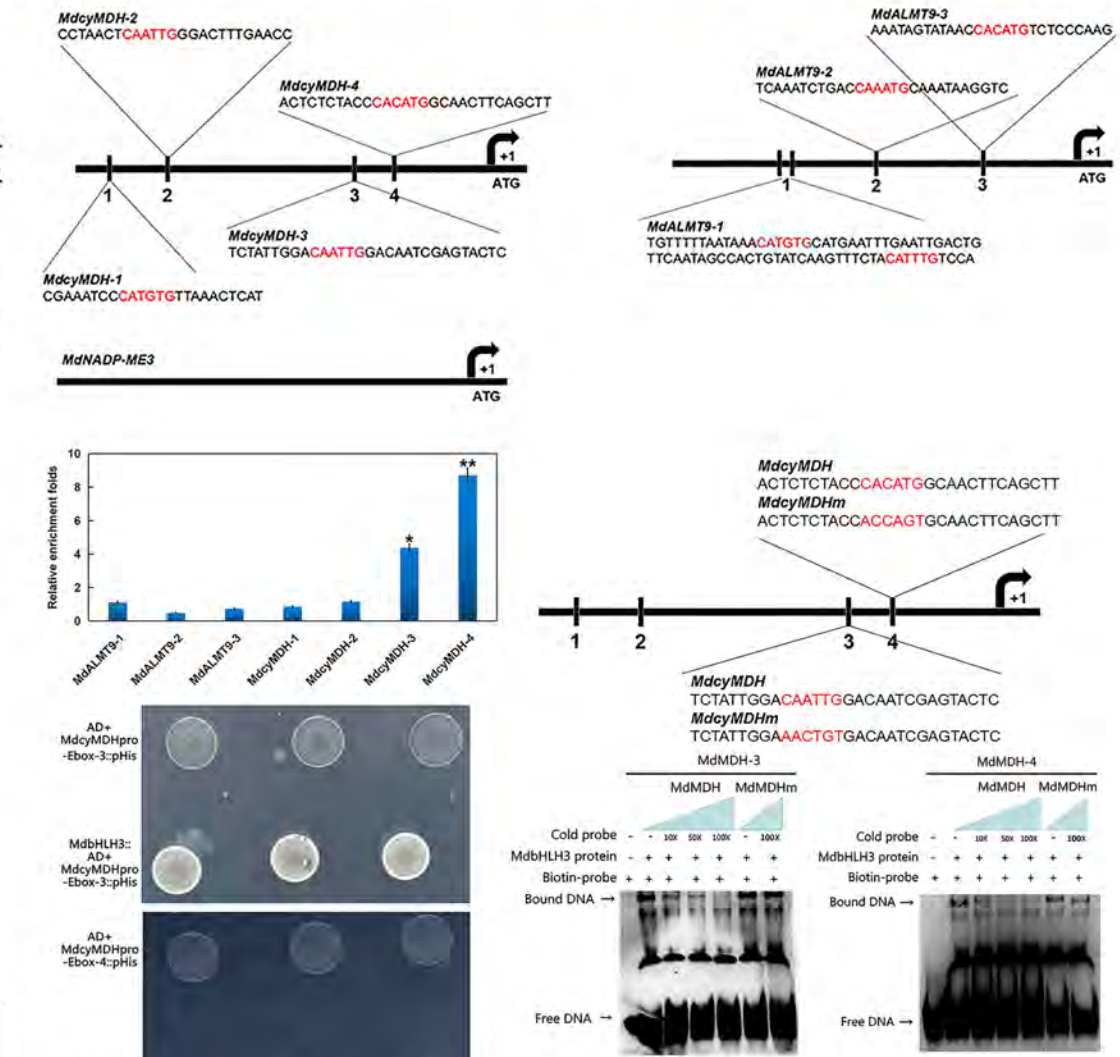
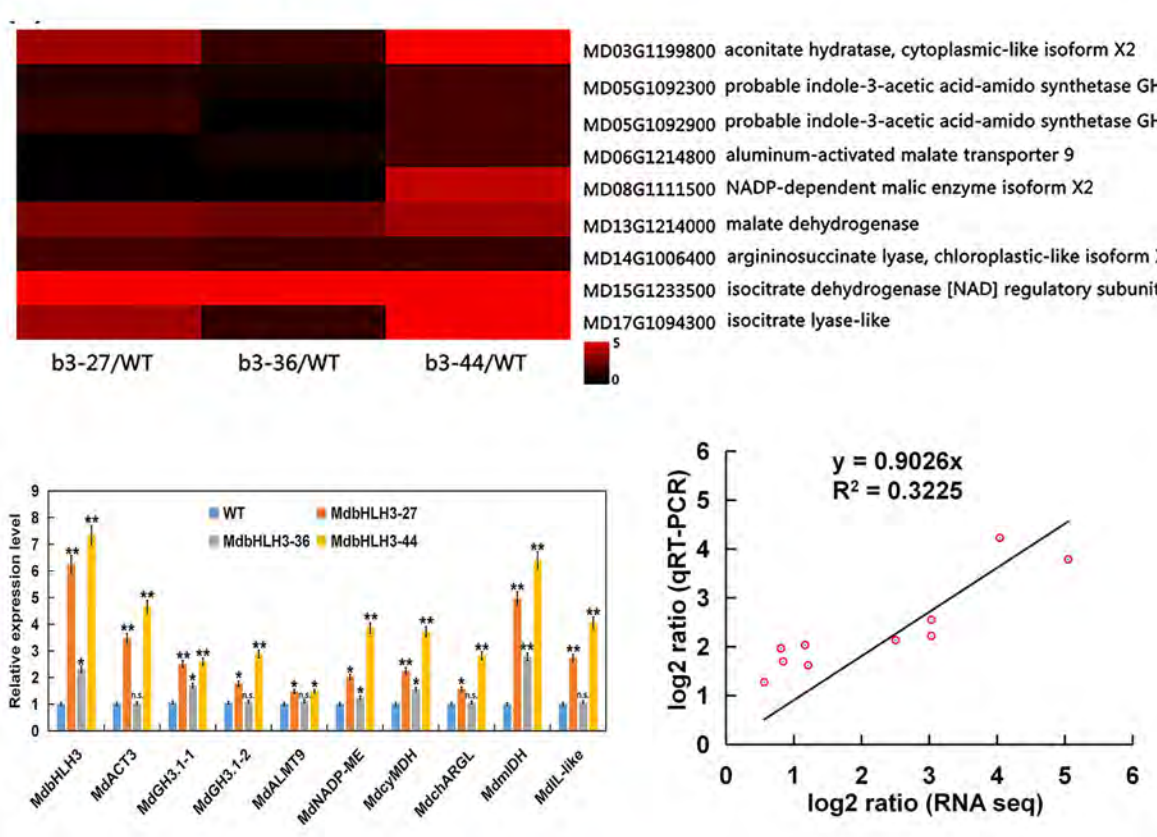
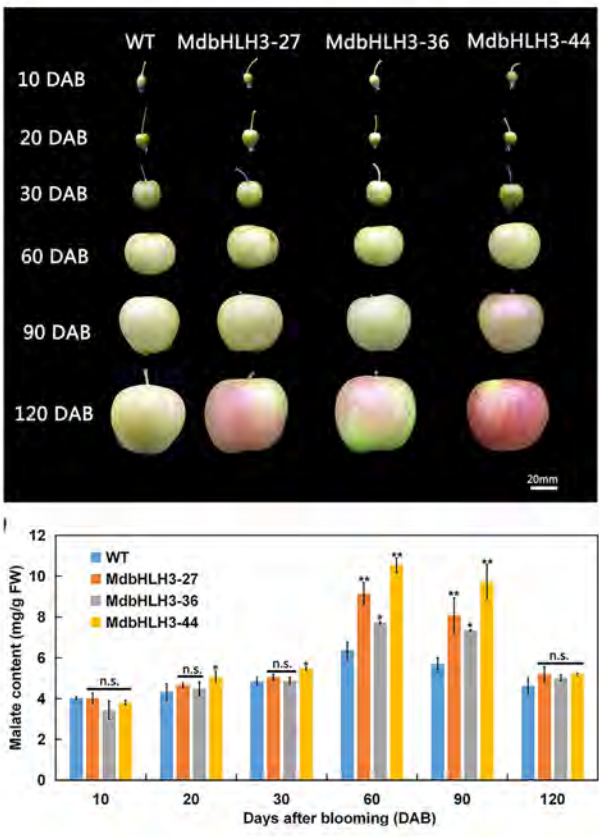


Figure 1 over-expression of MdbHLH3 promotes malate accumulation in apple.

Figure 2 MdbHLH3 regulates malate-associated genes in apple fruits.

Figure 3 MdbHLH3 binds to the promoter of MdcyMDH.

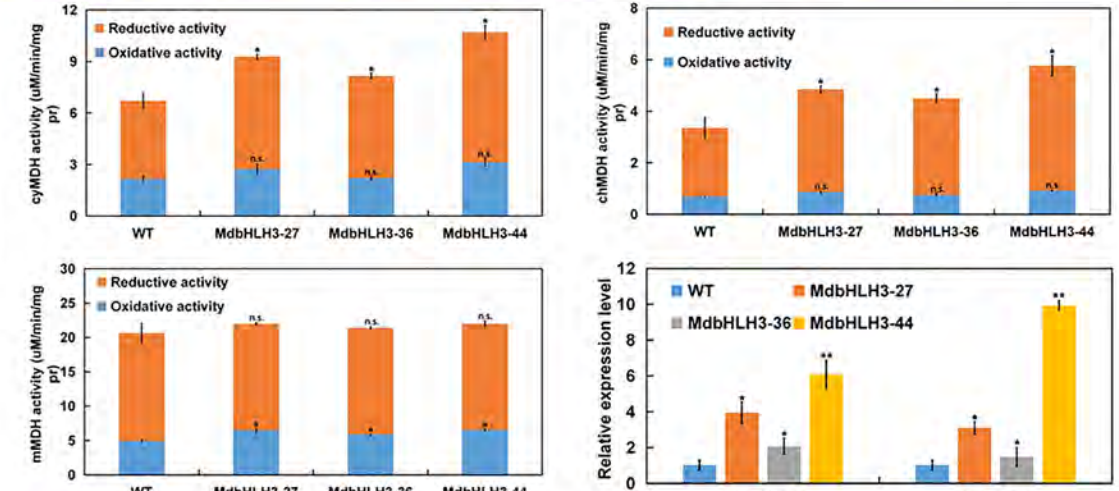
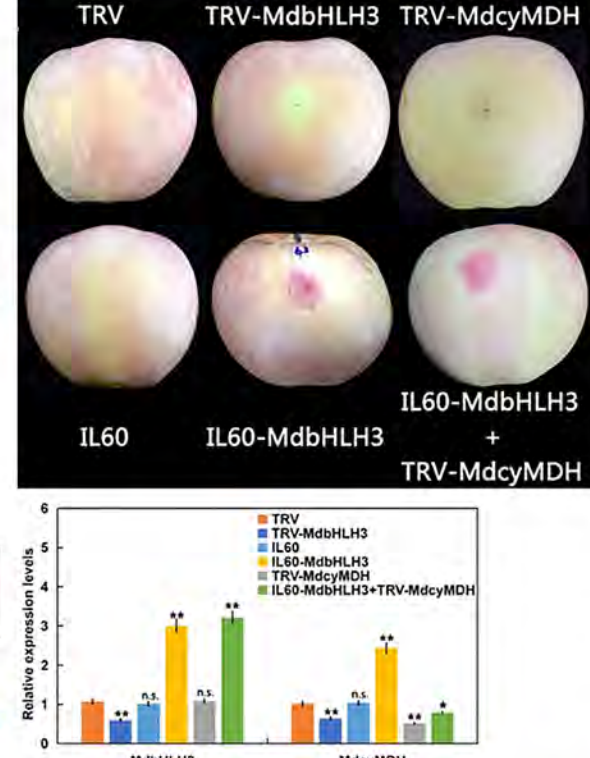
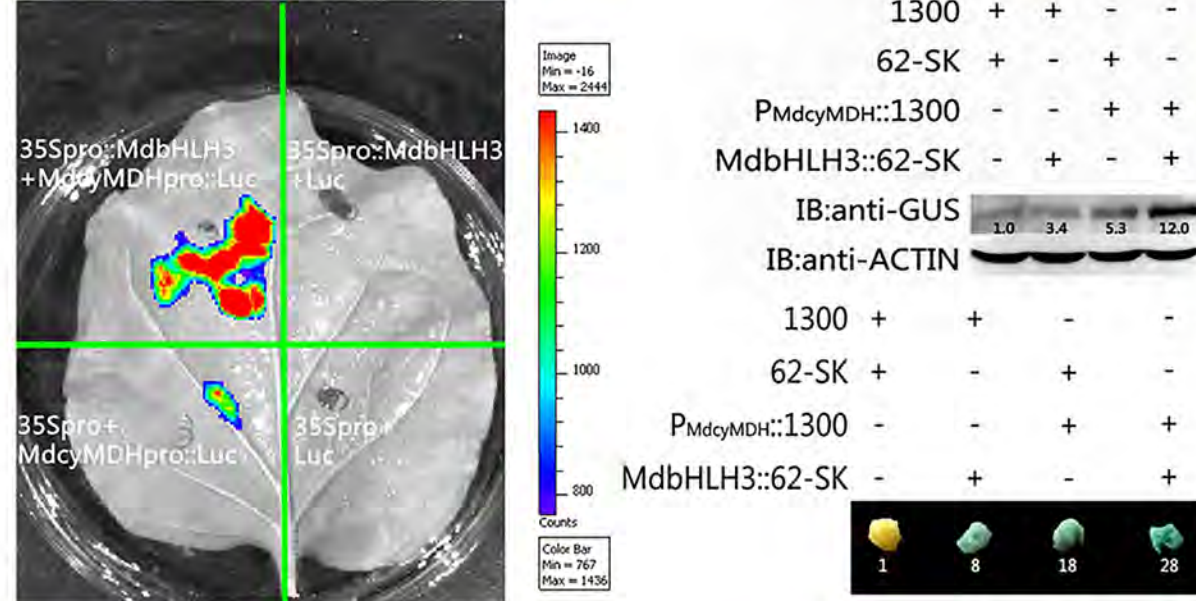


Figure 4 MdbHLH3 enhances its expression by activating the transcription of MdcyMDH.

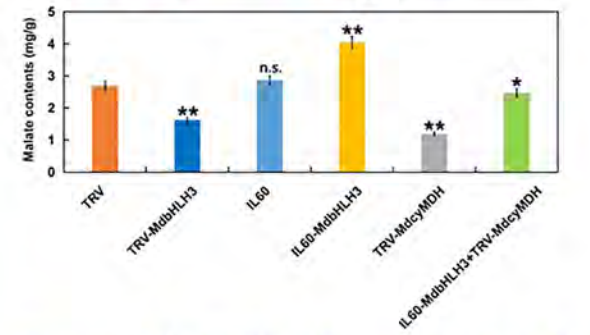


Figure 6 MdbHLH3-over-expression alters mitochondrial metabolism in the apple trees.

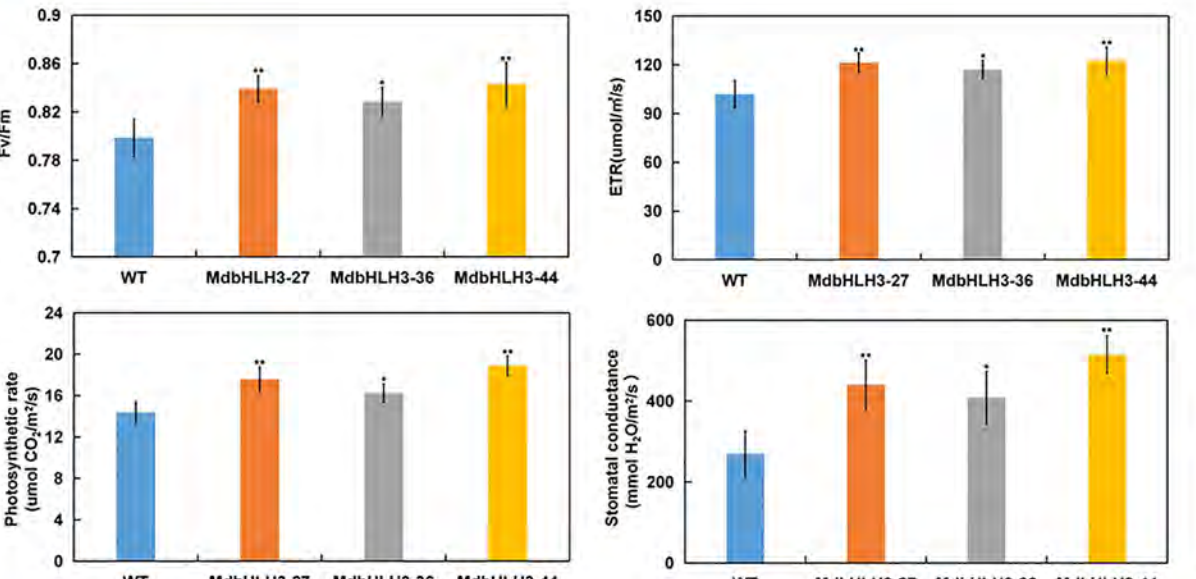


Figure 5 MdbHLH3 promotes malate accumulation via activating MdcyMDH expression.

Figure 7 MdbHLH3-over-expression alters chloroplast metabolism in the apple trees.

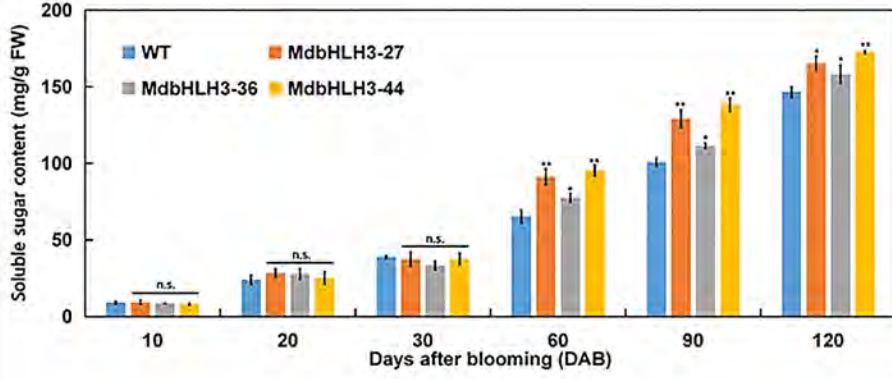
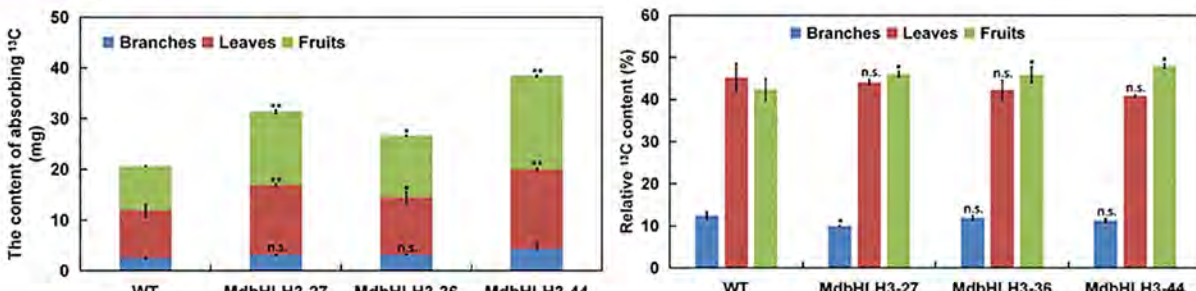


Figure 8 MdbHLH3-over-expression increases sugar content in apple fruits.

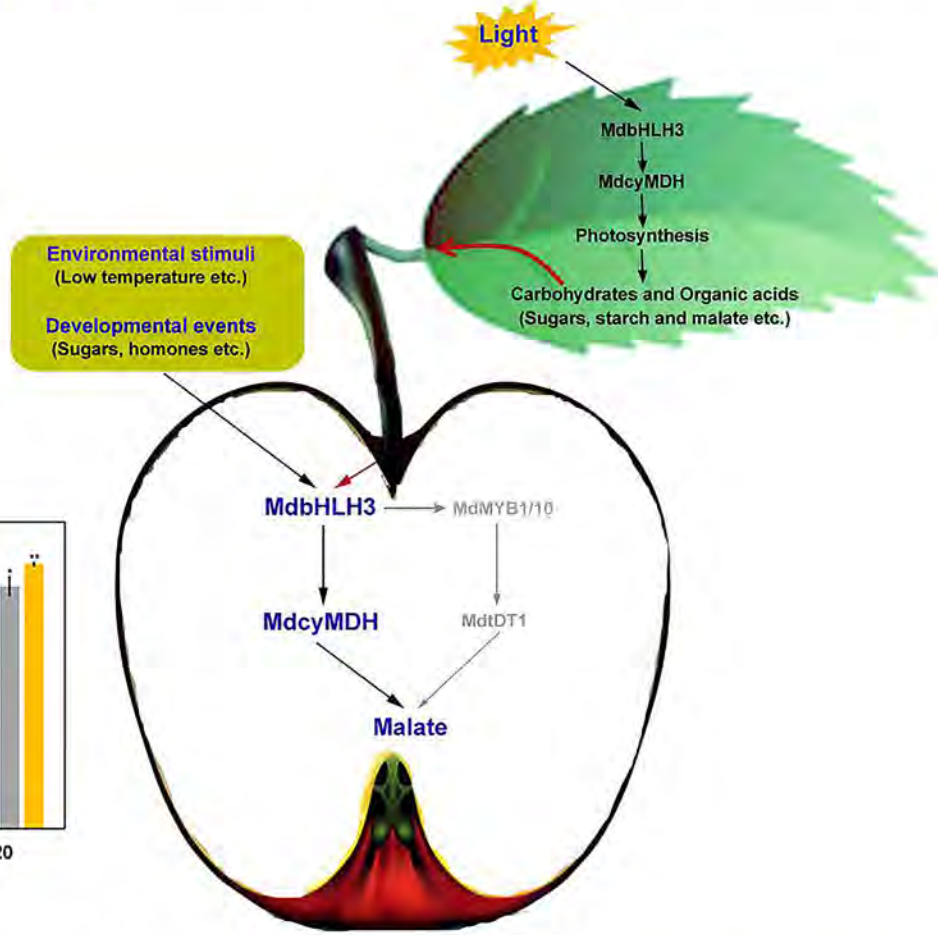


Figure 9 Working model for MdbHLH3 function in the regulation of fruit quality.

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### Conclusion:

We demonstrating that an apple bHLH transcription factor MdbHLH3 activates the transcription of the MdcyMDH and increases the content of malate. It enhances the conversion and transportation of carbohydrates from source to sink, and ultimately promotes the accumulation of soluble sugars.