

LtMYB305 transcription factor regulates the expression of *LtLCYB* gene to mediate carotenoids synthesis in *Liriodendron* petals

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Abstract: Carotenoids are a group of naturally occurring tetraterpenoid with great industrial and pharmacological value. The carotenoids biosynthesis was extensively elucidated in model plants, such as tomato and monkey flower. However, little is known about the regulation mechanism of carotenoids biosynthesis. Here, we used *L. chinense* and *L. tulipifera* petals as materials to study the differences in carotenoid accumulation. We seek to unveil the mystery via morphologic, metabolomic, transcriptomic, bioinformatics and transgenic approaches. The paraffin section of petals showed that cell morphology was almost the same in *Liriodendron* genus, while the quantification of carotenoids content displayed a different pattern in petals between two *Liriodendron* species, with high concentrations of carotenoids accumulated on the orange band (OB) in *L. tulipifera* petals. Correspondingly, KEGG analysis showed that carotenoids biosynthesis pathway was significantly enriched in *L. tulipifera*. HPLC and transcriptome analyses indicated that *LtLCYB* might be a key gene functioning in OB coloration, thus further characterization was conducted. RT-qPCR and subcellular localization showed that *LtLCYB* was highly expressed in OB, and the *LtLCYB* was a chloroplast-located protein. Overexpression of *LtLCYB* in tobacco promoted the carotenoid synthesis. Furthermore, we identified R2R3-MYB TFs and found a potential member that maybe related to OB coloration. Y1H assay proved that *LtMYB305* bind to *LCYB* promoter. Overexpression of *LtMYB305* in tobacco increased the carotenoid content. Collectively, our findings proposed that the OB coloration in *Liriodendron* stems from the interspecies variation in the differential expression of CBP genes, and *LtMYB305* takes a pivotal role in OB coloration. This study establishes a robust foundation for investigating the regulation mechanism of carotenoid synthesis, and offers valuable insights for the genetic improvement of petal colors in *Liriodendron*.