

## Transcriptomic analysis reveals key genes involved in petal color fading in Nuclumbo nucifera

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## Abstract

Flower color was the most important trait of aquatic plant lotus (*Nuclumbo* nucifera), and color fading significantly decreased the ornamental value. However, the molecular mechanism underlying lotus petal decoloration remains largely unknown. In this study, 'Q3S', a lotus cultivar with quick color fading was used to explore the mechanism of color change by analyzing the anthocyanin and global transcriptional profiling of petals at four distinct coloration stages during blooming. Five anthocyanins were detected in 'Q3S' petals, and delphinine 3-O-glucoside was the most abundant one. The continuous reduction of total anthocyanin content resulted the petal color changed from red to nearly white. The pH value increased at early stages and peaked at stage 3 when the anthocyanin content sharply decreased. Moreover, MDA content and POD activity increased during color fading. The expression pattern of anthocyanin biosynthesis genes (CHS, F3H, ANS, and UFGT) negatively correlated with anthocyanin content in lotus petals after flowering. On the contrary, the expression of putative regulatory transcription factors, such as MYBs, WRKYs and bHLHs increased after flower open, probably suppressing the expression of structure genes and reduced the anthocyanin biosynthesis. One the contrary, DEGs associated with anthocyanin degradation and vacuolar pH related genes, including peroxidase, proton pumps and their regulators, like WRKY and MYB, exhibited significant upregulation at late stages of flowering. This study for the first time elucidated the transcriptional dynamics during lotus petal color fading, and proposed the involvement of anthocyanin biosynthesis repressor and anthocyanin degrading genes and pH regulators during color fading. These findings will provide valuable information and candidate genes for improving the flower color breeding of lotus.





## Figure 3. Gene clusters of total DEGs and the TFs in them. a, Five clusters of DEGs grouped by K-means, Log(TPM,10) used as expression. b, The percentage of different TF families identified in total DEGs.



Figure 1. The color of petal and anthocyanin content in four developmental stages after flowering. a, The total anthocyanins concentration in petals. b, Distribution of five anthocyanins in four petal stages. c, petal color change in four stages, measured by the CIELAB color space.



Rich factor

Figure 4. Expression of genes involved in anthocyanin biosynthesis and transport progress. a, Diagram of anthocyanin biosynthesis. b, gene expression of structural genes in four stages of lotus petal. c, gene expression of transport genes in four stages of lotus petal.



Figure 5. Physiological analysis four petal at four tages. a, Cell sap pH value. **b**, MDA content. **c**, POD enzyme activity in four petal stages.

Figure 2. Transcriptome analysis of DEGs between petal stages. a, DEGs of pairwise comparison of petal stages. **b**, Venn diagram of DEGs between comparison. c, GO enrichment of total DEGs between stages.

Figure 6. Peroxidase genes in lotus and their expression at four petal stages. a, phylogenetic tree of peroxidase in lotus. b, The expression level of peroxidase showed differential expression at four stages.



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