

附件 1
申报格式参考



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Full-length transcriptome sequences and the identification of putative genes for flavonoid biosynthesis in safflower

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Summary

Background: The flower of the safflower (*Carthamus tinctorius* L.) has been widely used in traditional Chinese medicine for the ability to improve cerebral blood flow. Flavonoids are the primary bioactive components in safflower, and their biosynthesis has attracted widespread interest. Previous studies mostly used second-generation sequencing platforms to survey the putative flavonoid biosynthesis genes. For a better understanding of transcription data and the putative genes involved in flavonoid biosynthesis in safflower, we carry our study.

Results: High-quality RNA was extracted from six types of safflower tissue. The RNAs of different tissues were mixed equally and used for multiple size-fractionated libraries (1-2, 2-3 and 3-6k) library construction. Five cells were carried (2 cells for 1-2 and for 2-3k libraries and 1 cell for 3-6k libraries). 10.43Gb clean data and 38,302 de-redundant sequences were captured. 44 unique isoforms were annotated as encoding enzymes involved in flavonoid biosynthesis. The full length flavonoid genes were characterized and their evolutionary relationship and expression pattern were analyzed. They can be divided into eight families, with a large differences in the tissue expression. The temporal expressions under MeJA treatment were also measured, 9 genes are significantly up-regulated and 2 genes are significantly down-regulated. The genes involved in flavonoid synthesis in safflower were predicted in our study. Besides, the SSR and lncRNA are also analyzed in our study.

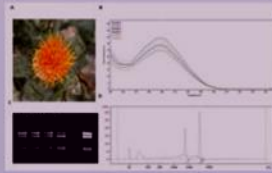


Fig. 1 Extraction and validation of high quality RNA. A: The safflower at third day after sowing (DAS3). B: Readmap map of the RNA. Sample 1 to 6 represent the RNA of different tissues (1: root, 2: stem, 3: leaf, 4: flower bud, 5: petal, 6: stamen). C: Gel electrophoresis of RNA samples. 1 to 6 represent the RNA of different tissues. The size was marked on the figure.



Fig. 2 Phylogenetic relationships of flavonoid synthase genes with safflower, rice and Arabidopsis thaliana. The phylogenetic tree was constructed using MEGA 4.0 with the neighbor-joining method. The safflower flavonoid genes were divided into 8 families: CHS, CHS1, CHS2, LAD, BHT, CHS, CHS and F3H. According to the scale bar, the scale is 0.1 substitutions per site.

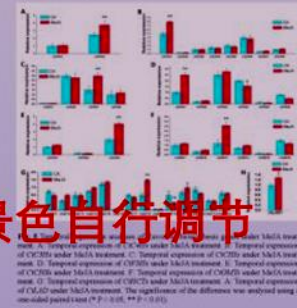


Fig. 3 Temporal expression of flavonoid biosynthesis genes under MeJA treatment. A: Temporal expression of CHS under MeJA treatment. B: Temporal expression of CHS1 under MeJA treatment. C: Temporal expression of CHS2 under MeJA treatment. D: Temporal expression of LAD under MeJA treatment. E: Temporal expression of BHT under MeJA treatment. F: Temporal expression of CHS3 under MeJA treatment. G: Temporal expression of CHS4 under MeJA treatment. H: Temporal expression of CHS5 under MeJA treatment. I: Temporal expression of CHS6 under MeJA treatment. The significance of the differences was analyzed using a one-tailed paired t-test (**P < 0.01, ***P < 0.001).

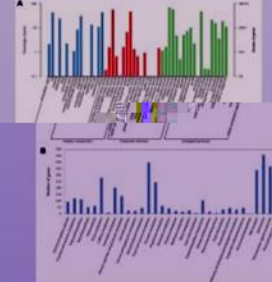


Fig. 3 (continued) Temporal expression of flavonoid biosynthesis genes under MeJA treatment. The significance of the differences was analyzed using a one-tailed paired t-test (**P < 0.01, ***P < 0.001).

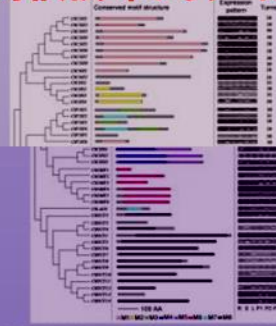


Fig. 4 The structure and expression analysis of flavonoid biosynthesis genes. A: The phylogenetic relationships of flavonoid biosynthesis genes in safflower. The phylogenetic tree was constructed by MEGA 4.0. B: The conserved motif structure analysis. The protein sequences were analyzed on Pfam (http://pfam.sanger.ac.uk). CHS represents Chalcone Synthase, CHS1 represents Chalcone Synthase, CHS2 represents Chalcone Synthase, CHS3 represents Chalcone Synthase, CHS4 represents Chalcone Synthase, CHS5 represents Chalcone Synthase, CHS6 represents Chalcone Synthase, LAD represents Luteolin 4-O-glucosyltransferase, BHT represents Biotin Holo-Tetrahymena, CHS3 represents Chalcone Synthase, CHS4 represents Chalcone Synthase, CHS5 represents Chalcone Synthase, CHS6 represents Chalcone Synthase. C: Expression analysis for representative CHS genes. 0 represents roots, 1 represents stems, 2 represents leaves, 3 represents buds at the first, MeJA+2 DAS3 days after sowing (DAS3).



Fig. 4 Flavonoid metabolic pathway in safflower and the genes significantly respond to MeJA treatment. The diagram was drawn combined with the chemical composition and gene expression (including the tissue expression and the expression under MeJA treatment). Red arrow represents MeJA induce the synthesis of the flavonoid. The genes inside the red circle were the significantly respond to MeJA treatment based on the gene expression analysis.

Conclusion

PacBio was used to sequence the full-length transcriptome for safflower. Clean data, 10.43Gb, were obtained and 38,302 de-redundant sequences were captured. 44 unique isoforms were annotated as encoding enzymes involved in flavonoid biosynthesis and analyzed their expression patterns. Forty-four genes were divided into eight families that were mainly distributed in the flavonoid biosynthesis, and these genes showed large differences in expression. The genes involved in flavonoid biosynthesis in safflower were predicted in our study. Besides, the SSR and lncRNA are also analyzed in our study.



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