



附件 1  
申报格式参考



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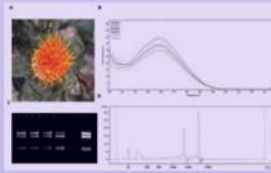
**Full-length transcriptome sequences and the identification of putative genes for flavonoid biosynthesis in safflower**

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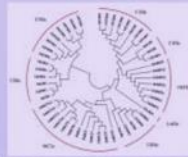
**Summary**

**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.

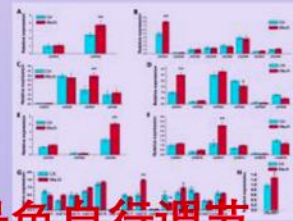
**Results:** High-quality RNA was extracted from six types of safflower tissue. The RNAs of different tissues were mixed equally and used for multiple size-fractionated libraries (1-2, 2-3 and 3-6k) library construction. Five cells were carried (2 cells for 1-2 and for 2-3k libraries and 1 cell for 3-6k 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.



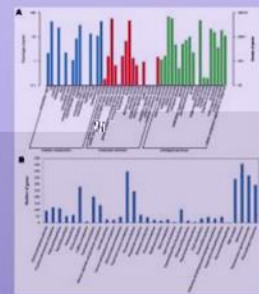
**Fig. 1** Extraction and validation of high quality RNA. A: The safflower at third day after sowing (D3AS). B: Banding map of the RNA. C: Electropherogram of the RNA. D: Banding map of the RNA. E: Gel electrophoresis of the RNA. F: Gel electrophoresis of the RNA. This was worked on the figure.



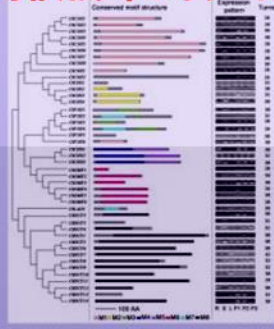
**Fig. 2** Phylogenetic relationships of flavonoid biosynthesis genes with safflower, rice and Arabidopsis thaliana. The phylogenetic tree was constructed using MEGA 5.0 with the neighbor-joining method. The safflower flavonoid genes were divided into 8 families: CHS, F3H, F3H5, LDOX, F3H7, F3H8, F3H9 and F3H10. According to the phylogenetic tree, the safflower flavonoid genes were divided into 8 families.



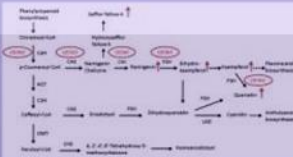
**Fig. 3** Temporal expression of flavonoid biosynthesis genes in safflower under MeJA treatment. A: Temporal expression of CHS. B: Temporal expression of F3H. C: Temporal expression of F3H5. D: Temporal expression of LDOX. E: Temporal expression of F3H7. F: Temporal expression of F3H8. G: Temporal expression of F3H9. H: Temporal expression of F3H10. The significance of the differences was analyzed using a one-tailed paired t-test (\*\*P < 0.01, \*\*\*P < 0.001).



**Fig. 4** GC and GC30 content of the safflower de-redundant sequences. A: GC content. B: GC30 content. GC and GC30 content of all the safflower de-redundant sequences. Three groups: GC<sub>10-20</sub> and GC<sub>30-40</sub> (clustered group) were constructed into GC. GC30 content of the safflower de-redundant sequences. Only some of the significant pictures was listed in the figure.



**Fig. 5** 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 5.0. B: The conserved motif structure analysis. The protein sequences were analyzed on Pfam (http://pfam.sanger.ac.uk). M1 represents Chalcone synthase domain, M2 represents Chalcone synthase domain, M3 represents 3-OH-flavonol 3-O-methyltransferase domain, M4 represents Chalcone and flavone oxidase domain, M5 represents flavonol 3-O-methyltransferase domain, M6 represents 3-O-methyltransferase domain, M7 represents two basic domains in myricetin synthase, M8 represents transketolase family domain. C: Expression analysis by semi-quantitative RT-PCR. A represents root, B represents stem, C represents flower, D represents petiole at the first, third and fifth days after sowing (D1AS).

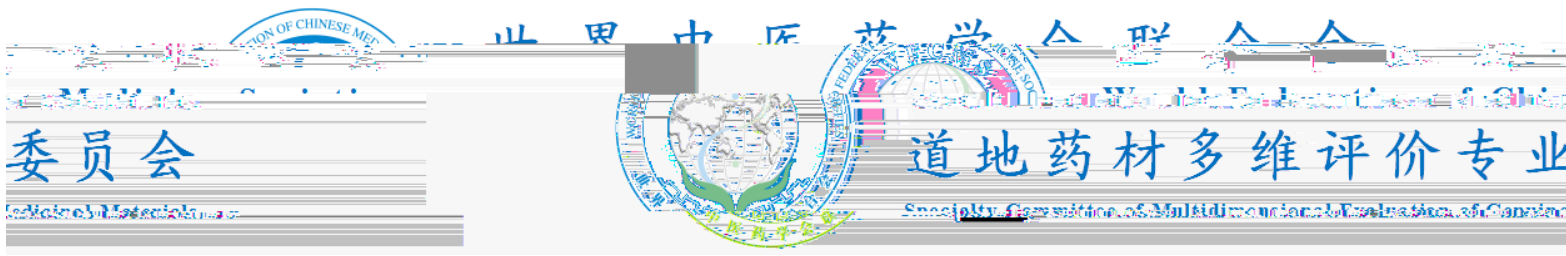


**Fig. 6** Flavonoid metabolic pathway in safflower and the genes significantly regulated by MeJA treatment. The diagram was drawn combined with the chemical composition and gene expression (including the tissue expression and the expression under MeJA treatment). Red arrow represents MeJA induces the expression of the flavonoid. The genes inside the red circle were the significantly regulated by MeJA treatment based on the gene expression analysis.

**Conclusion**

PacBio **RS II** was used to sequence the full-length transcriptome for safflower. Clean data, 10.43Gb, were obtained and 38,302 de-redundant sequences were captured. We screened all genes involved in the biosynthesis of flavonoids and analyzed their expression patterns. Forty-four genes were divided into eight families that were annotated for involvement in the biosynthesis of flavonoids, and these genes showed large differences in expression. The genes involved in flavonoid synthesis in safflower were predicted in our study. The temporal expression of these genes under MeJA treatment was also measured, 9 genes are significantly up-regulated and 2 genes are significantly down-regulated. 3 genes are mainly predicted in MeJA promoting the synthesis of flavonoids. Besides, the SSR and lncRNA were also analyzed in our study. Our results also provide a new perspective for the study on safflower.





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