



附件 1
壁报格式参考



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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.

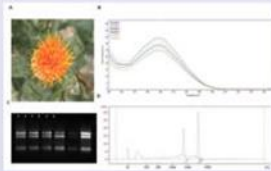


Fig. 1 Detection and validation of high quality RNA. A: The safflower at third day after anthesis (DAA). B: sequencing map of the RNA. Sample 1 is to represent the RNA of different tissues (1-2, 2-3 and 3-6k). C: Gel electrophoresis. 1-4 represent samples 1, 2 and 3 DAA. The arrow marked in the figure.

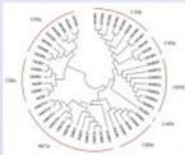


Fig. 2 Phylogenetic relationships of flavonoid biosynthesis genes with safflower, rice and Arabidopsis thaliana. The phylogenetic tree was constructed using MEGA 5 with the neighbor-joining method. The safflower flavonoid genes were divided into 8 families: CHS, F3H, F3H5, LAD, F3H7, CHS, F3H5 and F3H7. According to the phylogenetic tree, the genes were divided into 8 families in safflower.

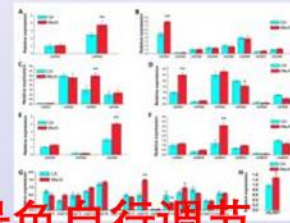


Fig. 3 Temporal relationships of flavonoid biosynthesis genes with safflower, rice and Arabidopsis thaliana. A: Temporal expression of CHS under MeJA treatment. B: Temporal expression of F3H5 under MeJA treatment. C: Temporal expression of F3H7 under MeJA treatment. D: Temporal expression of F3H5 under MeJA treatment. E: Temporal expression of F3H7 under MeJA treatment. F: Temporal expression of F3H5 under MeJA treatment. G: Temporal expression of F3H7 under MeJA treatment. H: Temporal expression of F3H5 under MeJA treatment. I: Temporal expression of F3H7 under MeJA treatment. The significance of the differences was analyzed using a one-tailed paired t-test (* P < 0.05, ** P < 0.01).

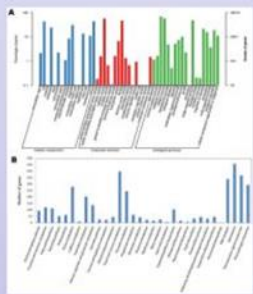


Fig. 3 Clustering analysis of the safflower de-redundant sequences. A: Clustering analysis of all the safflower de-redundant sequences. Three primary (CC, CCG and C) sub-clusters (horizontal groups) were constructed into C1, C2, C3 (vertical groups) of the safflower de-redundant sequences. Only some of the significant pathways were listed in the figure.

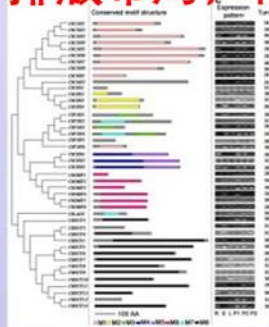


Fig. 4 The structure and expression analysis of flavonoid biosynthesis genes. A: The phylogenetic relationships of flavonoid biosynthesis genes in safflower. The phylogenetic tree was constructed by MEGA 5. B: The predicted motif structure analysis. The protein sequences were analyzed on Pfam (http://pfam.sanger.ac.uk). S10 represents Transmembrane (TM) domain, S12 represents Chalcone-Flavanone isomerase domain, S13 represents 2OG-Fe(II) oxygenase superfamily domain, S14 represents Chalcone and stilbene synthase domain, S15 represents 3-oxoacyl-CoA synthase domain, S16 represents 3-oxoacyl-CoA synthase domain, S17 represents alpha-ketolactonase domain, S18 represents transketolase domain. C: Expression analysis by semi-quantitative RT-PCR. S1 represents roots, S2 represents stems, S3 represents leaves, S4-S11 represents joints at the first, third and fifth days after anthesis (DAA).



Fig. 5 Flavonoid metabolic pathway in safflower and the genes significantly regulated by MeJA treatment. The diagram was drawn combined with the obtained comparisons and gene expression (including the leaves expression and the expression under MeJA treatment). Red arrow represents MeJA induces the content of the flavonoid. The genes marked in 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 analysed 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. 5 genes are mainly participated in MeJA promoting the synthesis of flavonoids. Besides, the SSR and lncRNA are also analyzed in our study. Our results also provided a valuable resource for further study on safflower.





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