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Expressing Genes of Safflower (*Carthamus tinctorius* L.): A Review

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Abstract

Safflower is of potential interest for agriculture mainly due to its commercial utility as oil, animal feed, and pharmacologically significant secondary metabolites. Knowledge of a metabolome of plant is crucial to optimize the crop traits, yields and quality, and ensure nutritional and health factors to produce functional food or feeds. Gene expression studies provide a theoretical molecular biology foundation for improving new traits and developing new cultivars.Here reader may find a review of articles on different regulators controling gene expression in safflower, including transcription factors and microRNAs in relation to flavonoid metabolism pathway, oil production and abiotic stress tolerance.

Keywords: Genes, expression, safflower, *Carthamus tinctorius* L., biotechnology

INTRODUCTION

Because of the fatty acid content variations in the seed oil, safflower (*Carthamus tinctorius* L.) is of potential agricultural relevance (La Bella et al., 2019). Alternative diesel fuels can be made from alcohols and biofuels derived from oils (Celebi and Aydn, 2018). Because of environmental concerns, biodiesel is rising in popularity. As a result, alternative and sustainable crops are needed as new biofuel sources. Safflower could be a sustainable biodiesel source material, but it does have one drawback (as do many biodiesels made from vegetable oils), namely, a limited oxidative stability (Nogales-Delgado et al., 2019). The safflower plant is a promising energy crop that thrives in arid and semi-arid climates (Khounani et al., 2019). Human health is primarily concerned with the phenol content of vegetable oil and its antioxidant activity. On a wide scale, oilseed species are regarded key sources of these chemicals with therapeutic benefits (Zemour et al., 2019).

Safflower omics

Safflower has long been grown as a crop due to its commercial utility as oil, animal feed, and pharmacologically significant secondary metabolites. The integration of omics approaches, including genomics, transcriptomics, metabolomics, and proteomics datasets, has provided more comprehensive knowledge of the chemical composition of crop plants for multiple applications. Knowledge of a metabolome of plant is crucial to optimize the evolution of crop traits, improve crop yields and quality, and ensure nutritional and health factors that provide the opportunity to produce functional food or feedstuffs. Safflower contains numerous chemical components that possess many pharmacological activities including central nervous, cardiac, vascular, anticoagulant, reproductive, gastrointestinal, antioxidant, hypolipidemic, and metabolic activities, providing many other human health benefits (Mani et al., 2020). Gene expression studies provide a theoretical molecular biology foundation for improving new traits and developing new cultivars. Real-time quantitative PCR (RT-qPCR) has become a crucial approach for gene expression analysis. Appropriate reference genes (RGs) are essential for accurate and rapid relative quantification analysis of gene expression (Li et al., 2015). Environmental stresses influence the growth and development of plants by influencing patterns of gene expression. Different regulators control gene expression, including transcription factors (TFs) and microRNAs. MicroRNAs (miRNAs: ~21 nucleotides long) are encoded by miRNA genes transcribed by RNA polymerase II (RNP-II) and play key roles in plant development and physiology (Kouhi et al., 2020).

Flavonoid metabolism pathway

Safflower is cultivated mainly for medicinal use, with its dried tubular flowers being the medicinal part and its seeds being commonly consumed as vegetable oil in many countries. Flavonoids are the dominant active medical compounds. UDP-glycosyltransferase (UGT) plays an essential role in the biosynthesis and storage of flavonoids in safflower. 45 UGT unigenes were screened from transcriptomic database of safflower by Guo et al., (2016). The phylogenetic tree showed that CtUGT3 and CtUGT16 were classified under the UGT71 subfamily involved in metabolite process, whereas CtUGT25 has high identities with PoUGT both catalyzing the glycosylation of flavonoids and belonging to the UGT90 subfamily. To

functionally characterize UGT in safflower, CtUGT3, CtUGT16 and CtUGT25 were cloned and analyzed. Subcellular localization suggested that the three UGTs might be located in the cell cytoplasm and chloroplast. The co-expression relation of expression pattern and metabolite accumulation demonstrated that CtUGT3 and CtUGT25 were positively related to kaempferol-3-O-β-D-glucoside and CtUGT16 was positively related to quercetin-3-O-β-D-glucoside in yellow line, whereas CtUGT3 and CtUGT25 were positively related to quercetin-3-O-β-D-glucoside in white line. This study indicates that the three CtUGTs play a significant and multiple role in flavonoids biosynthesis with presenting different functional characterization in safflower. Anthocyanin reductase (ANR) is a key enzyme for the biosynthesis of proanthocyanidins downstream of flavonoid metabolism pathway, which has a negative regulatory effect on anthocyanin content. Dandan et al. (2022) investigated the sequence characteristics of ANR gene in Carthamus tinctorius L. and its relationship with flower color. The open reading frame (ORF) of CtANR gene was 1020 bp in length, encoding an unstable hydrophilic protein. CtANR gene showed the lowest abundance in the early stage of fruiting bulb formation, followed by root and stem, whereas exhibited relatively high expression in flower. expression level of CtANR gene in red flower variety was decreased first, then increased and then decreased, while that in white flower variety was decreased first and then increased sustainably. The MYB transcription factors (TFs) is a plant TF families, which involves in hormone signal transduction, and abiotic stress tolerance, etc. MYB transcription factors are involved in the regulation of flavonoids. The cloning and expression analysis of MYB transcription factor genes in safflower is of great significance, not only for clarifying the regulation mechanism of flavonoids biosynthesis in safflower, but also for the artificial regulation of flavonoid biosynthesis in safflower. The MYB transcription factors were cloned and their sequences were analyzed by Chen et al. (2018). Eight long fragment MYB transcription factors were screened and six MYB transcription factors was successfully cloned, named CtMYB-TF1, CtMYB-TF2, CtMYB-TF4, CtMYB-TF5, CtMYB-TF6 and CtMYB-TF7, respectively. The six MYB transcription factors had the core domain of MYB transcription factor family, and evolutionary analysis showed that the CtMYB-TF7 transcription factor was closely related to the factors AtMYBL2 and AtMYB12. Expression analysis showed that the expression of CtMYB-TF5, CtMYB-TF6 and CtMYB-TF7 was low in roots, stems and leaves, and was high in the flower.

Oil content

Safflower varieties were commonly divided into high, low and middle linoleic acid (LA) types according to their LA relative percentage contents in the seed oil. Fatty acid desaturase 2 (FAD2) plays a key role for LA content in seed. The sequence variations of FAD2 (CtFAD2-2, CtFAD2-10, CtFAD2-11) genes which could express in developmental seed of safflower were analyzed in 15 different LA-type materials by Li et al. (2019). The CtFAD2-2 sequences were the same in all materials, and the CtFAD2-10, CtFAD2-11 sequences formed into two haplotypes independent of the LA-type of safflower seed. Two haplotypes of CtFAD2-10 had the function of oleic acid desaturase. CtFAD2-2, CtFAD2-10

and CtFAD2-11 expressed mainly at 10 days after flower (DAF) for two different LA-type materials and the accumulation of few mRNA was detected in 14–22 DAF. For low-LA type, the accumulation of CtFAD2-1 mRNA was extremely low during seed development stages. The gene structure and expression level of CtFAD2-1 may be the main factor affecting the differentiation of LA-type for safflower materials.

Abiotic stress tolerance

The basic leucine zipper (bZIP) is a widely known transcription factors family in eukaryotes. In plants, the role of bZIP proteins are crucial in various biological functions such as plant growth and development, seed maturation, response to light signal and environmental stress. To date, bZIP protein family has been comprehensively identified in Arabidopsis, castor, rice, ramie, soybean and other plant species. Li et al., (2020) identified 52 putative bZIP genes from Carthamus tinctorius using a draft genome assembly and further analyzed them. Based on the common bZIP domain, CtbZIP family were clustered into 12 subfamilies renamed as (A–J, S, X), of which the X is a unique subfamily to Carthamus tinctorius. A total of 20 conserved protein motifs were found in CtbZIP proteins. Their transcription regulation could be highly influenced by light intensity and hormones. Cysteine protease (EC3.4.22), also known as mercaptan protease, is a large class of proteolytic enzymes. Cysteine protease (CP) plays an important role in plant senescence. CPs was analyzed by using safflower, and the function of CtCP1 under l abiotic stress was analyzed by Hong et al. (2021). qRT-PCR at different florescence showed that the expression of CPs gene was the highest in the decline period, and CtCP1 gene changed significantly under abiotic stress. Expression of CtCP1 was the highest in the decline stage and low temperature. The results of transgenic lines under low temperature stress showed that inhibition of CtCP1 expression enhanced the resistance of Carthamus tinctorius to low temperature, and overexpression of CtCP1 weakened the resistance of Carthamus tinctorius to low temperature. The tocopherol cyclase was one of the key enzymes in plant vitamin E biosynthesis pathway. Tocopherol cyclase gene was obtained using RT-PCR techniques and named CtTC by Guan et al., (2016). The putative protein contained 507 amino acids and peptide analysis showed that it was a non secretory protein, and there was no signal peptide. The subcellular localization showed that the CtTC protein was located in the chloroplast. The expression of CtTC gene in safflower seeds at different development stages was determined by quantitative real-time PCR, it was found that the highest expression level of CtTC gene was detected in 50 days after flowering. Quantitative RT-PCR analysis suggested that expression of CtTC is induced and strengthened by drought stresses. Kouhi et al. (2020) investigated the expression profiles of seven conserved miRNAs related to drought, salinity, heat, and Cd stress in the leaf and root organs in safflower. Gene Ontology (GO) analysis found that target genes of miRNAs are often TFs such as AP2/ERF and HD-ZIP as well as NAC domain-containing proteins. Expression analyses confirmed that miRNAs can play a vital role in keeping safflower stress-tolerant. Differential expression of miR156, miR162, miR164, miR166, miR172, miR398, and miR408 regulate the expression of their respective target genes. These genes activate several pathways leading to physiological and

biochemical responses to abiotic stresses. Some conserved miRNAs were regulated by abiotic stresses. Proline (Pro) accumulation under water stress was measured in safflower drought tolerant cv. A1 and sensitive cv. Nira by Thippeswamy et al. (2010). Activities of pyrroline-5-carboxylate reductase (P5C reductase) and pyrroline-5-carboxylate synthetase (P5C synthetase), two enzymes involved in the Pro biosynthetic pathway were also estimated. Water stress resulted in a reduction in the leaf dry mass and chlorophyll content along with a gradual accumulation of Pro. RT-PCR results show higher expression of Δ1-pyrroline-5-carboxylate synthetase (p5cs) gene in correlation with up-regulated Pro accumulation in cv. A1. P5C reductase was found to be the Pro synthesis rate limiting whereas P5C synthetase did not show any specific response to the drought stress in both cultivars. The basic helix–loop–helix (bHLH) family is the second largest superfamily of transcription factors that belongs to all three eukaryotic kingdoms. The key function of this superfamily is the regulation of growth and developmental mechanisms in plants. Hong et al., (2019) identified 41 bHLH genes in Carthamus tinctorius that were classified into 23 subgroups and identified 10 conserved protein motifs found in the safflower bHLH family.

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