Biochemical Systematics and Ecology 51 (2013) 251–258

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

Molecular structure and the second introns variation of gene *F3'H* of two medicinal *Chrysanthemum morifolium* populations

Weiping Zhao, Jun Zhao, Lan He, Yanhui Sun, Hua Cai*

College of Biological and Food Engineering, Chuzhou University, Chuzhou, 239000 Anhui, China

ARTICLE INFO

Article history:
Received 10 July 2013
Accepted 21 September 2013
Available online 11 October 2013

Keywords:
Allelic variation
Flavonoids
F3'H gene
Medicinal chrysanthemum
Flower color
Intron

ABSTRACT

The gene *F3'H* encodes one of the key enzymes controlling the biosynthesis of flavonoids in higher plants. In this study, two methods—long fragment cloning and homology cloning—were used to obtain full-length cDNAs and partial genomic DNA sequences for the *F3'H* genes in two medicinal chrysanthemum populations, 'Chu-ju' and 'Gong-ju'. Analysis of gene structure showed that in both populations the *F3'H* gene contained three exons and two introns, with the 5′ and 3′ ends of the introns conforming to the GT/AG rule. The *F3'H* genes of the two medicinal chrysanthemum populations both encoded 509 amino acids in their open reading frames (ORF) with 98.88% homology, which indicated the gene is highly conserved. Analysis of intron structure showed that the second intron of the *F3'H* genes in the two populations contained motifs closely related to gene transcription that were similar to TATA-box, CAAT-box, AT-rich element, MYB binding site, HD-Zip, and HSE motifs. This suggested that introns may be involved in expression regulation of medicinal chrysanthemum *F3'H* genes. The two medicinal chrysanthemum populations had abundant allelic variation in their intron regions. Besides 23 single-nucleotide polymorphisms (SNPs), 'Gong-ju' had a 39 bp insertion sequence compared with 'Chu-ju', and there were four or six structures similar to the TATA-box and one CAAT-box in this sequence. In addition, in another site, 'Gong-ju' had two G-box structures related to light response regulation that 'Chu-ju' did not have. These intron variations may be related to physiological differences between the two populations; in 'Gong-ju' the expression of *F3'H* is higher, the yellow tubular florets are smaller, the content of flavonoids like apigenin is higher, and the content of luteolin is lower than in 'Chu-ju'. Therefore, it can be speculated that the intron sequence of the *F3'H* gene in medicinal chrysanthemum is a positive control element for expression of the gene.

1. Introduction

Chrysanthemum (*Chrysanthemum morifolium* (Ramat.) Tzvelv.) is a popular traditional Chinese medicinal plant. Due to its rich flavonoids and other biologically active substances, chrysanthemum has significant pharmacological effects on human health, with notable curative effects for treating the common cold, headaches, and dizziness (Pharmacopoeia of the People's Republic of China, 2010). The main domestic varieties of medicinal chrysanthemum are 'Chu-ju' and 'Gong-ju', which are divided according to their different growing regions and processing methods (Mandal et al., 2000; Willits and Bailey, 2000;

* Corresponding author. Tel./fax: +86 550 3088968.
E-mail address: chczh@163.com (H. Cai).

0305-1978/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bse.2013.09.012

Shao et al., 2010). These two populations have different chemical compositions and active substance contents (Gu and Qin, 2004).

Flavonoids, a large class of plant secondary metabolites ubiquitously present in fruits, flowers and vegetables, include the well-known characteristic red, blue, and purple anthocyanin pigments of plant tissues (Winkel-Shirley, 2001, 2002). As natural compounds, flavonoids have received a lot of attention in present-day society because of their possible benefits for human health, such as neuro-protective and cardiovascular protective effects (Schroeter et al., 2001). The synthesis of flavonoids in plants is extremely complex and involves more than 20 important enzymes, among which flavonoid 3′-hydroxylase (flavanone 3′-hydroxylase, F3'H) is a key enzyme for the synthesis of downstream compounds (Xu et al., 2007; Hou et al., 2011). The F3′H enzyme encoded by the *F3′H* gene, which belongs to the cytochrome P450 superfamily, catalyzes hydroxylation at the 3′ position of the B-ring in flavonoids (Ayabe and Akashi, 2006; Tanaka, 2006). This leads to the production of red cyanidin-based pigments (Ayabe and Akashi, 2006; Tanaka, 2006). *F3′H* plays an important role in flavonoid biosynthesis and regulation. At the same time, as a main structural gene encoding enzymes for flavonoid biosynthesis, it acts as a regulatory factor in an important branch point between flavonol and anthocyanin biosynthesis (Olsen et al., 2010).

The F3'H enzyme can also participate in the acetate-malonate pathway through flavanone substances, protecting plant cells from ultraviolet radiation damage and pathogenic microorganisms (Huang et al., 2007; Sun et al., 2010; Kanako et al., 2012). Research has shown that the *F3′H* gene has different temporal and spatial expression patterns in different varieties, tissues of the same variety, and growth stages (Brugliera et al., 1999; Huang et al., 2007). For instance, the expression of *F3'H* in petunia is highest in petals compared with other organs (Brugliera et al., 1999), while it is lower in the petals of Crofton weed than in leaves (Huang et al., 2007). In single-flower cut chrysanthemum and cineraria, the expression of *F3'H* in red or pink varieties is a little higher than in other varieties, while there is no *F3'H* expression in blue cineraria (Hu et al., 2009; Han et al., 2012). Additionally, the *F3'H* gene in chrysanthemum has high expression in the initial period of inflorescence opening, which then gradually decreases so that by the end of inflorescence opening, *F3'H* expression is extremely low or even non-existent (Chen et al., 2011). The above results suggest that there is diversity in *F3'H* genes. Allelic variation in the *F3'H* gene may exist in a wide range of different species or varieties.

To investigate the relationship of gene structure with flavonoid content and petal color in 'Chu-ju' and 'Gong-ju', the structure of the *F3'H* gene was analyzed. *F3'H* genes from the two main populations of medicinal chrysanthemum, 'Chu-ju' and 'Gong-ju', were cloned. The results showed that some allelic variation existed in the exon and intron regions. Our results suggested a relationship between the *F3'H* gene and flavonoid content (as well as color) in medicinal chrysanthemum, which lays a foundation for further study of the anabolic mechanisms of *F3'H* in the flavonoid biosynthesis of medicinal chrysanthemum.

2. Materials and methods

2.1. Plant materials

'Chu-ju' and 'Gong-ju', the two populations of medicinal chrysanthemum (*Chrysanthemum morifolium* (Ramat.) Tzvelv.) used for these experiments, are local cultigens of Chuzhou and Huangshan in Anhui and both are propagated by cuttings. For our experiments, whole seedlings were transplanted from the field to pots in the lab.

2.2. Extraction of genomic DNA and total RNA and first-strand cDNA synthesis

Genomic DNA was extracted from young, fresh leaves of 'Chu-ju' and 'Gong-ju' using the EasyPure Plant Genomic DNA Kit (TransGen Biotech, Beijing, China) according to the manufacturer's instructions. Total RNA was extracted from young leaves using the RNAiso Plus Kit (TaKaRa Biotech, Dalian, China) according to the manufacturer's instructions. First-strand cDNA of 'Chu-ju' and 'Gong-ju' was reverse transcribed using the PrimeScript 1st Strand cDNA Synthesis Kit (TaKaRa Biotech) according to the manufacturer's instructions.

2.3. Primer design and PCR amplification

Taking the genomic DNA sequence of the *F3'H* gene in *Chrysanthemum morifolium* (GenBank: GU249553.1) as a template, the Primer 5.0 software was used to design primers to clone *F3'H* in 'Chu-ju' and 'Gong-ju'. The primers were synthesized by the Shanghai Sango Biotechnology Company. Among the five pairs of DNA primers designed, it was found that only one pair (L1) was effective in an amplification test. The sequences of the L1 pair are as follows: forward primer 5′-TAGGAGGAGGTAGCAGTC-3′; reverse primer 5′-CGTTAGCCAGTTCCCAAT-3′. The total volume of the PCR reaction system was 50 μL, containing 5 μL of 10 × PCR Buffer (Mg^{2+} Plus), 4 μL of 2.5 mM dNTPs, 1 μL of each 1 μM primer, 0.5 μL of 5 μg μL^{-1} DNA Polymerase, 10 μL template DNA and made up with sterilized ultrapure water. The PCR reaction process consisted of an initial denaturation at 94 °C for 5 min, followed by 35 cycles of 94 °C for 45 s, 54 °C for 45 s and 72 °C for 1.5 min, with a final elongation step of 72 °C for 10 min.

Full-length cDNAs of the *F3'H* genes in 'Chu-ju' and 'Gong-ju' were amplified by Long-range PCR technology. Since the template 5′-UTR was only 15 bp and was not sufficient to design a primer, the 3′-UTR of 137 bp in *Chrysanthemum morifolium* (GenBank: GU249553.1) was used as a template to design a specific reverse primer. In the test, the mRNA sequence of