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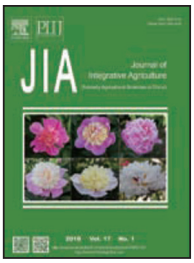

RESEARCH ARTICLE

Molecular characterization of chalcone isomerase (*CHI*) regulating flower color in herbaceous peony (*Paeonia lactiflora* Pall.)

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Abstract

Chalcone isomerase (CHI) is a key enzyme that converts yellow chalcone to colorless naringenin, playing an important regulatory role in color formation of ornamental flowers. We determined the coding sequence of *CHI* in herbaceous peony using rapid-amplification of cDNA ends (RACE) technology, and subsequently detected the expression pattern of *CHI* in the inner and outer petals at different developmental stages using qRT-PCR. We cloned the upstream promoter sequences of *CHI* using genome walking technology and predicted the location of CpG islands and 5′ truncation. In addition, we constructed five dual-luciferase reporter gene carriers and detected the promoter activities of different fragments. Our results showed that the full-length cDNA sequence of *CHI* was 898 bp, and the 5′-upstream core promoter was located at –1 651 to –2 050 bp region, where contained one CpG island (–1 897 to –2 010 bp) and several important binding sites of transcription factor, such as Sp1, serum response factor (SRF), activating protein (AP)-2alpha and CCAAT/enhancer binding protein (C/EBP)alpha. Expression results showed that the expression of *CHI* at different developmental stages was generally higher in inner petals than those in outer petals, and the maximum at the bud stage (S1). Thus, this study will provide theoretical basis for an in-depth study of *CHI* gene function and expression regulation.

Keywords: herbaceous peony, *CHI* gene, cloning, promoter, transcriptional activity

1. Introduction

Flower color affects the ornamental value of plants and directly associates with its commercial development value, so those ornamental plants with novel colors have more market prospects. *Paeonia lactiflora* Pall. is a traditional famous flower in China. However, although there are rich resources in herbaceous peony cultivars with diverse colors such as pink, red, purple and other cultivars, there is only one yellow cultivar. Therefore, breeding yellow peony cultivars with novel colors has vital significance and market prospects.

Currently, conventional cultivation methods cannot fundamentally solve the deficiency of yellow herbaceous peony cultivars, suggesting the urgency to start from studying the molecular mechanism of formation and regulation of herbaceous peony color and finally improve color by molecular designing, to promote the sustainable development of the flower industry. Research has found that carotenoids and flavonoids are two categories of pigments mainly involved in yellow color formation. Yellow color formation is associated with the chalcone isomerase (*CHI*) gene (Zhou *et al*. 2009; Zhao *et al*. 2014), as a key enzyme catalyzing the

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formation of colorless naringenin from yellow chalcone. The expression level of *CHI* directly affects the accumulation of yellow chalcone, a colorless phenotype or light yellow anthoxanthin and red anthocyanin. Therefore, *CHI* gene plays a very important role in the improvement of yellow flowers (Zhao *et al*. 2012). The important regulatory role of *CHI* in color formation has attracted the attention of many researchers. Initially, the researchers isolated the cDNA sequence of *CHI* from bean (Mehdy and Lamb 1987), *Glycine max* (Chen *et al*. 2012), *Antirrhinum majus* (Sparvoli *et al*. 1994), and *Callistephus chinensis* (Li *et al*. 2006), respectively. Subsequently, *CHI* from ornamental plants and herbs also gained attention, such as, the full-length cDNA (725 bp) of *CHI* in *Lonicera japonica* was cloned successfully (Qiao 2012); the full-length *CHI* gene (1 163 bp) in *Camellia sinensis* was cloned with expressed sequence tag (EST) sequencing technology and T_4 RNA ligase-mediated 5′ rapid-amplification of cDNA ends (RACE) technology (Ma *et al*. 2007). However, research on herbaceous peony *CHI* is still in its early stages, as we are still unclear about its coding sequence, expression pattern and regulatory mechanism of upstream promoter regions affecting gene expression.

In this study, we firstly cloned the cDNA sequence of herbaceous peony *CHI* gene with RACE technology and revealed the expression pattern of *CHI* gene in inner and outer petals of Xiangyangqihua (pink outer petals, and yellow inner petals) and Liantai (pink outer petals, and milk white inner petals) at different developmental stages (bud stage (S1), initial bloom stage (S2), blooming stage (S3), and decline stage (S4)) using qRT-PCR. In addition, we further cloned the upstream promoter region of *CH1* using genome walking technology, analyzed the promoter region, explored its transcriptional regulatory mechanism through 5′ end truncation treatment by directionally cloning different promoter fragments and detecting promoter activity using a dual luciferase detection system. In this study, we further clarified the key regulatory regions and candidate transcription factor binding sites of *CHI* promoter, which provides certain guidance for further study of *CHI* gene function.

2. Materials and methods

2.1. Plant materials

Petals of herbaceous peony cultivars Xiangyangqihua (anemone type) and Liantai (anemone type) at different developmental stages, S1, S2, S3, and S4, were selected as the experimental materials (Fig. 1). Xiangyangqihua has pink outer petals and yellow inner petals, while Liantai has pink outer petals and milk white inner petals, and their flower color gradually becomes light with the extension of stages from S1 to S4. Samples were collected from the germplasm nursery of herbaceous peony at the School of Horticulture and Plant Protection, Yangzhou University, China from April to July 2015. All materials were immediately frozen in liquid nitrogen after collection, brought back to the laboratory, and stored at –80°C for later use.

Fig. 1 Petal changes at different stages of herbaceous peony. A, Xiangyangqihua. B, Liantai. S1, S2, S3, S4 are the bud, initial bloom, blooming and decline stages, respectively.

2.2. Reagents

3′- and 5′-Full RACE Core Set Kits, SMARTer™ RACE cDNA Amplification Kit, Genome Walking Kit, and MiniBEST Agarose Gel DNA Extraction Kit were purchased from TaKaRa (Dalian, China). pEASY™-T5 Zero Cloning Kit, HEK 293T and DH5α competent cells were bought from TransGen Biotech (Beijing, China). Lipofectamine® 3000 Transfection Reagent came from Thermo Fisher Scientific (Waltham, MA). Dual-Luciferase Assay System was bought from Promega Promega (Madison, WI, USA). Primers were synthesized by Sangon Biotech (Shanghai, China).

2.3. Primers design

Partial mRNA sequence of *CHI* gene in herbaceous peony was obtained from previous transcriptome sequencing (Zhao *et al*. 2014). Specific RACE amplification primers for 5′- and 3′-end sequences were designed according to the instructions of 3′- and 5′-Full RACE Core Set Kits. Fluorescence quantitative primers were designed after obtaining the full-length coding sequence of *CHI* by Primer 3.0 Software (http://primer3.ut.ee/). Specific primers were designed according to the Genome Walking Kit for genome walking twice. All primers' information was shown in Table 1.

2.4. 3′ and 5′ RACE

Total RNA was isolated from petals using the RNA Isolation Kit (Invitrogen Life Technologies, China) according to the