Scientia Horticulturae 236 (2018) 158–165

Contents lists available at ScienceDirect

Scientia Horticulturae

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

Identification of DFR as a promoter of anthocyanin accumulation in poinsettia (*Euphorbia pulcherrima*, willd. ex Klotzsch) bracts under short-day conditions

Zhanying Gu, Hao Chen*, Ruonan Yang, Meihui Ran

Key Laboratory of Cultivation and Protection for Non-Wood Forest Trees (Ministry of Education), Central South University of Forestry and Technology, Changsha, 410004, PR China

ARTICLE INFO

Keywords:
Poinsettia bracts
Photoperiod
Anthocyanin metabolism
DFR

ABSTRACT

Poinsettia (*Euphorbia pulcherrima*, Willd. ex Klotzsch) originated in Mexico is an important ornamental tree in all over the world because its bract color can change from green to red under short-day conditions. In view of this, poinsettia not only has high ornamental value but also is an important model plant in studies on anthocyanin metabolism regulated by photoperiod. In this research, we compared the content of metabolic products in anthocyanin biosynthesis pathway and transcriptome sequencing data between green and red-turning bracts of poinsettia to clarify the mechanism of color change. The results of metabolic product analysis suggested that far downstream genes such as dihydroflavonol 4-reductase (DFR) gene in anthocyanin biosynthesis pathway could be inhibited in green bracts. A total of 91,917 uni-transcripts were identified through transcriptome sequencing. Seventy-two uni-transcripts were assigned to flavonoid biosynthesis pathways. Through a correlation analysis of gene expression profiles and color compound contents, *DFR* was taken into account as a candidate gene promoting anthocyanin accumulation in poinsettia bracts under short-day conditions. Transgenic research showed that overexpression of poinsettia *DFR* significantly increased the anthocyanin content in *Arabidopsis* (*Arabidopsis thaliana*). Based on these results, this research identified DFR as a promoter of anthocyanin accumulation in poinsettia bracts under short-day conditions. Moreover, the results of this research will shed light on elucidation of anthocyanin biosynthesis mechanism of plants and provide candidate genes for genetic improvement on poinsettia.

1. Introduction

Poinsettia (*Euphorbia pulcherrima*, Willd. ex Klotzsch) originated in Mexico has high ornamental value due to its bracts with a variety of bright colors. Plants with red bracts are most popular all over the world. The color of poinsettia bracts changes from green to red under short-day conditions. Thus, poinsettia not only has high economic value but also is an ideal model species in researches on anthocyanin metabolism regulated by photoperiod (Meng and Liu, 2015).

Specific anthocyanins are key determinants of plant tissue color. For example, delphinidin leads to varying shades in the blue flowers of *Muscari latifolium*, while cyanidin produces reddish hues (Qi et al., 2013). Purple-fleshed sweet potatoes have attractive reddish-purple color with high levels of anthocyanins (Lee et al., 2013). Previous study showed that red color of poinsettia bracts also depends on the extensive accumulation of anthocyanins (Bennett et al., 2008). As the most in-depth studied kinds of compounds in plants, the metabolic pathway of anthocyanins has been extensively described (Grotewold, 2006; Tanaka et al., 2008). However, the anthocyanin metabolism is very complicated in terms of researches in higher plants because many pathways interlink and overlap in their synthesis (Clark and Verwoerd, 2011). These diverse pathways through which plant anthocyanin can be synthesized make it more difficult to deal with this matter. In view of this, the natural color transformation in poinsettia bracts provides opportunities to elucidate the complex network of anthocyanin metabolism regulated by photoperiod.

For consumer acceptance, the key of cultivating poinsettia lies in producing plants with bright color foliage. Breeding efficiency of poinsettia can be greatly improved by molecular approaches based on identification of the genes involved in anthocyanin biosynthesis. As is known, RNA sequencing (RNA-Seq) approaches will make researches on the molecular mechanism of metabolite variation in some species without reference genome sequence more efficient. However, whether there is a direct correlation between the level of transcript and the

* Corresponding author.
E-mail address: chenhao198223@outlook.com (H. Chen).

https://doi.org/10.1016/j.scienta.2018.03.032
Received 1 November 2017; Received in revised form 20 January 2018; Accepted 16 March 2018

abundance of respective metabolite is still uncertain.

In this study, RNA sequencing was performed to obtain transcriptome data of poinsettia green and red-turning bracts. Through comprehensive analysis on transcriptome and chemical compound data, the major pathways involved in poinsettia anthocyanin metabolism were deduced and the key enzyme gene *DFR* contributing to color transformation under short-day conditions was identified. The results will shed light on elucidation of molecular mechanism of anthocyanin metabolism in plants and provide genic resources applied in genetic improvement of poinsettia.

2. Materials and methods

2.1. Plant materials

The rooted cuttings of poinsettia variety "Sonora Red" used in this study were grown in a 22 °C chamber with 16 h light/8 h dark photoperiod (long-day conditions). When they reaches 30 cm high, some of the plants used to sample red-turning bracts were transferred into a 22 °C chamber with 8 h light/16 h dark photoperiod (short-day conditions). After 60-day growth, green and red-turning bracts with the same size were taken from plants grown under long-day and short-day conditions, respectively (Fig. 1). In order to reduce the error, the tissues with equal weight of these green or red-turning bracts from different individuals were respectively mixed for RNA sequencing, gene expression analysis and flavonoid analysis. Seven-day-old seedlings of wild type and transgenic *Arabidopsis* plants (Col-0 background) grown under long-day conditions (16 h light/8 h dark) were sampled for relative gene expression analysis and anthocyanin measurement.

2.2. Flavonoids measurement and anthocyanin content assay

High-performance liquid chromatography (HPLC) was used to analyze the anthocyanin profiles in poinsettia bracts as previously described (Slatnar et al., 2013). In order to analyze other flavonoids, 50 mg frozen bract tissue was finely grounded with liquid nitrogen and then mixed with 500 µl MeOH at 4 °C for 48 h in darkness. After centrifuging the mixture, supernatants were immediately transferred to fresh tubes, and then incubated in 500 µl 1% MeOH at 4 °C for 24 h. HPLC analysis for flavonoids was performed as previously described (Qi et al., 2013). For anthocyanin content assay in *Arabidopsis*, blot-dried 10-day-old seedlings were ground into powder after quick-freezing in liquid nitrogen and then mixed with 1 ml extraction buffer (18% isopropanol (v/v), 0.12 mol/l HCl). The mixture was incubated in boiling water for 2 min and then centrifuged at 1600 × *g* for 15 min. The supernatant was collected and then measured at OD_{535} (A535) and OD_{650} (A650). Anthocyanin content was calculated as (A535-2.2 × A650)/fresh weight (Lange et al., 1971; Wu et al., 2014).

Fig. 1. Green and red-turning bracts of poinsettia. These kinds of bracts were sampled for RNA sequencing and flavonoid analysis (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

2.3. RNA extraction and validation of gene expression

Total RNA of each sample was purified using RNeasy Plant Mini Kit (Qiagen). The RIN (RNA integrity number) values (> 8.0) of total RNA were tested on Agilent 2100 Bioanalyzer (Santa Clara). The expression profiles of all color pigmentation-related uni-transcripts were validated by quantitative real-time PCR (qPCR). cDNA synthesis and qPCR were performed as previously described (Chen et al., 2012a,b, 2015). The qPCR primers are listed in Table S2. Three biological replicates were performed to confirm the reliability and reproducibility of the results. The poinsettia *actin* gene was used as an endogenous control for normalization of gene expression levels.

2.4. cDNA library preparation and transcriptome sequencing

Oligo (dT)-rich magnetic beads were used to performance mRNA purification. First- and second-strand cDNA were synthesized using SuperScript™ II RT (Invitrogen) and *E. coli* DNA polymerase I (Invitrogen), respectively. The paired-end cDNA libraries used for RNA sequencing were prepared according to Illumina's protocols. RNA sequencing was performed on a HiSeq 2000 platform.

2.5. De novo assembly and annotation of transcriptome data

The clean reads were screened out from the raw reads through removing the low quality reads (adaptors and unknown nucleotides > 5%). Trinity platform (http://trinityrnaseq.sourceforge.net/) (Grabherr et al., 2011) was used to *de novo* assemble the clean reads with the parameters of 'K-mer = 25, group pairs distance = 300'. Short reads were assembled into no longer extended sequences based on their overlap regions. These kinds of sequences were clustered using the TGI Clustering tool to construct uni-transcript sequences (Pertea et al., 2003). In order to obtain protein annotation information, uni-transcript sequences were aligned against online protein databases such as NCBI nr (non-redundant), Swiss-Prot, KEGG (Kyoto Encyclopedia of Genes and Genomes) and COG (Cluster of Orthologous Groups of proteins). Blast2GO software was used to categorize uni-transcripts into gene ontologies after alignments (Conesa et al., 2005).

2.6. Expression annotation

SOAPaligner (http://soap.genomics.org.cn/soapaligner.html) was used to realign all usable reads to each uni-transcript sequence to calculate their coverage depth subsequently normalized into RPKM (reads per kb per million reads) values (Mortazavi et al., 2008). The expression levels of all uni-transcripts were calculated with RPKM algorithm in order to find out differentially expressed uni-transcripts. False discovery rates (FDR) were evaluated to identify the *P*-value thresholds in multiple tests used to determine the significance of differences between gene expression levels (Benjamini and Yekutieli 2001). Only uni-transcripts with FDR value < 0.001 and absolute value of log2 ratio ≥ 2 were subsequently analyzed.

2.7. Gene amplification, sequence alignment and vector construction

The full-length cDNA sequence of poinsettia *DFR* gene (GenBank: MF943249) was amplified using SMARTer® RACE 5′/3′ Kit (Clontech) with gene specific primers (GSP) for 5′ RACE (GSP1: GAAATATCCCT TGCCTTACT, GSP2: GATGTATGCAATGCTCACAT) and 3′ RACE (GSP1: TGTCGTTGGATGACAACGGT, GSP2: TGACCTCATGATGTCTAGAA). To produce *Arabidopsis* plants overexpressing poinsettia *DFR*, the full-