Scientia Horticulturae 189 (2015) 132–138

Contents lists available at ScienceDirect

Scientia Horticulturae

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

Isolation and characterization of differentially expressed genes in petals of chrysanthemum mutant cultivars developed by irradiation

Sang Hoon Kim[a,1], Sang Yeop Sung[a,1], Ye-Sol Kim[a], Yeong Deuk Jo[a], Si-Yong Kang[a], Jin-Baek Kim[a], Joon-Woo Ahn[a], Bo-Keun Ha[b,*], Dong Sub Kim[a,*]

[a] Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute, Jeongeup 580-185, Republic of Korea
[b] Division of Plant Biotechnology, College of Agriculture and Life Sciences, Chonnam National University, Gwangju 500-757, Republic of Korea

ARTICLE INFO

Article history:
Received 13 February 2015
Received in revised form 1 April 2015
Accepted 4 April 2015

Keywords:
Anthocyanin
Chrysanthemum
Mutation
Radiation
Suppression subtractive hybridization

ABSTRACT

Chrysanthemum is one of the most popular ornamental plants, whose petal colors are primarily determined by pigments including flavonoids/anthocyanins and carotenoids. To develop chrysanthemum cultivars with various petal colors, mutation breeding targeting alteration of pigmentation pattern has been performed. A radiation-induced mutant line, 'ARTI-purple', showed a flower color altered from the original bright pink to purple. In this study, we used suppression subtractive hybridization to analyze transcripts and characterize the differential gene expression of chrysanthemum petals between the mutant 'ARTI-purple' and its wild-type *Chrysanthemum* × *morifolium* cultivar 'Argus'. One hundred and seventy-six genes were identified (*e*-value ≤ 1e − 5) and classified based on sequence homology to genes with known or putative functions. The genes were categorized functionally by gene ontology analysis and their tentative pathways were confirmed using the TAIR database. The analyses revealed that these genes were related to carbohydrate metabolism, biosynthesis of secondary metabolites, and lipid metabolism. Six genes in a Kyoto encyclopedia of genes and genomes (KEGG) pathway which included the largest number of differentially expressed genes were selected for validation by quantitative PCR, and most of them showed higher expression levels compared with the wild-type. In addition, we isolated two novel clones (PC06E06 and PC08C09) having glutathione *S*-transferase (GST) family conserved domains and one clone (PC02G08) having a Multidrug and toxic compound extrusion (MATE) family conserved domain based on analysis using conserved domain database (CDD). The expressions of PC08C09 and PC02G08 were upregulated in 'ARTI-purple', which implies that anthocyanin accumulation pattern might be altered in mutant. In this study, we identified several differentially expressed genes between 'Argus' and 'ARTI-purple'. The analysis suggested that several metabolic genes as well as glutathione *S*-transferases and MATEs might be involved in the control of flower pigmentation in chrysanthemum.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Chrysanthemum (*Chrysanthemum* × *morifolium*) is a popular ornamental plant whose flower colors are the most important factors affecting their market value. Flower color of chrysanthemum is determined by the compositions and concentrations of pigments including flavonoids and carotenoids. Among those pigments, anthocyanins are grouped as a class of flavonoids and represent various colors such as red, purple, orange, and blue following the expressions of species-specific enzymes of their biosynthetic pathway such as chalcone synthase (CHS), flavanone 3-hydroxylase (F3H), dihydroflavonol 4-reductase (DFR), anthocyanidin synthase (ANS), and UDP-glucose: anthocyanidin 3-*O*-glucosyltransferase (3GT) (Aharoni et al., 2001; Kobayashi et al., 2001; Suzuki et al., 2001; Shelagh et al., 2001; Kitamura et al., 2004; Tanaka et al., 2008; Davies, 2009). Flavonoid derivatives

Abbreviations: 3GT: UDP-glucose, anthocyanidin 3-*O*-glucosyltransferase; 5′3GT, anthocyanidin 5′3-*O*-glycosyltransferase; ANS, anthocyanidin synthase; CDD, conserved domain database; CHS, chalcone synthase; Cy3G, cyanidin 3-*O*-glucoside; DFR, dihydroflavonol 4-reductase; E3′G, epicatechin 3′-*O*-glucoside; EC number, enzyme commission number; F3H, flavanone 3-hydroxylase; GO, gene ontology; GSTs, glutathione *S*-transferases; KEGG, kyoto encyclopedia of genes and genomes; MAT, malonyl-CoA: anthocyanin 5-*O*-glucoside-6″-*O*-malonyltransferase; MATE, multidrug and toxic compound extrusion; SSH, suppression subtractive hybridization; TAIR, The Arabidopsis Information Resource.

* Corresponding authors at: Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute, Jeongeup 580-185, Republic of Korea/Division of Plant Biotechnology, College of Agriculture and Life Sciences, Chonnam National University, Gwangju 500-757, Republic of Korea.
Tel.: +82 62 530 205/+82 63 570 3311; fax: +82 63 570 3319.
E-mail addresses: shkim80@kaeri.re.kr (S.H. Kim), sungssy@gmail.com (S.Y. Sung), yesol@kaeri.re.kr (Y.-S. Kim), jyd@kaeri.re.kr (Y.D. Jo), sykang@kaeri.re.kr (S.-Y. Kang), jbkim74@kaeri.re.kr (J.-B. Kim), joon@kaeri.re.kr (J.-W. Ahn), bkha@chonnam.ac.kr (B.-K. Ha), bioplant@kaeri.re.kr (D.S. Kim).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.scienta.2015.04.007
0304-4238/© 2015 Elsevier B.V. All rights reserved.

(e.g. Cy3G, and E3′G) are modified by glycosylation, acylation and/or methylation and accumulated in the vacuole by mediations of transporter proteins such as glutathione *S*-transferases (GSTs) or a multidrug and toxic compound extrusion (MATE) transporters (Kitamura et al., 2004; Conn et al., 2008; Zhao and Dixon, 2010). The biosynthetic pathway for anthocyanins and their regulation by a R2R3-MYB transcription factor have been well studied, yet the location and catabolism of the synthesized anthocyanins are still not clearly established (Zhao and Dixon, 2010).

Mutation breeding is a useful technology for plant breeders and biological researchers (Broertjes, 1966; Dowrick and Bayoumi, 1966). Since the 1930s, plant breeders have used gamma-ray irradiation for mutation breeding in asexual and sexual ornamental plants (Ahloowalia et al., 2004). Irradiation at lower dose rate is useful especially for radiation breeding of vegetatively propagated crops because the obtained mutants would be directly used as new cultivars (Yamaguchi et al., 2008). Irradiated plants have been selected based on their visible characteristic, such as flower color, structure and size, or leaf shape and growth patterns. Among them, a flower color mutant can be characterized by accumulation of various pigments including flavonoids/anthocyanins, betalains, and carotenoids (Tanaka et al., 2008). In chrysanthemum, flavonoids/anthocyanins have been reported to be responsible for pink-purple coloration (Sung et al., 2013; Noda et al., 2013). A spray-type chrysanthemum cultivar 'Argus' and its derived mutant 'ARTI-purple' have been studied for their expressions of genes for anthocyanin biosynthetic enzymes, namely *CmCHS*, *CmCHI*, *CmF3H*, *CmDFR*, *CmANS*, *Cm3GT*, *Cm5′3GT*, and *CmMAT* and anthocyanin concentrations. Although no differences in the expressions of these genes were observed, the concentration of anthocyanin in 'ARTI-purple' was approximately 3.5-fold higher than that of 'Argus' (Sung et al., 2013). The results indicated that the expression of *CmMYB1*, a repressive regulator of anthocyanin, was reduced in 'ARTI-purple', which inhibited a metabolic process affecting anthocyanin content, rather than the direct transcriptional inhibition of anthocyanin biosynthetic genes (Sung et al., 2013).

Suppression subtractive hybridization (SSH) is a useful method to identify and isolate genes differentially expressed between two individuals. It is based primarily on a technique called suppression PCR, and combines normalization and subtraction in a single procedure. The normalization step equalizes the abundance of cDNAs within the target population, and the subtraction step excludes the common sequences between the target and driver populations. Based on this principle, only one round of subtractive hybridization is needed and the subtracted library is normalized in terms of an abundance of different cDNAs. It dramatically increases the probability of obtaining low-abundance differentially expressed cDNAs and simplifies the analysis of the subtracted library (Diatchenko et al., 1996, 1999). This technology has been successfully applied to the analysis of the transcriptomes of several plant species during development and in response to stress (Xu et al., 2007; Galla et al., 2009). The identified ESTs can be characterized by an analysis of the Kyoto encyclopedia of genes and genome (KEGG) pathways, gene ontology (GO), and the conserved domain database (CDD) family. Such analyses can expand our understanding by predicting differences in biological compounds and gene expression networks (Nabors, 1976; Xu et al., 2007; Galla et al., 2009; Sung et al., 2013).

In this study, we aimed to analyze cDNA libraries by PCR-based SSH to characterize the differentially expressed genes in chrysanthemum petals between a mutant with purple-colored flowers and its wild-type cultivar with bright pink flowers. This approach successfully identified differentially expressed genes and enhanced our understanding of anthocyanin accumulation in chrysanthemums.

2. Materials and methods

2.1. Plant materials

The Chrysanthemum cultivar 'Argus' with bright pink flowers and its mutant 'ARTI-purple' with purple flowers which was derived by a gamma-ray mutagenesis program (40 Gy to plantlets which were regenerated *in vitro*) employed by us (Sung et al., 2013) were used as plant materials. 'Argus' and 'ARTI-purple' were developed by vegetative propagation of irradiated mutant lines for three generations under natural conditions in a plastic greenhouse at the Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute. Visual inspection indicated that the mutant's purple color had been stably inherited for three years. The petals of each genotype were collected at 10 days after flowering from November to December. For sampling, flower development was divided into two stages after flowering. Stage 1 (ray floret stretched and pigmented) and 2 (ray florets fully developed) were defined as 13 and 25 days after the initiation of flowering, respectively (Supplementary Fig. S1). The stage 1 and 2 ray florets were mixed and frozen with liquid nitrogen to be preserved at $-80\,^{\circ}C$ before being used as a resource for SSH procedure and expression analysis.

2.2. mRNA isolation and construction of SSH libraries

Total RNA was isolated from the ray florets of 'Argus' and 'ARTI-purple' using the TRIzol Reagent (Invitrogen, USA) according to the manufacturer's protocol. The chrysanthemum petals contained high levels of polysaccharides; therefore, the extracted total RNA in diethylpyrocarbonate was precipitated for 3 h using a constant concentration of 3 M $LiCl_2$ at $-20\,^{\circ}C$. Poly A^+ mRNA was then isolated using a Poly Tract mRNA isolation kit (Promega, USA). A NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, USA) was used to quantify the total RNA and mRNA at wavelengths of 230, 260, and 280 nm. The mRNA was adjusted to a final concentration of 1 $\mu g/\mu l$. Electrophoresis on 1.2% agarose gels was used to verify the integrity of the total RNA and mRNA.

The PCR-Select cDNA Subtraction Kit (Clontech, USA) was used to construct two subtraction cDNA libraries (forward and reverse) according to the manufacturer's protocol. As the forward subtraction cDNA library, mRNA from both 'Argus', the tester, and 'ARTI-purple', the driver, was used for cDNA synthesis by a suppression PCR using subtractive hybridization. The reverse subtraction cDNA library was derived from 'ARTI-purple' as the tester and cDNA from 'Argus' as the driver. Two rounds of PCR using oligonucleotide primers complementary to the adapters amplified the subtractive hybridized products. A QIAquick PCR Purification Kit (Qiagen, China) was used to purify the products of the second nested PCR. The purified products were ligated into a vector pGEM-T (Invitrogen, USA) and transformed into *Escherichia coli* DH-5α cells. The SSH cDNA libraries were sequenced and edited to remove any vector and ambiguous sequences.

2.3. General molecular procedures and GO analysis

To assign putative functions to the encoded proteins of the genes represented by the cDNAs, the sequences obtained by SSH were compared with sequenced deposited in the NCBI GenBank database using BLASTX in the Blast2GO tool. For the annotation, the default settings of filtration at an *e*-value of 1.0e − 3 (annotation cut-off of 33) were used. In addition, gene ontology, functional annotation, enzyme commission number (EC number), and PATHWAY were analyzed by Blast2GO. The tentatively identified KEGG pathways were confirmed by searching TAIR database. The sequences with homologous sequence shorter than 50 bp were classified as an

Download English Version:

https://daneshyari.com/en/article/6407039

Download Persian Version:

https://daneshyari.com/article/6407039

Daneshyari.com