Saudi Journal of Biological Sciences xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Medically important carotenoids from *Momordica charantia* and their gene expressions in different organs

Do Manh Cuong [a,1], Mariadhas Valan Arasu [b,1], Jin Jeon [a], Yun Ji Park [a], Soon-Jae Kwon [c], Naif Abdullah Al-Dhabi [b,*], Sang Un Park [a,*]

[a] Department of Crop Science, Chungnam National University, 99 Daehak-ro, Yuseong-gu, Daejeon 34134, Republic of Korea
[b] Addiriyah Research Chair for Environmental Studies, Department of Botany and Microbiology, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia
[c] Korea Atomic Energy Research Institute, Advanced Radiation Technology Institute, 29 Geumgu-gil, Jeongeup-si, Jeollabuk-do 580-185, Republic of Korea

ARTICLE INFO

Article history:
Received 16 October 2017
Revised 9 November 2017
Accepted 12 November 2017
Available online xxxx

Keywords:
Medically important carotenoids
Momordica charantia
Transcriptome level
HPLC analysis

ABSTRACT

Carotenoids, found in the fruit and different organs of bitter melon (*Momordica charantia*), have attracted great attention for their potential health benefits in treating several major chronic diseases. Therefore, study related to the identification and quantification of the medically important carotenoid metabolites is highly important for the treatment of various disorderes. The present study involved in the identification and quantification of the various carotenoids present in the different organs of *M. charantia* and the identification of the genes responsible for the accumulation of the carotenoids with respect to the transcriptome levels were investigated. In this study, using the transcriptome database of bitter melon, a partial-length cDNA clone encoding geranylgeranyl pyrophosphate synthase (*McGGPPS2*), and several full-length cDNA clones encoding geranylgeranyl pyrophosphate synthase (*McGGPPS1*), zeta-carotene desaturase (*McZDS*), lycopene beta-cyclase (*McLCYB*), lycopene epsilon cyclases (*McLCYE1* and *McLCYE2*), beta-carotene hydroxylase (*McCHXB*), and zeaxanthin epoxidase (*McZEP*) were identified in bitter melon. The expression levels of the mRNAs encoding these eight putative biosynthetic enzymes, as well as the accumulation of lycopene, α-carotene, lutein, 13Z-β-carotene, E-β-carotene, 9Z-β-carotene, β-cryptoxanthin, zeaxanthin, antheraxanthin, and violaxanthin were investigated in different organs from *M. charantia* as well as in the four different stages of its fruit maturation. Transcripts were found to be constitutively expressed at high levels in the leaves where carotenoids were also found at the highest levels. Collectively, these results indicate that the putative *McGGPPS2, McZDS, McLCYB, McLCYE1, McLCYE2,* and *McCHXB* enzymes might be key factors in controlling carotenoid content in bitter melon. In conclusion, the over expression of the carotenoid biosynthetic genes from *M. charantia* crops to increase the yield of these medically important carotenoids.

1. Introduction

Momordica charantia (family Cucurbitaceae), commonly known as bitter gourd or bitter melon, is a popular herb found in Asia, Africa, and the Caribbean. As a medicinal plant, bitter melon is used in the treatment of several diseases or conditions including diabetes, HIV, viral infections, cancer, inflammation, ulcers, and sepsis (Chao et al., 2014; Liaw et al., 2015). Researchers have found that, with respect to its pharmaceutical applications, the important components of bitter melon are the phenolic, flavonoid, triterpene, and carotenoid compounds, including alpha and beta-carotene, lycopene, and zeaxanthin (Liaw et al., 2015).

The Carotenoids derivative present in the vegetables and fruits were known for their medical applications especially in controlling the chronic and vascular diseases (Khoo et al., 2011). Till today more than 600 types of Carotenoids are identified from various plant specieses (Khoo et al., 2011). The color of carotenoids also attracts both pollinators and seed dispersal agents to flowers and fruit and also the starting molecules for the synthesis of abscisic acid which is mainly involved in the plant stress regulations. Vita-

* Corresponding authors.
E-mail addresses: naldhabi@ksu.edu.sa (N.A. Al-Dhabi), supark@cnu.ac.kr (S.U. Park).
[1] These two authors contributed equally to this work
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2017.11.039
1319-562X/

min A is synthesized from the intermediary molecules of carotenoids such as such as α-carotene and β-carotene (Tuan et al., 2011a,b). In human vitamin A deficiency causes various visionary diseases such as xerophthalmia, and blindness. Also, consumption of carotenoids help in reducing the risks of cancer, cataract formation and heart related disease in human. In carotenoid biosynthetic pathway, dimethylallyl pyrophosphate (DMAPP) catalysis by geranylgeranyl pyrophosphate synthetase (GGPPS) (Fig. 1) (Tuan et al., 2011a,b). The genes responsible for the metabolic pathways enzymes were already amplified and characterized in *Arabidopsis* (Ruiz-Sola and Rodriguez-Concepcion, 2012), *Brassica rapa* (Li et al., 2015), tomato (Namitha et al., 2011), carrot (Clotault et al., 2008), *Momordica cochinchinensis* (Hyun et al., 2012), rice (Beyer et al., 2002), maize (Messias et al., 2014), and *Scutellaria baicalensis* Georgi (Tuan et al., 2015).

In recent years, several genes in the *M. charantia* carotenoid biosynthesis pathway have been cloned and characterized including phytoene synthase (*McPSY*), phytoene desaturase (*McPDS*), carotenoid cleavage dioxygenase 1 (*McCCD1*), carotenoid cleavage dioxygenase 4 (*McCCD4*), 9-cis-epoxycarotenoid dioxygenase (*McNCED*) (Tuan and Park, 2013). However, there are still a number of genes that remain uncharacterized, including geranylgeranyl pyrophosphate synthase (*McGGPPS*), zeta-carotene desaturase (*McZDS*), lycopene beta-cyclase (*McLCYB*), lycopene epsilon cyclase (*McLCYE*), beta-carotene hydroxylase (*McCHXB*), and zeaxanthin epoxidase (*McZEP*) (Tuan and Park, 2013). To date, no comparative studies have been performed examining the genes from *M. charantia*. Here, we have examined the levels of *McGGPPS, McZDS, McLCYE, McLCYB, McCHXB,* and *McZEP* in different organs, as well fruit at different stages of maturation, from *M. charantia.* This is the first description of these enzymes in *M. charantia*, and it marks a first step toward possible bioengineering of *M. charantia* crops to increase the yield of these medically important carotenoids.

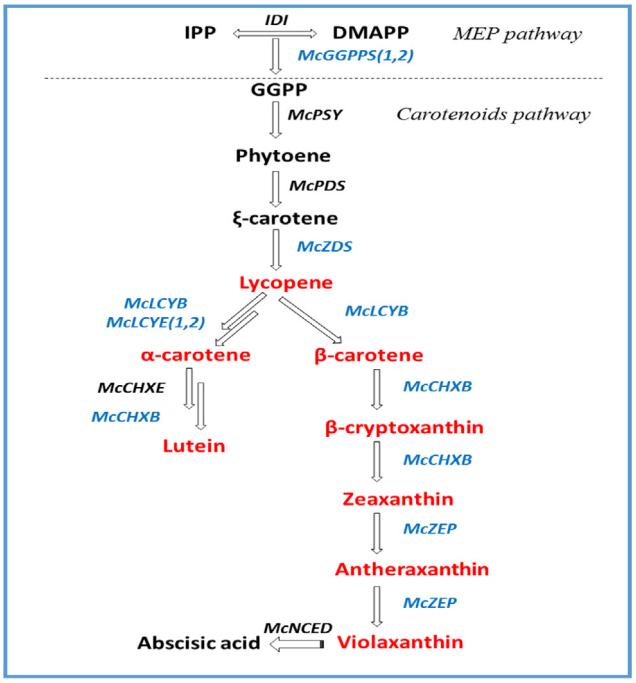

Fig. 1. Carotenoid biosynthetic pathway in plants. Red color denotes the carotenoids measured in this study by HPLC analysis and blue color indicates enzymatic activities for which gene expression was monitored via real time-PCR. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2. Materials and methods

2.1. Plant material

Seeds of a Chinese cultivar of bitter melon (*Momordica charantia L.)* were purchased from Beijing Namo Tech.-Trade Co. Ltd (Beijing, China). After three months, different bitter melon organs including roots, stems, old leaves, young leaves, male flowers, female flowers, and fruit at four different stages of maturation (Table S1) were collected and harvested.

2.2. RNA isolation and cDNA synthesis

RNeasy Plant mini kit (QIAGEN, Valencia, CA, USA) was used for the extraction and purification of the total RNA from different organs of *M. charantia* (Tuan and Park, 2013). After extraction, 1 μg of high-quality total RNA was used for the preparation of cDNA synthesis. The cDNA was synthesized using the ReverTra Ace-α-kit (Toyobo Co. Ltd., Osaka, Japan).

2.3. Sequence analysis

Using sequence data from the sequencing of complementary DNA (cDNA) libraries obtained from *M. charantia* seedlings (data not shown). The genes that showed maximum identity and similarity were selected for further study (Tuan et al., 2011a,b).

2.4. Quantitative real-time polymerase chain reaction (qRT- PCR) analysis

Real-time PCR primers (Table 1) were designed using the Primer 3 website (http://frodo.wi.mit.edu/primer3/) based on the sequences of geranylgeranyl pyrophosphate synthase (*McGGPPS1* and *McGGPPS2*), zeta-carotene desaturase (*McZDS*), lycopene beta-cyclase (*McLCYB*), lycopene epsilon cyclase (*McLCYE1* and *McLCYE2*), beta-carotene hydroxylase (*McCHXB*), zeaxanthin epoxidase (*McZEP*), and based on the published gene sequences of phytoene synthase (*McPSY*) (GenBank Accession Number: AY494789), and phytoene desaturase (*McPDS*) (GenBank Accession Number: AY494790.1). The levels of gene expression were calculated by relative quantification using the *M. charantia* cyclophilin gene (*McCYP*) (GenBank Accession Number HQ171897) as reference. Standard amplification procedures such as initial denaturation 95 °C for 5 min, 95 °C for 15 s, template annealing at 65 °C for 15 s, and final extension 72 °C for 20 s, respectively. To the PCR mixture SYBR Green was added for the quantification of the expression level of the individual genes (Tuan et al., 2011a,b).

2.5. Extraction and high performance liquid chromatography (HPLC) analysis of carotenoids from M. charantia

The extraction method used for carotenoid analysis in bitter melon was similar to that described by Tuan et al. (2011a,b) . Gradient elution system was used for the complete separation of the individual carotenoid components. For the mobile solvent preparation 10 mM ammonium acetate was dissolved in (92% of methanol and 8% of water (Solvent A) and 100% methyl *tert*-butyl ether (MTBE) (Solvent B). Initially the column was eluted with 83% A and 17% B for 23 min, after that 70% A and 30% B for 29 min, 59% A and 41% B for 35 min, 30% A and 70% B for 40 min, 30% A and

Please cite this article in press as: Cuong, D.M., et al. Medically important carotenoids from *Momordica charantia* and their gene expressions in different organsMomordica charantia –>. Saudi Journal of Biological Sciences (2017), https://doi.org/10.1016/j.sjbs.2017.11.039