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

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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-

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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.

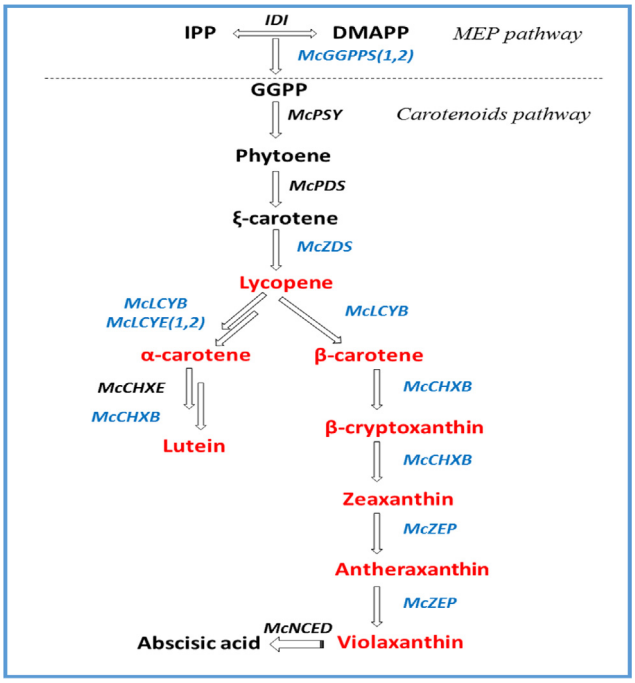

Fig. 1. Carotenoid biosynthetic pathway in plants. Red color denotes the carotenoids measured in this study by HPLC analysis and blue color indicates enzymatic activities for which gene expression was monitored via real time-PCR. (For interpretation of the references to color in this figure legend, the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

Table 1
Sequences of specific primers used for quantitative real-time PCR.

Primers name	Forward primer sequences (5′–3′)	Reverse primer sequences (5′–3′)	Size (bp)
McGGPPS1	GGCACCAATTCGATGTTCTT	GATCTCGTCGGGGTACTGAA	154
McGGPPS2	GCCGTGTGTGGAGGACTAAT	GCTTCCCCTTCTTCGTTCTT	154
McPSY	GCTTCATCGTTGGTTGTCTCTCT	TGCTCCATTTCTGCCTCTTACTC	154
McPDS	TTTGCTTGGATTACCCTAGACCA	TGCACCAGCGATCACTACTTTTA	128
McZDS	TCTTGGCTTTATTCCCATCG	AGTTGCTCCTTCCATGCTGT	194
McLCYB	CGGGAGGGTTAATAGGAAGC	GGACAAGGCATCGAGAGAAG	194
McLCYE1	AAGCGTTTTGAAGCAAGGAA	AGTGCAAGCCCAAAGAGAAA	141
McLCYE2	CTTGTAGCCTGCGAACATGA	TCCTACCTCGACCTCCACAC	147
McCHXB	ACGATGTTTTCGCCATTACC	GGACCCACAGGGAATCTTTT	178
McZEP	GAGCGTGCTGTGCATTAGAA	ACAACTGGGTTCTCCCACTG	193
McCYP	GGCAAACCCTAAAGTTTTCTTCG	GATGAGCCCTTGTAATGAAGTGG	174

70% B for 44 min, 83% A and 17% B for 55 min respectively (Howe and Tanumihardjo, 2006)

3. Results

3.1. Sequence analyses of carotenoid biosynthetic genes from M. charantia

In our study, complementary DNA (cDNA) libraries from *M. charantia* seedlings were sequenced using the Illumina Next Seq500 platform. Out of 68,073,862 total reads, approximately 88,703 unigenes were identified (data not shown). Among the unigenes from the *M. charantia* database, several full-length cDNAs encoding *McGGPPS1, McZDS, McLCYB, McLCYE1, McLCYE2, McCHXB,* and *McZEP*, and a partial-length cDNA clone encoding*McGGPPS2* were identified (Table 2). Their homology was confirmed by BLAST program, and the cDNAs were designated as *McGGPPS1* (335 amino acids, aa), *McGGPPS2* (261 aa), *McZDS* (579 aa), *McLCYB* (504 aa), *McLCYE1* (249 aa), *McLCYE2* (308 aa), *McCHXB* (311 aa), *McZEP* (450 aa).

3.2. Gene expression of carotenoid biosynthesis genes in different M. charantia organs

The expression levels of the studied genes in roots, stems, young leaves, old leaves, male flowers, female flowers, and four fruit stages from *M. charantia* using real-time PCR was presented in Figs. 2 and 3. Among the carotenoid biosynthetic genes of *M. charantia*, considerable levels of almost all the genes were detected in leaves, whereas only *McCHXB* was expressed at high levels in the roots. Considerable levels of *McGGPPS1* and *McGGPPS2* were detected in leaves (Fig. 2), whereas only small levels were found in other organs. However, expression levels of these two GGPPSs in leaves varied depending on the age of the leaf; *McGGPPS1* had high expression levels in young leaves, whereas *McGGPPS2* had high expression levels in old leaves. The transcription patterns of *McZDS, McLCYB, McLCYE1,* and *McZEP* were all essentially similar, with high expression observed in old leaves, intermediate levels in young leaves, and low levels in male flowers, female flowers, stems, and roots. Considerable levels of *McLCYE2* were detected in leaves, lower levels in stems and flowers, and trace levels in the roots.

With the exception of *McLCYB and McLCYE2,* the *M. charantia* expression patterns of *McGGPPS1, McGGPPS2, McPSY, McPDS, McZDS, McLCYE1, McCHXB,* and *McZEP* were similar during the four stages of fruit maturation (Fig. 3). Specifically, expression barely changed from stage 1 to stage 3.In contrast, *McLCYE2* decreased from stage 1 to stage 4, whereas expression of *McLCYB* increased from stage 1 to stage 3, but then decreased at stage 4.

3.3. HPLC analyses of carotenoid in different M. charantia organs

Carotenoids in the roots, stems, young leaves, old leaves, male flowers, female flowers, and the four stages of fruit maturation, were determined by HPLC analysis of tissue extracts (Tables 3 and 4). Carotenoid accumulation was lowest in the roots, the only abundant carotenoid in roots being lutein (4.81 μg/gdry weight (DW)); however, this accumulation was significantly lower than that observed in other organs of bitter melon. These high levels of lutein may explain why low levels of its precursor, α- carotene, were found in *M. charantia*. α-carotene accumulation was highest in old leaves (41.06 μg/g DW), whereas only small amounts were found in young leaves, stems, male flowers, and female flowers (18.77 μg/g DW, 7.29 μg/g DW, 5.75 μg/g DW, and 5.34 μg/g DW, respectively); there were no detectable levels in roots. β-carotene, including E-β-carotene, 9z-β-carotene, and 13z-β-carotene, synthesized high amounts in *M. charantia*. Especially the the content of E-β-carotene was higher in old leaves and young leaves (287.03 μg/g DW and 220.12 μg/g DW, respectively), comparable contents were documented the stems, male flowers, and female flowers (51.11 μg/g DW, 65.13 μg/g DW and 56.64 μg/g DW, respectively), and not detected in roots. The accumulation of the isomers 9z-β-carotene and 13z-β-carotenewassimilar, being abundant in old leaves (49.16 μg/g DW and 45.62 μg/g DW, respectively), and young leaves (41.54 μg/g DW and 33.22 μg/g DW, respectively), with low accumulation in male flowers (13.9 μg/g DW and 16 μg/g DW, respectively), female flowers (9.38 μg/g DW and 8.26 μg/g DW, respectively), and stems (8.10 μg/g DW and 8.6 μg/g DW, respectively), and undetectable in roots. *M. charantia* also contained a small amount of violaxanthin and antheraxanthin in the male flowers (48.91 μg/g DW and 32.29 μg/g DW, respectively) whereas only trace amounts of these carotenoids were found in other organs.

In the fruit of *M. charantia*, the noted amount of the carotenoids was varied between covering stage 1 to stage 3 to orange (stage 4) (Table 4). For example, zeaxanthin was highest in stage 4 fruit (45.91 g/gDW), followed by stage 3 fruit (9.82 μg/gDW), but undetectable in stage 1 and stage 2 fruit. Lycopene, β-cryptoxanthin, and antheraxanthin only accumulated at the final stage of fruit maturation (stage 4) (being 12.4 μg/gDW, 69.05 μg/gDW, and 7.07 g/gDW, respectively).The accumulation of lutein decreased from stage 1 (41.16 g/gDW) to stage 3 (17.43 μg/gDW), and then increased again at stage 4 (21.89 μg/gDW). The α-carotene content decreased from stage 1 (12.67 μg/gDW) to stage 4 (2.48 μg/gDW). The β-carotene content, including 13Z-β-carotene, e-β-carotene, 9Z-β-carotene, varied during fruit maturation from stage 1 to stage 4. 13Z-β-carotene and 9Z-β-carotene contents decreased from stage 1 (4.44 μg/gDW, and 3.84 μg/gDW, respectively) to stage 2 (1.79 μg/gDW, and 2.03 g/gDW, respectively), and then further increased at stage 3 (1.93 μg/gDW, and 2.23 g/gDW, respectively) and stage 4 (5.89 μg/gDW, and 2.83 μg/gDW, respectively). In

Table 2
Comparison of carotenoid genes of *M. charantia* with the most orthologous genes.

Genes of *M. charantia*	Length (amino acid)	Orthologous genes	Accession no.	Identity (%)
McGGPPS1	335	*Cucumis melo*	XP_008447910.1	88
		Cucumis sativus	XP_004144868.1	87
		Populus euphratica	XP_011046617.1	80
McGGPPS2	261	*Cucumis sativus*	XP_004144868.1	82
		Morus notabilis	XP_010102651.1	82
		Cucumis melo	XP_008447910.1	81
McZDS	579	*Cucurbita moschata*	AEK86566.1	90
		Cucumis melo	XP_008462722.1	89
		Cucumis sativus	XP_004142522.1	89
McLCYB	504	*Citrullus lanatus*	ABM90917.1	96
		Cucumis sativus	XP_004150761.1	95
		Cucurbita moschata	AEN94902.1	91
		Cucumis melo	XP_008457615.1	95
McLCYE1	249	*Cucumis sativus*	XP_004141172.1	95
		Cucurbita moschata	AEN94903.1	92
		Cucumis melo	XP_008459489.1	94
McLCYE2	308	*Cucumis melo*	XP_008459489.1	87
		Cucumis sativus	XP_004141172.1	84
		Cucumis melo var. makuwa	AID51466.1	87
McCHXB	311	*Cucurbita moschata*	AEK86567.1	87
		Cucumis sativus	XP_004140758.1	88
		Cucumis melo	XP_008439286.1	88
McZEP	450	*Cucumis sativus*	XP_004148358.2	88
		Morus notabilis	XP_010104831.1	72
		Vitis vinifera	XP_002265622.3	74

contrast E-β-carotene content decreased from stage 1 (26.13 g/gDW) to stage 3 (10.84 g/gDW), and then increased at stage 4 (28.08 μg/g DW).

4. Discussion

In our study, two genes encoding geranylgeranyl pyrophosphate synthase (*McGGPPS1* and *McGGPPS2*) were identified. However, the expression level of *McGGPPS2* was higher than *McGGPPS1* in all different *M. charantia* organs. Moreover, the gene expression level of *McGGPPS2* correlated with the accumulation of total carotenoids being highest in old leaves, followed by young leaves, male flowers, female flowers, stems, and lowest in roots. This result suggests that *McGGPPS2* is involved in the production of carotenoids in *M. charantia.* This result was similar to what has previously been described in a range of other plants. For example, a statistical analysis of the correlation between carotenoid content and candidate gene transcript levels in a maize germ plasam collection revealed that the expression of only one of the three plastidial *GGPPS* enzymes (*GGPPS1*) positively correlated with endosperm carotenoid content (Vallabhaneni and Wurtzel, 2009). Similarly, the Arabidopsis genome contains a family of 12 genes encoding putative *GGPPS* isoforms (Lange and Ghassemian, 2003); however, only one *Arabidopsis* gene, encoding *GGPPS1*, has been suggested to be involved in the production of carotenoids (Meier et al., 2011).

The first committed step in plant carotenoid biosynthesis is the synthesis of phytoene from GGPP; this reaction is catalyzed by phytoene synthase (*PSY*). The number of *PSY* genes differs in different plants; *Arabidopsis* (Ruiz-Sola and Rodriguez-Concepcion, 2012), and Tartary buckwheat (Tuan et al., 2013) contain one *PSY* gene, tobacco (Busch et al., 2002) and tomato (Giorio et al., 2008) contain two *PSY* genes, and maize (Li et al., 2008), rice (Welsch et al., 2008), and cassava (Arango et al., 2010) contain three *PSY* genes. Some *PSY* isoforms are involved in the biosynthesis of carotenoids in other tissues. For example, tomato *PSY1* participates in the production of carotenoids in fruit, the seed endosperm (maize *PSY1*), or the root (maize and rice *PSY3*). In our study, a single *M. charantia PSY* gene (*McPSY*) was expressed in virtually all tissues, and positively correlated with *McGGPPS1* expression levels showing high expression levels in young leaves and stage 4 fruit compared to other organs (Figs. 2 and 3).

The α-carotene content was highest in old leaves, followed by young leaves and lowest in roots, and this correlated well with *McLCYE1* expression across the different *M. charantia* organs. Similarly the expression level of *McLCYB* correlated well with β-carotene content. Accordingly, we propose that *McLCYE1* and *McLCYB* are involved in the production of carotenoids in *M. charantia.*

During the maturation of *M. charantia* fruit, of the contents of zeaxanthin and β-cryptoxanthin, was higher. When the color of the fruit changed from green (stage 1-3) to orange (stage 4), the concomitant increase in zeaxanthin and β-cryptoxanthin content is likely brought about because of increased *McCHXB* expression. However, in other organs, such as the roots, which have high *McCHXB* expression, the levels of β-cryptoxanthin and zeaxanthin content did not correspond with *McCHXB* expression. Based on this, we proposed that *McCHXB* causes accumulation of β-cryptoxanthin and zeaxanthin in fruit. We also suggested that there is another isoform of *CHXB* in *M. charantia*, which has direct influence on the synthesis of β-cryptoxanthin and zeaxanthin in the different organs. It is also evidenced that the two isoforms of the *CHXB* gene was specific for chromoplasts in flowers and/or the fruit (Galpaz et al., 2006).

The expression of *McZDS* was highest in stage 4 fruit and corresponded with lycopene accumulation. Similarly, the α-carotene content decreased from stage 1 to stage 4 and corresponded with the level of *McLCYE2* expression in fruit. Based on this, we suggested that *McZDS* and *McLCYE2*cause the accumulation of lycopene and α-carotene, respectively, in the fruit of *M. charantia.* In this study, we have also reported the levels of *McZEP* gene expression in different organs and, unlike for several of the previously discussed genes, we found that carotenoid content did not correlate with the levels of *McZEP* gene expression (Clotault et al., 2008).

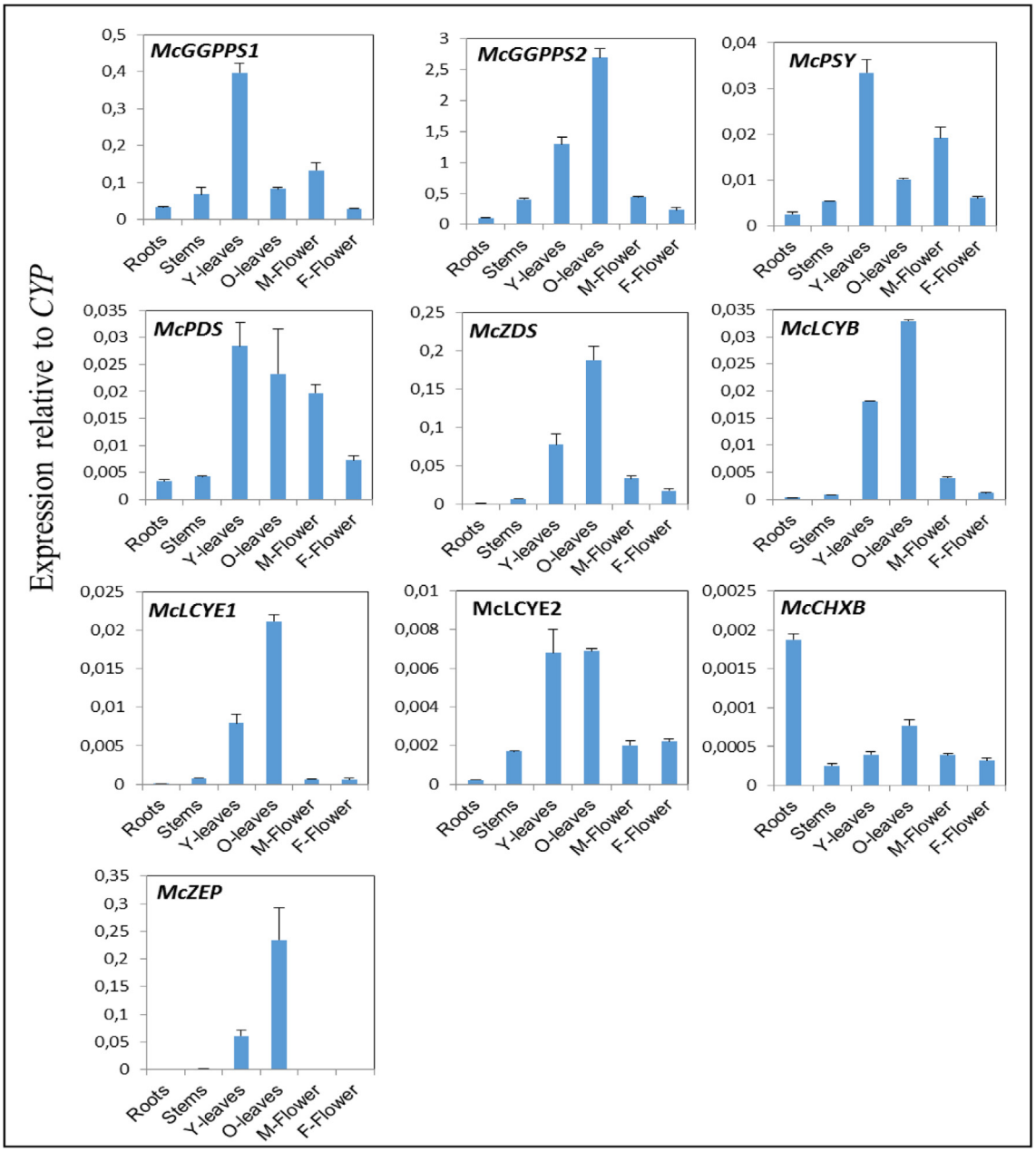

Fig. 2. Expression levels of genes in carotenoid biosynthesis pathway in different organs of *M. charantia*.

In our study, carotenoids were generally found to be at high levels in organs that are exposed to direct light (e.g. leaves, flowers, and stems). This finding is similar to previous studies conducted in *M. charantia* (Tuan et al., 2011a,b), *Allium sativum* (Tuan et al., 2012), and Chinese cabbage (Fraser et al., 2000) which also found that the levels of carotenoids were very high in the leaves, but very low in the organs not exposed to light (e.g. roots).

5. Conclusion

Summary, eight genes related to the carotenoid biosynthetic pathway in bitter melon including *McGGPPS1, McGGPPS2, McZDS, McLCYB, McLCYE1, McLCYE2, McCHXB*, and *McZEP* were identified and their expression levels and carotenoid accumulation were high in leaves and comparatively lower in roots. Alternatively, the obtained results have guided to propose that the putative *McGGPPS2, McZDS, McLCYB, McLCYE1, McLCYE2* and *McCHXB* might be the key factors for controlling carotenoid content in *M. charantia*. Collectively, the obtained results were evidenced and were marked as a first step toward possible bioengineering of medically important *M. charantia* crops to increase the yield of these medically important carotenoids. In the future, the production rate of carotenoids might be increased by the bioengineering tools.

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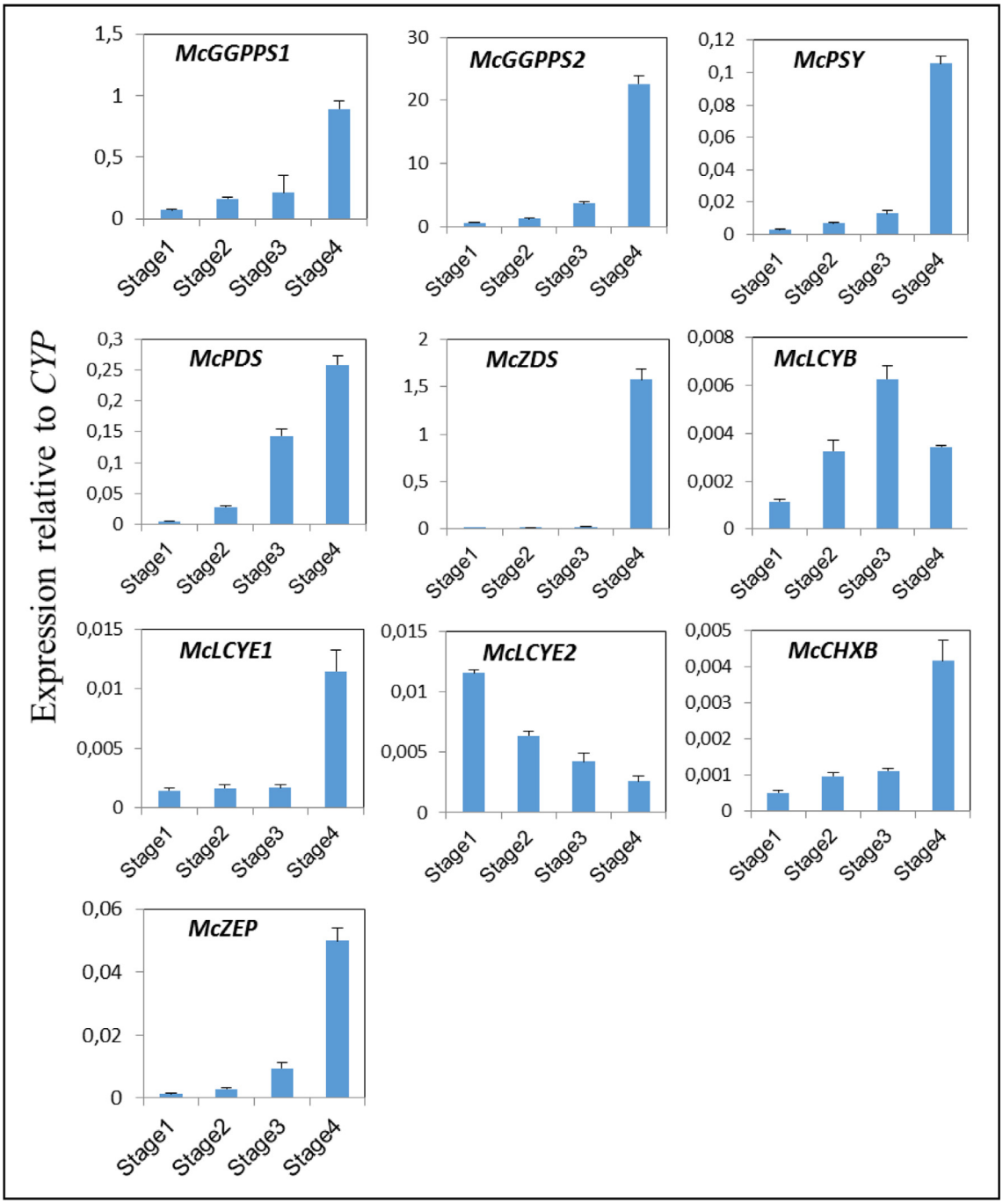

Fig. 3. Expression levels of genes in carotenoid biosynthesis pathway during fruit maturation of *M. charantia* fruit. CYP, *M. charantia* cyclophilin.

Table 3
Carotenoid content in different organs of *M. charantia* (μg/g dry weight).

	Roots	Stems	Y-leaves	O-leaves	M-Flower	F-Flower
Lycopene	ND	ND	ND	ND	ND	ND
α-carotene	ND	7.29 ± 0.57	18.77 ± 2.13	41.06 ± 1.03	5.75 ± 1.18	5.34 ± 0.44
Lutein	4.81 ± 0.83	93.64 ± 7.69	314.83 ± 48.78	412.33 ± 59.89	57.33 ± 2.70	86.12 ± 3.83
13Z-β-carotene	ND	8.60 ± 1.23	33.22 ± 6.62	45.62 ± 6.27	16.00 ± 1.27	8.26 ± 1.13
E-β-carotene	ND	51.11 ± 6.80	220.12 ± 14.50	287.03 ± 21.81	65.13 ± 4.92	56.64 ± 6.71
9Z-β-carotene	ND	8.10 ± 0.22	41.54 ± 0.64	49.16 ± 0.89	13.90 ± 2.97	9.38 ± 1.23
β-cryptoxanthin	ND	8.07 ± 0.05	10.97 ± 1.11	10.87 ± 0.13	15.26 ± 1.09	9.12 ± 0.33
Zeaxanthin	ND	11.78 ± 1.17	28.15 ± 2.67	15.38 ± 1.17	13.42 ± 0.57	9.63 ± 0.35
Antheraxanthin	ND	1.70 ± 0.20	12.08 ± 3.39	4.79 ± 0.63	32.29 ± 1.06	4.95 ± 0.24
Violaxanthin	ND	ND	17.32 ± 1.20	21.02 ± 1.96	48.91 ± 1.27	12.37 ± 0.34
Total carotenoids	4.81 ± 0.83	190.29 ± 17.92	697.01 ± 81.04	887.26 ± 93.77	267.98 ± 17.04	201.82 ± 14.60

Table 4
Carotenoid content in different fruit of *M. charantia* (μg/g dry weight).

	Stage 1	Stage 2	Stage 3	Stage 4
Lycopene	ND	ND	ND	12.44 ± 3.20
α-carotene	12.67 ± 1.26	4.54 ± 0.39	3.19 ± 0.36	2.48 ± 0.18
Lutein	41.16 ± 3.21	22.66 ± 1.26	17.43 ± 0.61	21.89 ± 1.33
13Z-β-carotene	4.44 ± 0.70	1.79 ± 0.16	1.93 ± 0.32	5.89 ± 0.64
E-β-carotene	26.13 ± 2.59	12.25 ± 1.00	10.84 ± 0.89	28.08 ± 1.64
9Z-β-carotene	3.84 ± 0.25	2.03 ± 0.15	2.23 ± 0.36	2.83 ± 0.09
β-cryptoxanthin	ND	ND	ND	69.05 ± 15.76
Zeaxanthin	ND	ND	9.82 ± 0.17	45.91 ± 3.81
Antheraxanthin	ND	ND	ND	7.07 ± 1.54
Violaxanthin	ND	ND	ND	ND
Total carotenoids	88.24 ± 8.00	43.28 ± 2.97	45.44 ± 2.71	195.63 ± 28.20

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.sjbs.2017.11.039.

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