January 2016. Horticultural Plant Journal, 2 (1): 9–15.

Horticultural Plant Journal

Available online at www.sciencedirect.com

The journal's homepage: http://www.journals.elsevier.com/horticultural-plant-journal

Characterization of Carotenoid Accumulation and Carotenogenic Gene Expression During Fruit Development in Yellow and White Loquat Fruit

ZHANG Ling [a,1], ZHANG Zhike [a,1], ZHENG Tingting [a,b], WEI Weilin [a], ZHU Yunmei [a], GAO Yongshun [a], YANG Xianghui [a,*], and LIN Shunquan [a,*]

[a] *College of Horticulture, South China Agricultural University, Guangzhou 510642, China*

[b] *Shangrao City Forest Resources Monitoring Center, Shangrao, Jiangxi 334000, China*

Received 25 November 2015; Received in revised form 23 December 2015; Accepted 3 January 2016

Available online 19 February 2016

Abstract

Accumulation of carotenoids in peel and pulp of the yellow-fleshed loquat 'Zaozhong 6' (ZZ6) and the white-fleshed loquat 'Baiyu' (BY) were tracked during different fruit development stages, and the expression of 15 carotenogenic genes were analyzed. During loquat fruit ripening the fresh weight content of β-carotene in peel and pulp of ZZ6 increased gradually and peaked at the fully ripe stage, reaching 68.53 μg·g^{-1} FW in the peel and 11.92 μg·g^{-1} FW in the pulp. In BY, the content of β-carotene in the peel increased and peaked at the fully ripe stage, reaching 38.89 μg·g^{-1} FW, while it decreased in the pulp from the original 0.47 μg·g^{-1} FW and reduced to 0.29 μg·g^{-1} FW. The content of β-cryptoxanthin in the peel and pulp of ZZ6 and BY both increased steadily, and peaked at the fully ripe stage; however, the content of lutein decreased in the peel of ZZ6 and increased in the pulp, but in BY, it dropped and then rose in the peel. There was no significant change of β-cryptoxanthin in the pulp of BY. After the breaker stage, the mRNA levels of phytoene synthase (*PSY*) and chromoplast-specific lycopene β-cyclase (*CYCB*) were higher in the peel, while *CYCB* and β-carotene hydroxylase (*BCH*) mRNAs were higher in the flesh of ZZ6, compared with BY. The results showed that the expression level of *PSY*, *CYCB*, and *BCH* appeared to cooperatively regulate the accumulation of carotenoids.

Keywords: *Eriobotrya japonica*; loquat; carotenoids; gene expression

1. Introduction

Gene cloning and function research of carotenoids biosynthesis pathway have been the focus of recent research. The separation and functional verification of the phytoene synthase (*PSY*) gene in tomato began in 1987. The main chain pathways of carotenoids biosynthesis were elucidated in 1990. In 2000, 1-deoxy-D-xylulose-5-phosphate-synthase (DXS), the first synthase gene of the plant carotenoids biosynthesis, was considered to be a key enzyme for the synthesis of plant carotenoids and other terpenoid substances (Lois et al., 2000). The coding gene was first obtained from *Arabidopsis thaliana* (Mandel et al., 1996) and then isolated and functionally identified in pepper (Bouvier et al., 1998), mint (Lange and Croteau, 1999), and tomato (Lois et al., 2000). Lycopene is a branch point of the further anabolism of carotenoids. Lycopene can be cyclized by lycopene β-cyclase (LCYb) and lycopene ε-cyclase (LCYe) to synthesize α-carotene or cyclized by a lycopene β-cyclase (LCYb)/chromoplast-specific lycopene β-cyclase (CYCB) alone to synthesize β-carotene. Alphacarotene and β-carotene are hydroxylated to produce lutein and zeaxanthin, respectively, catalyzed by β-ring hydroxylase (BCH) and ε-ring hydroxylase (ECH). The other carotenogenic genes, such as 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXP) reductoisomerase (*DXR*), isopentenyl pyrophosphate synthase (*IDS*), lycopene cyclase (*LCY*), phytoene desaturase (*PDS*), ζ-carotene desaturase (*ZDS*), have been cloned early and their functions were studied. A positive correlation was found between enhanced isoprenoid biosynthesis and accumulation of transcripts encoding DXR, which means that it plays a very important role in the carotenoid synthesis (Rodríguez-Concepción and Boronat, 2002). Research on tomato showed that *IDS* is limiting for

[1] Co-first author.

[*] Corresponding authors. Tel.: +86 20 38294596.

E-mail addresses: gzyxh@scau.edu.cn, loquat@scau.edu.cn.

Peer review under responsibility of Chinese Society for Horticultural Science (CSHS) and Institute of Vegetables and Flowers (IVF), Chinese Academy of Agricultural Sciences (CAAS)

http://dx.doi.org/10.1016/j.hpj.2016.02.006

carotenoids biosynthesis during tomato fruit ripening, and its expression might be coordinated with *DXS* and *PSY*. Transferring *IDS* into *A. thaliana* increased carotenoids by 50% (Botella-Pavía et al., 2004). Similarly, studies on the DXPS, PSY1, and PDS expression of tomato demonstrated that the expressions of these genes were significantly enhanced during maturation. For example the expression of *PSY1* increased more than 20 times, which resulted in a 10-fold increase in total carotenoid content (Giuliano et al., 1993; Lois et al., 2000). During fruit ripening, the expression of carotenoid biosynthetic genes in *citrus* fruit shows the same trend as in tomato: a simultaneous increase in the expression of genes (*PSY*, *PDS* and *ZDS*) leading to a massive carotenoids accumulation in the flavedo of 'Valencia' orange (Kato et al., 2004). However, recent researches showed that the carotenoids accumulation may be irrelevant to carotenogenic gene expression. Li et al. (2006) found that the cauliflower containing the *Or* gene confers a high level of β-carotene accumulation in flower buds, turning them orange, but β-carotene accumulation did not result from an increased capacity of carotenoid biosynthesis. Fu et al. (2012) studied plastids and plastid lipid–associated protein (PAP) expression at different ripening stages in 'Luoyangqing' and 'Baisha' loquat, and discovered differences in the quantity and structure of chromoplasts in flesh and peel, as well as PAP expression. Their results indicated that the inability to form chromoplasts in 'Baisha' flesh is the mostly likely explanation for the low carotenoids accumulation. Fu et al. (2014) reported that the failure of carotenoids accumulation in white-fleshed loquat can be due to the non-functional mutant *EjPSY2A*.

In the present study we used yellow-fleshed and white-fleshed loquat cultivars to study the carotenoid dynamics of their peel and flesh; as well as the expression of carotenegenic genes during fruit developmental stages. This study should help to clarify the molecular mechanism of the carotenoids accumulation of loquat and provide a theoretic foundation for scientific regulation of loquat carotenoid biosynthesis.

2. Materials and methods

2.1. Materials

'Zaozhong 6' (ZZ6, yellow-fleshed) and 'Baiyu' (BY, white-fleshed) loquat (*Eriobotrya japonica* Lindl.) were sampled from the Loquat Germplasm Resources Garden in College of Horticulture, South China Agricultural University (SCAU). The fruits were divided into 5 developmental stages: stage I, immature green, 92–98 d after full bloom (DAFB); stage II, breaker, 112–114 DAFB; stage III, degreening, 115–120 DAFB; stage IV, yellow mature, 118–122 DAFB; stage V, full mature, 124–128 DAFB (Zhang et al., 2013a). After the sample was collected, the top and the base of the fruit were quickly removed, the peel and pulp were separated and immediately frozen in liquid nitrogen and then stored at −70 °C for further research.

2.2. Carotenoid extraction and HPLC analysis

Carotenoids were extracted from fruits and analyzed by HPLC, according to a method previously described by Xiong et al. (2007). Components identification and quantitative analysis were done using Agilent 1200 HPLC-DAD analysis system, 5 μm C_{18} m reverse phase column (250 mm × 4.6 mm) and 20 mm × 4.6 mm C_{18} pre column, using external standard method. The chromatographic conditions refer to Hui's (2005). The compounds β-carotenoid, β-cryptoxanthin and lutein at different stages were detected. The standard samples of β-carotenoid, β-cryptoxanthin and lutein were purchased from Sigma Company.

2.3. Expression analysis of carotenogenic genes

Total RNA of peel and pulp from ZZ6 and BY at five stages was extracted from frozen samples following our previously published protocol (Zhang et al., 2013b). cDNA was synthesized according to the manual of M-MLV reverse transcriptase (TaKaRa).

Based on the primer design principle of quantitative real-time PCR (qRT-PCR), *DXS*, *DXR*, *IDI*, *PSY*, *LCYb*, *LCYe*, *BCH* and *ECH* were cloned and sequenced (GenBank number: JX097047, JX089590, JX097049, JX097048, JX089591, JX097050, JX097051 and JX097052, respectively). Primers for these genes (Table 1) were designed by Primer premier 6.0. And the amplification efficiency (E) was detected by qRT-PCR, and the standard curve was produced to check whether the amplification efficiency is in the range of 90%–110%. The primers *PDS*, *ZDS*, *CRTISO*, *CYCB*, *ZEP*, *VDE*, *CCD* stems were from Fu et al. (2012).

The qRT-PCR reactions were performed in a total volume of 20 μL, including 0.5 μL of each primer (10 μmol·L^{-1}), 1.0 μL cDNA, 8 μL ddH_2O, and 10 μL of SsoAdvanced SYBR Green Supermix (BIO-RAD) on an iQ5 BIO-RAD fluorescence qRT-PCR. The PCR programme was initiated with a preliminary step of 30 s at 94 °C, followed by 40 cycles at 94 °C for 15 s, 50 °C for 15 s, and 72 °C for 25 s. Three replicates were performed with ddH_2O as the negative control. A melting curve was generated for each sample at the end of each run to ensure the purity of the amplified products. The data were analyzed by

Table 1 Primers for quantitative real-time PCR

Gene	Upstream primers (5′–3′)	Downstream primers (5′–3′)	Amply sequence length/bp
DXS	CATAGATGACCGACCAAGT	AGGAGGCAGCCAAACAG	179
DXR	ATCCGCAGTCCATTATACATTC	CAGCAGCATAGGCAAGGT	231
IDI	TCGCCTTGGTTCAGACTTG	AAGCCGCAATCAAAGTTTCC	147
PSY	ACATTCAGCCCTTCAGA	ATCCAACAGTTCCAGCA	117
LCYb	CGGTATGGCTATTCTGCTTA	AGTCATCCTAATCTCTATCCTG	251
LCYe	TTCCTCGGCTCTACATTGT	GGCAGTGACCTCATCTCTT	209
BCH	GCCTTGTTCCTGGTCTTTGTT	ATGAGCCGCAGCAACCTT	152
ECH	AGTTTGCTCTGCTGGAAG	CGAAGACGAGGATGATGT	185

$2^{-\Delta\Delta Ct}$ to calculate the expression of carotenoid biosynthesis related genes.

3. Results

3.1. Carotenoid content and composition analysis

As shown in Fig. 1, the content of β-carotene in peel and flesh of ZZ6 increased gradually as the fruit ripened continuously and peaked at 68.53 μg·g^{-1} FW and 11.92 μg·g^{-1} FW respectively at stage V. In BY, the content of β-carotene in peel also increased and peaked at 38.90 μg·g^{-1} FW; while in the flesh it decreased slightly, from 0.47 μg·g^{-1} FW to 0.29 μg·g^{-1} FW. The β-cryptoxanthine levels in peel and flesh of both cultivars steadily increased and reached the highest when fruit was fully ripened, but its ratio in the total carotenoid content is relatively low. The content of lutein in peel of ZZ6 decreased continuously, but its content in flesh decreased slightly at earlier stages and increased quickly at later stages and peaked at 3.83 μg·g^{-1} FW, but lower than the content of the peel. The content of lutein in the peel of BY also decreased firstly and then increased to 7.94 μg·g^{-1} FW at stage V, even lower than at stage I, while it keeps low level in the flesh.

3.2. Analysis of carotenogenic gene expression in peel and flesh of ZZ6 and BY fruits at different developmental stages

The mRNA levels of 15 genes of the carotenoid pathway were analyzed in the peel and flesh of ZZ6 and BY at five developmental and ripening stages. As shown in Fig. 2, *DXS* showed similar expression patterns in ZZ6 and BY tissues. In ZZ6, mRNA levels first increased then decreased in both peel and pulp, and peaked at stage II; in BY, mRNA levels first increased and then decreased and rebounded at stage V, mRNA levels also peaked at stage II. In the peel and flesh of both cultivars, mRNA level of *DXR* showed a major increase at stage III and then decreased continuously until it increased slightly at stage V, its expression decreased steadily in the flesh with fruit development, and increased at stage V. *IDI* expression in the flesh was similar to the peel in both genotypes, peaked at stage I and then decreased quickly, remained low level thereafter.

In general, genes of *PSY*, *PDS* and *ZDS* showed a similar expression profile in ZZ6 and BY fruit tissues, their expression increased at earlier stages and then decreased, or decreased until it increased slightly at stage V. In the peel, mRNA level of *PSY* is higher than in the flesh (Fig. 2).

In the peel of ZZ6, mRNA level of *CYCB* increased rapidly and then decreased slightly, peaked at stage V, which was higher than in the other tissues, while in the flesh, its expression increased and peaked at stage III, then decline. In the peel and flesh of BY, the *CYCB* gene mRNA level reached the top at stage II, then declined until it increased slightly at stage V (Fig. 3).

In the peel of ZZ6, the mRNA level of *LCYb* was abundantly expressed, and then decreased dramatically and remained constant after stage II, but in the flesh of ZZ6 and in BY, its expression kept low and showed a gradual decrease. *LCYe* showed similar expression patterns in the peel and flesh of ZZ6, it expressed abundantly at earlier stages and then decreased quickly and kept low level at later stages; however, it expressed low during ripening in the peel and flesh of BY (Fig. 3).

The gene expression of *BCH* in the peel of both cultivars increased gradually during fruit ripening, and its expression level increased and then decreased in flesh, but the transcript of *BCH* was more abundant in flesh than in peel (Fig. 3). The *ECH* mRNA level has a similar expression pattern as *LCYe*, increased greatly at earlier stages and then decreased, remained constant in ZZ6; however, its expression level maintained low and showed a gradual decrease in BY (Fig. 3). In ZZ6, the mRNA level of *ZEP* increased firstly and then decreased, and its level was higher in flesh than in peel. At later stages, the *ZEP* expression decreased in the peel of BY, but in the flesh its expression decreased

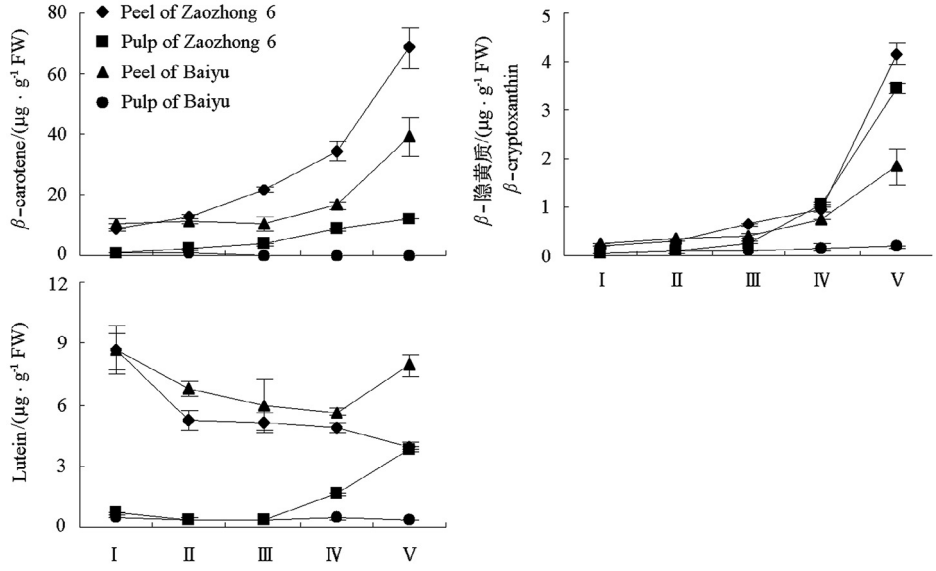

Fig. 1 The analysis of pigment content in loquat fruit during different developmental stages
I. Immature green; II. Breaker; III. Degreening; IV. Yellow mature; V. Full mature.

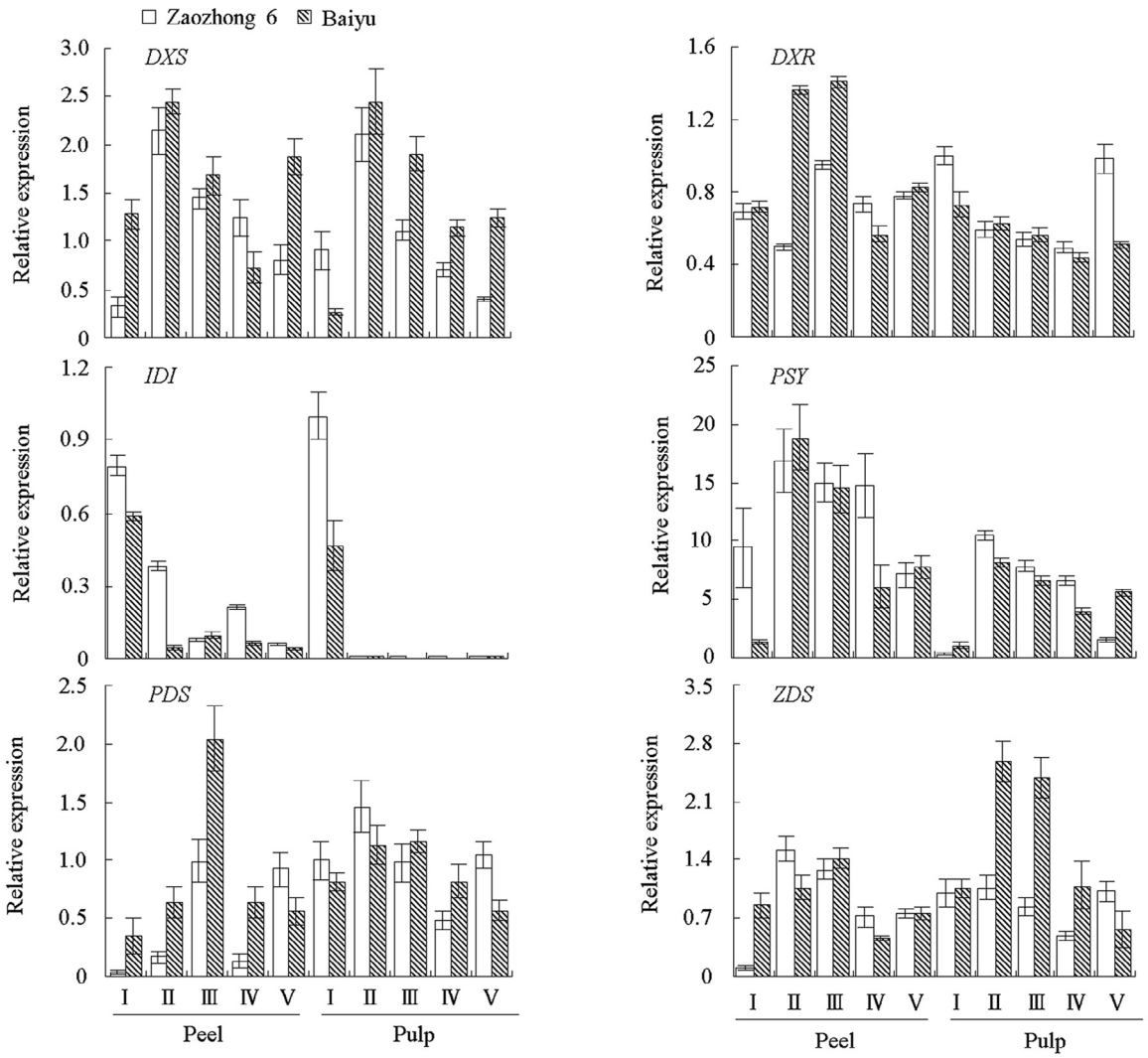

Fig. 2 Expression patterns of carotenoids biosynthetic genes *DXS*, *DXR*, *IDI*, *PSY*, *PDS* and *ZDS* during different developmental stages

firstly and then increased during fruit ripening, and its expression value was higher than that in peel (Fig. 3). *VDE* expression showed a similar expression profile to *CRTISO*, only abundantly expressed at stage II in the peel of ZZ6, and it kept low expression at the rest stages in peel and flesh of both cultivars, especially in flesh. The mRNA level of *CCD* was expressed the lowest at stage I in both genotypes, and then increased with the fruit ripening and expressed the highest at stage V in the flesh of BY (Fig. 3).

4. Discussion

4.1. Changes of carotenoids accumulation pattern in fruit during different developmental stages

The observed differences in carotenoid content and composition of loquat fruit have been reported previously. There are common conclusions that during fruit ripening the content of β-carotene and β-cryptoxanthin rise; however the content of lutein decreased, only increased slightly when fruit fully ripened. The main carotenoids accumulated were β-carotene and lutein in the peel, and β-carotene as well as β-cryptoxanthin in the flesh (Liu, 2007; Zhou, 2007).

The changes in carotenoid content and composition of ZZ6 and BY during fruit development and ripening were as found in previous studies. The content of β-carotene in the peel and flesh of ZZ6, as well as in the peel of BY, increased along with fruit development and peaked at stage V, but decreased slightly in the flesh of BY. In peel and flesh of both cultivars, the content of β-cryptoxanthin increased slightly with fruit ripening, especially in ZZ6. However, β-cryptoxanthin increased dramatically in the peel of BY at stage V, but increased relatively gently in BY flesh and remained at low level. The content of lutein of both cultivars is higher in the peel than in the flesh, but their variation is different: in the peel, the lutein content of ZZ6 declined while in BY it decreased but increased slightly as fruit fully ripened. In the pulp of ZZ6, lutein decreased at first and then increased rapidly, reaching a maximum at stage V, while in BY it continually decreased. Compared with previous studies, the

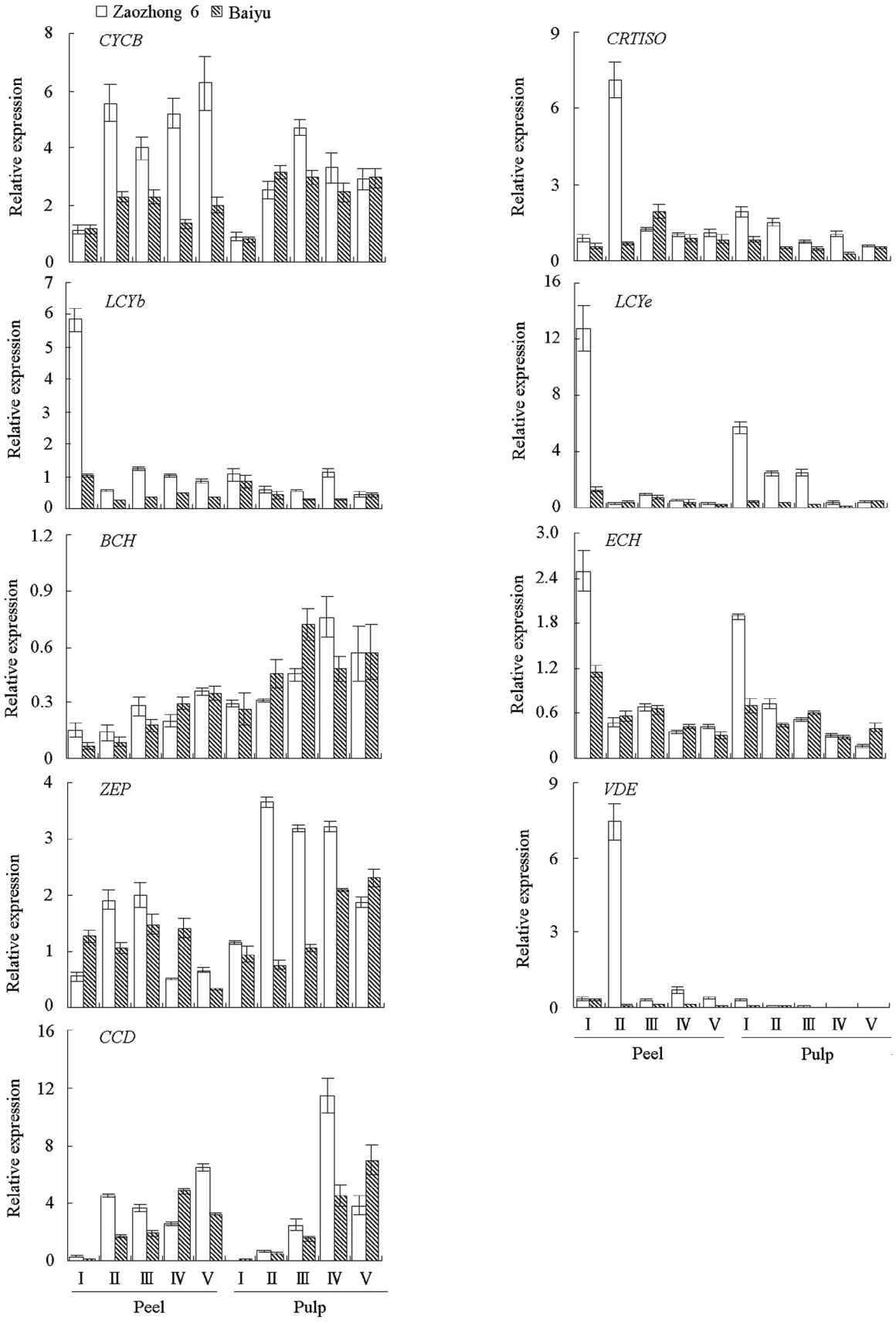

Fig. 3 Expression patterns of carotenoids biosynthetic genes *CYCB*, *LCYb*, *LCYe*, *BCH*, *ECH*, *ZEP*, *VDE*, *CRTISO* and *CCD* during different developmental stages

content of lutein in the flesh is slightly higher than the content of β-cryptoxanthin in the peel at the same period, perhaps because of cultivar and sampling period differences. Moreover, the carotenoid biosynthesis in plants is susceptible to environmental factor such as light and temperature (Tao et al., 2003).

4.2. Expression of carotenogenic genes

At immature green stage (stage I), β-carotene and β-cryptoxanthin in peel and flesh of both cultivars started to accumulate, which might have resulted from the high expression levels of *LCYe*, *LCYb*, *ECH* and *IDI*, although the total carotenoid levels in ZZ6 and BY fruit are similar. With fruit ripening, the expression level of *IDI* decreased rapidly and then remained low level, which indicates that carotenoids accumulation of ZZ6 and BY are mainly synthesized from IPP or DMAP. Further study is needed to explain the relationship between the decreased expression level of *IDI* and a decline in total carotenoid in the flesh of BY.

At breaker stage (stage II), a decrease in lutein occurred in peel and flesh of both cultivars, which was consistent with the decreased expression levels of *LCYe*, *LCYb* and *ECH*. From the breaker stage, the increased expression levels of *DXS*, *DXR*, *PSY*, *PDS*, *CYCB* and *ZDS* of both cultivars might explain the higher level of β-carotene and β-cryptoxanthin, as observed in previous studies by Fu et al. (2012).

During ripening, the β-carotene level in the peel of ZZ6 increased sharply at stage V mainly due to the higher gene expression levels of *PSY* and increased expression levels of *CYCB* and *BCH*. Phytoene synthase (PSY) synthesizes the first carotenoids-lycopene, which is recognized as a key enzyme in the carotenoid pathway, and plays an important role in the biosynthesis of various compositions of carotenoids (Wang et al., 2009; Zhu et al., 2011). The higher *PSY* expression level in the peel as compared to the flesh of both cultivars might explain the higher level of total carotenoids in peel than in flesh, indicating that PSY may regulate both the synthesis and accumulation of carotenoid in peel and flesh (Zhu et al., 2004; Alquézar et al., 2009; Blas et al., 2010).

CYCB is also important in β-carotene production (Zhu et al., 2004; Alquézar et al., 2009; Blas et al., 2010), is involved in the synthesize of β-carotene from lycopene, and is only found in the chromoplast (Ronen et al., 2000). Our study demonstrated that the expression level of *CYCB* in ZZ6 is higher than in BY in both peel and flesh. The *BCH* gene plays a major role in carotenoids accumulation in various plants, and is the first key enzyme in the synthesis of lutein (Xu and Zhang, 2002). β-cryptoxanthin accumulation may due to the increased expression level of *BCH* during fruit ripening, suggesting that the synthesis and accumulation of β-cryptoxanthin may be regulated by *BCH*.

Zeaxanthin is transformed into violaxanthin by zeaxanthin epoxidase (ZEP), and is reversed by violaxanthin deepoxidase (VDE) to give rise to the xanthophyll cycle, which has important effect on the carotenoid pathway (Zhou and Liu, 2011). *ZEP* and *VDE* have shown different results in different studies (Xiong, 2002). The expression level of *VDE* showed a similar expression profile to *CRTISO*, but is abundantly expressed at stage II in the peel of ZZ6, and its expression kept low at the other stages in peel and flesh of both cultivars, especially in the flesh. In ZZ6 and in the peel of BY, the mRNA level of *ZEP* first increased and then decreased, but in the flesh of BY its expression decreased at first and then increased during fruit ripening. The carotenoid composition identification showed that there are violaxanthin and zeaxanthin in loquat fruit, which suggests that *ZEP* and *VDE* may be related to the synthesis of these compounds. However, the proportion of violaxanthin and zeaxanthin in the total content of carotenoids is minor, so the effects of *ZEP* and *VDE* on the carotenoids accumulation require further study.

CCD is a dioxygenase of carotenoid oxidase, which can be oxidative cracking carotenoids at specific sites, which affect the carotenoid transformation (You and Yang, 2008). This study showed that the mRNA level of *CCD* increases with fruit ripening, which means the ability of carotenoid degradation strengthened. However, the increased expression level of *CCD* has little effect on carotenoids accumulation at different stages.

We assume that the increased content of β-carotene in the peel and flesh of ZZ6 and in the peel of BY was associated with the higher mRNA levels of *PSY*, *CYCB* in the peel and *BCH* in the flesh of ZZ6; which indicated that carotenoids accumulation at different stages of both cultivars was co-regulated by these genes. Fu et al. (2012) who analyzed the relationship between carotenoids accumulation and the carotenogenic gene expression in 'Luoyangqing' and 'Baisha' loquat, proposed similar results. Their study also claimed that the low content of carotenoids in the flesh of BY was related to the inability to form normal chromoplast. If there is a connection between the different carotenoids accumulation in peel and flesh of ZZ6 and BY, this needs to be investigated.

Acknowledgements

This research was partially supported by Guangzhou Science and Technology Program key projects (201504010028). Professor Janick helped with the language editing of this paper.

References

Alquézar, B., Zacarías, L., Rodrigo, M.J., 2009. Molecular and functional characterization of a novel chromoplast-specific lycopene β-cyclase from citrus and its relation to lycopene accumulation. J Exp Bot, 60: 1783–1797.

Blas, A.L., Ming, R., Liu, Z.Y., Veatch, O.J., Paull, R.E., Moore, P.H., Yu, Q.Y., 2010. Cloning of the papaya chromoplast-specific lycopene β-cyclase, CpCYC-b, controlling fruit flesh color reveals conserved microsynteny and a recombination hot spot. Plant Physiol, 152: 2013–2022.

Botella-Pavía, P., Besumbes, O., Phillips, M.A., Carretero-Paulet, L., Boronat, A., Rodríguez-Concepción, M.B., 2004. Regulation of carotenoid biosynthesis in plants: Evidence for a key role of hydroxymethylbutenyl diphosphate reductase in controlling the supply of plastidial isoprenoid precursors. Plant J, 40: 188–199.

Bouvier, F., D'harlingue, A., Backhaus, R.A., Kumagai, M.H., Camara, B., 1998. Identification of neoxanthin synthase as a carotenoid cyclase paralog. Eur J Biochem, 267: 6346–6352.

Fu, X.M., Feng, C., Wang, C.Y., Yin, X.R., Lu, P.J., Grierson, D., Xu, C.J., Chen, K.S., 2014. Involvement of multiple phytoene synthase genes in tissue and cultivar-specific accumulation of carotenoids in loquat. J Exp Bot, 65: 4679–4689.

Fu, X.M., Kong, W.B., Peng, G., Zhou, J.Y., Azam, M., Xu, C.J., Grierson, D., Chen, K.S., 2012. Plastid structure and carotenogenic gene expression in

red-and white-fleshed loquat (*Eriobotrya japonica*) fruits. J Exp Bot, 63: 341–354.

Giuliano, G., Tavazza, R., Diretto, G., Beyer, P., Taylor, M.A., 1993. Metabolic engineering of carotenoid biosynthesis in plants. Trends Biotechnol, 26: 139–145.

Hui, B.D., 2005. Carotenoid chemistry and biochemistry. China Light Industry Press, Beijing.

Kato, M., Ikoma, Y., Matsumoto, H., Sugiura, M., Hyodo, H., Yano, M., 2004. Accumulation of carotenoids and expression of carotenoid biosynthetic genes during maturation in citrus fruit. Plant Physiol, 134: 824–837.

Lange, B.M., Croteau, R., 1999. Isoprenoid biosynthesis via a mevalonate-independent pathway in plants: cloning and heterologous expression of 1-deoxy-D-xylulose-5-phosphate reductoisomerase from peppermint. Arch Biochem Biophys, 365: 170–174.

Li, L., Lu, S., Cosman, K.M., Earle, E.D., Garvin, D.F., O'Neill, J., 2006. β-Carotene accumulation induced by the cauliflower *Or* gene is not due to an increased capacity of biosynthesis. Phytochemistry, 67: 1177–1184.

Liu, X.M., 2007. Primary studies on carotenoid accumulation in loquat fruit [M. D. Dissertation]. Yangzhou: Yangzhou University. (in Chinese)

Lois, L.M., Rodríguez-Concepción, M., Gallego, F., Campos, N., Boronat, A., 2000. Carotenoid biosynthesis during tomato fruit development: regulatory role of 1-deoxy-D-xylulose 5-phosphate synthase. Plant J, 22: 503–513.

Mandel, M.A., Feldmann, K.A., Herrera-Estrella, L., Rocha-Sosa, M., Leon, P., 1996. CLA1, a novel gene required for chloroplast development, is highly conserved in evolution. Plant J, 9: 649–658.

Rodríguez-Concepción, M., Boronat, A., 2002. Elucidation of the methylerythritol phosphate pathway for isoprenoid biosynthesis in bacteria and plastids. A metabolic milestone achieved through genomics. Plant Physiol, 130: 1079–1089.

Ronen, G., Carmel-Goren, L., Zamir, D., Hirschberq, J., 2000. An alternative pathway to beta-carotene formation in plant chromoplasts discovered by map-based cloning of beta and old-gold color mutations in tomato. Proc Natl Acad Sci U S A, 97: 11102–11107.

Tao, J., Zhang, S.L., Zhang, L.C., An, X.M., Liu, C.R., 2003. Relationship between color formation and change in composition of carotenoids in peel of citrus fruit. J P1ant Physiol Mol Biol, 29: 121–126. (in Chinese)

Wang, L., Mu, P.Y., Xu, H.J., Zhuang, L., Sang, W., Nie, Y.B., Han, X.X., Zou, B., 2009. Allelic variations of *Psy-Al* gene for yellow pigment content in Xinjiang wheat cultivars. Journal of Triticeae Crops, 29: 782–786. (in Chinese)

Xiong, Y.L., 2002. Comparative analysis of carotenoid biosynthetic enzyme gene expression in juhongxin and popular heading Chinese cabbage [M. D. Dissertation]. Beijing: Capital Normal University. (in Chinese)

Xiong, Z.M., Zhou, C.H., Tao, J., 2007. Changes of carotenoid content in pulp of different loquat types during fruit coloring. Scientia Agricultura Sinica, 40: 2910–2914. (in Chinese)

Xu, C.J., Zhang, S.L., 2002. Advances in research of genes responsible for carotenoid biosynthesis in citrus. Acta Horticulturae Sinica, 29(S): 619–623. (in Chinese)

You, S.Z., Yang, H.Q., 2008. Carotenoid cleavage dioxygenases and their physiological function. Acta Botanica Boreali-Occidentalia Sinica, 28: 630–637. (in Chinese)

Zhang, L., Lin, S.Q., Hu, Y.L., Zheng, T.T., Yang, X.H., 2013a. Cloning of *DXR* gene and its expression analysis during fruit ripening in loquat. Journal of Fruit Science, 4: 563–566, 724. (in Chinese)

Zhang, L., Zhang, Z.K., Lin, S.Q., Zheng, T.T., Yang, X.H., 2013b. Evaluation of six methods for extraction of total RNA from loquat. Not Bot Horti Agrobot Cluj Napoca, 41: 313–316.

Zhou, C.H., 2007. Bioactive components and antioxidant capacity of loquat (*Eriobotrya Japonica* Lindl.) flower and fruit [Ph. D. Dissertation]. Hangzhou: Zhejiang University. (in Chinese)

Zhou, L., Liu, L., 2011. Regulation factors of carotenoid biosynthesis and their impacts on photosynthesis. Tianjin Agric Sci, 17: 5–8.

Zhu, C.F., Chen, X., Wang, Y.D., 2004. The application of plant carotenoid biosynthesis and its related genes in genetic engineering. J P1ant Physiol Mol Biol, 30: 609–618. (in Chinese)

Zhu, H.S., Li, Y.P., Wen, Q.F., 2011. Cloning and expression analysis of phytoene synthase gene in *Fragaria ananassa* Duch. Scientia Agricultura Sinica, 44: 349–357. (in Chinese)