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Distinct transcription profile of genes involved in carotenoid biosynthesis among six different color carrot (*Daucus carota* L.) cultivars

Jing Ma, Zhisheng Xu, Guofei Tan, Feng Wang, and Aisheng Xiong*

State Key Laboratory of Crop Genetics and Germplasm Enhancement, College of Horticulture, Nanjing Agricultural University, Nanjing 210095, China

*Correspondence address. Tel/Fax: +86-25-84396790; E-mail: xiongaisheng@njau.edu.cn

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Abstract

Carotenoid, a group of lipophilic molecules, is widely distributed in nature, and is important for plant photosynthesis and photoprotection. In carrot taproot, different types of dominant carotenoid accumulation lead to yellow, orange, and red colors. In this study, six different carrot cultivars were used to simultaneously analyze carotenoid contents by high performance liquid chromatography. The expression levels of genes involved in carotenoid biosynthesis of carrot were also detected by real-time quantitative PCR. It was found that genes involved in xanthophyll formation were expressed at high levels in yellow carrot cultivars. However, these genes were expressed at low levels in orange carrot cultivars. The contents of α- and β-carotene accounted for a large proportion in total carotenoid contents in orange carrot cultivars. These results indicate that α-carotene accumulation and xanthophyll formation may be related to the expression levels of carotene hydroxylase genes in carrot.

Key words: carotenoid, carotene hydroxylase genes, xanthophyll formation, HPLC, RT-qPCR, carrot

Introduction

Carrots (*Daucus carota* L.), one of the most popular taproot vegetable, is widely cultivated all over the world, which is rich in many bioactive pigments like lutein, α-carotene, and β-carotene [1–4]. Carrot is thought to originate in Afghanistan at about the ninth century. The spread of carrot cultivation generated the diversity of cultivars [5]. In the early 16th century, white and orange carrots first appeared in Western Europe [6], and almost simultaneously (1700 AD) a red-color kind of carrot was described in Asia [7,8]. In western countries, carrot is a main source of dietary provitamin A for human-beings [9]. In recent years, China has become the major carrot production country http://faostat3.fao.org/home/E (FAO, 2014). Carotenoid, an important group of lipophilic molecules, widely exists in plants, algae, and many bacteria [10–12]. Concerning the constitution of chloroplast, carotenoid plays an important functional role in the photosystems of higher plants [13]. Carotenoid has also been found in flowers and fruits of plant, and it can intensify the ability of pollination and the dispersal of seeds [14,15]. The carotenoid accumulated in carrot taproots serves as the major nutrition of carrot taproots and the contributor of the carrot taproot color.

Many studies of carotenoid biosynthesis focused on the development of different colors in fruits, seeds, or flower organs, such as citrus [16], tomato [17–19], pepper [20], maize [21,22], marigold [23], and chrysanthemum [24]. The genes and their encoded enzymes involved in carotenoid biosynthesis are well known nowadays. The steps of the carotenoid biosynthesis pathway are summarized in **Fig. 1** [14,15,28–31]. In plant, the biosynthesis of carotenoid is located in plasmids [31]. All the carotenoid molecules are derived from condensation of two C5 isoprene isomers isopentenyl diphosphate (IPP) and its allylic isomer dimethylallyl diphosphate (DMAPP) by GGPS to produce the precursor of carotenoid biosynthesis, geranylgeranyl diphosphate (GGPP) [32]. Then the phytoene synthase (PSY), which is the rate-limiting enzyme in most plant

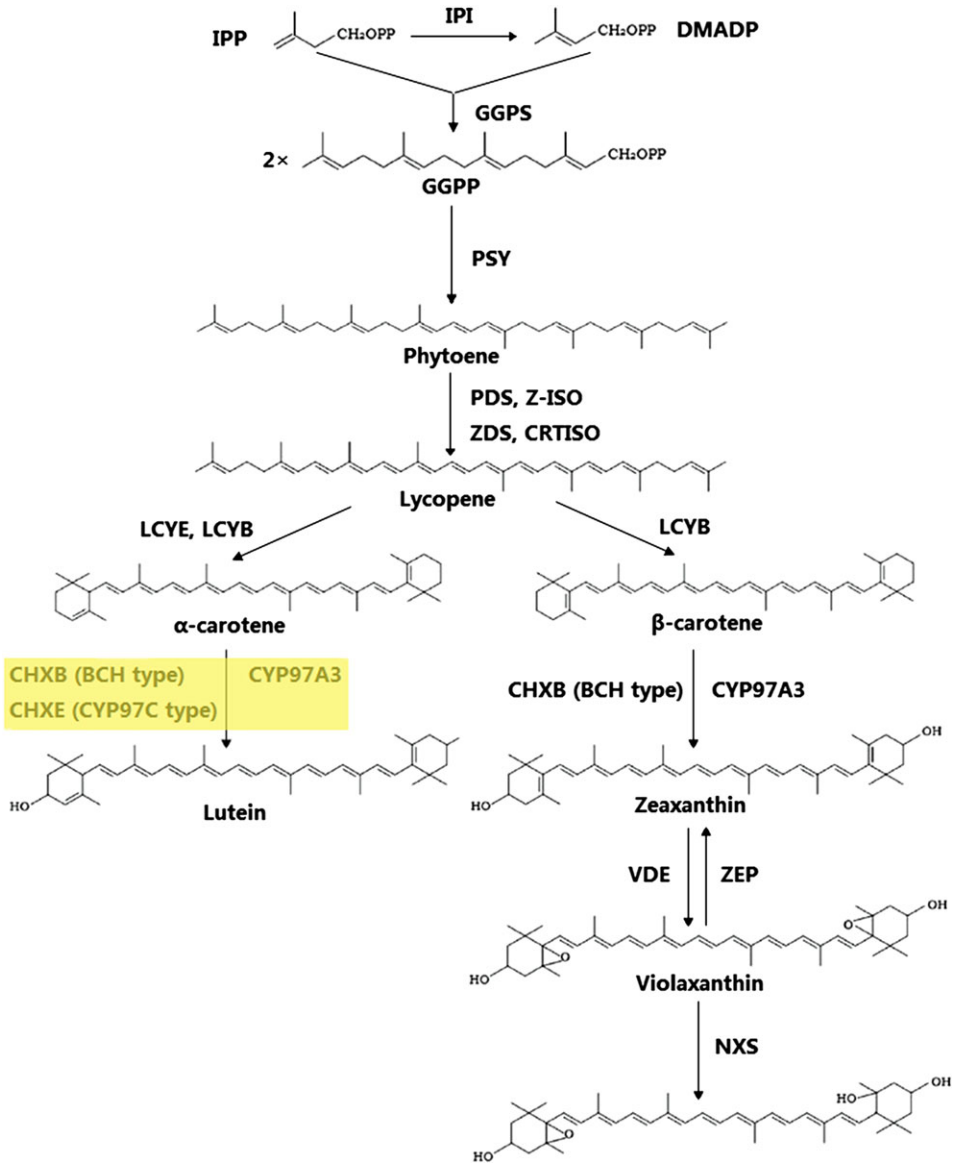

Figure 1. The carotenoid biosynthesis in plants The three key enzymes (CHXB, CHXE, and CYP97A3) were marked with shadow. IPI, isopentenyl diphosphate isomerase; GGPS, GGPP synthase; PSY, phytoene synthase; PDS, phytoene desaturase; Z-ISO, ξ-carotene isomerase; ZDS, ξ-carotene desaturase; CRTISO, carotenoid isomerase; LCYE, lycopene ε-cyclase; LCYB, lycopene β-cyclase; CHXB, β-carotene hydroxylase; CHXE, ε-carotene hydroxylase; CYP97A3, cytochrome P450-type monooxygenase 97A3; CYP97C, cytochrome P450-type monooxygenase 97 C; ZEP, zeaxanthin epoxidase; VDE, violaxanthin de-epoxidase; NXS, neoxanthin synthase. Adapted from [25–27].

carotenoid biosynthesis systems, catalyzes the first carotenoid-specific reaction to produce C40 hydrocarbon phytoene by condensation of two GGPP molecules [10,33]. The compound subsequently undergoes a series of desaturation and isomerization to form the red-colored all-trans-lycopene catalyzed by four enzymes, i.e. phytoene desaturase (PDS), ξ-carotene desaturase (Z-ISO), ξ-carotene isomerase (ZDS), and carotenoid isomerase (CRTISO) in plants [34–36]. The all-trans-lycopene is diversely cyclized by ε-ring (LCYE) and β-ring (LCYB) cyclic end cyclases to yield α and β-ring carotenes, respectively [18,37–39]. The carotene hydroxylase is the major enzyme which catalyzes carotene to xanthophylls, such as lutein and zeaxanthin [25].

Carrot is rich in carotenoid and some researchers have detected carotenoid contents in carrot, but little is known about the carotenogenesis in carrot taproots, especially lutein biosynthesis in taproots of different color carrots. Carrot taproot is also a storage tissue for various carotenes and xanthophylls [26]. The vivid colors of carotenoid are determined by the conjugated double-bond and the nature of end ring structures [40]. Moreover, carotenoids are the main compounds that determine the colors of carrot fleshy taproots, varying from red to yellow which is mainly caused by lycopene, α-carotene, β-carotene, and lutein, respectively [9,41].

In this study, the contents of three carotenoids were detected in taproots of six different color carrots, 'Junchuanhong' (orange), 'Kuroda' (orange), 'Sanhongliucun' (orange), 'Bejo1719' (yellow), 'Qitouhuang' (yellow) and 'Baiyu' (~white) [42]. The genes related to carotenoid biosynthesis in taproots of different color carrots were also analyzed. The transcript levels of those genes related to

carotenoid biosynthesis indicated that transcriptional regulation of the biosynthetic genes plays important roles in various carotenoid accumulation in taproots of different color carrots. The expression levels of three principal genes, i.e. *DcCHXB2*, *DcCHXE*, and *CYP97A3*, in yellow carrot taproots were higher than those in taproots of other color carrots. Further understanding of the expression profiles of carotenoid biosynthesis genes will provide insight into the downstream of carotene degradation in carrot.

Materials and Methods

Plant materials and growth

Six cultivars of carrots were selected based on their taproot colors: 'Junchuanhong' (orange), 'Sanhongliucun' (orange), 'Kuroda' (orange), 'Qitouhuang' (yellow), 'Bejo' (yellow) and 'Baiyu' (~white) [42]. Carrot seeds were preserved in the State Key Laboratory of Crop Genetics and Germplasm Enhancement, Nanjing Agricultural University (Nanjing, China). The seeds were grown in greenhouse with a photoperiod of 14 h of light and 10 h of darkness at 28°C after germination on filter paper for 3 days. The 60-day, 90-day and 120-day carrot taproots were harvested from soil and immediately frozen in liquid nitrogen and powdered, and then stored at −80°C for RNA isolation and carotenoid extraction.

Determination of carotenoid contents

The carotenoid extraction was performed according to the method of Chen *et al.* [43] with some modifications. Briefly, ~50 mg of powdered frozen taproots of different color carrots were extracted with 1 ml acetone at room temperature twice. Then the combined extraction supernatants (2 ml) were filtered through 0.45-μm filters and analyzed by HPLC on a Shimadzu LC-20A HPLC System (Shimadzu, Kyoto, Japan). Twenty microliter of supernatants was injected into a Hedera ODS-2 C18 analytical column (250 mm × 4.6 mm, 5 μm nominal particle size; Shimadzu) with mobile phase consisting of mixtures of methanol: acetonitrile (90:10, v/v). The flow rate was 1 ml min^{-1}, and elution was detected with a Shimadzu diode-array detector at 450 nm. All data were quantified on the basis of their standard curves. The lutein (purity ≥ 90%) standard used in this study was purchased from Shanghai Yuanye Bio-Technology Co., Ltd (Shanghai, China), and α- and β-carotene standard (purity ≥ 95%) used in this study were purchased from Wako Pure Chemical Industries, Ltd (Chuo-Ku, Osaka, Japan). The specific carotenoid contents were expressed in μg/g of fresh weight (μg/g FW). The values are expressed as the means of the three separate determinations in each case.

Total RNA extraction and quantitative RT-PCR analysis

The taproot materials were ground in liquid nitrogen. An RNA Simple Total RNA Kit (Tiangen, Beijing, China) was used to extract total RNA according to the manufacturer's instructions. The RNA was reverse-transcribed by using M-MLV reverse transcriptase (TaKaRa, Dalian, China). One microgram of total RNA was first treated at 42°C for 2 min with 2 μl of 5× gDNA Eraser Buffer and 1 μl of gDNA Eraser (TaKaRa) to remove genomic DNA, and then adjusted to 10 μl with RNase Free dH_2O. Then, 1 μl of PrimeScript RT Enzyme Mix I, 1 μl of RT Primer Mix, 4 μl of 5× PrimeScript Buffer 2 and 4 μl of RNase Free dH_2O were added into the reaction mixture. The reverse transcription was performed at 37°C for 15 min and then 85°C for 15 s. cDNA was eventually diluted 20 folds for SYBR Green RT-qPCR analysis.

RT-qPCR was carried out using the SYBR Premix *Ex Taq* (Tli RNAse H Plus) kit (TaKaRa) on the Applied Biosystems 7500 real-time PCR System. Each RT-qPCR reaction was performed in triplicate containing ~10 μl of SYBR Premix *Ex Taq*, 2 μl of diluted cDNA, 0.2 μM of each primer, and 7.2 μl of ddH_2O in a total volume of 20 μl. The PCR procedure was as follows: denaturation at 95°C for 30 s, followed by 40 cycles of 95°C for 10 s, 60°C for 20 s, and 65°C for 10 s. The gene-specific primer pairs for RT-qPCR were designed from unique gene regions using Primer Premier 5.0 and listed in **Table 1**. The $2^{-\Delta\Delta Cq}$ method was used to calculate relative genes expression as described by Pfaffl [44]. The procedure was as follows: Ratio = $2^{-\Delta\Delta Cq}$; ΔΔCq = ΔCq (sample) − ΔCq (control); $\Delta C_q = C_q$(target) − C_q (reference). The *DcActin1* gene was used as reference gene [45]. All primers used for the relative quantification were synthesized by Genscript Nanjing Inc. (Nanjing, China).

Table 1. Nucleotide sequences of primers used for RT-qPCR

Gene	Forward primer (5′-3′)	Reverse primer (5′-3′)
DcIPI	GGGTGAGGAAGGCTTGAAACTAT	ACTTCCTTGAGGGTTCCTTTCTG
DcGGPS	GACGCTTCAGTCTCACTCCAAGA	GCAATACTAAGAAGAGGACGGACA
DcPSY1	ATACCAAACGGACAGGATCTAAG	CCTCATTCAACAAATTCCAGCT
DcPSY2	GTGTTTGGGTGGTTCTGA	GAACTGTCTTTTGACCAACGC
DcPSY3	ATGATAGAAGGCATGAGGAT	TACCCATAACAGGAACACTC
DcPDS	AAGTCAAGTTTGCGTTGGGTCTC	TCAGGTATGCCCTGCTTTCTCAT
DcZDS1	CCCAGTTGGAGCACCATTACACGG	GGGCAAGGGCTACAGCATTTCTT
DcCRTISO	GAAAAGCCTCAGACCCAGATGTC	TCTCCAACTCTTTATCAACACTC
DcLCYE	GGATACTCAGTCTAAACTTGCCC	GCAGGACCACAACCAATAACCAC
DcLCYB	GCAGGGTTAGCGGTAGCACAACA	TCCATAGCCTCAAACTCATCCAC
DcCHXB1	CTTGGCATTACTTCTATGGCTGTAT	CGAATGTACCAATCATCTCCGAAT
DcCHXB2	TGAGGAAAGAATGGCGAGGAAGA	ACCTGGAGTAGACGGCGAAGACA
DcCHXE	GCTTGCTGCTGGGCCGAGAGATTTT	GAGCCAAACAAGAACTCAGAAACCT
DcZEP	CTTGATAAAGCATACAACCGAAGT	AAGTCCATGAATAACCGAGACAC
DcVDE	GGAGGTGGAGAAAGTGAAGGACA	TCTTTAGTCAGCCCATTGAGTGCG
DcCYP97A3	CCCGTCTAACTTTGGATA	GCTTTGGCGAAATGTC
DcCCD4	AACGACCGCCGAGACAGCATA	GCTCCCTTGCGACGAAGAATGG
DcCCD8	CAAGTGTGGAGGTTCCGCTGTT	TGTGGTGTCGGCACTATGTTCA
DcActin1	CGGTATTGTGTTGGACTCTGGTGAT	CAGCAAGGTCAAGACGGAGTATGG

Statistical analysis

Data are represented as the mean ± SD from three separate experiments. The carotenoid contents and RT-qPCR data were statistically analyzed by one-way ANOVA using SPSS Version 17.0. A statistical method (Duncan's multiple range test) for significant differences was analyzed at 0.05 probability.

Results

Taproot color of six carrot cultivars at three different developmental stages

Various carrot cultivars colors are attributed to different types of carotenoid accumulation in carrot taproots. Carotenoids accumulate in both xylem and phloem of all six carrot cultivars, as well as the cortex (**Fig.** 2). However, the color of xylem and phloem are different, causing to the uneven distribution of carotenoid in carrot taproots. The 'Junchuanhong', 'Kuroda' and 'Sanhongliucun' displayed an obvious orange color, while the 'Bejo1719' and 'Qitouhuang' showed a vivid yellow color, and the 'Baiyu' showed an extreme light yellow (~white) color (**Fig.** 2).

Carotenoid contents in six different carrot cultivars during root development

HPLC was used to analyze carotenoid contents in six different carrot cultivars during different root developmental stages. All the six cultivars of carrots showed colored taproots at all the three developmental stages that resulted from carotenoid accumulation. Among the three orange cultivars, both α- and β-carotene contents of 'Sanhongliucun' raised rapidly (>2 fold) during root development. The other two orange carrot cultivars ('Junchuanhong' and 'Kuroda') exhibited slower or no obvious alteration in α- and β-carotene (**Figs.** 3 and 4). However, the α- and β-carotene contents of 60 day- and 90 day-old 'Sanhongliucun' are less than those of the other two orange carrot cultivars ('Junchuanhong' and 'Kuroda') at the same stages. Besides the remarkable increase of carotenoid accumulation, 'Sanhongliucun' shared similar α- and β-carotene contents with the other two carrot cultivars ('Junchuanhong' and 'Kuroda') at 120-day stage (**Figs.** 3 and 4). In contrast to the high proportion of α- and β-carotene, an unexpected decrease of lutein content was found in the carrot taproot of 90-day-old 'Junchuanhong', but at the later stage, lutein content was recovered to 6.43 μg/g FW, which is higher than that in the 60-day-old 'Junchuanhong'. In the other two orange carrot cultivars ('Kuroda' and 'Sanhongliucun') the lutein contents were also rather low (<10 μg/g FW) with little alteration (**Fig.** 5). In

Figure 2. Growth status and transverse section of different cultivars of carrot taproots at three different stages The long and short black lines in the right lower corner in each image represent 3 cm and 1 cm, respectively.

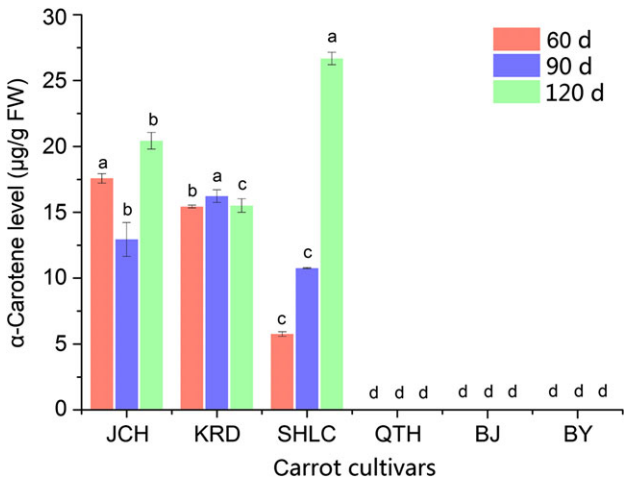

Figure 3. α-Carotene contents in six carrot cultivars during root development Cultivar abbreviations: JCH, Junchuanhong; KRD, Kuroda; SHLC, Sanhongliucun; BJ, Bejo1719; QTH, Qitouhuang; BY, Baiyu. Different lowercase letters indicate significant differences between six carrot cultivars at same developmental stages at $P < 0.05$.

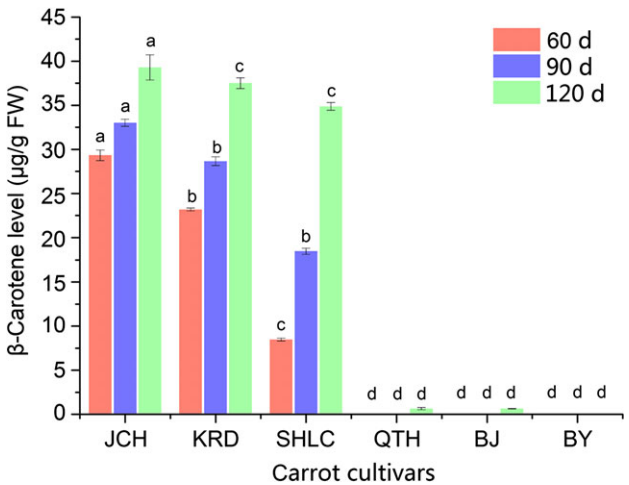

Figure 4. β-Carotene contents in six carrot cultivars during root development Cultivar abbreviations: JCH, Junchuanhong; KRD, Kuroda; SHLC, Sanhongliucun; BJ, Bejo1719; QTH, Qitouhuang; BY, Baiyu. Different lowercase letters indicate significant differences between six carrot cultivars at same developmental stages at $P < 0.05$.

two yellow cultivars ('Qitouhuang' and 'Bejo1719'), lutein was found to be the predominant carotenoid with an increasing content during three stages. No α-carotene was detected and only trace amount of β-carotene was detected in 120-day-old taproots (**Figs. 3–5**). In addition, the lutein content in 'Bejo1719' reached 15.55–26.93 μg/g FW, which was much higher than that in 'Qitouhuang' (3.39–6.54 μg/g FW). But in 'Baiyu' taproot, no α- or β-carotene was detected, only very little lutein was detected in 90-day-old taproot (0.38 μg/g FW) and 120-day-old taproot (1.49 μg/g FW). These results revealed that the three orange carrot cultivars ('Junchuanhong', 'Kuroda' and 'Sanhongliucun') were abundant with α-carotene and β-carotene, but lack of lutein, and in yellow carrot cultivars ('Qitouhuang' and 'Bejo1719'), lutein became the major contenoid that provide yellow color.

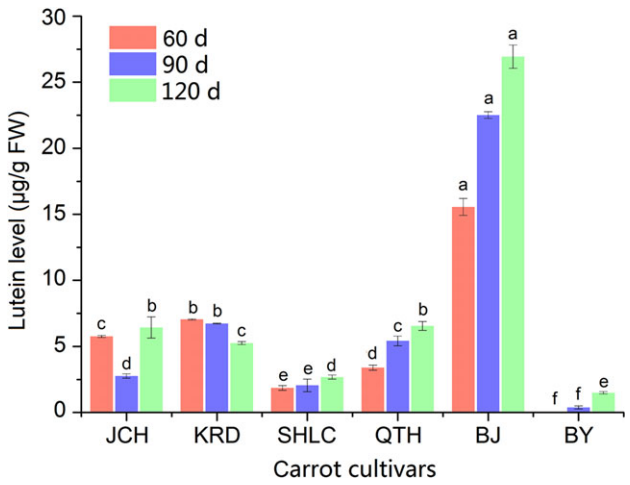

Figure 5. Lutein contents in six carrot cultivars during root development
Cultivar abbreviations: JCH, Junchuanhong; KRD, Kuroda; SHLC, Sanhongliucun; BJ, Bejo1719; QTH, Qitouhuang; BY, Baiyu. Different lowercase letters indicate significant differences between six carrot cultivars at same developmental stages at $P < 0.05$.

Transcript levels of carotenoid biosynthetic genes in six carrot cultivars taproots at three developmental stages

Almost all the carotenoid biosynthetic genes in carrot have been annotated, and the sequences have been assembled [46,47]. To examine the molecular mechanism of carotenoid accumulation in carrot taproots, 18 carotenoid biosynthesis-related genes were selected to investigate their transcript levels in the taproots of six carrot cultivars at three developmental stages by RT-qPCR (**Tables 2–4**, and **Fig. 6**). All these genes were detected in the taproots of six carrot cultivars at three developmental stages, including 'Baiyu', even though only trace amount of lutein was detected in taproots of 'Baiyu'.

Besides *DcPSY2*, *DcPSY3*, and *DcZDS1*, the upstream carotenoid biosynthesis-related genes were expressed relatively stably among six carrot cultivars. The expression level of *DcPSY2* was higher in 60- and 90-day-old 'Sanhongliucun' taproots than in other cultivars, and at 60-day and 90-day stages, *DcPSY3* expressed more actively in orange carrot cultivars (**Tables 2–4**) than in others. And *DcZDS1* showed an extremely high expression in 90-day-old 'Qitouhuang', just like the downstream gene *DcZEP*. In the downstream carotenoid biosynthesis, more genes showed various expression patterns in different color carrot cultivars. For example, *DcCHXB2*, *DcCYP97A3* and *DcCHXE* exhibited low expression levels in orange carrot cultivars, while they were actively expressed in yellow carrot cultivars (**Fig. 6**). *DcCHXB1* and *DcCHXB2*, both enconding non-heme hydroxylase (BCH type), showed inverse expression profiles between yellow and orange cultivars of carrots. *DcCYP97A3* and *DcCHXE* are translated to carotene hydroxylase belonging to CYP97A and CYP97C, respectively [27,48]. And *DcVDE* was stably expressed in six carrot cultivars taproots at three developmental stages (**Tables 2–4**). As a consequence, the expression levels of carotene hydroxylase genes of yellow carrots were higher than those of orange carrots. The major genes involved in lutein synthesis are summarized in a yellow box (**Fig. 1**). The expression levels of *DcCCD4* and *DcCCD8* were also detected, and the two genes were identified to involve in the carotenoid cleavage (**Tables 2–4**). Comparing with other cultivars, the *DcCCD4* showed an obviously higher expression level in 'Kurada' and 'Baiyu' with a decreasing tendency during the developmental stages, especially at the

Table 2. The expression levels of carotenoid biosynthesis-related genes at 60-day developmental stage

Gene*	Relative expression levels in different cultivars					
	JCH	KRD	SHLC	QTH	BJ	BY
IPI	1.01 ± 0.15^{a}	0.48 ± 0.08^{b}	1.07 ± 0.12^{a}	0.49 ± 0.09^{b}	0.54 ± 0.06^{b}	0.68 ± 0.06^{b}
GGPS1	1.02 ± 0.29^{bc}	1.21 ± 0.19^{abc}	1.26 ± 0.09^{ab}	1.53 ± 0.24^{a}	0.81 ± 0.13^{c}	1.50 ± 0.29^{a}
PSY1	1.39 ± 0.13^{c}	2.88 ± 0.78^{b}	5.11 ± 0.79^{a}	4.59 ± 0.05^{a}	1.66 ± 0.35^{c}	0.82 ± 0.21^{c}
PSY2	1.04 ± 0.32^{d}	5.65 ± 0.37^{b}	17.66 ± 1.30^{a}	6.32 ± 0.37^{b}	0.42 ± 0.12^{d}	3.82 ± 0.43^{c}
PSY3	1.00 ± 0.05^{b}	0.57 ± 0.04^{c}	1.54 ± 0.15^{a}	0.89 ± 0.02^{b}	0.34 ± 0.04^{d}	0.13 ± 0.01^{e}
PDS	1.02 ± 0.27^{c}	2.64 ± 0.34^{b}	3.10 ± 0.34a	1.37 ± 0.11c	0.44 ± 0.10^{d}	1.11 ± 0.17c
ZDS1	1.08 ± 0.45^{bcd}	2.06 ± 0.63^{b}	3.95 ± 0.56^{a}	1.65 ± 1.08^{bc}	0.44 ± 0.12^{d}	0.58 ± 0.06^{cd}
CRTISO	1.01 ± 0.15^{c}	2.84 ± 0.32^{b}	3.64 ± 0.57^{a}	2.47 ± 0.26^{b}	0.36 ± 0.09^{d}	1.30 ± 0.23^{c}
LCYE	1.01 ± 0.20^{cd}	1.52 ± 0.07^{ab}	1.75 ± 0.51^{a}	1.24 ± 0.17^{bc}	0.64 ± 0.25^{d}	0.83 ± 0.18^{cd}
LCYB	1.01 ± 0.15^{bc}	1.03 ± 0.17^{b}	1.44 ± 0.25^{a}	1.34 ± 0.08^{a}	0.27 ± 0.03^{d}	0.71 ± 0.04^{c}
ZEP	1.01 ± 0.15^{b}	3.03 ± 0.52^{a}	3.20 ± 0.30^{a}	0.96 ± 0.16^{b}	0.25 ± 0.01^{c}	0.91 ± 0.17^{b}
VDE	1.03 ± 0.34^{b}	2.20 ± 0.32^{a}	2.05 ± 0.04^{a}	1.03 ± 0.10^{b}	0.48 ± 0.02^{c}	0.85 ± 0.14^{b}
CCD4	1.00 ± 0.09^{d}	145.27 ± 2.73^{b}	5.39 ± 0.19^{cd}	12.87 ± 1.19^{c}	7.10 ± 1.77^{cd}	293.01 ± 9.52^{a}
CCD8	1.00 ± 0.09^{c}	5.49 ± 0.46^{a}	0.94 ± 0.05^{c}	0.89 ± 0.07^{c}	5.14 ± 0.73^{a}	1.88 ± 0.15^{b}

Cultivar abbreviations: JCH, Junchuanhong; KRD, Kuroda; SHLC, Sanhongliucun; BJ, Bejo1719; QTH, Qitouhuang; BY, Baiyu. Different lowercase letters indicate significant differences between six carrot cultivars at same developmental stages at $P < 0.05$.

*Carotene hydroxylase genes were not included. The expression levels of carotene hydroxylase genes are shown in **Fig. 6**.

Table 3. The expression levels of carotenoid biosynthesis genes at 90-day developmental stage

Gene*	Relative expression levels in different cultivars					
	JCH	KRD	SHLC	QTH	BJ	BY
IPI	0.28 ± 0.01^{e}	0.51 ± 0.07^{c}	0.38 ± 0.01^{d}	0.28 ± 0.05^{e}	0.74 ± 0.02^{a}	0.65 ± 0.02^{b}
GGPS1	0.21 ± 0.08^{de}	0.56 ± 0.09^{bc}	0.09 ± 0.01^{e}	0.65 ± 0.12^{b}	2.61 ± 0.18^{a}	0.39 ± 0.05^{cd}
PSY1	1.03 ± 0.31^{d}	4.94 ± 0.27^{a}	1.42 ± 0.55^{d}	2.68 ± 0.63^{bc}	3.10 ± 0.36^{b}	2.28 ± 0.19^{c}
PSY2	2.49 ± 0.27^{bc}	3.27 ± 0.57^{b}	10.01 ± 2.16^{a}	2.66 ± 0.90^{bc}	1.23 ± 0.42^{bc}	1.99 ± 0.30^{bc}
PSY3	0.57 ± 0.05^{b}	0.88 ± 0.01^{a}	0.09 ± 0.03^{d}	0.27 ± 0.07^{c}	0.46 ± 0.09^{b}	0.12 ± 0.01^{d}
PDS	1.31 ± 0.10^{b}	1.62 ± 0.10^{ab}	1.96 ± 0.15^{a}	1.87 ± 0.19^{a}	1.26 ± 0.26^{b}	1.86 ± 0.07^{a}
ZDS1	5.36 ± 0.45^{c}	5.97 ± 0.73^{c}	15.31 ± 2.69^{b}	59.33 ± 6.42^{a}	3.05 ± 0.59^{c}	2.97 ± 1.04^{c}
CRTISO	1.82 ± 0.34^{bc}	1.86 ± 0.25^{bc}	3.00 ± 0.49^{b}	4.62 ± 1.47^{a}	1.66 ± 0.16^{d}	0.76 ± 0.07^{d}
LCYE	0.65 ± 0.17^{cd}	0.99 ± 0.22^{cd}	3.61 ± 0.17^{b}	7.10 ± 1.01^{a}	1.35 ± 0.31^{c}	0.41 ± 0.10^{d}
LCYB	0.16 ± 0.06^{c}	0.87 ± 0.10^{a}	0.31 ± 0.03^{c}	0.50 ± 0.05^{b}	0.57 ± 0.11^{b}	0.86 ± 0.14^{a}
ZEP	2.75 ± 0.43^{b}	2.81 ± 0.39^{b}	3.69 ± 0.71^{b}	14.3 ± 0.80^{a}	1.60 ± 0.14^{c}	0.92 ± 0.26^{c}
VDE	1.22 ± 0.01^{c}	1.25 ± 0.17^{c}	3.09 ± 0.19^{a}	0.32 ± 0.22^{d}	2.03 ± 0.32^{b}	1.22 ± 0.06^{c}
CCD4	1.33 ± 0.18^{d}	119.29 ± 5.17^{a}	7.71 ± 0.08^{cd}	34.56 ± 0.71^{b}	13.21 ± 1.87^{c}	123.12 ± 5.92^{a}
CCD8	0.14 ± 0.04^{c}	14.02 ± 1.75^{a}	0.14 ± 0.03^{c}	0.37 ± 0.03^{c}	1.49 ± 0.25^{b}	0.20 ± 0.02^{c}

Cultivar abbreviations: JCH, Junchuanhong; KRD, Kuroda; SHLC, Sanhongliucun; BJ, Bejo1719; QTH, Qitouhuang; BY, Baiyu. Different lowercase letters indicate significant differences between six carrot cultivars at same developmental stages at $P < 0.05$.

*Carotene hydroxylase genes were not included. The expression levels of carotene hydroxylase genes are shown in **Fig. 6**.

Table 4. The expression levels of carotenoid biosynthesis genes at 120-day developmental stage

Gene*	Relative expression levels in different cultivars					
	JCH	KRD	SHLC	QTH	BJ	BY
IPI	0.33 ± 0.01^{c}	0.24 ± 0.03^{c}	0.43 ± 0.05^{b}	0.50 ± 0.03^{ab}	0.49 ± 0.01^{ab}	0.55 ± 0.06^{a}
GGPS1	0.22 ± 0.08^{b}	0.29 ± 0.06^{b}	0.61 ± 0.05^{a}	0.68 ± 0.19^{a}	0.64 ± 0.04^{a}	0.37 ± 0.01^{b}
PSY1	1.22 ± 0.31^{c}	1.01 ± 0.18^{c}	2.70 ± 0.35^{b}	3.63 ± 0.51^{a}	1.31 ± 0.17^{c}	2.25 ± 0.34^{b}
PSY2	2.03 ± 0.35^{a}	1.05 ± 0.19^{bc}	0.50 ± 0.16^{c}	1.37 ± 0.81^{ab}	0.89 ± 0.19^{bc}	0.64 ± 0.19^{bc}
PSY3	0.34 ± 0.02^{bc}	0.53 ± 0.11^{b}	0.09 ± 0.03^{c}	0.17 ± 0.02^{c}	2.63 ± 0.27^{a}	0.29 ± 0.04^{bc}
PDS	3.19 ± 0.04^{a}	0.85 ± 0.01^{c}	1.20 ± 0.13^{b}	0.62 ± 0.05^{d}	1.41 ± 0.19^{b}	0.56 ± 0.14^{d}
ZDS1	0.69 ± 0.14^{c}	0.57 ± 0.24^{c}	0.69 ± 0.19^{c}	7.89 ± 0.23^{a}	0.95 ± 0.34^{c}	5.35 ± 2.06^{b}
CRTISO	0.89 ± 0.04^{ab}	1.06 ± 0.13^{ab}	1.35 ± 0.40^{ab}	1.56 ± 0.45^{a}	0.66 ± 0.02^{b}	1.08 ± 0.57^{ab}
LCYE	1.65 ± 0.52^{c}	0.64 ± 0.15^{d}	1.86 ± 0.28^{c}	7.46 ± 0.42^{a}	1.74 ± 0.42^{c}	5.35 ± 0.34^{b}
LCYB	0.37 ± 0.09^{b}	0.59 ± 0.11^{b}	0.62 ± 0.14^{b}	1.44 ± 0.29^{a}	0.52 ± 0.07^{b}	0.64 ± 0.08^{b}
ZEP	1.14 ± 0.07^{ab}	1.07 ± 0.16^{ab}	1.09 ± 0.15^{ab}	1.94 ± 0.15^{ab}	0.86 ± 0.23^{b}	2.50 ± 0.65^{a}
VDE	2.36 ± 0.29^{a}	1.00 ± 0.12^{c}	2.42 ± 0.29^{a}	1.54 ± 0.23^{b}	1.44 ± 0.19^{b}	1.61 ± 0.15^{b}
CCD4	32.24 ± 5.21^{c}	89.21 ± 16.45^{b}	26.03 ± 1.74^{cd}	9.68 ± 1.65^{de}	5.08 ± 0.57^{e}	116.29 ± 11.87^{a}
CCD8	1.01 ± 0.10^{b}	1.19 ± 0.09^{a}	0.07 ± 0.01^{cd}	0.18 ± 0.00^{c}	1.04 ± 0.05^{b}	0.03 ± 0.02^{d}

Cultivar abbreviations: JCH, Junchuanhong; KRD, Kuroda; SHLC, Sanhongliucun; BJ, Bejo1719; QTH, Qitouhuang; BY, Baiyu. Different lowercase letters indicate significant differences between six carrot cultivars at same developmental stages at $P < 0.05$.

*Carotene hydroxylase genes were not included. The expression levels of carotene hydroxylase genes are shown in **Fig. 6**.

60-day stage of 'Baiyu' with its relative expression level reaching ~290 folds. Moreover, the expression level of *DcCCD8* varied among these six carrot cultivars. *DcCCD8* expressed much more actively in 'Kurada' and 'Bejo-1719' than in the other four carrot cultivars.

Discussion

Carotenoids are the major pigments supplying red, orange, and yellow colors for flowers and also nutrient for fruits and vegetables. Carotenoid accumulation is a complicated network consisting of a series of biosynthesis, degradation, stable storage, and so on [10,49]. Previous studies have reported that in many horticultural plants, including tomato, citrus, watermelon, and papaya, transcriptional regulation of biosynthetic genes is a crucial determinant for carotenoid production [26]. Specific carotenoid accumulation of some mutants (*Cucumis melo CRTISO* mutant, *Arabidopsis thaliana lut1/lut5* mutant, Chinese cabbage *CRTISO* mutant, etc.) also indicated that the transcriptional regulation of carotenogenesis is involved in controlling carotenoid production [25,50,51].

Although all carotenoid biosynthetic genes are located on the nuclear genome, carotenoids and their biosynthetic enzymes are known to locate in the plastid. The synthesis, store and retain of carotenoids require many kinds of plant organelles. The most important plastids are chloroplasts and chromoplasts which are responsible for the synthesis and storage of carotenoid metabolites [52]. All plastid types are differentiated originally from the colorless pluripotent progenitor proplastid in undifferentiated meristem tissue. The colorless proplastids have the potential to form differentiated plastids capable of storing chlorophylls and carotenoids [53]. Carotenoids are synthesized in many types of plastids, such as chloroplasts and chromoplasts, and carotenoids are stored in plastids of fruits, flowers, roots, and seeds. So carotenoids are synthesized directly and in carrot taproots [54]. In carrot, carotenoids are not only important for its

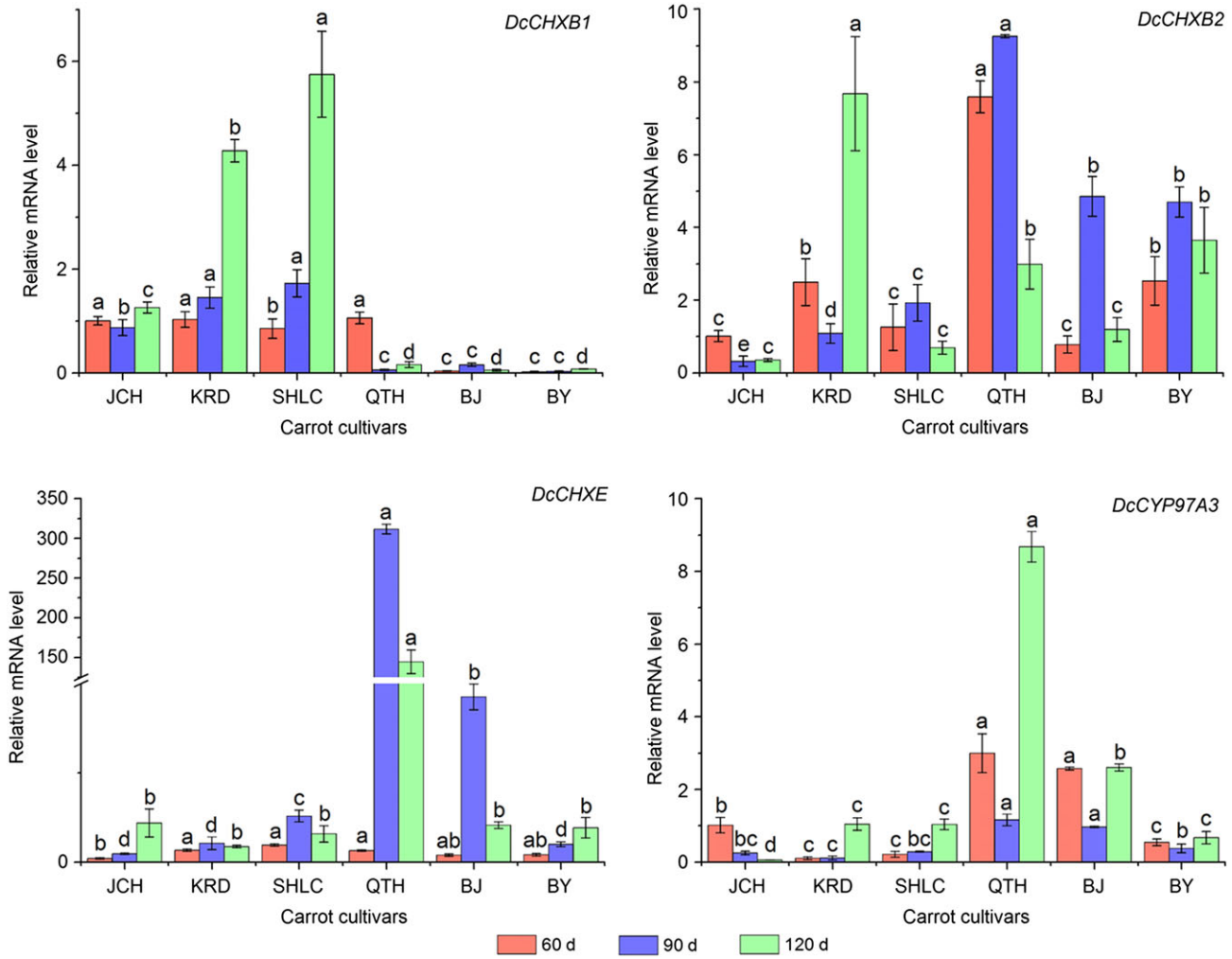

Figure 6. Transcript levels of carotene hydroxylase genes (*DcCHXB1, DcCHXB2, DcCHXE* and *DcCYP97A3*) in six carrot cultivars during three developmental stages Cultivar abbreviations: JCH, Junchuanhong; KRD, Kuroda; SHLC, Sanhongliucun; BJ, Bejo1719; QTH, Qitouhuang; BY, Baiyu. Different lowercase letters indicate significant differences between six carrot cultivars at same developmental stages at $P < 0.05$.

physiology, but also the providers for diverse colors of taproots [9,41]. In orange carrots, α-carotene and β-carotene are the major accumulated carotenoid, while in yellow carrots, lutein is the predominate carotenoid [25,41]. At the beginning of germination, carrot taproots are colorless, and after growing for a month, color starts to change and carotenoid is accumulated, finally reaching the highest levels shortly before the secondary growth is completed [41,55]. The 'Junchuanhong', 'Kuroda' and 'Sanhongliucun' have different level of orange colors, while the 'Bejo1719' and 'Qitouhuang' show a bright yellow color. The 'Baiyu' displays a very light yellow (~white) color. α-Carotene and β-carotene lead to a vivid orange color, which enhances the nutritional contents and epicurean experience of carrot, as well as its visual attraction. Lutein is the primary carotenoid that provides the yellow color in yellow carrot cultivars. In this study, carotenoid composition and contents were found to vary between different colored carrot cultivars. We detected the expression levels of 18 carotenogenesis-related genes to further determine their specific roles in carotenoid biosynthesis in carrot. Our results revealed that the upstream genes (*DcIPI* and *DcGGPS*) were expressed steadily in different carrot cultivars. However, the expressions of the downstream genes strongly varied among six carrot cultivars, especially for *DcCHXB1*, *DcCHXB2*, *DcCHXE*, *DcCYP97A3*, *DcCCD4*, and *DcCCD8*. Moreover, these genes act synergistically to yield xanthophyll and degrade carotenoid to apocarotenoid. Two yellow carrot cultivars produce lutein as the major carotenoid composition instead of α- and β-carotene, and several carotene hydroxylase genes, such as *CHXB2*, *CHXE*, and *CYP97A3*, are expressed at high levels during development in contrast to orange carrot cultivars. In plants, the synergy of carotene ring hydroxylases drives the formation of lutein from α-carotene [56]. The analyses of lutein biosynthetic mutants (*lut1*, *lut2*, *ccr2*, *lut5*, etc.) have further proved that carotene hydroxylase are involved in lutein biosynthesis [35,36,57–60]. These results indicate that the transcriptional regulation of carotene hydroxylase genes modulate lutein formation in yellow carrot taproots.

In carrot cultivar ('Baiyu'), no α-carotene was detected and only little β-carotene was detected at later developmental stages, even though lutein content is extremely low. However, the relative expressions of the selected carotenegonesis-related genes were detected in 'Baiyu', the levels of some genes were not lower than those in other carrot cultivars. This result indicates that the rare carotenoid accumulation may not be caused by the carotenoid biosynthesis genes expression [41]. Moreover, transcriptional levels can not always explain the alterations of functional proteins involved in

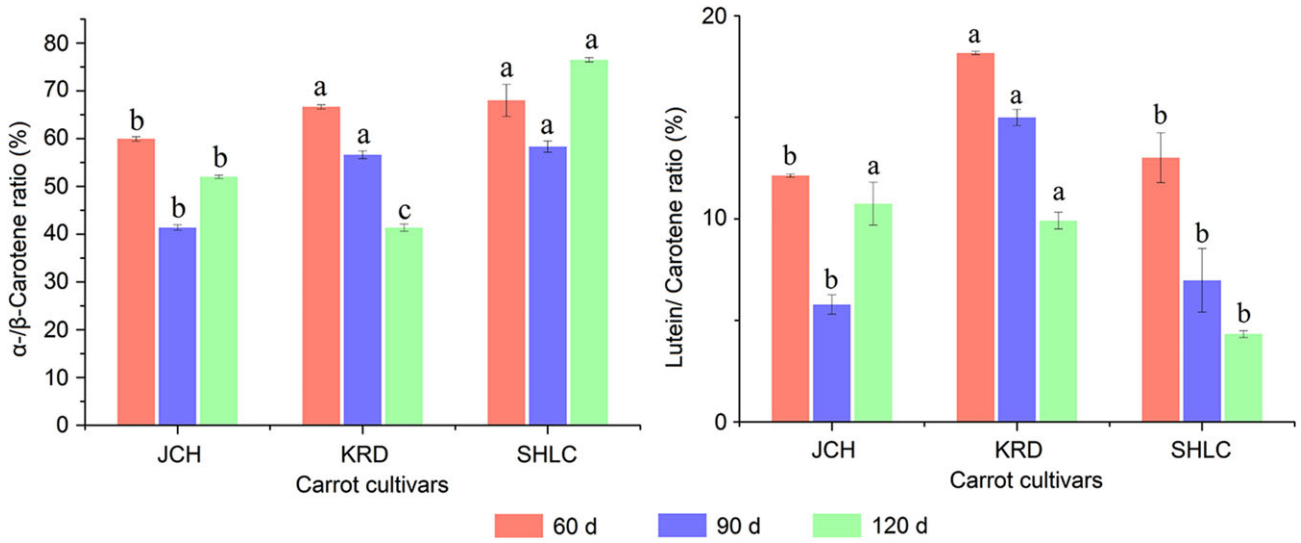

Figure 7. α-/β-Carotene ratio and lutein/carotene ratio (%) of three orange carrot cultivars Different lowercase letters indicate significant differences between six carrot cultivars at same developmental stages at $P < 0.05$.

carotenoid biosynthesis; and carotenoid cleavage may be another reason for the absence of carotenoid in 'Baiyu'. Previous studies have revealed that carotenoid cleavage dioxygenase (CCD) are the major factors involved in the carotenoid degradation [10,26,47]. In this study, we detected the expression levels of *DcCCD4* and *DcCCD8* in six carrot cultivars. *CCD4* was indentified to be mainly responsible for the majority of apocarotenoid formed [61]. And *CCD8* plays an essential role in strigolactone biosynthesis pathway [62]. We found that the expression of *DcCCD4* was much higher in 'Baiyu' than in other carrot cultivars, which may explain the phenomenon that all carotenoid biosynthesis-related genes were expressed but only trace amount of lutein was detected. These results indicate that in white carrot 'Baiyu', carotenoid may be rapidly degraded for the synthesis of apocarotenoid or other carotenoid cleavage derivatives, leading to trace amounts of carotenoid.

A high level of β-carotene content was detected in orange carrot cultivars, as well as α-carotene. These results suggest that β-carotene is accumulated in orange carrots probably by the lower transcript levels of carotene hydroxylase genes in these carrots. These carotene hydroxylase genes show specific and overlapping activities in carotene hydroxylation (**Figs.** 3 and 4). However, in yellow carrot cultivars, only trace amounts of β-carotene were detected, and carotene hydroxylase genes expressed at lower levels may result in β-carotene accumulation in orange carrot. Our results (**Figs.** 3 and 7**A**) are consistent with previous studies demonstrating that the proportion of α-carotene in total carotenoid content and the α-/β-carotene ratio are higher in most orange carrot cultivars than in other species [41,63,64]. For example, in *Arabidopsis* leaves, the proportion of α-carotene in total carotenoid content was less than 1% and the α-/β-carotene ratio was ~0.02 [48]. In tomato fruits, the proportion of α-carotene was only 4% and the α-/β-carotene ratio was 0.02 [48]. In addition, the lutein/carotene ratio was detected to explain the proportion of lutein in orange carrot cultivars. All the orange carrots showed very low proportion of lutein contents (**Fig.** 7**B**). The low expression levels of the genes involved in lutein synthesis (*CHXB2*, *CHXE*, and *CYP97A3*) may be responsible for the high contents of α- and β-carotene and high α-/β-carotene ratio in orange carrot taproots. CYP97A3 hydroxylase defect resulted from nucleotide insertion of *CYP97A3* gene was found to lead to α-carotene accumulation in orange carrot [25]. Our data suggest that the relative expression of *CYP97A3* is also down-regulated in orange carrots in contrast to that in yellow ones.

In orange carrot, over-accumulation of α-carotene is an obvious character which is different from other carrot cultivars. In this study, high levels of α- and β-carotene was also observed in orange carrot, while in yellow carrot cultivars, lutein is the major carotenoid (**Fig.** 7). β-Carotene content was enhanced in the orange carrot cultivars as taproot developed. Lutein content was also enhanced as taproot grew in the yellow carrot cultivars. RT-qPCR analysis results revealed that, out of the 18 selected genes three candidate genes (*CHXB2*, *CHXE* and *CYP97A3*) were up-regulated in yellow carrot cultivars, while they were down-regulated in orange carrot cultivars. These genes may involve in carotenoid biosynthesis in carrot taproots by encoding functional carotenoid biosynthesis enzymes. The different transcript levels between orange and yellow carrot cultivars may lead to different extent of dominant carotenoid accumulation.

In summary, the carotenoid contents and the expression profiles of carotenoid biosynthesis-related genes were detected in six carrot cultivars with different colors (orange, yellow, and white) in this study. Both carotenoid contents and the expression levels of carotenoid biosynthesis-related genes were found to vary between orange and yellow cultivars. Three carotene hydroxylase genes (*CHXB2*, *CHXE* and *CYP97A3*) were expressed at higher levels in yellow carrot cultivars, which may cause the degradation of α-carotene and β-carotene into different types of xanthophyll. In contrast, the low expression levels of these three carotene hydroxylase genes in orange carrot cultivars may result in the accumulation of α-carotene and β-carotene.

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References

1. Fraser PD, Bramley PM. The biosynthesis and nutritional uses of carotenoids. *Prog Lipid Res* 2004, 43: 228–265.
2. Krinsky NI, Johnson EJ. Carotenoid actions and their relation to health and disease. *Mol Aspects Med* 2005, 26: 459–516.
3. Luby CH, Maeda HA, Goldman IL. Genetic and phenological variation of tocochromanol (vitamin E) content in wild (Daucus carota L. var. carota) and domesticated carrot (*D. carota* L. var. sativa). *Hortic Res* 2014, 1: 14015.
4. Wang GL, Xu ZS, Wang F, Li MY, Tan GF, Xiong AS. Regulation of ascorbic acid biosynthesis and recycling during root development in carrot (*Daucus carota* L.). *Plant Physiol Biochem* 2015, 94: 10–18.
5. Mackevic V. The carrot of Afghanistan. *Bull Appl Bot, Genet Plant Breed* 1929, 20: 517–562.
6. Banga O. Origin and distribution of the western cultivated carrot. *Genetica Agraria* 1963, 17: 357–370.
7. Laufer B. Sino-Iranica: Chinese contributions to the history of civilization in ancient Iran: With special reference to the history of cultivated plants and products by Berthold Laufer Ch'eng Wen Publishing. *Add1* 1919, 12: 662–664.
8. Shinohara S. *Vegetable Seed Production Technology of Japan Elucidated With Respective Variety Development Histories, Particulars* Vol 1, Tomie Press (Japan), 1984, 55–81.
9. Surles RL, Weng N, Simon PW, Tanumihardjo SA. Carotenoid profiles and consumer sensory evaluation of specialty carrots (Daucus carota, L.) of various colors. *J Agric Food Chem* 2004, 52: 3417–3421.
10. Nisar N, Li L, Lu S, Khin NC, Pogson BJ. Carotenoid metabolism in plants. *Mol Plant* 2015, 8: 68–82.
11. Britton G, Liaaen-Jensen S, Pfander H. Carotenoids handbook. *J Appl Cosmetol* 2004, 22: 118–120.
12. Britton G, Liaaen-Jensen S, Pfander H. *Carotenoids Volume 5: Nutrition and Health*. Springer Science & Business Media (Germany), 2009.
13. Lee AI, Thornber JP. Analysis of the pigment stoichiometry of pigment-protein complexes from barley (*Hordeum vulgare*). The xanthophyll cycle intermediates occur mainly in the light-harvesting complexes of photosystem I and photosystem II. *Plant Physiol* 1995, 107: 565–574.
14. Howitt CA, Pogson BJ. Carotenoid accumulation and function in seeds and non-green tissues. *Plant Cell Environ* 2006, 29: 435–445.
15. Hirschberg J. Carotenoid biosynthesis in flowering plants. *Curr Opin Plant Biol* 2001, 4: 210–218.
16. Kato M, Ikoma Y, Matsumoto H, Sugiura M, Hyodo H, Yano M. Accumulation of carotenoids and expression of carotenoid biosynthetic genes during maturation in citrus fruit. *Plant Physiol* 2004, 134: 824–837.
17. Giuliano G, Bartley GE, Scolnik PA. Regulation of carotenoid biosynthesis during tomato development. *Plant Cell* 1993, 5: 379–387.
18. Ronen G, Cohen M, Zamir D, Hirschberg J. Regulation of carotenoid biosynthesis during tomato fruit development: expression of the gene for lycopene epsilon-cyclase is down-regulated during ripening and is elevated in the mutant Delta. *Plant J* 1999, 17: 341–351.
19. Fraser PD, Truesdale MR, Bird CR, Schuch W, Bramley PM. Carotenoid biosynthesis during tomato fruit development (evidence for tissue-specific gene expression). *Plant Physiol* 1994, 105: 405–413.
20. Hornero-Mendez D, Gomez-Ladron De Guevara R, Minguez-Mosquera MI. Carotenoid biosynthesis changes in five red pepper (*Capsicum annuum* L.) cultivars during ripening. Cultivar selection for breeding. *J Agr Food Chem* 2000, 48: 3857–3864.
21. Li Z-H, Matthews PD, Burr B, Wurtzel ET. Cloning and characterization of a maize cDNA encoding phytoene desaturase, an enzyme of the carotenoid biosynthetic pathway. *Plant Mol Biol* 1996, 30: 269–279.
22. Wurtzel ET. Chapter five Genomics, genetics, and biochemistry of maize carotenoid biosynthesis. *Recent Adv Phytochem* 2004, 38: 85–110.
23. Moehs CP, Tian L, Osteryoung KW, Dellapenna D. Analysis of carotenoid biosynthetic gene expression during marigold petal development. *Plant Mol Biol* 2001, 45: 281–293.
24. Kishimoto S, Ohmiya A. Regulation of carotenoid biosynthesis in petals and leaves of chrysanthemum (*Chrysanthemum morifolium*). *Physiol Plantarum* 2006, 128: 436–447.
25. Arango J, Jourdan M, Geoffriau E, Beyer P, Welsch R. Carotene hydroxylase activity determines the levels of both alpha-carotene and total carotenoids in orange carrots. *Plant Cell* 2014, 26: 2223–2233.
26. Yuan H, Zhang J, Nageswaran D, Li L. Carotenoid metabolism and regulation in horticultural crops. *Hortic Res* 2015, 2: 15036.
27. Kim J, Smith JJ, Tian L, Dellapenna D. The evolution and function of carotenoid hydroxylases in Arabidopsis. *Plant Cell Physiol* 2009, 50: 463–479.
28. Romer S, Fraser PD. Recent advances in carotenoid biosynthesis, regulation and manipulation. *Planta* 2005, 221: 305–308.
29. Taylor M, Ramsay G. Carotenoid biosynthesis in plant storage organs: recent advances and prospects for improving plant food quality. *Physiol Plantarum* 2005, 124: 143–151.
30. DellaPenna D, Pogson BJ. Vitamin synthesis in plants: tocopherols and carotenoids. *Annu Rev Plant Biol* 2006, 57: 711–738.
31. Cunningham FX, Gantt E. Genes and enzymes of carotenoid biosynthesis in plants. *Annu Rev Plant Physiol Plant Mol Biol* 1998, 49: 557–583.
32. Eisenreich W, Rohdich F, Bacher A. Deoxyxylulose phosphate pathway to terpenoids. *Trends Plant Sci* 2001, 6: 78–84.
33. Santos CAF, Senalik D, Simon PW. Path analysis suggests phytoene accumulation is the key step limiting the carotenoid pathway in white carrot roots. *Genet Mol Biol* 2005, 28: 287–293.
34. Britton G. Structure and properties of carotenoids in relation to function. *FASEB J* 1995, 9: 1551–1558.
35. Isaacson T, Ronen G, Zamir D, Hirschberg J. Cloning of tangerine from tomato reveals a carotenoid isomerase essential for the production of beta-carotene and xanthophylls in plants. *Plant Cell* 2002, 14: 333–342.
36. Park H, Kreunen SS, Cuttriss AJ, DellaPenna D, Pogson BJ. Identification of the carotenoid isomerase provides insight into carotenoid biosynthesis, prolamellar body formation, and photomorphogenesis. *Plant Cell* 2002, 14: 321–332.
37. Cunningham FX Jr., Gantt E. One ring or two? Determination of ring number in carotenoids by lycopene epsilon-cyclases. *Proc Natl Acad Sci USA* 2001, 98: 2905–2910.
38. Cunningham FX Jr., Chamovitz D, Misawa N, Gantt E, Hirschberg J. Cloning and functional expression in *Escherichia coli* of a cyanobacterial gene for lycopene cyclase, the enzyme that catalyzes the biosynthesis of beta-carotene. *FEBS Lett* 1993, 328: 130–138.
39. Pecker I, Gabbay R, Cunningham FX Jr., Hirschberg J. Cloning and characterization of the cDNA for lycopene beta-cyclase from tomato reveals decrease in its expression during fruit ripening. *Plant Mol Biol* 1996, 30: 807–819.
40. Frank HA, Trautman JK, Shrevez AP, Owens TG, Albrecht AC, Frank HA, Trautman JK, *et al*. Carotenoids in photosynthesis: structure and photochemistry. *Pure Appl Chem* 1991, 63: 109–114.
41. Clotault J, Peltier D, Berruyer R, Thomas M, Briard M, Geoffriau E. Expression of carotenoid biosynthesis genes during carrot root development. *J Exp Bot* 2008, 59: 3563–3573.
42. Pertea G, Huang X, Liang F, Antonescu V, Sultana R, Karamycheva S, Lee Y, *et al*. TIGR Gene Indices clustering tools (TGICL): a software system for fast clustering of large EST datasets. *Bioinformatics* 2003, 19: 651–652.
43. Chen MD, Zhu HS, Wen QF, Hong-Qi MA, Lin YZ. Determination of carotenoids in strawberry by UPLC. *J Fruit Sci* 2013, 30: 706–711.
44. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* 2001, 29: e45.
45. Xu ZS, Huang Y, Wang F, Song X, Wang GL, Xiong AS. Transcript profiling of structural genes involved in cyanidin-based anthocyanin biosynthesis between purple and non-purple carrot (*Daucus carota* L.) cultivars reveals distinct patterns. *BMC Plant Biol* 2014, 14: 262.
46. Xu ZS, Tan HW, Wang F, Hou XL, Xiong AS. CarrotDB: a genomic and transcriptomic database for carrot. *Database (Oxford)* 2014, 2014: 1229–1245.
47. Iorizzo M, Ellison S, Senalik D, Zeng P, Satapoomin P, Huang J, Bowman M, *et al*. A high-quality carrot genome assembly provides new

insights into carotenoid accumulation and asterid genome evolution. *Nat Genet* 2016, 48: 657–666.
48. Stigliani AL, Giorio G, D'Ambrosio C. Characterization of P450 carotenoid beta- and epsilon-hydroxylases of tomato and transcriptional regulation of xanthophyll biosynthesis in root, leaf, petal and fruit. *Plant Cell Physiol* 2011, 52: 851–865.
49. Li L, Yuan H. Chromoplast biogenesis and carotenoid accumulation. *Arch Biochem Biophy* 2013, 539: 102–109.
50. Galpaz N, Burger Y, Lavee T, Tzuri G, Sherman A, Melamed T, Eshed R, *et al*. Genetic and chemical characterization of an EMS induced mutation in Cucumis melo CRTISO gene. *Arch Biochem Biophy* 2013, 539: 117–125.
51. Zhang J, Yuan H, Fei Z, Pogson BJ, Zhang L, Li L. Molecular characterization and transcriptome analysis of orange head Chinese cabbage (*Brassica rapa* L. ssp. pekinensis). *Planta* 2015, 241: 1381–1394.
52. Lopez-Juez E, Pyke KA. Plastids unleashed: their development and their integration in plant development. *Int J Dev Biol* 2005, 49: 557–577.
53. Li L, Yuan H, Zeng Y, Xu Q. Plastids and carotenoid accumulation. *Subcell Biochem* 2016, 79: 273–293.
54. Alvarez-Buylla ER, Benítez M, Aldana M, Escalera-Santos GJ, Chaos Á, Liu CM, Yaish MWF, *et al*. Plant developmental biology – biotechnological perspectives. *Procampower Conf* 2010, 34: 182–187.
55. Phan CT, Hsu H. Physical and chemical changes occurring in the carrot root during growth. *Can J Plant Sci* 1973, 53: 635–641.
56. Quinlan RF, Shumskaya M, Bradbury LM, Beltrán J, Ma C, Kennelly EJ, Wurtzel ET. Synergistic interactions between carotene ring hydroxylases drive lutein formation in plant carotenoid biosynthesis. *Plant Physiol* 2012, 160: 204–214.
57. Tian L, Musetti V, Kim J, Magallanes-Lundback M, DellaPenna D. The Arabidopsis LUT1 locus encodes a member of the cytochrome p450 family that is required for carotenoid epsilon-ring hydroxylation activity. *Proc Natl Acad Sci USA* 2004, 101: 402–407.
58. Cunningham FX Jr., Pogson B, Sun Z, McDonald KA, DellaPenna D, Gantt E. Functional analysis of the beta and epsilon lycopene cyclase enzymes of Arabidopsis reveals a mechanism for control of cyclic carotenoid formation. *Plant Cell* 1996, 8: 1613–1626.
59. Pogson B, McDonald KA, Truong M, Britton G, DellaPenna D. Arabidopsis carotenoid mutants demonstrate that lutein is not essential for photosynthesis in higher plants. *Plant Cell* 1996, 8: 1627–1639.
60. Kim J, DellaPenna D. Defining the primary route for lutein synthesis in plants: the role of Arabidopsis carotenoid beta-ring hydroxylase CYP97A3. *Proc Natl Acad Sci USA* 2006, 103: 3474–3479.
61. Latari K, Wust F, Hubner M, Schaub P, Beisel KG, Matsubara S, Beyer P, *et al*. Tissue-specific apocarotenoid glycosylation contributes to carotenoid homeostasis in Arabidopsis leaves. *Plant Physiol* 2015, 168: 1550–1562.
62. Alder A, Jamil M, Marzorati M, Bruno M, Vermathen M, Bigler P, Ghisla S, *et al*. The path from beta-carotene to carlactone, a strigolactone-like plant hormone. *Science* 2012, 335: 1348–1351.
63. Simon PW, Tanumihardjo SA, Clevidence BA, Novotny JA. Role of color and pigments in breeding, genetics, and nutritional improvement of carrots. *Acs Sym Ser* 2008, 983: 151–166.
64. Arscott SA, Tanumihardjo SA. Carrots of many colors provide basic nutrition and bioavailable phytochemicals acting as a functional food. *Compr Rev Food Sci Food Safety* 2010, 9: 223–239.