Invited Review

Molecular Basis of Carotenoid Accumulation in Horticultural Crops

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Carotenoids are isoprenoid pigments, which are widely distributed in nature. In fruits and flowers, carotenoids are responsible for bright yellow, orange, and red colors and provide a substrate to form flavor compounds, which attract pollinators and seed dispersers. In leaves, carotenoids play an essential role in photosynthesis. When carotenoids are ingested in the diet, they play a vital role in human nutrition and health as a precursor of vitamin A, antioxidants, and anti-inflammatory agents. It is therefore important to control carotenoid accumulation to improve the commercial value of horticultural crops. Carotenoid accumulation is regulated by flux through the carotenoid biosynthetic pathway, and also by degradation and sequestration into plastids, which function as sink organelles. These processes are mostly controlled at the transcriptional levels of relevant genes. In this review, we summarize recent advances in studies on the molecular mechanisms that regulate carotenoid accumulation in vegetables, fruits, and ornamental flowers.

Key Words: carotenoid biosynthesis, degradation, horticultural crop, plastid, sink capacity.

Introduction

Carotenoids are C40 lipophilic isoprenoid pigments biosynthesized from 5-carbon isoprene units. More than 750 naturally occurring carotenoids, which are widely distributed in microorganisms, animals, and plants, have been identified to date (Britton et al., 2004).

Carotenoids play indispensable roles in plant growth and development. In photosynthetic tissues, carotenoids are essential components of the photosynthetic antenna and reaction center complexes and assist in harvesting light energy (Green and Durnford, 1996; Niyogi, 2000). They also function as protectants against potentially harmful photooxidative processes. Enzymatic cleavage products called apocarotenoids serve as precursors for plant hormones including abscisic acid (ABA) and strigolactone (Dun et al., 2009; Nambara and Marion-Poll, 2005).

Carotenoid is one of the key factors that determine the commercial value of horticulture crops. In fruits and flowers, carotenoids are responsible for bright yellow, orange, and red colors. In addition, volatile apocarotenoids are important components of flavor and aroma (Yuan et al., 2015). Carotenoids are indispensable components for the human diet as precursors of vitamin A, potent antioxidants, and anti-inflammatory agents. They play an important role in decreasing the risk of several diseases such as cancer, cardiovascular diseases, and eye-related disorders (Rao and Rao, 2007).

Carotenoid profiles in green tissues are well conserved: carotenoids essential for photosynthesis such as lutein and β-carotene are predominantly accumulated (Raju et al., 2007; Saini et al., 2015; Yuan et al., 2015).

In contrast, fruits and flowers show considerable diversity in the carotenoid profiles of different plant species or even within the same plant species (Ohmiya, 2011; Yuan et al., 2015). This difference is attributed to a variety of factors such as transcriptional regulation of the carotenoid metabolic pathway, sink capacity, developmental signals, and environmental effects.

In this review, we introduce recent progress regarding the molecular basis of carotenoid accumulation,
which causes a diverse range of carotenoid profiles in vegetables, fruits, and ornamental flowers. This review provides valuable knowledge that assists traditional breeding programs, as well as targeted genetic engineering to achieve quantitative and/or qualitative manipulation of carotenoids, which is an important breeding target to improve nutritional value and the aesthetic appeal of horticultural crops.

1. A general overview of carotenoid accumulation

1) Carotenoid biosynthetic pathway

The carotenoid biosynthetic pathway in plants is summarized in Figure 1 (for reviews, see Cazzonelli and Pogson, 2010; Nisar et al., 2015; Tanaka et al., 2008). The initial step of carotenoid biosynthesis involves one isoprene unit, which is C5 isopentenyl diphosphate (IPP). Four IPPs are condensed to form C20 geranylgeranyl diphosphate (GGPP) by the action of GGPP synthase (GGPS), and two GGPP molecules are converted into the first C40 carotenoid, phytoene, through a reaction catalyzed by phytoene synthase (PSY). Phytoene is then converted to lycopene, via the formation of ζ-carotene, by the addition of conjugated double bonds, and the conversion of cis- to trans-configurations through the action of two desaturases and two isomerases, namely, phytoene desaturase (PDS), ζ-carotene desaturase (ZDS), ζ-carotene isomerase (Z-ISO), and carotenoid isomerase (CRTISO). Cyclization of lycopene, which can occur at one end or both ends, is a branch point of the carotenoid biosynthetic pathway. The formation of two β-rings leads to β-carotene and its derivatives (ββ-carotenoids), whereas the formation of one β-ring and one ε-ring at either end leads to α-carotene and its derivatives (βε-carotenoids). Formation of β- and ε-rings is catalyzed by lycopene β-cyclase (LCYB) and lycopene ε-cyclase (LCYE), respectively. Hydroxylation of the β-rings of β-carotene is catalyzed by a non-heme di-iron enzyme, β-ring

![Fig. 1. Schematic representation of the carotenoid biosynthetic pathway in plants. IPP: isopentenyl diphosphate; IPI: IPP isomerase; GGPP: geranylgeranyl diphosphate; GGPS: GGDP synthase; PSY: phytoene synthase; PDS: phytoene desaturase; Z-ISO: ζ-carotene isomerase; ZDS: ζ-carotene desaturase; CRTISO: carotenoid isomerase; LCYE: lycopene ε-cyclase; LCYB: lycopene β-cyclase; CHYE/CYP97C: ε-ring hydroxylase; CHYB/CYP97A: β-ring hydroxylase, ZEP: zeaxanthin epoxidase; VDE: violaxanthin deepoxidase; NSY: neoxanthin synthase; CCS: capsanthin-capsorubin synthase.](image-url)
hydroxylase (CHYB). Hydroxylations of the ε- and β-rings of β,ε-carotenoids are catalyzed by the heme-containing cytochrome P450-type enzymes, CHYE/CYP97C and CHYB/CYP97A, respectively. Epoxidation of the β-rings of zeaxanthin, catalyzed by zeaxanthin epoxidase (ZEP), yields violaxanthin via a singly-epoxidized intermediate, antheraxanthin. Zeaxanthin is regenerated by the action of violaxanthin de-epoxidase (VDE), which catalyzes de-epoxidation of violaxanthin. Opening of one epoxide ring in violaxanthin by the action of NSY yields neoxanthin (Al-Babili et al., 2000). Carotenoids can be classified into two groups: carotenoids that contain only the parent hydrocarbon chain called "carotenes", and oxygenated derivatives of carotenes called "xanthophylls".

Differences in carotenoid content and composition are related to various gene expression levels of biosynthetic enzymes.

2) Carotenoid cleavage

The steady-state level of carotenoids reflects the balance of biosynthesis and degradation. Carotenoid cleavage dioxygenases (CCDs) catalyze the specific enzymatic cleavage of carotenoids and produce diverse apocarotenoid compounds involved in plant growth and reproduction (Fig. 2) (Hou et al., 2016; Ohmiya, 2009). In Arabidopsis, CCD enzyme families consist of nine members (Tan et al., 2003). Seven of these enzymes are involved in the plant hormone precursor production; 9-cis-epoxycarotenoid dioxygenases (NCEDs: NCED2, NCED3, NCED5, NCED6, and NCED9) cleave 9-cis-violaxanthin and 9-cis-neoxanthin to yield xanthoxin, which is further modified to ABA (Schwartz et al., 1997). CCD7 and CCD8 are involved in the synthesis of strigolactones from β-carotene via carlactones (Alder et al., 2012). The remaining two CCDs (CCD1 and CCD4) play an important role in carotenoid degradation in fruits and flowers. Some apocarotenoids produced by these enzymes provide unique aromas and colors in flowers and fruits to attract pollinators and seed dispersers, respectively.

3) Plastid as a sink organelle of carotenoid

Plastids are the primary site of the biosynthesis and accumulation of carotenoids. In leaves, the carotenoid biosynthesis predominantly takes place in the envelope and thylakoid membrane of chloroplasts (Pogson and Albrecht, 2011). Carotenoids are present in the form of chlorophyll-carotenoid-protein complexes to perform a photosynthetic function. In fruits and flowers, carotenoids are accumulated in chromoplasts. Carotenoid storage substructures, such as globular, tubular, membranous, and crystalloid, were previously reported (Li and Yuan, 2013). These substructures play important roles in stimulating continuous biosynthesis by removing carotenoids from the site of biosynthesis to avoid overaccumulation (Sun et al., 2018). Carotenoid accumulation is closely associated with the differentiation of plastids during development of fruits and flowers. At an early stage of development, fruits and flowers display a pale-green color and carotenoids are accumulated in chloroplasts along with chlorophylls. As the development progresses, a transition from chloroplasts to chromoplasts takes place (Egea et al., 2010) and changes in pigment composition concomitantly occur. Chlorophyll content decreases and carotenoid composition changes from a chloroplastic composition (mainly lutein and β-carotene) to a chromoplastic (plant-specific) composition. Therefore, they are referred to as chloroplast-type and chromoplast-type carotenoids, respectively (Yamamizo et al., 2010).

The amount of carotenoids is determined by the rate of biosynthesis and degradation, and also by the storage capacity in the sink structure. The elevated carotenoid level in fruits is accompanied by changes in the size, ultrastructure, and number of chromoplasts (Kolotilin et al., 2007; Galpaz et al., 2008; Azari et al., 2010). It is, therefore, important to regulate plastid biogenesis to enhance carotenoid accumulation. A number of studies examined the regulatory mechanism of plastid biogenesis and development, and several factors involved in the process were identified. Among these factors, ORANGE (OR) protein is the best-characterized and recognized key player in the chromoplast biogenesis. In cauliflower (Brassica oleracea), OR is a single-locus gain-of-function mutation that confers an orange pigmentation and a high level of β-carotene accumulation in curd tissues (floral meristems), where carotenoids are normally absent (Li et al., 2001; Paolillo...
et al., 2004). The *Or* gene encodes a novel protein containing a cysteine-rich zinc-finger domain in its C-terminus (Lu et al., 2006). This domain is found in DnaJ-like molecular chaperones, which participate in protein folding, assembly, disassembly, and translocation into organelles. Zhou et al. (2015) showed that OR protein interacts directly with PSY and positively regulates its enzymatic activity.

2. Vegetables

1) Carotenoid composition

Vegetables are a rich source of carotenoids in human diets. Green leafy vegetables show similar carotenoid profiles: carotenoids essential for photosynthesis such as lutein and β-carotene are predominantly accumulated, followed by violaxanthin, neoxanthin, and zeaxanthin (Raju et al., 2007; Saini et al., 2015; Yuan et al., 2012). Roots also accumulate a negligible amount of *lYCopersicum* as lutein and β-carotene are predominantly accumulated, followed by violaxanthin, neoxanthin, and zeaxanthin (Raju et al., 2007; Saini et al., 2015; Yuan et al., 2015). In contrast, fruit vegetables show considerable diversity in the carotenoid profiles of different plant species. For example, red pepper (*Capsicum annuum*) fruits accumulate unique red ketocarotenoids: capsanthin and capsorubin (Kim et al., 2016). The major carotenoid contained in red-fleshed tomato (*Solanum lycopersicum*) fruits is lycopene, consisting of 85% of its total carotenoids (Tadmor et al., 2005). Squash fruits (*Cucurbita maxima*) predominantly accumulate β-carotene, lutein, and violaxanthin (Nakkanong et al., 2012). Roots also accumulate a negligible amount of carotenoids. Orange-rooted carrots (*Daucus carota*) and the storage roots of sweet potato (*Ipomoea batatas*) exceptionally accumulate high levels of β-carotene in their storage roots, which are rich sources for vitamin A (Burri, 2011; Simon and Wolff, 1987).

In the following section, we discuss the genetic elements responsible for specific carotenoid accumulation, with a special focus on the fruits of tomato and pepper, which are widely used as model plants to investigate carotenogenesis.

2) Regulation of carotenoid accumulation via biosynthesis

Studies on tomato mutants, in which different steps of the carotenoid biosynthetic pathway were individually silenced, provide evidence that carotenoid accumulation is mainly determined by transcriptional regulation of carotenogenic genes. For example, in fruits of *tangerine*, a loss-of-function mutant of *CRTISO* displays an orange color due to the accumulation of prolycopene instead of all-trans-lycopene (Isaacson et al., 2002) (Fig. 3A). Moreover, *Beta* mutant accumulates a large amount of β-carotene due to up-regulation of chromoplast-specific *LCYB* (*LCYB2*) expression (Ronen et al., 2000) (Fig. 3A). In contrast, null mutation in *LCYB2* (old-gold) causes abolishment of β-carotene and increase in lycopene content, resulting in deep-red fruits (Fig. 3A). Increased *LCYE* transcription in *Delta* mutant leads to the conversion of lycopene to δ-carotene and the mutant displays an orange fruit color (Ronen et al., 1999). In addition, yellow-flesh, which is a loss-of-function mutant of chromoplast-specific *PSY*, causes a complete lack of carotenoids in ripe fruits (Fray and Grierson, 1993) (Fig. 3A).

Spatio-temporal carotenoid accumulation in tomato is transcriptionally regulated. An increased production of lycopene during fruit color change from green to red at fruit ripening can be preceded by enhanced transcription of upstream genes for lycopene biosynthesis (i.e., *PSY, PDS, CRTISO, and DXS*) and downregulation of downstream genes (i.e., *LCYE, LCYB*, and *CHYB*) (Isaacson et al., 2002; Lois et al., 2000; Ronen et al., 1999, 2000). Multiple isoforms are present for the key enzymes of the carotenoid biosynthetic pathway. In tomato, two divergent isoforms for *GGPS, PDS, LCYB*, and *CHYB* were reported; one of the isoforms expresses specifically in fruit and flowers, which contributes to chromoplast-specific carotenoid accumulation (Ament et al., 2006; Fraser et al., 1999; Galpaz et al., 2006; Ronen et al., 2000).

The main carotenoids contained in red pepper fruit are capsanthin and capsorubin, which are red ketocarotenoids with a cyclopentanol ring at one (capsanthin) or both ends (capsorubin) (Kim et al., 2016). Capsanthin-capsorubin synthase (CCS) catalyzes the conversion of antheraxanthin and violaxanthin into capsanthin and capsorubin, respectively (Lefebvre et al., 1998) (Fig. 1). The accumulation of these ketocarotenoids during pepper fruit ripening from green to red is linked with transcriptional upregulation of *CCS, PSY, PDS*, and *CHYB* (Huguenev et al., 1996). The main carotenoids accumulated in orange pepper fruits vary depending on the cultivars; some cultivars express an orange fruit color by accumulating β-carotene, whereas others accumulate yellow xanthophylls and a small amount of red ketocarotenoids (Guzman et al., 2010). Yellow fruit cultivars accumulate a limited amount of carotenoids (mainly lutein and α- or β-carotene) (Ha et al., 2007). The loss or reduction in CCS expression in yellow or orange pepper fruit is responsible for the absence or low level of capsanthin (Guzman et al., 2010; Rodriguez-Uribe et al., 2012). In red pepper, CCS represents the most abundant protein in pepper chromoplast proteome (Wang et al., 2013; Siddique et al., 2006).

3) Effect of carotenoid sink capacity

Analysis of high pigment tomato mutants showed that elevated carotenoid content in fruits is associated with an increased number and/or size of chromoplast (Azari et al., 2010; Galpaz et al., 2008; Kolotilin et al., 2007). Pan et al. (2013) identified a transcription factor related to *ARABIDOPSIS PSEUDO RESPONSE REGULATOR 2*-like (APRR2-like), whose expression is upregulated during the ripening of tomato fruits. Overexpression of *APRR2-like* gene increases plastid
number, area, and carotenoid content without affecting PSY expression. These results suggest that sink capacity is important for carotenoid accumulation.

Fibrillin, also referred to as carotenoid-associated protein or plastid-associated protein, is a plastid-localized protein involved in the formation of the carotenoid-lipoprotein structure. Several lines of evidence support the role of fibrillin in enabling massive accumulation of carotenoids in chromoplasts. During pepper fruit ripening, the expression of fibrillin genes is well coordinated with carotenogenic genes, with maximal expression in red ripe fruit (Deruère et al., 1994; Pozueta-Romero et al., 1997). In tomato hpl mutant fruits, increased fibrillin expression is associated with enhanced carotenoid accumulation (Kilambi et al., 2013). In addition, the overexpression of pepper fibrillin in tomato fruits causes an increase in carotenoid levels (Simkin et al., 2007).

4) Hormonal and environmental factors affect carotenoid accumulation

Light is one of the key environmental factors regulating carotenoid biosynthesis (Pizarro and Stange, 2009). Downregulation of HY5, a positive regulator of light signaling, causes defects in light responses and decreases carotenoid accumulation in tomato fruits (Liu et al., 2004). Short red-light treatment increases lycopene accumulation in mature-green tomato fruits (Alba et al., 2000). In addition, red-light-induced lycopene accumulation is reversible by far-red light, suggesting that the accumulation is mediated by phytochrome. In pepper fruits, irradiation of light after harvest at the beginning of ripening increases carotenoid level by 2–3-fold (Fig. 3B) (Yoshida et al., 2014). Analysis of carotenogenic gene expression showed that expression levels in most of the carotenogenic genes are markedly increased by irradiation (Nagata et al., 2015).

Temperature also affects carotenoid accumulation in tomato fruits. Tomato fruits are often harvested at the unripe green stage and they are ripened during marketing. When harvested fruits are once kept at 30°C or higher, conversion from lycopene into β-carotene is stimulated and lycopene accumulation is strongly inhibited (Hamauzu et al., 1998). Temperatures below 12°C also suppress lycopene accumulation in tomato fruits (Dumas et al., 2003).
The effects of plant hormones on carotenoid accumulation are well studied in tomato fruits. Ethylene plays a key role to regulate ripening-associated processes including carotenoid accumulation. RIPENING INHIBITOR (RIN), a master regulator of ethylene-regulated fruit ripening, enhances lycopene accumulation during fruit ripening by positively regulating expression of PSY, PDS, ZDS, and CRTISO, and negatively regulating expression of LCYB and LCYE (Fujisawa et al., 2013; Martel et al., 2011). Fruits of Never ripe (Nr), an ethylene-insensitive mutant, are ripening-defective and fail to accumulate lycopene (Lanahan et al., 1994). Jasmonic acid and brassinosteroids are also involved in tomato fruit ripening. Application of either gaseous methyl jasmonate or brassinosteroid solution to Nr tomato mutants enhances the expression of lycopene biosynthetic genes and lycopene accumulation in fruits (Liu et al., 2012, 2014). The results indicate that both jasmonic acid and brassinosteroids participate in lycopene biosynthesis independent of ethylene signal transduction. Although an increasing number of studies have examined the effect of plant hormone on carotenoid accumulation, little is known as to whether they directly regulate the process in tomato and pepper fruits.

3. Fruits

1) Carotenoid components

Carotenoids are the major pigments accumulated in the fruits, providing attractive yellow, orange, and red colors. In fruits, carotenoid levels in the mature fruits greatly vary among different plant species (Lado et al., 2016). In the white or pale-yellow flesh of apple (Malus domestica), apricot (Prunus armeniaca), strawberry (Fragaria × ananassa Duch.), grape (Vitis vinifera), and peach (Prunus persica), a relatively low level of carotenoid is accumulated. However, in juice sacs of Satsuma mandarin (Citrus unshiu Marc.), red flesh of papaya (Carica papaya) and watermelon (Citrullus lanatus), and orange flesh of melon (Cucumis melo) and persimmon (Diospyros kaki), high amounts of carotenoids are accumulated. In addition, carotenoid composition also varies significantly among different plant species. For example, in melon, β-carotene is massively accumulated as the predominant pigment, which leads to an orange color in the flesh (Tzuri et al., 2015). In papaya, β-cryptoxanthin is the major carotenoid accumulated in the yellow flesh, whereas lycopene is predominantly accumulated in the red flesh (Schweiggert et al., 2011).

In citrus fruits, the carotenoid profile varies among species and cultivars, and approximately 115 different carotenoids were reported in citrus fruits (Stewart and Wheaton, 1973). Epidemiologic studies suggested that dietary intakes of β-cryptoxanthin reduce risks of eye diseases, certain cancers, and inflammation (Cerhan et al., 2003; Iskandar et al., 2013; Pouchieu et al., 2014; Takayanagi et al., 2011; Yamaguchi, 2012). Citrus fruits, particularly Satsuma mandarin and Ponkan mandarin, predominantly accumulate β-cryptoxanthin in the juice sacs and are the major sources of β-cryptoxanthin in nature (Kato et al., 2004). In contrast, Valencia orange (Citrus sinensis Osbeck) mainly accumulates violaxanthin isomers with 9-cis-violaxanthin as the principal carotenoid. Lisbon lemon (Citrus limon Burm. f.) also accumulates β-cryptoxanthin as the principal carotenoid, but at a much lower level than in Satsuma mandarin and Valencia orange.

Citrus fruits are one of the most popular and widely consumed fruits in the world, and are rich and complex sources of carotenoids (Gross et al., 1987). In recent years, there is significant progress in understanding the regulatory mechanism. In the following section, we introduce recent advances in studies on carotenoid metabolism and accumulation in citrus fruits.

2) Biosynthesis of carotenoid in citrus fruits

In citrus fruits, carotenoid biosynthesis in the flavedo is highly influenced by the stage of maturation (Fig. 3C) (Kato et al., 2004). At the immature stage, chloroplast-type carotenoids, such as β-carotene, α-carotene, and lutein, are mainly accumulated in the flavedo of citrus fruits. During the maturation, the conversion of chloroplast to chromoplast occurs along with a rapid decrease of β-carotene and lutein, and massive accumulation of β,β-xanthophylls (such as β-cryptoxanthin and violaxanthin) in the flavedo. In citrus fruits, the biosynthesis of β,β-xanthophylls is concomitant with the induction of a subset of carotenoid biosynthetic genes (PSY, PDS, ZDS, LCYB1, CHYB, and ZEP) during maturation (Kato et al., 2004). Meanwhile, the differences in the expression levels of the upstream biosynthesis genes (PSY, PDS, ZDS, and LCYB1) and downstream biosynthesis genes (CHYB and ZEP) lead to the differences in the β,β-xanthophyll compositions between Satsuma mandarin and Valencia orange (Kato et al., 2004). Higher expression of upstream biosynthesis genes (PSY, PDS, ZDS, and LCYB1), and lower expression of downstream biosynthesis genes (CHYB and ZEP) leads to a massive accumulation of β-cryptoxanthin in Satsuma mandarin (Kato et al., 2004, 2007). Esterification is an important process for the massive accumulation of carotenoids in chromoplasts, because it facilitates sequestration and enhances the stability of carotenoids in chromoplasts (Ariizumi et al., 2014). In citrus fruits, more than 80% of β-cryptoxanthin is esterified with fatty acids such as laurate, myristate, and palmitate (Ma et al., 2017).

In citrus fruits, carotenoid biosynthesis is influenced by environmental stimuli with dynamic changes in content and composition. Zhang et al., (2012) showed that β-cryptoxanthin accumulation was induced by a blue light-emitting diode (LED) light, sucrose, and mannitol treatments, but was suppressed by plant hormones (i.e.,...
ABA and gibberellin treatments). Low temperature was reported to be optimal to synthesize β-cryptoxanthin in citrus fruits (Yungyuen et al., 2018). For example, at 10°C, the accumulation of β-cryptoxanthin can be significantly induced along with a simultaneous increase in the expression of PSY, PDS, ZDS, LCYB1, LCYB2, and CHYB genes in the juice sacs of Satsuma mandarin in vitro.

3) Transcriptional regulation of citrus carotenoid biosynthetic genes

To clarify candidate genes involved in the considerable diversity among carotenoid profiles of citrus fruits, Sugiyama et al. (2011) examined quantitative trait locus (QTL) mapping for carotenoid composition using a F1 hybrid population (AG population) of ‘Okitsu 46 gou’ (A255) and ‘Kankitsu Chukanbohon Nou 5 gou’ (G434), with a wide distribution of carotenoid content and composition. A major QTL for β-cryptoxanthin was detected at the Gm0005 locus in linkage group 6 of G-map, and the logarithm (base 10) of odds (LOD) value was 3.4. DNA markers of Gm0005 were developed from a gene encoding pepper plastid-associated protein (Moriguchi et al., 1998). Various QTLs for other carotenoid components were also detected, but most of them expressed low LOD values. Sugiyama et al. (2014) extended the study to compare the genetic locus and expression QTL (eQTL) of carotenoid metabolic genes using AG population. Firstly, genetic loci of the major carotenoid metabolic genes were mapped as follows: PSY on linkage group (LG)-4, PDS on LG-3, ZDS on LG-9, CHYB on LG-3, and ZEP on LG-2. LCYB and NCED could not be assigned onto any linkage group owing to low genomic sequence polymorphism. Subsequently, eQTLs of PSY, CHYB, and ZEP were mapped on the loci of their corresponding genes, revealing that transcription would be regulated by cis-elements in their promoter regions. However, the eQTLs of PDS and ZDS were mapped on the same locus near Tr0271 DNA marker on LG-8, indicating that transcription would be regulated by trans-elements in this genetic region. A significant eQTL of LCYB and NCED was not detected in this population. Thus, the mode of transcriptional regulation may differ among carotenoid metabolic genes.

4) The cis-regulatory motif involved in citrus carotenoid metabolism

The eQTLs controlling expression levels of PSY, CHYB, and ZEP were located on the loci of their corresponding genes, where there were several allelic genes with different sequences. For example, the PSYs in an AG population comprised four alleles (PSY-a1 and PSY-a2 from A255, PSY-g1 and PSY-g2 from G434), which were derived from the parent lines at the PSY locus on LG-4, and F1 individuals with PSY-g2 tended to show low transcription levels in fruits (Sugiyama et al., 2017). Sequence analysis of four alleles in an AG population revealed that a 6-bp deletion in the upstream region of PSY-g2 altered the cis-regulatory motif from MYBPZM (CCWACC; Grotewold et al., 1994) to RAV1AAT (CAACA; Kagaya et al., 1999) at −329 bp from the initiation codon. MYBPZM is a core cis-regulatory motif for maize P gene, responsible for the red pigmentation of the kernel pericarp and flavonoid biosynthesis (Grotewold et al., 1994). RAV1AAT motif is a cis-regulatory motif of RAV1 protein, involved in immediate physiological responses and developmental adaptations to environmental stimuli (Kagaya and Hattori, 2009). The allelic genotypes based on the MYBPZM and RAV1AAT motifs were correlated with transcription levels among the pedigree varieties of AG population. Similar results were obtained in ZEP alleles, the transcription level of a ZEP-1m allele with MYBPZM was higher than that of a ZEP-2m allele without MYBPZM (Sugiyama et al., 2010). Recently, MYBPZM was reported to be one of the candidate cis-regulatory motifs affecting the gene expression of pepper CCS (Li et al., 2013). Therefore, MYBPZM may be an important cis-regulatory motif that influences the carotenoid metabolism in citrus fruit, although a direct demonstration by promoter assay was not conducted.

5) Transcription factors to promote citrus carotenoid metabolism

As described above, carotenoid biosynthesis in citrus fruits is affected by environmental stimuli and endogenous factors, implying that numerous transcription factors could be involved in the regulation of carotenoid biosynthesis in response to various factors. However, the transcription factors regulating the citrus carotenoid metabolism are not well characterized. Endo et al. (2015) explored the transcription factor genes associated with the carotenoid biosynthesis through microarray screening using citrus fruits treated with exogenous ethylene or gibberellin, which accelerate or delay carotenoid accumulation in the peel, respectively. CubHLH1, with a basic helix-loop-helix (bHLH) domain, was found to have transcriptionally similar responsive profiles to those of carotenoid biosynthetic genes. The amino acid sequence of CubHLH1 has homology to Arabidopsis activation-tagged bri1 suppressor 1 (ATBS1) interacting factor (AIF), which was functionally characterized as a negative regulator of the brassinosteroid signaling pathway (Wang et al., 2009). In the study, overexpression of CubHLH1 in tomato caused a dwarf phenotype, and the lycopene content in ripening fruits was reduced along with changes in carotenoid biosynthetic gene expression. The ABA content of all the transgenic tomato fruits was higher than that of the wild-type (Wang et al., 2009). Therefore, CubHLH1 had a similar function to Arabidopsis AIFs and may progress carotenoid biosynthesis in mature citrus fruits. Zhu et al. (2017) identified an R2R3-MYB
transcription factor (CrMYB68) that negatively regulates the expression of BCH2 (CHYB) and NCED5 in the flavedo of Ponkan mandarin (C. reticulata Blanco). Moreover, Lu et al. (2018) reported that CsMADS6 revealed multi-targeted regulation of citrus carotenoid metabolism and the sepals of CsMADS6-overexpressing tomato lines exhibited dramatic changes in carotenoid profiles, accompanied by changes in the plastid ultrastructure. However, there are few studies on the isolation and molecular characterization of the transcription factors regulating the carotenoid metabolism in citrus.

6) Catabolism of carotenoid in fruits

CCD family proteins (particularly CCD1 and CCD4) affect fruit color, aroma, and flavor via carotenoid degradation (Fig. 2) (Ohmiya, 2009). CCD1 is involved in the production of various apocarotenoids in fruits. Recombinant citrus CCD1 cleaves β-cryptoxanthin, zeaxanthin, all-trans-violaxanthin, and 9-cis-violaxanthin at 9,10 (9',10') double bond to yield C14 dialdehyde, 3-hydroxy-β-ionone, β-ionone, 5,6-epoxy-3-hydroxy-β-ionone, and C24 epoxy-apocarotenal (Kato et al., 2006). Recombinant grape CCD1 cleaves zeaxanthin and lutein to yield 3-hydroxy-β-ionone (Mathieu et al., 2005, 2007), ε-carotene to yield α-ionone, β-carotene to yield β-ionone, and lycopene to yield 6-methyl-5-hepten-2-one (Lashbrooke et al., 2013). In papaya, the expression level of CCD1 is closely associated with the accumulation of β-ionone and 6-methyl-5-hepten-2-one (Jing et al., 2015). These apocarotenoids are important constituents of aroma and may contribute to increased consumer preference for various fruits.

CCD4 greatly influences the pigmentation of fruits. In citrus, CCD4 is specifically expressed in the flavedo of only a few varieties such as ‘Yamashitabeni-wase’ (a variety of Satsuma mandarin) (Ma et al., 2013). Recombinant citrus CCD4 cleaves zeaxanthin and β-cryptoxanthin at 7,8 (7',8') double bond to form β-citraurin, which is responsible for the reddish flavedo color in ‘Yamashitabeni-wase’ (Ma et al., 2013). These results suggest that CCD4 is a major factor in determining the flavedo color of ‘Yamashitabeni-wase’. In peach, CCD4 expression level was lower in yellow-fleshed cultivars than in white-fleshed cultivars (Brandi et al., 2011; Falchi et al., 2013). Analysis of genomic CCD4 sequence showed that CCD4 gene in yellow-fleshed cultivars was disrupted by three independent mutations, namely, nucleotide substitution, a frameshift in the microsatellite sequence, and insertion of an LTR retrotransposon, which may cause suppressed CCD4 expression (Adami et al., 2013; Falchi et al., 2013; Fukamatsu et al., 2014). Transient suppression of CCD4 in the white flesh resulted in an increased accumulation of lutein, β-cryptoxanthin, zeaxanthin, and violaxanthin (Bai et al., 2016). These results strongly indicate that peach CCD4 is the Y gene that determines flesh color by carotenoid degradation (Bliss et al., 2002). Moreover, white-fleshed cultivars produced larger amounts of volatile apocarotenoids compared to yellow-fleshed cultivars, suggesting that CCD4 is also involved in aroma formation (Brandi et al., 2011). In pineapple, a specific allele of CCD4 was observed only in white-fleshed cultivars, and CCD4 expression level was negatively correlated with yellow pigmentation in the flesh (Fig. 3D) (Nashima, 2018; Nashima et al., 2017).

Only limited information is available as to whether NCED affects carotenoid accumulation in fruits. Kato et al. (2006) reported that, in flavedo and juice sacs of Mandarin orange and Lisbon lemon, NCED expression increased markedly during fruits maturation, which was associated with an increase in ABA level. They also demonstrated a reciprocal relationship between the levels of NCED transcript and 9-cis-violaxanthin (a substrate of NCED), suggesting that NCED activity affected carotenoid accumulation in citrus fruits via ABA synthesis.

4. Ornamental flowers

1) Carotenoid components

Carotenoids are important pigments for flower coloration ranging from yellow to red. Their main role in flowers is to attract insects and birds for pollination. The main carotenoid components in flowers are xanthophylls and β-carotene (Ohmiya, 2011).

Carotenoid components in petals differ depending on the species. For example, in chrysanthemum (Chrysanthemum molifolium), major carotenoid components are β,ε-carotenoids such as lutein and lutein-5,6-epoxide (Kishimoto et al., 2004). On the other hand, some plants preferentially accumulate β,β-carotenoids. The main carotenoids in petals of Oncidium are violaxanthin and neoxanthin (Hieber et al., 2006), and zeaxanthin in zinnia (Zinnia elegans) (Kishimoto et al., 2007). Some plants accumulate unique carotenoids, which are rarely found in other plants, in their petals: e.g., capsanthin in tiger lily (Lilium lancifolium) (Deli et al., 1998) and Asiatic hybrid lily (Lilium spp.) (Yamagishi et al., 2010), astaxanthin in Adonis annua (Seybold and Goodwin, 1958) and Adonis aestivalis (Maoka et al., 2011), eschscholtzaxanthin in California poppy (Eschscholzia californica) (Andrewes et al., 1979), and gazanixanthin [(5Z)-rubixanthin] in gazanxia (Gazania rigens) (Bartlett et al., 1969).

Carotenoids in petals commonly exist as all-trans-forms and cis-form carotenoids are rarely found. In chrysanthemum petals, various cis-form carotenoids, such as (9Z)-lutein and (9Z)-lutein-5,6-epoxide, are detected (Kishimoto et al., 2004). The cis-configuration is detected at the C-7 (C-7') and/or C-9 (C-9') positions. Orange petals of calendula (Calendula officinalis) contain various red carotenoids (mostly lycopenes and rubixanthins) with cis-configuration at the C-5 (C-5') position, which are rarely detected as natural products.
(Kishimoto et al., 2005). Kishimoto and Ohmiya (2012) reported that orange-flowered calendula cultivars are a loss-of-function mutation of petal-specific CRTISO that impairs the conversion of cis-lycopene to trans-lycopene, a prerequisite reaction for lycopene cyclization.

Some plants accumulate apocarotenoids in their flowers and express orange or red colors. For example, orange tepals of Crocosmia accumulate crocetin (Ootani and Hayashi, 1982) and orange petals of California poppy accumulate crocetin (Wakelin et al., 2003). The red stigma of crocus (Crocus sativus) accumulates apocarotenoids, which are responsible for the red color (crocetin glycosides and picrocrocin) and aroma (safaranal) of the spice saffron (Winterhalter and Rouseff, 2002).

2) Regulation of carotenoid accumulation via biosynthesis and cleavage

In flower petals, higher levels of transcripts of carotenoid biosynthesis enzymes are associated with higher carotenoid content (for a review, see Ohmiya, 2013). In marigold, orange petals accumulate much larger amounts of yellow xanthophyll lutein than yellow petals. The expression of most carotenogenic genes, particularly DXS and PSY, in orange petals was higher than in yellow petals (Moehs et al., 2001). Asiatic hybrid lily (Yamagishi et al., 2010) and Ipomoea species (Yamamizo et al., 2010) show higher expressions of carotenoid biosynthetic genes in carotenoid-rich yellow or orange petals than in white petals.

The difference in LCYB and LCYE activities profoundly affects the distinct balance between β,β- and β,ε-carotenoids. In Oncidium petals, a high proportion of β,β-carotenoids is associated with a higher expression of LCYB than of LCYE (Chiou et al., 2010). In contrast, chrysanthemum petals have a higher expression of LCYE than LCYB, which may be the cause of a high proportion of β,ε-carotenoids (Kishimoto and Ohmiya, 2006).

In some plant species, CCD4 has a significant impact on carotenoid accumulation in petals. The best-known example is chrysanthemum (Ohmiya et al., 2006). Expressions of carotenoid biosynthetic genes in petals of yellow and white petals are similar (Kishimoto et al., 2006), whereas CCD4a is expressed only in white petals. Suppression of CCD4 expression results in an increased carotenoid accumulation in petals (Fig. 3E) (Ohmiya et al., 2006, 2009). The results indicate that carotenoids are synthesized, but are subsequently degraded by CCD4a, which results in the white petal color in chrysanthemum. Involvement of CCD4 in white color formation was also reported in tepals of lily (Hai et al., 2012) and crocus (Rubio-Moraga et al., 2013), and petals of potato (Campbell et al., 2010) and Brassica (Zhang et al., 2015). CCD1 also cleaves carotenoids and contributes to the emission of important fragrance components (β- and α-ionones) in flowers of petunia and Osmanthus fragrans (Baldermann et al., 2010; Simkin et al., 2004). However, the lack of correlation between CCD1 expression and carotenoid accumulation was demonstrated in petals of Ipomoea nil (Yamamizo et al., 2010) and petunia (Kishimoto et al., 2018a), possibly because CCD1 is located in the cytoplasm and has limited access to carotenoids in chromoplasts (Bouvier et al., 2003; McCarty and Klee, 2006).

Analysis of biosynthetic and catabolic gene expressions showed that amounts of carotenoids in petals are determined by the balance of biosynthesis and degradation. In Eustoma, pale yellow petals show higher expressions of PSY, ZDS, LCYB, and LCYE, but lower expression of CCD4, than in white petals (Liu et al., 2013). In petunia, pale yellow petals show significantly higher expressions of GGPS1, PSY1, and LCYE, and completely lack CCD4a expression compared with white petals (Kishimoto et al., 2018a). In pale yellow-flowered petunia cultivars, there are two insertions in the presumed promoter and coding regions of CCD4a, which may cause complete loss of CCD4a transcript.

3) Role of xanthophyll esterification on carotenoid accumulation

In green leaves, all xanthophylls exist in free forms, whereas in petals, most are accumulated in esterified forms (Ariizumi et al., 2014; Breithaupt et al., 2002; Maoka et al., 2011; Moehs et al., 2001; Yamamizo et al., 2010). Several types of carotenoid-containing bodies such as fibrillar, globular, and tubular-types in chromoplasts were reported (Camara et al., 1995; Ljubesić et al., 1991), and those are mainly composed of carotenoids, polar lipids, and structural proteins (Austin et al., 2006; Vishnevetsky et al., 1999). Deruère et al. (1994) reported that esterified xanthophylls were more efficient than free xanthophylls for fibril assembly in vitro condition, and presumed that the formation of carotenoid-containing bodies prevents the harmful effects of excess carotenoids on cellular functions.

CHYB (an enzyme that catalyzes the hydroxylation of cyclic carotenoids) plays a key role in carotenoid accumulation in petals. Tomato white-flower (wf) mutants have loss-of-function mutation in the flower-specific CHYB gene. The petals of the wf mutant show large decreases in xanthophylls, whereas the expression of most carotenogenic genes is unaffected (Galpaz et al., 2006). During petal development, an increase in CHYB expression occurs along with an increase in carotenoid content in Ipomoea (Yamamizo et al., 2010), lily (Yamagishi et al., 2010), and marigold (Moehs et al., 2001). Xanthophylls in petals are esterified with fatty acids via oxygenated residues and therefore positive correlation between carotenoid content and CHYB transcript level supports the hypothesis that esterification is important for carotenoid accumulation in petals.

An enzyme catalyzing xanthophyll esterification
(XES: xanthophyll esterase) is first identified by analyzing pale yellow petal 1 (pyp1) tomato mutants (Fig. 3F) (Ariizumi et al., 2014). Petals of pyp1 mutants completely lack esterified xanthophylls and show a reduction in total carotenoid content and abnormal chromoplast development. Expression levels of carotenoid metabolic genes in the petals of wild-type and pyp1 mutants showed no significant differences. The results suggest that PYP1 has the ability to increase carotenoid accumulation without influencing the carotenoid metabolism at the transcriptional level, and esterification is an important process for the massive accumulation of carotenoid in petals. The importance of xanthophyll esterification for carotenoid accumulation in petals was also supported by a comparison of pale yellow-flowered petunia and deep yellow-flowered calibrachoa (Kishimoto et al., 2019). The expression of XES in petunia petals was significantly lower than that in calibrachoa petals. Moreover, petunia XES is presumed to have low substrate specificity in most xanthophylls accumulating in corollas. It is also assumed that a low level of XES activity is one of the key factors that petunia lacks in deep yellow-flowered cultivars. Recently, Kishimoto et al. (2018b) showed that heterologous expression of XESs isolated from petals of other plant species caused an increased accumulation of carotenoids in petunia corollas. There was a tendency that xanthophylls showing high substrate specificity to introduced XES to preferentially accumulate in corollas. These results indicate that the activity of XES strongly influences the amount and composition of carotenoids in flowers.

4) Transcriptional regulator of carotenoid biosynthesis

The carotenoid metabolic pathway is controlled in a tissue-specific manner. However, there are few findings on the transcription factors that control carotenoid metabolism in flowers. Reduced Carotenoid Pigmentation 1 (RCPI) from Mimulus lewisi is the only flower-specific transcription factor reported to date (Sagawa et al., 2016). A loss-of-function mutant of RCPI showed lower expression in all of the tested carotenoid biosynthetic genes and lower accumulation of carotenoids in the nectar guide of corollas than in wild-type lines. It was presumed that RCPI is a positive regulator of the whole carotenoid biosynthetic pathway, but it is unclear whether RCPI directly controls the expression of carotenoid biosynthetic genes. It is noteworthy that RCPI not only promotes carotenoid biosynthesis, but also represses anthocyanin biosynthesis in petals. Therefore, RCPI works as a genetic switch between anthocyanin and carotenoid production and may be involved in spatial patterning of these pigments in M. lewisi flowers. There is a considerable number of species with distinct pigmentation patterns with respect to carotenoid and anthocyanin, such as torenia (Torenia fournieri), orchids, and Iris. Further studies are required to examine whether orthologs of RCPI widely exist in plants and are involved in the formation of pigmentation pattern in flowers.

Conclusion and Perspectives

The steady-state level of carotenoids is partly dependent on the metabolic equilibrium between the biosynthesis and degradation of carotenoids. However, simply manipulating the carotenoid metabolic pathway does not always result in a drastic change in carotenoid accumulation (Watanabe et al., 2017, 2018). One possible explanation for this phenomenon is that there may be a factor affecting carotenoid accumulation besides carotenoid metabolism. In this review, we provide evidence showing the importance of chromoplasts as sites for carotenoid biosynthesis and accumulation. Induction of chromoplast formation may be necessary, as well as manipulating the carotenoid metabolism, for massive accumulation of carotenoids.

The carotenoid metabolic pathway has been extensively studied and almost all the enzymes involved in the pathway identified and characterized in many plant species (for reviews, see Cazzonelli and Pogson, 2010; Nisar et al., 2015; Ohmiya, 2009; Tanaka et al., 2008). However, the mechanism of carotenogenesis is poorly understood in terms of the regulation of the carotenoid metabolic pathway and chromoplast biogenesis. A number of regulators may be involved in these processes. Future studies should address the identification of key regulatory factors controlling the carotenoid metabolic pathway and chromoplast biogenesis. This information will provide further insight into carotenoid metabolic engineering to improve the nutritional and/or aesthetic value of horticultural crops.

Literature Cited

Adami, M., P. De Franceschi, F. Brandi, A. Liverani, D. Giovannini, C. Rosati, L. Dondini and S. Tarturini. 2013. Identifying a carotenoid cleavage dioxygenase (ccd4) gene controlling yellow/white fruit flesh color of peach. Plant Mol. Biol. Report. 31: 1166–1175.

Alba, R., M. M. Cordonnier-Pratt and L. H. Pratt. 2000. Fruit localized phytochromes regulate lycopene accumulation independently of ethylene production in tomato. Plant Physiol. 123: 363–370.

Al-Babili, S., P. Huguemey, M. Schledz, R. Welsch, H. Frohnmeyer, O. Laule and P. Beyer. 2000. Identification of a novel gene coding for neoxanthin synthase from Solanum tuberosum. FEBS Letters 485: 168–172.

Alder, A., M. Jamil, M. Marzorati, M. Bruno, M. Vermathen, P. Bigler, S. Ghisla, H. Bouwmeester, P. Beyer and S. Al-Babili. 2012. The path from β-carotene to carlactone, a strigolactone-like plant hormone. Science 335: 1348–1351.

Ament, K., C. C. Van Schic, H. J. Bouwmeester, M. A. Haring and R. C. Schuurink. 2006. Induction of a leaf-specific geranylgeranyl pyrophosphate synthase and emission of (E,E)-4,8,12-trimethyltrideca-1,3,7,11-tetraene in tomato are dependent on both jasmonic acid and salicylic acid signaling pathways. Planta 224: 1197–1208.

Andrewes, A. G., G. Engler, G. Borch, H. H. Strain and S. Liaaen-Jensen. 1979. Absolute configuration of eschscholtz-
xanthin. Phytochemistry 18: 303–309.

Suttle, G. Ramsay, G. J. Bryan, P. E. Hedley and M. A. Taylor. 2010. The metabolic and developmental roles of carotenoid cleavage dioxygenase 4 from potato. Plant Physiol. 154: 656–664.

Cazzonelli, C. and B. J. Pogson. 2010. Source to sink: regulation of carotenoid biosynthesis in plants. Trends Plant Sci. 15: 266–274.

Cerhan, J. R., K. G. Saag, L. A. Melino, T. R. Mikulus and L. A. Criswell. 2003. Antioxidant micronutrients and risk of rheumatoid arthritis in a cohort of older women. Am. J. Epidemiol. 157: 345–354.

Chiou, C. Y., H. A. Pan, Y. N. Chuang and K. W. Yeh. 2010. Differential expression of carotenoid-related genes determines diversified carotenoid coloration in floral tissues of Oncidium cultivars. Planta 232: 937–948.

Deli, J., P. Molnár, Z. Matus, G. Tóth, A. Steck and H. Pfander. 1998. Isolation and characterization of 3,5,6-trihydroxy-carotenoids from petals of Lilium tigrinum. Chromatographia 48: 27–31.

Deraère, J., S. Römer, A. d’Harlingue, R. A. Backhaus, M. Kuntz and B. Camara. 1994. Fibrib assembly and carotenoid overaccumulation in chromoplasts: a model for supramolecular lipoprotein structures. Plant Cell 6: 119–133.

Dumas, Y., M. Dadomo, G. Di Lucca and P. Grolier. 2003. Effects of environmental factors and agricultural techniques on antioxidant content of tomatoes. J. Sci. Food Agr. 83: 369–382.

Dun, E. A., B. Philip, P. B. Brewer and C. A. Beveridge. 2009. Strigolactones: discovery of the elusive shoot branching hormone. Trends Plant Sci. 14: 364–372.

Egea, I., C. Barsan, W. Bian, E. Pungatto, A. Latche, C. Chervin, M. Bouzayen and J. C. Pech. 2010. Chromoplast differentiation: current status and perspectives. Plant Cell Physiol. 51: 1601–1611.

Endo, T., H. Fujii, A. Sugiyama, M. Nakano, N. Nakajima, Y. Ikoma, M. Omura and T. Shimada. 2015. Overexpression of a citrus basic helix-loop-helix transcription factor (CubHLH1), which is homologous to Arabidopsis activation-tagged br1 suppressor 1 interacting factor genes, modulates carotenoid metabolism in transgenic tomato. Plant Sci. 243: 35–48.

Falchi, R., E. Vendramin, L. Zanon, S. Scalabrini, G. Cipriani, I. Verde, G. Vizzotto and M. Morgante. 2013. Three distinct mutational mechanisms acting on a single gene underpin the origin of yellow flesh in peach. Plant J. 76: 175–187.

Fraser, P. D., J. W. Kiano, M. R. Truesdale, W. Schuch and P. M. Bramley. 1999. Phytoene synthase-2 enzyme activity in tomato does not contribute to carotenoid synthesis in ripening fruit. Plant Mol. Biol. 40: 687–698.

Fray, R. G. and D. Grierson. 1993. Identification and genetic analysis of normal and mutant phytoene synthase genes of tomato by sequencing, complementation and co-suppression. Plant Mol. Biol. 22: 589–602.

Fujisawa, M., T. Nakano, Y. Shima and Y. Ito. 2013. A large-scale identification of direct targets of the tomato MADS box transcription factor RIPENING INHIBITOR reveals the regulation of fruit ripening. Plant Cell 25: 371–386.

Fukamatsu, Y., T. Tamura, S. Hihara and K. Oda. 2014. Mutations in the CD64 carotenoid cleavage dioxygenase gene of yellow-flesh peaches. Biosci. Biotechnol. Biochem. 77: 2514–2516.

Galpaz, N., G. Ronen, Z. Kalfia, D. Zamir and J. Hirschberg. 2006. A chromoplast-specific carotenoid biosynthesis pathway is revealed by cloning of the tomato white flower locus. Plant Cell 18: 1947–1960.
Galpaz, N., Q. Wang, N. Menda, D. Zamir and J. Hirschberg. 2008. Abscisic acid deficiency in the tomato mutant high-pigment 3 leading to increased plastid number and higher fruit lycopene content. Plant J. 53: 717–730.

Green, B. R. and D. G. Durnford. 1996. The chlorophyll-carotenoid proteins of oxygenic photosynthesis. Ann. Rev. Plant Physiol. Plant Mol. Biol. 47: 685–714.

Gross, J. 1987. Pigments in Fruits. Harcourt Brace Jovanovich, London.

Grotewold, E., B. J. Drummond, B. Bowen and T. Peterson. 1994. The myb-homologous P gene controls phlobaphene pigmentation in maize floral organs by directly activating a flavonoid biosynthetic gene subset. Cell 76: 543–553.

Guzman, I., S. Hamby, J. Romero, P. W. Bosland and M. A. O’Connell. 2010. Variability of carotenoid biosynthesis in orange colored Capsicum spp. Plant Sci. 179: 49–59.

Ha, S., J. Kim, J. Park, S. Lee and K. Cho. 2007. A comparison of the carotenoid accumulation in Capsicum varieties that show different ripening colours: deletion of the capsanthin-capsorubin synthase gene is not a prerequisite for the formation of a yellow pepper. J. Exp. Bot. 58: 3135–3144.

Hai, M. T. L., J. Masuda, I. Miyajima, N. Q. Thien, N. Mojahedi, M. Hiramatsu, J.-H. Kim and H. Okubo. 2012. Involvement of carotenoid cleavage dioxygenase 4 gene in tepal color change in Lilium brownii var. colchectori. J. Japan. Soc. Hort. Sci. 81: 366–373.

Hamauzu, Y., K. Chachin and Y. Ueda. 1998. Effects of postharvest temperature on the conversion of 4-C-mevalonic acid to carotenes in tomato fruit. J. Japan. Soc. Hort. Sci. 67: 549–555.

Huguenev, P., F. Bouvier, A. Badillo, J. Quennemet, A. d’Harlingue and B. Camara. 1996. Developmental and stress regulation of gene expression for plastid and cytosolic isoprenoid pathways in pepper fruits. Plant Physiol. 111: 619–626.

Hieber, A. D., R. G. Mudaalige-Jayawickrama and A. R. Kuehnle. 2006. Color genes in the orchid Oncidium Gower Ramsey: identification, expression, and potential genetic instability in an interspecific cross. Planta 223: 521–531.

Hou, X., J. Rivers, P. W. Bosland and M. A. Vivier. 2013. Functional characterisation of three

Kato, M., Y. Ikoma, T. Kuniga, N. Nakajima, T. Yoshida and M. Yano. 2007. Accumulation of carotenoids and expression of carotenoid biosynthetic genes and carotenoid cleavage dioxygenase genes during fruit maturation in the juice sacs of ‘Tamami’, ‘Kiyomi’ tangor, and ‘Wilking’ mandarin. J. Japan. Soc. Hort. Sci. 76: 103–111.

Kato, M., H. Matsumoto, Y. Ikoma, H. Okuda and M. Yano. 2006. The role of carotenoid cleavage dioxygenases in the regulation of carotenoid profiles during maturation in citrus fruit. J. Exp. Bot. 57: 2153–2164.

Kilambi, H. V., R. Kumar, R. Sharma and Y. Sreeakshmi. 2013. Chromoplast-specific carotenoid-associated protein appears to be important for enhanced accumulation of carotenoids in hp1 tomato fruits. Plant Physiol. 161: 2085–2101.

Kim, J.-S., C. G. An, J.-S. Park, Y. P. Lim and S. Kim. 2016. Carotenoid profiling from 27 types of paprika (Capsicum annuum L.) with different colors, shapes, and cultivation methods. Food Chem. 201: 64–71.

Kishimoto, S. and A. Ohmiya. 2006. Regulation of carotenoid biosynthesis in petals and leaves of chrysanthemum (Chrysanthemum morifolium Ramat.). Physiol. Plant. 128: 437–447.

Kishimoto, S. and A. Ohmiya. 2012. Carotenoid isomerase is key determinant of petal color of Calendula officinalis. J. Biol. Chem. 287: 276–285.

Kishimoto, S., T. Maoka, M. Nakayama and A. Ohmiya. 2004. Carotenoid composition in petals of chrysanthemum (Dendranthema grandiflorum (Ramat.) Kitamura). Phytochemistry 65: 2781–2787.

Kishimoto, S., T. Maoka, K. Sumitomo and A. Ohmiya. 2005. Analysis of carotenoid composition in petals of calendula (Calendula officinalis L.) Biosci. Biotechnol. Biochem. 69: 2122–2128.

Kishimoto, S., C. Oda-Yamamizo and A. Ohmiya. 2018a. Regulation of carotenoid pigmentation in corollas of petunia. Plant Mol. Biol. Rep. 36: 632–642.

Kishimoto, S., C. Oda-Yamamizo and A. Ohmiya. 2018b. Effect of introduction of carotenoid esterase gene into pale yellow-flowered petunia cultivar on carotenoid accumulation in corollas. Hort. Res. (Japan) 17 (Suppl. 1): 238 (In Japanese).

Kishimoto, S., C. Oda-Yamamizo and A. Ohmiya. 2019. Comparison of petunia and calibrachoa in carotenoid pigmentation of corollas. Breed. Sci. (In press)

Kishimoto, S., K. Sumitomo, M. Yagi, M. Nakayama and A. Ohmiya. 2007. Three routes to orange petal color via carotenoid components in 9 Compositae species. J. Japan. Soc. Hort. Sci. 76: 250–257.

Kolotilin, I., H. Koltai, Y. Tadmor, C. Baror, M. Reuveni, A. Meir, S. Nahon, H. Shlomo, L. Chen and I. Levin. 2007. Transcriptional profiling of high pigment-26 tomato mutant links early fruit plastid biogenesis with its overproduction of phytonutrients. Plant Physiol. 145: 389–401.

Lado, J., L. Zacarias and M. J. Rodorigo. 2016. Regulation of carotenoid biosynthesis during fruit development. p. 161–198. In: C. Stange (ed.). Carotenoid in Nature, Subcellular Biochemistry 79. Springer International Publishing, Switzerland.

Lanahan, M. B., H. C. Yen, J. J. Giovannoni and H. J. Klee. 1994. The never ripe mutation blocks ethylene perception in tomato. Plant Cell 6: 521–530.

Lashbrook, J. G., P. R. Young, S. J. Dockrall, K. Vasanth and M. A. Vivier. 2013. Functional characterisation of three
members of the *Vitis vinifera* L. carotenoid cleavage dioxygenase gene family. BMC Plant Biol. 13: 156. DOI: 10.1186/1471-2229-13-156.

Lefebvre, V., M. Kurtz, B. Camara and A. Palloix. 1998. The capsanthin-capsorubin synthase gene: a candidate gene for the y locus controlling the red fruit colour in pepper. Plant Mol. Biol. 36: 785–789.

Li, L. and H. Yuan. 2013. Chromoplast biogenesis and carotenoid accumulation. Arch. Biochem. Biophys. 539: 102–109.

Li, L., D. J. Paolillo, M. V. Parthasarathy, E. M. DiMuzio and D. F. Garvin. 2001. A novel gene mutation that confers abnormal patterns of β-carotene accumulation in cauliflower (*Brassica oleracea* var. *botrytis*). Plant J. 26: 59–67.

Li, Z., S. Wang, X. L. Gui, X. B. Chang and Z. H. Gong. 2013. A further analysis of the relationship between yellow ripe fruit color and the capsanthin-capsorubin synthase gene in pepper (*Capsicum* sp.) indicated a new mutant variant in *C. annuum* and a tandem repeat structure in promoter region. PLoS ONE 8: e61996. DOI: 10.1371/journal.pone.0061996.

Liu, H., S. Kishimoto, C. Yamamizo, N. Fukuta and A. Ohmiya. 2013. Carotenoid accumulations and carotenogenic gene expressions in the petals of *Eustoma grandiflorum*. Plant Breed. 132: 417–422.

Liu, L., C. Jia, M. Zhang, D. Chen, S. Chen, R. Guo, D. Guo and Q. Wang. 2014. Ectopic expression of a *BZR1-1D* transcription factor in brassinosteroid signalling enhances carotenoid accumulation and fruit quality attributes in tomato. Plant Biotechnol. J. 12: 105–115.

Liu, L., J. Wei, M. Zhang, L. Zhang, C. Li and Q. Wang. 2012. Ethylene independent induction of lycopene biosynthesis in tomato fruits by jasmonates. J. Exp. Bot. 63: 5751–5761.

Liu, Y., S. Roof, Z. Ye, C. Barry, A. van Tuinen, J. Vrebalov, C. Bowler and J. Giovannoni. 2004. Manipulation of light signal transduction as a means of modifying fruit nutritional quality in tomato. Proc. Natl. Acad. Sci. USA 101: 9897–9902.

Ljubesic, N., M. Wrischer and Z. Devidé. 1991. Chromoplasts—the last stages in plastid development. Int. J. Dev. Biol. 35: 251–258.

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

Lu, S., J. Van Eck, X. Zhou, A. B. Lopez, D. M. O’Halloran, K. M. Cosman, B. J. Conlin, D. J. Polillo, D. F. Garvin, J. Vrebalov, L. V. Kochian, H. Küpper, E. D. Earle, J. Cao and L. Li. 2006. The cauliflower *Oe* gene encodes a DnaJ cysteine-rich domain containing protein that mediates high levels of β-carotene accumulation. Plant Cell 18: 3594–3605.

Lu, S. W., Y. Zhang, K. Zhu, W. Yang, J. L. Ye, L. Chai, Q. Xu and X. Deng. 2018. The citrus transcription factor CsMADS6 modulates carotenoid metabolism by directly regulating carotenogenic genes. Plant Physiol. 176: 2657–2676.

Ma, G., L. C. Zhang, A. Matsuta, K. Matsutani, K. Yamawaki, M. Yahata, A. Wahyudi, R. Motohashi and M. Kato. 2013. Enzymatic formation of β-citraurin from β-cryptoxanthin and zeaxanthin by carotenoid cleavage dioxygenase 4 in the flavedo of citrus fruit. Plant Physiol. 163: 682–695.

Ma, G., L. Zhang, K. Iida, Y. Madono, W. Yungyuen, M. Yahata, M. Yamawaki and M. Kato. 2017. Identification and quantitative analysis of β-cryptoxanthin and β-citraurin esters in Satsuma mandarin fruit during the ripening process. Food Chem. 234: 356–364.

Maoka, T., T. Etoh, S. Kishimoto and S. Sakata. 2011. Carotenoids and their fatty acid esters in the petals of *Adonis aestivalis*. J. Oleo. Sci. 60: 47–52.

Martel, C., J. Vrebalov, P. Tafelmeyer and J. J. Giovannoni. 2011. The tomato MADS-box transcription factor RIPENING INHIBITOR interacts with promoters involved in numerous ripening processes in a COLORLESS NONRIPENING-dependent manner. Plant Physiol. 157: 1568–1579.

Mathieu, S., F. Bigey, J. Procureur, N. Terrier and Z. Gúnata. 2007. Production of a recombinant carotenoid cleavage dioxygenase from grape and enzyme assay in water-miscible organic solvents. Biotechnol. Lett. 29: 837–841.

Mathieu, S., N. Terrier, J. Procureur, F. Bigey and Z. Gúnata. 2005. A carotenoid cleavage dioxygenase from *Vitis vinifera* L.: functional characterization and expression during grape berry development in relation to C13-norisoprenoid accumulation. J. Exp. Bot. 56: 2721–2731.

McCarty, D. R. and H. J. Klee. 2006. Characterization of three members of the Arabidopsis carotenoid cleavage dioxygenase family demonstrates the divergent roles of this multifunctional enzyme family. Plant J. 45: 982–993.

Moehs, C. P., L. Tian, K. W. Osteryoung and D. DellaPenna. 2001. Analysis of carotenoid biosynthetic gene expression during marigold petal development. Plant Mol. Biol. 45: 281–293.

Moriguchi, T., M. Kita, T. Endo-Inagaki, Y. Ikoma and M. Omura. 1998. Characterization of a cDNA homologous to carotenoid-associated protein in citrus fruits. Biochem. Biophys. Acta 1442: 334–338.

Nagata, M., C. Yoshida and H. Matsunaga. 2015. Changes in the expression of carotenoid biosynthetic genes during light irradiation of sweet pepper (*Capsicum annuum* L.) fruit after harvest. Hort. Res. (Japan) 14: 391–396 (In Japanese with English abstract).

Nakkanong, K., J. H. Yang and M. F. Zhang. 2012. Carotenoid accumulation and carotenogenic gene expression during fruit development in novel interspecific inbred squash lines and their parents. J. Agric. Food Chem. 60: 5936–5944.

Nambara, E. and A. Marion-Poll. 2005. Abscisic acid biosynthesis and catabolism. Annu. Rev. Plant Biol. 56: 281–293.

Nashima, K., Y. Ohmiya, A. 2009. Carotenoid cleavage dioxygenases and their apocarotenoid products in plants. Plant Biotechnol. 26: 351–358.

Nisar, N., L. Li, N. C. Khin and B. J. Pogson. 2015. Carotenoid metabolism in plant. Mol. Plant 8: 68–82.

Niyogi, K. 2000. Safety valves for photosynthesis. Curr. Opin. Plant Biol. 3: 455–460.

Ohiyama, A. 2009. Carotenoid cleavage dioxygenases and their apocarotenoid products in plants. Plant Biotechnol. 26: 351–358.

Ohiyama, A. 2011. Diversity of carotenoid composition in flower petals. Jpn. Agric. Res. Q (JARQ) 45: 163–171.

Ohiyama, A. 2013. Quantitative and qualitative control of carotenoid accumulation in flower petals. Sci. Hort. 163: 10–19.

Ohiyama, A., S. Kishimoto, R. Aida, S. Yoshioka and K. Sumitomo. 2006. Carotenoid cleavage dioxygenase (*CmCCD4a*) contributes to white color formation in chrysanthemum petals. Plant Physiol. 142: 1193–1201.

Ohiyama, A., K. Sumitomo and R. Aida. 2009. “Yellow Jimba”:...
Suppression of carotenoid cleavage dioxygenase (CmCCD4a) expression turns white chrysanthemum petals yellow. J. Japan. Soc. Hort. Sci. 78: 450–455.

Ootani, S. and K. Hayashi. 1982. Further search for water-soluble carotenoids in yellow flowers of several plants. Res. Inst. Evolut. Biol. Sci. Rep. 1: 71–76 (In Japanese with English abstract).

Pan, Y., G. Bradley, K. Pyke, G. Ball, C. Lu, R. Fray, A. Marshall, S. Jayasuta, C. Baxter, R. van Wijk, L. Boydren, C. Cade, N. H. Chapman, P. D. Fraser, C. Hodgman and G. B. Seymour. 2013. Network inference analysis identifies an APRR2-like gene linked to pigment accumulation in tomato and pepper fruits. Plant Physiol. 161: 1476–1485.

Paolillo, Jr., D. J., D. F. Garvin and M. V. Parthasarathy. 2004. The chromoplasts of Or mutants of cauliflower (Brassica oleracea L. var. botrytis). Protoplasma 224: 245–253.

Pizarro, L. and C. Stange. 2009. Light-dependent regulation of carotenoid biosynthesis in plants. Cienc. Inv. Agr. 36: 143–162.

Pogson, B. J. and V. Albrecht. 2011. Genetic dissection of chloroplast biogenesis and development: an overview. Plant Physiol. 155: 1545–1551.

Pouchieu, C., P. Galan, V. Ducros, P. Latino-Martel, S. Hercberg and J. P. Pouchieu. 2014. Plasma carotenoids and retinol and overall and breast cancer risk: a nested case-control study. Nutr. Cancer 66: 980–988.

Pouzet-Urro, J., E. Schmelz, D. G. Clark and H. J. Klee. 2004. Circadian regulation of the PhCCD1 carotenoid cleavage dioxygenase controls emission of β-ionone, a fragrance volatile of petunia flowers. Plant Physiol. 136: 3504–3514.

Simon, P. W. and X. Y. Wolff. 1987. Carotenoid accumulation in tomato and orange carrots. J. Agric. Food Chem. 35: 1017–1202.

Stewart, I. and T. A. Wheaton. 1973. Conversion of β-cryptoxanthin to reticulatuxanthin and β-apo-8′-carotenal to citranaxanthin during the isolation of carotenoids from Citrus. Phytochemistry 12: 2947–2951.

Sugiyama, A., Y. Ikoma, H. Fujii, T. Endo, H. Nesumi, T. Shimada and M. Omura. 2017. Allelic diversity of phytoene synthase gene influences transcription level in citrus fruit among a citrus F1 hybrid population. Breed Sci. 67: 382–392.

Sugiyama, A., Y. Ikoma, H. Fujii, T. Shimada, T. Endo, T. Shimizu and M. Omura. 2010. Structure and expression levels of alleles of citrus zeaxanthin epoxidase genes. J. Japan. Soc. Hort. Sci. 79: 263–274.

Sugiyama, A., M. Omura, H. Muramoto, T. Shimada, H. Fujii, T. Endo, T. Shimizu, H. Nesumi and Y. Ikoma. 2011. Quantitative trait loci (QTL) analysis of carotenoid content in citrus fruit. J. Japan. Soc. Hort. Sci. 80: 136–144.

Sugiyama, A., M. Omura, T. Shimada, H. Fujii, T. Endo, T. Shimizu, H. Nesumi, K. Nonaka and Y. Ikoma. 2014. Expression quantitative trait loci analysis of carotenoid metabolism-related genes in Citrus. J. Japan. Soc. Hort. Sci. 83: 32–43.

Sun, T., H. Yuan, H. Cao, M. Yazdani, Y. Tadmor and L. Li. 2018. Carotenoid metabolism in plants: the role of plastids. Mol. Plant 11: 58–74.

Tadmor, Y., S. King, A. Levi, A. Davis, B. Wasserman, J. Hirschberg and E. Lewinsohn. 2005. Comparative fruit colouration in watermelon and tomato. Food Res. Int. 38: 837–841.

Takayanagi, K., S. Morimoto, Y. Shirakura, K. Mukai, T. Sugiyama, Y. Tokuiji and M. Omnishi. 2011. Mechanism of visceral fat reduction in Tsumura Suzuki obese, diabetes (TSOD) mice orally administered β-cryptoxanthin from Satsuma mandarin oranges (Citrus unshiu Marc), J. Agric. Food Chem. 59: 12342–12351.

Tan, B. C., L. M. Joseph, W. T. Deng, L. Liu, Q. B. Li, K. Cline and D. R. McCarthy. 2003. Molecular characterization of the Arabidopsis 9-cis epoxy-carotenoid dioxygenase gene family. Plant J. 35: 44–56.

Tanaka, Y., N. Sasaki and A. Ohmiya. 2008. Biosynthesis of plant pigments: anthocyanins, betalains and carotenoids. Plant J. 54: 733–749.
Tzuri, G., X. Zhou, N. Chayut, H. Yuan, V. Portnoy, A. Meir, U. Sää, F. Baumkoler, M. Mazourek, E. Lewinsohn, Z. Fei, A. A. Schaffer, L. Li, J. Burger, N. Katzir and Y. Tadmor. 2015. A ‘golden’ SNP in CmOr governs fruit flesh color of melon (Cucumis melo). Plant J. 82: 267–279.

Vishnevetsky, M., M. Ovadis and A. Vainstein. 1999. Carotenoid sequestration in plants: the role of carotenoid associated proteins. Trends Plant Sci. 4: 232–235.

Wakelin, A. M., C. E. Lister and A. J. Conner. 2003. Inheritance and biochemistry of pollen pigmentation in California poppy (Eschscholzia californica Cham.). Int. J. Plant Sci. 164: 867–875.

Wang, H., Y. Zhu, S. Fujioka, T. Asami, J. Li and J. Li. 2009. Regulation of Arabidopsis brassinosteroid signaling by atypical basic helix-loop-helix proteins. Plant Cell 21: 3781–3791.

Wang, Y. Q., Y. Yang, Z. Fei, H. Yuan, T. Fish, T. W. Thannhauser, M. Mazourek, L. V. Kochian, X. Wang and L. Li. 2013. Proteomic analysis of chromoplasts from six crop species reveals insights into chromoplast function and development. J. Exp. Bot. 64: 949–961.

Watanabe, K., C. Oda-Yamamizo, K. Sage-Ono, A. Ohmiya and M. Ono. 2017. Overexpression of carotenogenic genes in the Japanese morning glory Ipomoea (Pharbitis) nil. Plant Biotech. 34: 177–185.

Watanabe, K., C. Oda-Yamamizo, K. Sage-Ono, A. Ohmiya and M. Ono. 2018. Alteration of flower colour in Ipomoea nil through CRISPR/CAS9-mediated mutagenesis of carotenoid cleavage dioxygenase 4 . Transgenic Res. 27: 25–38.

Winterhalter, P. and R. L. Rousseff. 2002. Carotenoid-derived aroma compounds: An introduction. p.1–17. In: P. Winterhalter and R. L. Rousseff (eds.). Carotenoid derived aroma compounds, ACS Symp. Series 802. ACS, Washington, D.C.

Yamagishi, M., S. Kishimoto and M. Nakayama. 2010. Carotenoid composition and changes in expression of carotenogenic genes in tepals of Asiatic hybrid lily. Plant Breed. 129: 100–107.

Yamaguchi, M. 2012. Role of carotenoid β-cryptoxanthin in bone homeostasis. J. Biomed. Sci. 19: 36. DOI: 10.1186/1423-0127-19-36.

Yamamizo, C., S. Kishimoto and A. Ohmiya. 2010. Carotenoid composition and carotenogenic gene expression during Ipomoea petal development. J. Exp. Bot. 61: 709–719.

Yoshida, C., M. Takahashi, Y. Iwasaki, S. Furuno, H. Matsunaga and M. Nagata. 2014. Factors affecting color development in sweet pepper (Capsicum annuum L.) fruit harvested at breaker stage of mature-green fruit. Hort. Res. (Japan) 13: 155–160 (In Japanese with English abstract).

Yuan, H., J. Zhang, D. Nageswaran and L. Li. 2015. Carotenoid metabolism and regulation in horticultural crops. Hort. Res. 2: 15036. DOI: 10.1038/hortres.2015.36.

Yungyuen, W., G. Ma, L. C. Zhang, M. Futamura, M. Tabuchi, K. Yamawaki, M. Yahata, S. Ohta, T. Yoshioka and M. Kato. 2018. Regulation of carotenoid metabolism in response to different temperatures in citrus juice sacs in vitro. Sci. Hortic. 129: 349–356.

Zhang, B., C. Liu, Y. Wang, X. Yao, F. Wang, J. Wu, G. J. King and K. Liu. 2015. Disruption of a CAROTENOID CLEAVAGE DIOXYGENASE 4 gene converts flower colour from white to yellow in Brassica species. New Phytol. 206: 1513–1526.

Zhang, L. C., G. Ma, M. Kato, K. Yamawaki, T. Takagi, Y. Kiriiwa, Y. Ikoma, H. Matsumoto, T. Yoshioka and H. Nesumi. 2012. Regulation of carotenoid accumulation and the expression of carotenoid metabolic genes in citrus juice sacs in vitro. J. Exp. Bot. 63: 871–886.

Zhou, X., R. Welsch, Y. Yang, D. Alvarez, M. Riediger, H. Yuan, T. Fish, J. Liu, T. W. Thannhauser and L. Li. 2015. Arabidopsis OR proteins are the major posttranscriptional regulators of phytoene synthase in controlling carotenoid biosynthesis. Proc. Natl. Acad. Sci. USA 112: 3558–3563.

Zhu, F., T. Luo, C. Liu, Y. Yang, H. Yang, W. Yang, L. Zheng, X. Xiao, M. Zhang, R. Xu, J. Xu, Y. Zeng, J. Xu, Q. Xu, W. Guo, R. M. Larkin, X. Deng and Y. Cheng. 2017. An R2R3-MYB transcription factor represses the transformation of α- and β-branch carotenoids by negatively regulating expression of CrBCH2 and CrNCED5 in flavedo of Citrus reticulate. New Phytol. 216: 178–192.