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REVIEW ARTICLE

Carotenoid metabolism and regulation in horticultural crops

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Carotenoids are a diverse group of pigments widely distributed in nature. The vivid yellow, orange, and red colors of many horticultural crops are attributed to the overaccumulation of carotenoids, which contribute to a critical agronomic trait for flowers and an important quality trait for fruits and vegetables. Not only do carotenoids give horticultural crops their visual appeal, they also enhance nutritional value and health benefits for humans. As a result, carotenoid research in horticultural crops has grown exponentially over the last decade. These investigations have advanced our fundamental understanding of carotenoid metabolism and regulation in plants. In this review, we provide an overview of carotenoid biosynthesis, degradation, and accumulation in horticultural crops and highlight recent achievements in our understanding of carotenoid metabolic regulation in vegetables, fruits, and flowers.

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INTRODUCTION

Carotenoids are a subgroup of isoprenoids with more than 750 members distributed in plants, algae, fungi, and bacteria. Carotenoids typically contain 40 carbons in their polyene backbones with conjugated double bonds and rings at the ends. The extensively conjugated double bonds allow carotenoids to absorb visible light, yielding yellow, orange, and red colors that make them the most conspicuous pigments in plants.

Color is an important quality trait for fruits and vegetables and a critical agronomic trait for flowers. The vivid yellow, orange, and red colors in many horticultural crops are attributed to high levels of carotenoid accumulation in chromoplasts.[1,2] In addition to giving horticultural crops their visual appeal, carotenoids also enhance the nutritional and health benefits of fruits and vegetables for humans in providing precursors for vitamin A synthesis and antioxidants for reducing various chronic diseases, such as cancer, cardiovascular disease, and age-related eye diseases.[3,4] As such, there is considerable interest in improving the color traits of horticultural crops.

Carotenoids are present in both photosynthetic and non-photosynthetic tissues of horticultural crops. In photosynthetic green tissues, carotenoids fulfill essential functions in photosynthesis for photosystem assembly, light harvesting, and photoprotection.[5] In non-photosynthetic tissues, carotenoids provide bright colors and produce scents and flavors to attract insects and animals for pollination and seed dispersal. Carotenoids also serve as precursors for two important phytohormones, abscisic acids (ABA) and strigolactones, which are key regulators for plant development and stress response.[6] Due to the indispensable roles of carotenoids in plant growth and development and in human nutrition and health, significant progress has been made in our understanding of carotenoid metabolism in plants.[7–11] Many of the major advances in carotenoid research have been achieved using some carotenoid-enriched horticultural crops as models. While carotenoid content and composition are relatively consistent in green leaf tissues of plant species, carotenoid levels and constituents vary greatly in non-green tissues of horticultural crops even within the same species. Therefore, various and multifaceted regulatory mechanisms are expected to exist in horticultural crops to control carotenoid metabolism and accumulation. Carotenoid research in the majority of horticultural crop species has focused largely on the regulation of carotenoid content and composition at transcriptional level of carotenogenic genes. There is an increasing amount of information available related to other aspects of regulation of carotenoid accumulation. This review provides an overview of carotenogenesis and discusses recent advances in our understanding of the multiple levels of regulation of carotenoid accumulation in fruits, vegetables, and flowers.

GENERAL PATHWAY OF CAROTENOID METABOLISM

Carotenoids are synthesized in all types of differentiated plastids but accumulate in high levels in the chloroplasts of green tissues and the chromoplasts of roots, fruits, and flower petals.[1,8,11,12] The biochemical steps of carotenoid biosynthetic pathway have long been established by labeling and inhibition studies and mutant analysis. However, the identification of the genes encoding carotenogenic enzymes is a more recent development from the past two decades. All the genes and enzymes that catalyze the core reactions of carotenoid biosynthesis and degradation have been identified in plants (Figure 1). A large number of the pathway genes from various horticultural crops have been cloned and studied.[13–17]

Carotenoids, along with other plastid-synthesized isoprenoids, arise from the condensation of the 5-carbon precursors isopentenyl diphosphate and dimethylallyl diphosphate, which are produced via the plastidial 2-*C*-methyl-*D*-erythritol 4-phosphate (MEP) pathway in plastids.[18] The specific carotenoid biosynthetic pathway starts with the head-to-head condensation of two geranylgeranyl diphosphates by phytoene synthase (PSY) to produce the first colorless carotenoid 15-*cis*-phytoene. This step is considered to be the primary bottleneck in carotenogenesis. Horticultural crops normally contain 2-3 *PSY* genes that exhibit tissue-specific expression, such as *PSY1* in fruits, *PSY2* in leaves, *PSY3* in the roots of tomato and citrus,[19,20] *PSY-A* in all tissues including fruits, and *PSY-B* in the leaves and roots of watermelon.[21] Uncolored phytoene is converted *via* a series of desaturations by phytoene desaturase (PDS) and ζ-carotene desaturase (ZDS) to introduce *cis* double bonds, and isomerizations by ζ-carotene isomerase (Z-ISO) and carotenoid

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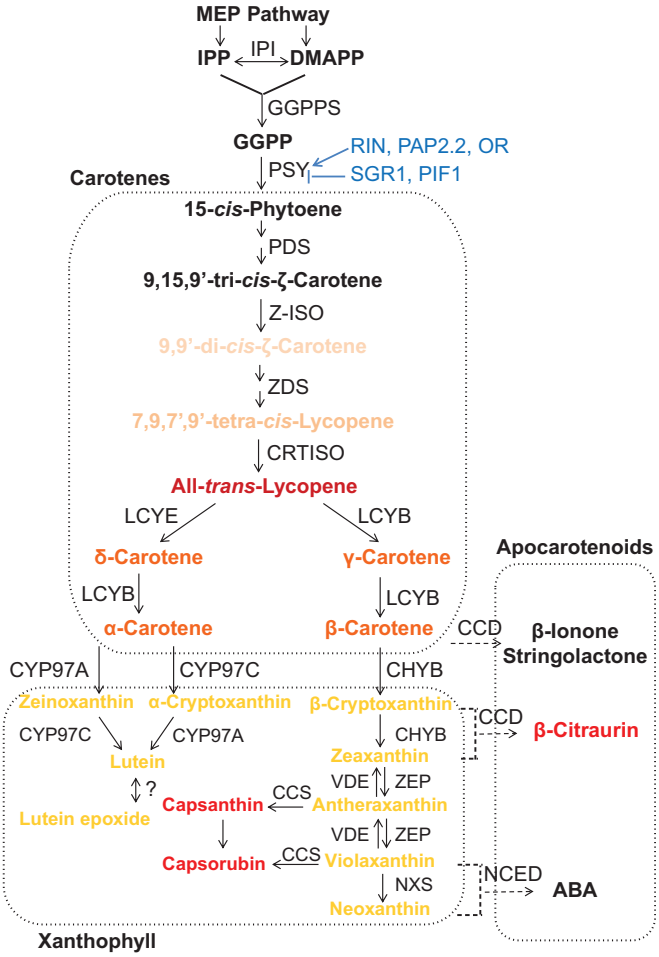

Figure 1. General carotenoid metabolic pathway in horticultural crops. PSY catalyzes the first committed condensation step from GGPP to produce the first C40 carotene, phytoene. Following several desaturation and isomerization steps, lycopene is produced. The next cyclization yields the α-carotene and β-carotene branches. A wide range of carotenoids are degraded by CCDs or NCEDs to produce apocarotenoids. IPP, isopentenyl diphosphate; DMAPP, dimethylallyl diphosphate; GGPP, geranylgeranyl diphosphate; IPI, isopentenyl diphosphate isomerase; GGPPS, GGPP 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; CHYB, β-carotene hydroxylase; CYP97C, cytochrome P450-type monooxygenase 97C; ZEP, zeaxanthin epoxidase; VDE, violaxanthin de-epoxidase; CCS, capsanthin-capsorubin synthase; NXS, neoxanthin synthase; CCD, carotenoid cleavage dioxygenase; NCED, 9-*cis*-epoxycarotenoid dioxygenase. Metabolites are bolded and colored according to their compound colors, whereas black indicates no color. Enzymes and regulators are not bolded. Solid arrows indicate biosynthesis and dashed arrows indicate degradation PSY regulators are colored in blue. Dotted rectangles separate different groups of carotenoids.

isomerase (CRTISO) to convert the *cis* configuration back into the *trans* configuration, thus yielding red-colored all-*trans*-lycopene, the predominant pigment in red tomato and watermelon fruits. Two *PDS* and at least three *ZDS* genes exist in most citrus species.[22]

The subsequent cyclization of the lycopene carbon chain ends starts the branch point of the pathway and represents a crucial step in carotenoid metabolism for generating carotenoid diversity (Figure 1). The addition of a β-ring and an ε-ring forms the β,ε branch of carotenoids, including α-carotene and its derivatives; the formation of two β-rings creates the β,β branch of carotenoids, containing β-carotene and its derivatives. Lycopene ε-cyclase (LCYE) and lycopene β-cyclase (LCYB) mediate carbon flow into the two different branches in the carotenoid biosynthesis. The red-colored all-*trans*-lycopene is cyclized by LCYE or LCYB to introduce ε- or β-rings, respectively, generating orange α-carotene or β-carotene, the predominant provitamin A carotenoids abundant in carrot, sweet potato, and orange melon fruit. Two *LCYB* genes are found in tomato: *LCYB1* (*LCY-B*) is abundant in vegetative tissues, and *LCYB2* (*CYC-B*) is significant in fruits and flowers.[23] Similarly, two copies of *LCYB* and *LYCE* exist in papaya and citrus species.[24–26] Carotenoids that contain only hydrocarbons are grouped as carotenes, which include phytoene and all types of carotenes.

The addition of oxygen by hydroxylases and epoxidases to cyclic carotenes produces xanthophylls (Figure 1). There are two different types of hydroxylases: the CHYB (BCH) type, which hydroxylates the β-ring of cyclic carotenes, and the cytochrome P450 type. Of the latter type, hydroxylases CYP97A and CYP97C hydroxylate the β- and ε-rings, respectively. The orange-colored α-carotene is sequentially catalyzed primarily by CYP97-type hydroxylases to produce yellow lutein, which is abundant in the yellow flowers of marigold and daffodil and in dark green leafy vegetables. Lutein is the classic final product of the β,ε-branch in the carotenoid biosynthesis pathway. However, in some plants, lutein can be converted into lutein epoxides by epoxidation, although the enzymes involved are not clear.[27] The orange-colored β-carotene in the β,β-branch is hydroxylated by CHYB to produce yellow zeaxanthin. Zeaxanthin is epoxidized to yield antheraxanthin and then violaxanthin. Violaxanthin can be converted back to zeaxanthin by violaxanthin de-epoxidase, forming the ubiquitous violaxanthin cycle that is essential for plants to adapt to different light conditions. In red pepper and tiger lily, antheraxanthin and violaxanthin are converted by capsanthin-capsorubin synthase (CCS) into capsanthin and capsorubin, the main carotenoids that generate the characteristic red and orange colors of these species.[28,29] CCS has high sequence identity with LCYB and belongs to the lycopene cyclase family.[28] The final step of the β,β-branch in the classic carotenoid biosynthetic pathway is to convert yellow-colored violaxanthin into neoxanthin *via* neoxanthin synthase,[30] another lycopene cyclase family protein. In tomato, LCYB2 was also found to have CCS activity.[23]

Carotenoids are catabolized enzymatically by a family of carotenoid cleavage dioxygenases (CCDs; sometimes referred to as carotenoid cleavage oxygenases, CCOs) to produce apocarotenoids, which control carotenoid turnover and contribute to the colors or aromas of flowers and fruits and the production of two important phytohormones, ABA and the strigolactones. In various plant species, members of the CCD enzyme family are denominated according to their sequence similarity to the *Arabidopsis* CCD family and are generally divided into two groups: four CCDs (CCD1, 4, 7, and 8) and five 9-*cis*-epoxycarotenoid dioxygenases (NCED 2, 3, 5, 6, and 9).[31] Different CCDs and NCEDs recognize different carotenoid substrates and cleave at different sites, producing various apocarotenoids.[6] CCD1 and CCD4 have wide substrate specificities, from phytoene to neoxanthin, to produce cleavage products such as bixin, crocin, saffron, α-ionone, β-ionone, β-cyclocitral, and β-citraurin.[32–38] CCD7 and CCD8 are involved in the synthesis of strigolactones from β-carotene *via* carlactones.[39] NCEDs specifically cleave 9-*cis*-violaxanthin and 9-*cis*-neoxanthin to yield xanthoxin, which is further modified to ABA.[40] A recent study identified a novel dioxygenase from *Crocus sativus*, designated as CCD2, that is distinct from and closely related to CCD1 and cleaves zeaxanthin.[37] Although many *CCD* family genes with multiple members have been identified in fruits, vegetables, and flowers, their precise roles in carotenoid turnover remain to be fully elucidated.

MAJOR CAROTENOIDS ACCUMULATED IN CAROTENOID-ENRICHED HORTICULTURAL CROPS

Carotenoid composition and content vary widely in fruits, vegetables, and flowers (Table 1) and have been subjected to extensive analysis. In many cases, the genetic elements responsible for specific carotenoid accumulation are unknown. Varieties with different colors in a single species are often seen.

Vegetables are one of the major sources of carotenoids for human consumption. Tomato is widely used as a model system for carotenoid research. This crop displays diverse color variation in its fruit (i.e., yellow, tangerine, orange, orange-red, and red) with different carotenoid profiles. Red tomato is rich in lycopene, which constitutes *ca*. 85% of its total carotenoids.[41] The other colored tomato fruits accumulate primarily different types of carotenoids. The pale-yellow fruit contains low levels of β-carotene with an almost complete lack of lycopene.[42] The orange *tangerine* fruit (*t* mutant) accumulates pro-lycopene.[43] The orange/red fruit (*Delta* mutant) contains high levels of δ-carotene.[44] A different orange fruit (*Beta* mutant) produces enhanced β-carotene levels at the expense of lycopene.[23] A deep crimson fruit (b^{OG} mutant) produces elevated lycopene and is nearly devoid of β-carotene.[23]

Pepper synthesizes carotenoids to give its fruits a range of red, yellow, and orange colors across diverse pepper species. Red fruit predominantly produces the characteristic carotenoid capsanthin. The yellow fruit varieties contain primarily lutein and α- or β-carotene without capsanthin. In contrast, the orange fruit varieties accumulate various main carotenoids, such as capsanthin, lutein, and/or β-carotene, depending on the variety.[29,45] In general, considerably higher levels of total carotenoids are observed in red varieties than in non-red ones.[29,46] A three-locus model (*c, y1*, and *y2*) has been proposed for pepper fruit color inheritance.[47] *CCS* appears to be responsible for *y* and *PSY* for *c2* in red pepper.[48,49]

Other vegetables that contain high levels of carotenoids include carrot, sweet potato, winter squash, orange cauliflower, and many dark green leafy vegetables. Carrot and sweet potato accumulate massive amounts of carotenoids, with β-carotene as the major component and α-carotene as the minor component. Two major loci, *Y* and Y_2, control much of the variation in carotenoid accumulation in carrot.[50] In winter squash, carotenoid composition varies, and β-carotene, lutein, and violaxanthin predominate in most varieties.[51] Cauliflower curd normally contains negligible amounts of carotenoids, but an orange cauliflower accumulates high levels of β-carotene,[52] which is due to the *Orange* (*OR*) gene mutation.[53] Additionally, broccoli and many dark leafy vegetables such as spinach, mustard green, and kale are rich in carotenoids with lutein, β-carotene, neoxanthin, and violaxanthin in decreasing order of abundance as other photosynthetic leaf tissues (Table 1).

Fruits are another major source of carotenoids for human consumption. A large number of fruits accumulate various carotenoids. Melon fruit typically has white-, green-, or orange-colored flesh with relatively simple carotenoid compositions. The orange-fleshed cultivars (cantaloupe and a newly developed orange-fleshed honeydew) accumulate β-carotene as the principal carotenoid, and its quantitative difference determines the variation in color intensity.[54] The white- and green-fleshed varieties have negligible or low levels of carotenoids. The orange *versus* non-orange flesh color trait

Table 1 Carotenoids accumulated in some major carotenoid-enriched horticultural crops

	Common name	Variety	Color	Major carotenoid	Minor carotenoid	Reference
Vegetable	Tomato	Wild type	Red	Lycopene	β-carotene, phytoene, ζ-carotene	41
		Delta	Orange/red	δ-carotene, lycopene	Lutein, α-carotene	44
		Beta	Orange	β-carotene, lycopene	phytoene	41, 23
		Old-gold	Golden	Lycopene	β-carotene, phytoene	41, 23
	Pepper		Red	Capsanthin	zeaxanthin, cryptoxanthin, β-carotene	45
			Orange	Zeaxanthin, capsanthin, lutein	β-carotene, cryptoxanthin	45
			Yellow	Lutein, β-carotene	Zeaxanthin, α-carotene	46
	Carrot		Orange	β-carotene	α-carotene, lutein	14
			Red	Lycopene, β-carotene	Lutein	14
			Yellow	Lutein	β-carotene	14
	Sweet potato		Orange	β-carotene	α-carotene	
	Cauliflower	*Or*	Orange	β-carotene		52
	Dark green vegetables		Green	Lutein, β-carotene	α-carotene, zeaxanthin	
Fruit	Melon	Cantaloupe	Orange	β-carotene		54
	Citrus	Orange	Orange	Violaxanthin	β- cryptoxanthin, phytoene	15
		Mandarin	Orange	β- cryptoxanthin	Phytoene, violaxanthin, β-carotene	15
		Grapefruit	Red	β-carotene or lycopene, phytoene	Phytofluene	
	Watermelon		Red	Lycopene	β, ζ-carotene, Violaxanthin	21, 41
			Yellow	Violaxanthin or Neoxanthin	Lutein	21
			Orange	β-carotene	Phytoene, lycopene, ζ-carotene	41
	Peach	Redhaven	Yellow	Antheraxanthin, luteoxanthin, zeaxanthin	β-cryptoxanthin, β-carotene	59
	Papaya		Red	Lycopene	β-cryptoxanthin, β-carotene	58
			Yellow	β-cryptoxanthin, β-carotene	Lycopene	58
Flower	Marigold		Yellow to orange	Lutein		94
	Chrysanthemum		Yellow to orange	Lutein and its epoxide	Violaxanthin, β-carotene	65
	Oncidium	Gower Ramsey	Yellow	Violaxanthin	Lutein	66
		Sunkist	Orange	Violaxanthin, β-carotene	Lutein	
	Osmanthus	Zi Yingui	Butter yellow	β-carotene		67
		Jingui	Golden yellow	β-carotene, lutein	α-carotene	67
		Chenghong Dangui	Orange-red	α-carotene, β-carotene	Lutein	67
	Lily	Connecticut king	Yellow	Antheraxanthin, violaxanthin, lutein	β-carotene	68
		Saija and tiger lily	Red	Capsanthin	Antheraxanthin	28, 68
	Adonis		Red	Astaxanthin, adonirubin	β-carotene, Astaxanthin	69

inheritance is controlled by a single gene, *green-flesh*, which was recently revealed to be the melon *Or* gene.[55] Watermelon is predominately red-fleshed but can be orange, yellow, or white. Generally, red-fleshed watermelon has the highest carotenoid content and contains lycopene as the major carotenoid. Orange-fleshed watermelon usually has lycopene, β/ε-carotene, and phytoene, with different amounts depending on the variety.[41] Yellow-fleshed watermelon commonly contains violaxanthin and/or neoxanthin as the main carotenoids, and some varieties also have lutein or neochrome. White-fleshed watermelon has only trace amount of carotenoids.[21] A number of gene loci, i.e., *B*, *C*, *i-C*, *Wf*, *y*, and *y-o*, are known to control watermelon flesh color.[56] However, their identities remain to be fully elucidated.

Citrus has the most diverse carotenoid composition with the largest number of carotenoid species found in any fruit.[15] Citrus accumulates carotenoids in both the flavedo and the fruit sac, with their contents varying greatly in different varieties. Among various citrus fruits, mandarin, orange, and clementine are usually rich in several carotenoids including β-cryptoxanthin, violaxanthin, lutein, and zeaxanthin; grapefruit and pummelo accumulate primarily phytoene, phytofluene, ζ-carotene, and β-carotene; and citron, lemon, and lime contain low levels of carotenoids.[15] Citrus mutants with altered carotenoid compositions and contents also exist, such as mutants with pink to red flesh that accumulate lycopene, phytoene, or β-cryptoxanthin.[57] Papaya is commonly observed with red or yellow flesh. Its yellow-fleshed fruit accumulates mainly β-carotene, β-cryptoxanthin, and ζ-carotene, and the red-fleshed variety also contains high levels of lycopene.[58] In peach, the yellow-fleshed varieties exhibit variability in carotenoid compositions and accumulate mainly violaxanthin, lutein, β-cryptoxanthin, zeaxanthin, antheraxanthin, and/or luteoxanthin.[59] A recessive mutation at the *Y* locus is responsible for the yellow flesh color in peach, which was recently found to be due to a mutation in *CCD4*.[59–62] There are other fruits such as mango, apricot, and persimmon that are also rich in carotenoids.

In flowers, carotenoids and anthocyanins govern flower petal color.[63] The carotenoid compositions of some flowers have been listed in earlier reviews.[9,16,64] Depending on carotenoid composition and content, flowers exhibit light yellow, yellow or even orange, and red colors. The majority of carotenoids present in flower petals are xanthophylls (Table 1). Yellow flowers generally contain large amounts of xanthophylls, along with their epoxides, and traces of carotenes, whereas orange flowers contain carotenes as their main carotenoids. For example, lutein accumulates in high abundance in marigold and daffodil petals to yield an intense yellow color. Lutein and its epoxide are predominant in yellow chrysanthemum,[65] and violaxanthin and neoxanthin are predominant in the yellow *Oncidium* orchid.[66] An orange petal color is caused by α- and β-carotene.[67] Some flower petals have the red carotenoids capsanthin and capsorubin, which yield an orange to red color, such as the red Saija lily and orange tiger lily.[28,68] Adonis is the only known plant species to accumulate red-colored ketocarotenoid astaxanthin, yielding its distinctive blood-red-colored petals.[69] Although flowers contain both the β,β and β,ε-branches of carotenoids, many prefer one branch over the other.[64] In addition to their beautiful colors, some flowers also have unique aromas that arise from the production of apocarotenoids. *Crocus sativus* stigmas emit β-cyclocitral and safranal, which originate from the enzymatic cleavage of β-carotene and zeaxanthin.[70] *Osmanthus fragrans* releases β-ionone from the cleavage of α- or β-carotene.[71]

In many horticultural crops, carotenoids accumulate in chromoplasts, which are classified as crystalline, globular, fibrillary, membranous, or reticulo-tubular based on various sequestering substructures within the chromoplasts.[1,2,72] Often, more than one chromoplast type coexists in a crop species, although some favor a particular type of chromoplasts.[73] The high-level accumulation of specific carotenoids sometimes accompanies specific pigment-bearing substructures. For example, carotenoid crystals usually form only from the massive accumulation of lycopene and β-carotene, such as in red tomato, watermelon,[74,75] carrot, and orange cauliflower.[76,77]

TRANSCRIPTIONAL REGULATION OF THE CAROTENOID BIOSYNTHETIC GENES IN HORTICULTURAL CROPS

Carotenoid accumulation in chromoplasts is a net result of biosynthesis, degradation, and stable storage.[1,11] Thus, regulation of these three processes represents the major mechanisms underlying carotenoid accumulation in horticultural crops. Most studies on carotenoid regulation in non-model horticultural crops focus on the transcriptional regulation of carotenoid pathway genes. Although new insights into various aspects and levels of regulation are emerging, the mechanisms underlying carotenoid biosynthesis and accumulation in the majority of horticultural crops are not well understood.

Horticultural crops synthesize and accumulate diverse carotenoids with a wide range of contents in non-green organs. The first level of regulation of carotenoid biosynthesis in many vegetables, fruits, and flowers is *via* the control of biosynthetic gene transcription. Transcriptional regulation is a major determinant for carotenoid production in the classical model systems of tomato and pepper during fruit ripening in response to developmental signals. In tomato fruit, the increased production of lycopene during fruit color change from green to red is preceded by the enhanced transcription of upstream genes for lycopene biosynthesis, i.e., *PSY*, *PDS*, *CRTISO*, and *DXS*, and the downregulation of downstream genes *LCYB*, *LCYE*, and *CHYB*.[43,44,78,79] The accumulation of capsanthin during pepper fruit ripening from green to red is linked with the transcriptional upregulation of *CCS*, along with *PSY*, *PDS*, and *BCH*.[80] Mutant studies have provided further evidence for the key role of transcriptional regulation of carotenoid biosynthetic genes in controlling carotenoid production in tomato and pepper. The increased transcription of *LCYE* or *LCYB* in the tomato mutants *Delta* or *Beta*, respectively, leads to the conversion of lycopene into δ-carotene or β-carotene, respectively.[23,44] Decreased expression of *PSY* and *CRTISO* in the tomato mutants *r* and *tangerine* (*t*) results in the dramatically reduced production of carotenoids in *r* and the accumulation of lycopene precursor pro-lycopene in *t*.[42,43] The loss or reduction of *CCS* expression in the yellow or orange pepper fruit is responsible for the absence or low level of capsanthin.[29,45]

Transcriptional regulation of biosynthetic genes also appears to play a central role in the control of carotenoid production for many other horticultural crops because of the correlated changes. During orange melon fruit ripening, the production of massive amounts of β-carotene is concomitant with the dramatically increased expression of almost all the upstream genes, including those in the MEP pathway (i.e., *DXS*, *DXR*, *GGR*, *PSY1*, *ZDS*, *PDS*, and *LCYB*), and the reduced expression of the downstream genes *CHYB* and *LCYE*, channeling metabolic flux away from the α,β branch toward β-carotene synthesis (Chayut et al., 2015, manuscript submitted). Low levels of carotenoids in the juice sac of lemon compared with that of other citrus species are consistent with the lowest transcriptional levels of nearly all the carotenogenic genes at various stages of ripening; likewise, the predominant accumulation of β-cryptoxanthin or violaxanthin in mandarins and oranges is in agreement with the coordinately high expression of some upstream carotenogenic genes, such as *CitPSY*, *CitPDS*, and *CitLCYb*, and the low expression of downstream genes, such as *CitHYb* and *CitZEP*, in some varieties.[15,19,57,81] In watermelon, the trace level of carotenoids observed in white-fleshed fruit was found to be linked to the low transcription of most biosynthetic genes; the accumulation of lycopene during red and pink watermelon fruit ripening is associated with the upregulation of *GGPS* and *PSY*, and the production of violaxanthin and lutein in yellow-fleshed fruits is positively correlated with *CHYB* and *ZEP* transcript abundance, respectively.[21]

Carotenoid production in colored carrot is associated with the progressively increased expression of most biosynthetic genes during root development, and the high transcript levels of *LCYE* and *ZDS* in red and yellow carrot cultivars partially explain the accumulation of lutein and lycopene, respectively.[82] The transcriptional regulation of some carotenoid biosynthetic genes has also been suggested to play a role in mediating carotenoid production and specific carotenoid accumulation in other vegetables and fruits, such as *CHYB* and *ZEP* for violaxanthin and lutein synthesis and *LCYE* for carotenoid content in squash;[51,83] *PSY* and *PDS* for β-carotene formation in bitter melon;[84] and *LCYB* for β-carotene production in kiwifruit.[85]

The important role of transcriptional regulation of biosynthetic genes in controlling carotenoid production is also evident in non-model horticultural crop mutants that accumulate specific carotenoids. A mutation in papaya *LCYB2* dramatically reduces its expression, resulting in the accumulation of lycopene, and is responsible for the difference between red- and yellow-fleshed papaya.[24,86] Similarly, mutations in melon and Chinese cabbage *CRTISO* lead to the accumulation of pro-lycopene in mature fruit and inner head leaves, respectively.[87–89] A recent study in orange carrots revealed that a loss of function in the carotene hydroxylase *CYP97A3* gene is responsible for high α-carotene content.[90]

The transcriptional regulation of carotenogenesis in flower petals has been discussed in previous reviews.[9,16,64] A comparison of carotenogenic gene expression shows that the carotenoid types and contents of flower petals are closely linked with biosynthetic gene expression in some flowers. In the white flowers of *Ipomoea nil*, lily and marigold, the expression of carotenoid biosynthetic genes is much lower than that of their pale-yellow and yellow petal varieties.[68,91] The relative expression levels of *LCYB* and *LCYE* determine the branches of xanthophylls accumulated as major carotenoids in the flower petals. In the Asiatic hybrid lily[68] and *Mimulus lewisii*,[92] *LCYB* is highly expressed and the β,β branch products are preferred, whereas in yellow chrysanthemum,[65] *LCYE* is highly expressed and the α,β branch products are preferred. The transcription of *CHYB* has also been suggested to be critical for the carotenoid differences between the white or pale-yellow and yellow flowers of *Ipomoea* plants[91] and in the stigmas of different *Crocus* species.[93] The lower transcript levels of *CHYB* and *ZEP* have been proposed to be partially responsible for the higher β-carotene contents of orange orchid and *Osmanthus*.[66,67]

Gene promoters represent a critical element for the transcriptional regulation of gene expression. The functional analysis of carotenogenic gene promoters provides insights into the regulatory basis of carotenoid gene expression during fruit and flower development. The developmental upregulation of some carotenoid genes in both fruits and flowers appears to be linked with the specific activities of carotenogenic gene promoters in chromoplast-containing tissues.[94–98] The *ShLcyB* promoter from a green-fruited tomato (*Solanum habrochaites*) exhibits over fivefold activity in flowers and fruits but shows only a basal level of activity in leaves.[96] High *PDS* promoter activity was also found in the organs and developmental stages where chromoplasts are formed in tomato.[97] Similarly, the *GlLcyB, GlBCH*, and *GlZEP* promoters from *Gentiana lutea* are highly active in chromoplast-containing organs but have minimal activity in non-chromoplast-containing tissues.[94,95] The promoter of *CmCCD4a-5* was revealed to drive petal-specific transcription in the developing chrysanthemum flower.[98] The examination of carotenogenic promoters also provides a basis for the developmental, coordinated upregulation of carotenogenic genes. The Great Yellow Gentian accumulates carotenoids with the synchronized upregulation of several carotenogenic genes during flower petal development. An examination of their promoters identified three common *cis*-acting motifs, which were suggested to be responsible for co-regulating the carotenogenic genes.[94]

Although transcriptional regulation is important for carotenoid production in horticultural crops in response to developmental signals, the amount and type of carotenoids accumulated in some vegetables and fruits are not correlated with carotenogenic gene expression. Indeed, the coordinated, high-level transcription of upstream biosynthetic genes for specific and/or massive carotenoid production, as in tomato and pepper, is not observed in many species. In orange curd cauliflower, the massive accumulation of β-carotene was not found to be associated with the increased expression of biosynthetic genes in comparison with white curd cauliflower.[52] Although β-carotene production during melon fruit ripening is associated with the differential regulation of carotenogenic genes, similar levels and patterns of gene expression are observed between white and orange fruit (Chayut et al., 2015, manuscript submitted). Carotenoid accumulation disjoint from differential gene transcription has also been reported in many other cases, such as between white- and orange-fleshed squash,[51] during the fruit ripening of normal and red orange,[57] in yellow- *versus* red-fleshed watermelon,[21] and in flowers, such as between white and yellow marigold,[99] between white chrysanthemum and its yellow bud mutant,[65] and in lilies,[68] indicating the existence of different, additional regulatory mechanisms.

MODULATION OF BIOSYNTHETIC ENZYMES

In comparison with the transcriptional regulation of carotenogenic genes, not much is known about the regulation of carotenoid biosynthetic enzymes in plants in general. A number of mechanisms have been shown to modulate carotenogenic enzymes and their activities in regulating carotenoid biosynthesis, which include changes in amino acid sequences, membrane association, protein-protein interactions, suborganelle localization, and cofactors.

The most common modulation of biosynthetic enzyme activity is the alteration of enzyme amino acid sequences, which results in enzymes with either enhanced or reduced activities. For example, in the non-horticultural crop cassava, changing a single amino acid in a highly conserved region of PSY results in increased catalytic activity, leading to enhanced carotenoid production in yellow-rooted cultivars.[100] The introduction of a premature stop codon the mutation of enzyme sequences can cause enzymatic inactivation and the accumulation of precursor carotenoids, which is often observed as a control mechanism in some horticultural crops. Some examples include the formation of truncated proteins in PSY in carotenoid-lacking white flesh loquat,[101] in LCYB2 for the accumulation of lycopene in red flesh papaya,[24,86] in CYP97A3 for the high α-carotene content of orange carrot,[90] in CRTISO for the production of pro-lycopene in mature melon fruit,[87] and in CCS for the formation of orange-colored pepper fruit.[45] The insertion of additional amino acids in CRTISO also disrupts its activity and results in the formation of orange head Chinese cabbage.[88,89] Similarly, amino acid substitution in or deletion of CRTISO1-OR results in lost activity and the accumulation of 5-*cis*-carotenoids in orange calendula flower varieties.[102]

Carotenoid biosynthesis occurs in the plastidial membrane.[1,8,12] Association with the membrane has been shown to be a mechanism for regulating biosynthetic enzyme activity in a few cases. In daffodil flowers, PSY and PDS exist in two forms in chromoplasts: a soluble form and a membrane-bound form. The soluble form is enzymatically inactive in large HSP70-containing complexes in the stroma and only becomes active to induce carotenoid accumulation in flower petals when it is bound to the membrane.[103–105] More evidence to support this comes from the study of carotenogenesis during photomorphogenesis. Dramatically increased carotenoid content during de-etiolation is associated with phytochrome-regulated *PSY* expression.[106,107] In etiolated seedlings, in addition to low gene expression, most PSY proteins are located within the prolamellar bodies, which exhibit low enzymatic activity

because they lack a competent membrane. During de-etiolation, the topological relocalization of PSY to the thylakoid membranes of chloroplasts leads to enzymatic activation and carotenoid biosynthesis.[108]

Recent studies have also revealed other regulatory mechanisms to control biosynthetic enzyme protein levels and activities in model plants, such as post-transcriptional regulation via protein–protein interaction, which likely operates in horticultural crops. The OR protein was discovered in the orange curd cauliflower. OR variants regulate carotenoid accumulation in both cauliflower and melon fruit.[53,55] OR has been shown to physically interact with PSY in plastids and functions as a major post-transcriptional regulator to positively regulate PSY protein abundance and enzymatic activity in the control of carotenoid biosynthesis.[109] A tomato STAY-GREEN protein, SlSGR1, regulates lycopene accumulation during fruit maturation *via* direct interaction with PSY1 in the nucleus to negatively control PSY1 activity by suppressing its transcription.[110] Another example is the post-translational regulation of DXS, the key enzyme in the MEP pathway. DXS interacts directly with J-protein J20, which regulates DXS protein level and activity by recognizing inactive DXS and delivering it to the HSP70 chaperone system either for proper folding and enzyme activation or for proteolytic degradation under stress.[111] Suborganelle localization represents another method to control the competence of biosynthetic enzymes in mediating carotenoid biosynthesis, probably by affecting enzyme stability.[112,113] Recently, it was discovered that Z-ISO depends on a redox-regulated heme cofactor for enzyme activity to control carotenogenesis.[114]

REGULATION OF CAROTENOID DEGRADATION GENES

Carotenoid cleavage into apocarotenoids by CCDs or CCOs represents another important control to regulate carotenoid accumulation in horticultural crops. NCEDs are involved with ABA production, and CCD7 and CCD8 participate in strigolactone formation. Their activities most likely do not dramatically affect carotenoid levels in plants. In contrast, CCD1 and CCD4 mediate carotenoid homeostasis and aroma/flavor compound production in fruits and flowers.[6,115] Interestingly, unlike other CCDs, NCEDs, and all carotenoid biosynthetic enzymes, *Crocus sativus* CCD1 and a novel CCD2 and tomato LeCCD1 are localized in the cytosol instead of plastids, suggesting that the cleavage of carotenoids is localized in the outer envelopes of plastids.[37] Both *CCD1* and *CCD4* have been identified as gene families in many horticultural crops.[33,66,67,93,116,117] Investigation of these *CCDs* in horticultural crops, particularly in flowers, provides direct evidence for the critical role of regulating carotenoid turnover in controlling carotenoid levels in plants.

Our current understanding of the regulation of CCDs in mediating carotenoid content is primarily at the transcriptional level. Carotenoid accumulation in several yellow flowers and fruits has been shown to be negatively associated with *CCD1* or *CCD4* expression. The best example is in chrysanthemum: although the transcription of most carotenoid biosynthetic genes is similar in white and yellow petals, *CmCCD4a* is highly expressed in white flower petals but nearly undetectable in yellow petals.[116] The suppression of *CmCCD4a* expression by RNAi and mutations in *CmCCD4a* in white flower cultivars changed the flower color from white to yellow.[116,118,119] These findings indicate that in chrysanthemum, *CmCCD4a* is the single most important factor that regulates carotenoid accumulation by mediating carotenoid turnover. A negative correlation between *CCD4* expression and carotenoid accumulation has also been observed in other flowers such as *Crocus sativus*[33,117] and *fragrans*,[34,67] although further study is needed to functionally verify the specific role of *CCD4* in controlling their flower petal color.

In fruits, the white and yellow flesh colors of peach are a monogenic trait controlled by the *Y* gene. *PpCCD4* is expressed much less in yellow-fleshed fruit than in its white-fleshed mutant.[59] Recent genetic studies have provided evidence to support *PpCCD4* as the *Y* gene that controls fruit flesh color in peach. Various mutations in *PpCCD4* lower its transcript level and produce a truncated protein that reduces carotenoid degradation, resulting in yellow-flesh varieties.[60–62] The attractive orange-reddish color in the peal of citrus fruit is due to the accumulation of the carotenoid cleavage product β-citraurin from β-cryptoxanthin and zeaxanthin. The transcription of *CCD4b*, one of the five *CCD4* genes in citrus, is directly related to C_{30} apocarotenoid production during fruit development and affected by ripening regulators, indicating its important role in regulating carotenoid turnover in citrus fruit.[35,36,38] A correlation between the transcriptional regulation of *CCD4* gene expression and carotenoid levels has also been observed in other fruits, such as goji.[120] However, functional verification of the critical role of *CCD4* as a determinant is lacking in the majority of cases.

CCD1 contributes apocarotenoid flavor and aroma production in flowers and fruits. The *in vitro* expression of *CCD1* from a number of horticultural crops, such as tomato fruit, saffron flower, and melon fruit, in *E. coli* indicates that CCD1 cleaves β-carotene and other carotenoids into a range of volatiles.[121] *CCD1* transcription is associated with carotenoid levels in some horticultural crops. The undetectable level of carotenoids in white orchid is probably caused by the high expression of *OgCCD1* in comparison with the yellow and orange orchid varieties.[66] A correlation between increased *FaCCD1* transcription and decreased lutein content is observed during strawberry ripening.[122] Although a negatively correlated change between *CCD1* or *CCD4* and carotenoid content has been observed in various fruits and vegetables, the regulation of *CCD* expression is not well understood. Furthermore, it is believed that in some cases, even when *CCD* gene expression is not observed to be negatively correlated with carotenoid accumulation, CCD activity might play an important role in the control of carotenoid levels. This hypothesis is supported by a $^{14}CO_2$ pulse-chase labeling study that showed continuous carotenoid turnover at much high rates than expected in *Arabidopsis* leaves.[123] High levels of continuous turnover may also explain the low level of carotenoids found in the white tissues of some horticultural crops that contain carotenoid metabolic gene expression patterns comparable to their carotenoid-accumulating counterparts.

REGULATION OF CAROTENOID SEQUESTRATION AND STORAGE IN CHROMOPLASTS

Carotenoid sequestration and stable storage in plastids is another critical control point for carotenoid accumulation.[1,124,125] Although carotenoids are synthesized in various plastids, they accumulate in high levels in chromoplasts, which generate the attractive color of many horticultural crops. Chromoplasts possess a unique mechanism to sequester the synthesized carotenoids into carotenoid lipoprotein-sequestering substructures to promote continuous carotenoid biosynthesis and stable storage, leading to massive accumulation.[1,126] High variation in carotenoid lipoprotein substructures within chromoplasts is believed to contribute to the various carotenoid accumulation profiles found in fruits, vegetables, and roots.

Chromoplasts serve as a metabolic sink for carotenoid accumulation. Thus, the regulation of chromoplast biogenesis and development strongly affects carotenoid biosynthesis and accumulation. However, little is known about the genes and factors that govern these processes. The *Or* gene represents the only known gene that acts as a *bona fide* molecular switch to initiate chromoplast biogenesis. *Or* was originally cloned from an orange curd mutant of cauliflower and encodes a DnaJ cysteine-rich domain-containing protein.[53] Its mutation triggers the differentiation of non-colored plastids into chromoplasts with an increased capacity to accumulate β-carotene in cauliflower and potato.[53,127] Recently, the

Or gene was found to be *green flesh* (*gf*), the gene that differentiates between green and orange fruit flesh color in melon and is responsible for carotenoid accumulation in the melon fruit. A single SNP that leads to arginine to histidine change in the CmOr protein is the key reason for β-carotene accumulation in the orange-fleshed fruit.[55] Noticeably, the transcript and protein levels of CmOr from both green and orange flesh melon fruits are similar, indicating another level of regulation with the change of arginine to histidine in the CmOr protein. Members of the OR family have recently been found to be the major post-transcriptional regulators of PSY.[109] Whereas OR from both the wild type and the mutant regulates PSY at the post-transcriptional level, the OR mutant protein with the histidine substitution specifically promotes chromoplast formation, leading to massive carotenoid accumulation.[128] However, how OR regulates chromoplast biogenesis and what regulates OR expression remain unknown.

In addition, a few non-carotenogenic proteins are thought to be associated with chromoplast formation. Membrane proliferation and remodeling of the internal membrane represent the most prominent changes during chromoplast biogenesis.[1,2] A plastid fusion and/or translocation factor from pepper fruits was found to be involved in chromoplast membrane biogenesis.[72] Functional evidence indicates that a small molecular chaperone protein, HSP21, exerts a role in fruit reddening and the transition from chloroplasts to chromoplasts during tomato fruit ripening.[129]

The regulation of plastid number and size alters sink strength to affect carotenoid levels. Enhanced plastid number and size are considered main characteristics of tomato *high-pigment* (*hp*) mutants with enhanced fruit carotenoid contents.[2] *hp1* contains a mutation in *UV-DAMAGED DNA-BINDING PROTEIN 1* (*DDB*) that causes a 30% increase in plastid number in tomato fruits.[130] *hp2* has a defect in *DE-ETIOLATED1* (*DET1*) and dramatically enhances fruit chloroplast number and size,[131,132] which has also been observed via the fruit-specific suppression of *DET1*.[133] The tomato mutant *hp3* has a mutation in the *ZEP* gene that results in an increased plastid number per cell and an enlargement of the plastid compartment in *hp3* mature fruit, thus enabling higher levels of carotenoid accumulation.[134] Overexpressing *APRR2-Like* in tomato also enhances plastid number and area, enhancing carotenoid accumulation in mature red tomato fruit.[135]

Part of the mechanism underlying plastid storage sink-stimulated carotenoid accumulation is associated with the formation of carotenoid sequestration substructures. Thus, controlling the synthesis of the substructure components correlates directly with carotenoid accumulation. Carotenoid-associated proteins play important roles in producing carotenoid lipoprotein structures.[124] Both fibrillins from pepper fruits[136] and carotenoid-associated protein (CHRC) from cucumber[137] are involved in the sequestration of carotenoids and positively associated with carotenoid overaccumulation in chromoplasts. Indeed, overexpression of the pepper fibrillin gene in tomato fruit increases carotenoid levels and carotenoid-derived volatiles.[138] The transcript level of fibrillin was recently noted to be differentially correlated with specific carotenoids in pepper fruit.[139] The suppression of tomato *CHRC* produces flowers with decreased carotenoids, indicating a role for CHRC in mediating carotenoid storage in flower chromoplasts.[139] Enhanced abundance of the CHRC protein has been found in all tomato *hp* and *Intense pigment* mutants, which suggests a mechanism for the coupling increased plastid number and size with enhanced carotenoid accumulation in these high-pigment tomato fruits.[141] In transgenic potato tubers expressing the cauliflower *Or* mutant gene, increased carotenoid accumulation is clearly associated with the formation of carotenoid lipoprotein-sequestering substructures in chromoplasts.[126,127] Winter squash increases carotenoid accumulation during storage, which is accompanied by the conversion of amylochromoplasts into chromoplasts.[142] Increased sequestration is suggested as one possible basis for this observed phenomenon. In addition, the regulation of carotenoid esterification by *PYP1* (*Pale Yellow Petal 1*) has been observed to modulate carotenoid level and chromoplast development in tomato flower,[143] likely *via* increasing sequestration and limiting degradation.

REGULATORY GENES THAT CONTROL CAROTENOID ACCUMULATION

Although all the genes in the main carotenoid metabolic pathway have been isolated and well characterized from various horticultural crops, not much is known about the regulatory genes that directly regulate pathway genes and enzyme expression, although an intricate transcriptional network may exist in plants. A large number of transcription factors, particularly from tomato, have been shown to affect carotenoid accumulation through the regulation of fruit ripening. These transcription factors include *RIN*, *TAGL1*, *AP2a*, *ERF6*, *DET1*, *APRR2-Like*, *SGR*, *BZR1-1D*, etc.

Only a few regulators have been demonstrated to directly regulate the expression of carotenogenic genes or enzymes in mediating carotenoid biosynthesis and accumulation (Figure 1). Tomato *RIN* encodes a MADS box transcription factor and represents a global master regulator of fruit ripening.[144] Chromatin immunoprecipitation (ChIP) and Chip-chip analyses have revealed a number of carotenoid pathway genes as targets of RIN, and RIN regulates carotenoid accumulation *via* positively regulating *PSY1*, *ZISO*, and *CRTISO* in a direct manner, as well as positively controlling *PSY2* and *ZDS*, and negatively regulating *LYCB* and *LYCE* by an indirect effect in tomato fruit.[145,146] *SlSGR1* encodes a STAY-GREEN protein that regulates chlorophyll degradation during fruit ripening in tomato. SlSGR1 physically interacts with PSY1 in the nucleus to suppress *PSY1* expression and inhibit PSY activity, thus negatively regulating lycopene production.[110] Recently, we discovered that OR functions as a positive regulator and post-transcriptionally regulates PSY in plastids via direct protein–protein interaction to control carotenoid biosynthesis.[109] Phytochrome-interacting factor 1 has been documented to physically bind the promoter of *PSY* and repress *PSY* expression, thus negatively regulating carotenoid biosynthesis in dark-grown *Arabidopsis* seedlings.[107] Similarly, a member of the APETALA2 (AP2)/ethylene-responsive element-binding protein transcription factor family, AtPAP2.2, binds to the promoters of *PSY* and *PDS* to regulate carotenoid biosynthesis in planta.[147]

OTHER MECHANISMS OF REGULATION

Feedback regulation has long been proposed as a mechanism for the regulation of carotenoid levels in plants. In addition to ABA shown in non-horticultural crops,[148,149] a number of carotenoid metabolites such as *cis*-carotene and some uncharacterized signal molecules have been found to feedback-regulate transcript abundance of carotenoid genes in horticultural crops. Tomato *tangerine* locus *t* is epistatic over locus *r*, which codes *PSY1*. The expression of *PSY1* is partially recovered in the *tangerine* mutant, indicating the involvement of *cis*-carotene produced by *t* in the feedback regulation of the expression of the early pathway gene *PSY1*.[150] The overexpression of carotene hydroxylase *CYP97A3* in orange carrot causes a decrease in PSY protein level and reduced carotenoid levels, suggesting the negative feedback regulation of PSY by uncharacterized signals.[90]

Light signaling is known to regulate carotenoid accumulation.[11,12] Light regulates *PSY* to modulate carotenoid biosynthesis during deetiolation.[107] Functional analysis of two tomato light signaling genes, *HY5* and *COP1LIKE*, has revealed that the suppression of *HY5* expression leads to decreased carotenoid levels, and the downregulation of *COP1LIKE* causes increased carotenoid accumulation, indicating *HY5* and *COP1LIKE* play positive and negative roles, respectively, in controlling fruit pigment accumulation.[130] Orange head Chinese cabbage results from a defect in CRTISO.[88,89] When orange head Chinese cabbage is exposed to light, light-induced isomerization results in the production of lycopene, which indicates that CRTISO activity can be partially replaced by light, although the molecular basis of

photoisomerization is still unknown. In citrus, blue light enhances carotenoid accumulation by upregulating *CitPSY* expression.[26]

Phytohormones, such as ethylene, auxin, jasmonates (JA), and ABA, affect carotenoid accumulation in fruits *via* regulating fruit ripening.[17] Ethylene plays a critical role in climacteric fruit ripening. Many transcription factors have been shown to affect carotenoid accumulation in tomato fruit through the regulation of ethylene biosynthesis and signaling.[146,144] JA is also of importance in positively controlling carotenoid accumulation. In tomato, lycopene content is greatly reduced in the fruits of JA-deficient mutants and increased in transgenic lines with enhanced JA levels.[151] Exogenous MeJA treatment of an ethylene-insensitive tomato mutant (*Nr*) can dramatically enhance lycopene accumulation in fruits.[151] Similarly, ABA is involved in fruit ripening and affects carotenoid accumulation in fruits such as tomato, strawberry, and grape.[152–154] However, whether phytohormones directly regulate carotenoid pathway gene expression remains unknown.

Given the interactive nature of plant metabolic pathways, carotenoid metabolism is likely to be modulated by or interconnected with other cellular and metabolic processes. Recent advances in omics technologies have enabled the examination of global cellular and metabolic changes associated with carotenoid accumulation in a large number of horticultural crops, such as citrus,[155] watermelon,[156] orange head Chinese cabbage,[88] tomato[157] chili pepper[158] and oil palm.[159] In addition, chromoplast proteomes from various carotenoid-rich horticultural crops have been investigated.[157,160,161] Although omics analyses generally are not effective in identifying the causative genes that control carotenoid accumulation in these crops, these studies indicate a number of cellular and metabolic processes are associated with carotenoid accumulation, including sugar metabolism, lipid metabolism, molecular chaperones, energy/metabolite transport, and redox systems.[1,2] An intriguing but as yet unanswered question is whether these processes represent control points for the normal endogenous regulation of carotenoid accumulation. However, the dominant transposon-tagged tomato mutant *Orr*, which has a defect in the NDH (NADH dehydrogenase) complex leading to an altered plastid redox status, has reduced carotenoid accumulation in the chromoplasts during fruit ripening.[162]

CONCLUSIONS

Carotenoid metabolism has been extensively studied in horticultural crops. The molecular mechanisms underlying carotenoid biosynthesis and accumulation in many horticultural crops are poorly understood, although investigating model crops such as tomato yields fundamental knowledge of various aspects of carotenoid metabolism and regulation. Multiple levels of regulation are known to affect carotenoid biosynthesis and accumulation in response to metabolic, developmental, and environmental signals. The transcriptional regulation of carotenoid pathway genes is a main focus of investigation in horticultural crops and has been shown to be important in regulating carotenoid content and composition. However, as seen in the QTL analyses of many fruits and vegetables, carotenogenic metabolic genes are often not the major genetic loci that determine carotenoid levels; the pathway genes do not colocalize with the major QTLs of carotenoid content and/or composition. Several regulatory genes and proteins that directly control carotenogenesis to mediate carotenoid accumulation have been identified, whereas a large number of transcription factors have been shown to affect carotenoid accumulation *via* regulating fruit ripening. Horticultural crops synthesize and accumulate diverse levels and varieties of carotenoids even within the same species. Thus, horticultural crops provide unique and excellent genetic resources for novel gene discovery and for the dissection of new biochemical and molecular mechanisms underlying carotenoid biosynthesis and accumulation in plants.

COMPETING INTERESTS

The authors declare no conflict of interest.

REFERENCES

1 Li L, Yuan H. Chromoplast biogenesis and carotenoid accumulation. *Arch Biochem Biophys* 2013; **539**: 102–109.
2 Egea I, Barsan C, Bian W, Purgatto E, Latché A *et al.* Chromoplast differentiation: current status and perspectives. *Plant Cell Physiol* 2010; **51**: 1601–1611.
3 Fraser PD, Bramley PM. The biosynthesis and nutritional uses of carotenoids. *Prog Lipid Res* 2004; **43**: 228–265.
4 Fiedor J, Burda K. Potential role of carotenoids as antioxidants in human health and disease. *Nutrients* 2014; **6**: 466–488.
5 Domonkos I, Kis M, Gombos Z, Ughy B. Carotenoids, versatile components of oxygenic photosynthesis. *Prog Lipid Res* 2013; **52**: 539–561.
6 Walter MH, Strack D. Carotenoids and their cleavage products: biosynthesis and functions. *Nat Prod Rep* 2011; **28**: 663–692.
7 Lu S, Li L. Carotenoid metabolism: biosynthesis, regulation, and beyond. *J Integr Plant Biol* 2008; **50**: 778–785.
8 Cazzonelli CI, Pogson BJ. Source to sink: regulation of carotenoid biosynthesis in plants. *Trends Plant Sci* 2010; **15**: 266–274.
9 Zhu C, Bai C, Sanahuja G, Yuan D, Farré G *et al.* The regulation of carotenoid pigmentation in flowers. *Arch Biochem Biophys* 2010; **504**: 132–141.
10 Moise AR, Al-Babili S, Wurtzel ET. Mechanistic aspects of carotenoid biosynthesis. *Chem Rev* 2014; **114**: 164–193.
11 Nisar N, Li L, Lu S, Khin NC, Pogson BJ. Carotenoid metabolism in plants. *Mol Plant* 2015; **8**: 68–82.
12 Ruiz-Sola MÁ, Rodríguez-Concepción M. Carotenoid biosynthesis in Arabidopsis: a colorful pathway. *Arabidopsis Book* 2012; **10**: e0158.
13 Farré G, Sanahuja G, Naqvi S, Bai C, Capell T *et al.* Travel advice on the road to carotenoids in plants. *Plant Sci* 2010; **179**: 28–48.
14 Rodriguez-Concepcion M, Stange C. Biosynthesis of carotenoids in carrot: an underground story comes to light. *Arch Biochem Biophys* 2013; **539**: 110–116.
15 Kato M. Mechanism of carotenoid accumulation in citrus fruit. *J Japanese Soc Hortic Sci* 2012; **81**: 219–233.
16 Ohmiya A. Qualitative and quantitative control of carotenoid accumulation in flower petals. *Sci Hortic (Amsterdam)* 2013; **163**: 10–19.
17 Liu L, Shao Z, Zhang M, Wang Q. Regulation of carotenoid metabolism in tomato. *Mol Plant* 2015; **8**: 28–39.
18 Rodríguez-Concepción M. Supply of precursors for carotenoid biosynthesis in plants. *Arch Biochem Biophys* 2010; **504**: 118–122.
19 Peng G, Wang C, Song S, Fu X, Azam M *et al.* The role of 1-deoxy-d-xylulose-5-phosphate synthase and phytoene synthase gene family in citrus carotenoid accumulation. *Plant Physiol Biochem* 2013; **71**: 67–76.
20 Fantini E, Falcone G, Frusciante S, Giliberto L, Giuliano G. Dissection of tomato lycopene biosynthesis through virus-induced gene silencing. *Plant Physiol* 2013; **163**: 986–998.
21 Lv P, Li N, Liu H, Gu H, Zhao W. Changes in carotenoid profiles and in the expression pattern of the genes in carotenoid metabolisms during fruit development and ripening in four watermelon cultivars. *Food Chem* 2015; **174**: 52–59.
22 Fanciullino AL, Dhuique-Mayer C, Luro F, Morillon R, Ollitrault P. Carotenoid biosynthetic pathway in the Citrus genus: number of copies and phylogenetic diversity of seven genes. *J Agric Food Chem* 2007; **55**: 7405–7417.
23 Ronen G, Carmel-Goren L, Zamir D, Hirschberg J. An alternative pathway to beta-carotene formation in plant chromoplasts discovered by map-based cloning of beta and old-gold color mutations in tomato. *Proc Natl Acad Sci USA* 2000; **97**: 11102–11107.
24 Devitt LC, Fanning K, Dietzgen RG, Holton TA. Isolation and functional characterization of a lycopene β-cyclase gene that controls fruit colour of papaya (Carica papaya L.). *J Exp Bot* 2010; **61**: 33–39.
25 Mendes AF, Chen C, Gmitter FG, Moore GA, Costa MG. Expression and phylogenetic analysis of two new lycopene β-cyclases from Citrus paradisi. *Physiol Plant* 2011; **141**: 1–10.
26 Zhang L, Ma G, Kato M, Yamawaki K, Takagi T *et al.* Regulation of carotenoid accumulation and the expression of carotenoid metabolic genes in citrus juice sacs in vitro. *J Exp Bot* 2012; **63**: 871–886.
27 Förster B, Pogson BJ, Osmond CB. Lutein from deepoxidation of lutein epoxide replaces zeaxanthin to sustain an enhanced capacity for nonphotochemical chlorophyll fluorescence quenching in avocado shade leaves in the dark. *Plant Physiol* 2011; **156**: 393–403.

28 Jeknić Z, Morré JT, Jeknić S, Jevremović S, Subotić A *et al*. Cloning and functional characterization of a gene for capsanthin-capsorubin synthase from tiger lily (lilium lancifolium thunb. 'splendens'). *Plant Cell Physiol* 2012; **53**: 1899–1912.
29 Guzman I, Hamby S, Romero J, Bosland PW, O'Connell MA. Variability of carotenoid biosynthesis in orange colored Capsicum spp. *Plant Sci* 2010; **179**: 49–59.
30 Neuman H, Galpaz N, Cunningham FX, Zamir D, Hirschberg J. The tomato mutation nxd1 reveals a gene necessary for neoxanthin biosynthesis and demonstrates that violaxanthin is a sufficient precursor for abscisic acid biosynthesis. *Plant J* 2014; **78**: 80–93.
31 Tan BC, Joseph LM, Deng WT, Liu L, Li QB *et al.* Molecular characterization of the Arabidopsis 9-cis epoxycarotenoid dioxygenase gene family. *Plant J* 2003; **35**: 44–56.
32 Ibdah M, Azulay Y, Portnoy V, Wasserman B, Bar E *et al.* Functional characterization of CmCCD1, a carotenoid cleavage dioxygenase from melon. *Phytochemistry* 2006; **67**: 1579–1589.
33 Rubio A, Rambla JL, Santaella M, Gómez MD, Orzaez D *et al*. Cytosolic and plastoglobule-targeted carotenoid dioxygenases from Crocus sativus are both involved in β-ionone release. *J Biol Chem* 2008; **283**: 24816–24825.
34 Baldermann S, Kato M, Fleischmann P, Watanabe N. Biosynthesis of α-and β-ionone, prominent scent compounds, in flowers of osmanthus fragrans. *Acta Biochim Pol* 2012; **59**: 79–81.
35 Ma G, Zhang L, Matsuta A, Matsutani K, Yamawaki K *et al*. Enzymatic formation of β-citraurin from β-cryptoxanthin and Zeaxanthin by carotenoid cleavage dioxygenase4 in the flavedo of citrus fruit. *Plant Physiol* 2013; **163**: 682–695.
36 Rodrigo MJ, Alquézar B, Alós E, Medina V, Carmona L *et al.* A novel carotenoid cleavage activity involved in the biosynthesis of Citrus fruit-specific apocarotenoid pigments. *J Exp Bot* 2013; **64**: 4461–4478.
37 Frusciante S, Diretto G, Bruno M, Ferrante P, Pietrella M *et al.* Novel carotenoid cleavage dioxygenase catalyzes the first dedicated step in saffron crocin biosynthesis. *Proc Natl Acad Sci USA* 2014; **111**: 12246–12251.
38 Zheng X, Xie Z, Zhu K, Xu Q, Deng X *et al*. Isolation and characterization of carotenoid cleavage dioxygenase 4 genes from different citrus species. *Mol Genet Genomics* 2015; **290**: 1589–1603.
39 Ruyter-Spira C, Al-Babili S, van der Krol S, Bouwmeester H. The biology of strigolactones. *Trends Plant Sci* 2013; **18**: 72–83.
40 Schwartz SH, Tan BC, Gage DA, Zeevaart JA, McCarty DR. Specific oxidative cleavage of carotenoids by VP14 of maize. *Science* 1997; **276**: 1872–1874.
41 Tadmor Y, King S, Levi A, Davis A, Meir A *et al.* Comparative fruit colouration in watermelon and tomato. *Food Res Int* 2005; **38**: 837–841.
42 Fray RG, Grierson D. Identification and genetic analysis of normal and mutant phytoene synthase genes of tomato by sequencing, complementation and co-suppression. *Plant Mol Biol* 1993; **22**: 589–602.
43 Isaacson T, Ronen G, Zamir D, Hirschberg J. Cloning of tangerine from tomato reveals a carotenoid isomerase essential for the production of β-carotene and xanthophylls in plants. *Plant Cell* 2002; **14**: 333–342.
44 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.
45 Rodriguez-Uribe L, Guzman I, Rajapakse W, Richins RD, O'Connell MA. Carotenoid accumulation in orange-pigmented *Capsicum annuum* fruit, regulated at multiple levels. *J Exp Bot* 2012; **63**: 517–526.
46 Ha SH, Kim JB, Park JS, Lee SW, Cho KJ. 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* 2007; **58**: 3135–3144.
47 Hurtado-Hernandez H, Smith P. Inheritance of mature fruit color in *Capsicum annuum* L. *J Hered* 1985; **76**: 211–213.
48 Lefebvre V, Kuntz M, Camara B, Palloix A. The capsanthin-capsorubin synthase gene: a candidate gene for the y locus controlling the red fruit colour in pepper. *Plant Mol Biol* 1998; **36**: 785–789.
49 Huh JH, Kang BC, Nahm SH, Kim S, Ha KS *et al*. A candidate gene approach identified phytoene synthase as the locus for mature fruit color in red pepper (*Capsicum spp.*). *Theor Appl Genet* 2001; **102**: 524–530.
50 Just BJ, Santos CAF, Yandell BS, Simon PW. Major QTL for carrot color are positionally associated with carotenoid biosynthetic genes and interact epistatically in a domesticated x wild carrot cross. *Theor Appl Genet* 2009; **119**: 1155–1169.
51 Nakkanong K, Yang JH, Zhang MF. Carotenoid accumulation and carotenogenic gene expression during fruit development in novel interspecific inbred squash lines and their parents. *J Agric Food Chem* 2012; **60**: 5936–5944.
52 Li L, Paolillo DJ, Parthasarathy MV, Dimuzio EM, Garvin DF. A novel gene mutation that confers abnormal patterns of β-carotene accumulation in cauliflower (*Brassica oleracea* var. botrytis). *Plant J* 2001; **26**: 59–67.
53 Lu S, Van Eck J, Zhou X, Lopez AB, O'Halloran DM *et al.* The cauliflower *Or* gene encodes a DnaJ cysteine-rich domain-containing protein that mediates high levels of β-carotene accumulation. *Plant Cell* 2006; **18**: 3594–3605.
54 Burger Y, Paris HS, Cohen R, Katzir N, Tadmor Y *et al.* Genetic diversity of *Cucumis Melo*. *Hort Rev* 2009; **36**: 165–198.
55 Tzuri G, Zhou X, Chayut N, Yuan H, Portnoy V *et al.* A 'golden' SNP in CmOr governs fruit flesh color of melon (*Cucumis melo*). *Plant J* 2015; **82**: 267–279.
56 Guner N, Wehner T. Gene list for watermelon. *Cucurbit Genet Coop Rep* 2007; **30**: 96–120.
57 Wei X, Chen C, Yu Q, Gady A, Yu Y *et al.* Comparison of carotenoid accumulation and biosynthetic gene expression between Valencia and Rohde Red Valencia sweet oranges. *Plant Sci* 2014; **227**: 28–36.
58 Schweiggert RM, Steingass CB, Heller A, Esquivel P, Carle R. Characterization of chromoplasts and carotenoids of red- and yellow-fleshed papaya (*Carica papaya* L.). *Planta* 2011; **234**: 1031–1044.
59 Brandi F, Bar E, Mourgues F, Horváth G, Turcsi E *et al.* Study of 'Redhaven' peach and its white-fleshed mutant suggests a key role of CCD4 carotenoid dioxygenase in carotenoid and norisoprenoid volatile metabolism. *BMC Plant Biol* 2011; **11**: 24–37.
60 Falchi R, Vendramin E, Zanon L, Scalabrin S, Cipriani G *et al.* Three distinct mutational mechanisms acting on a single gene underpin the origin of yellow flesh in peach. *Plant J* 2013; **76**: 175–187.
61 Adami M, De Franceschi P, Brandi F, Liverani A, Giovannini D *et al.* Identifying a carotenoid cleavage dioxygenase (*ccd4*) gene controlling yellow/white fruit flesh color of peach. *Plant Mol Biol Report* 2013; **31**: 1166–1175.
62 Fukamatsu Y, Tamura T, Hihara S, Oda K. Mutations in the CCD4 carotenoid cleavage dioxygenase gene of yellow-flesh peaches. *Biosci Biotechnol Biochem* 2014; **77**: 2514–2516.
63 Grotewold E. The genetics and biochemistry of floral pigments. *Annu Rev Plant Biol* 2006; **57**: 761–780.
64 Ohmiya A. Diversity of carotenoid composition in flower petals. *Japan Agric Res Q* 2011; **45**: 163–171.
65 Kishimoto S, Ohmiya A. Regulation of carotenoid biosynthesis in petals and leaves of chrysanthemum (*Chrysanthemum morifolium*). *Physiol Plant* 2006; **128**: 436–447.
66 Chiou CY, Pan HA, Chuang YN, Yeh KW. Differential expression of carotenoid-related genes determines diversified carotenoid coloration in floral tissues of Oncidium cultivars. *Planta* 2010; **232**: 937–948.
67 Han Y, Wang X, Chen W, Dong M, Yuan W *et al.* Differential expression of carotenoid-related genes determines diversified carotenoid coloration in flower petal of Osmanthus fragrans. *Tree Genet Genomes* 2014; **10**: 329–338.
68 Yamagishi M, Kishimoto S, Nakayama M. Carotenoid composition and changes in expression of carotenoid biosynthetic genes in tepals of Asiatic hybrid lily. *Plant Breed* 2010; **129**: 100–107.
69 Cunningham FX, Gantt E. Elucidation of the pathway to astaxanthin in the flowers of *Adonis aestivalis*. *Plant Cell* 2011; **23**: 3055–3069.
70 Ángela LG, Ahrazem RO. Understanding carotenoid metabolism in saffron stigmas: unravelling aroma and colour formation. *Funct Plant Sci Biotechnol* 2010; **4**: 56–63.
71 Baldermann S, Kato M, Kurosawa M, Kurobayashi Y, Fujita A *et al.* Functional characterization of a carotenoid cleavage dioxygenase 1 and its relation to the carotenoid accumulation and volatile emission during the floral development of Osmanthus fragrans Lour. *J Exp Bot* 2010; **61**: 2967–2977.
72 Camara B, Hugueney P, Bouvier F, Kuntz M, Monéger R. Biochemistry and molecular biology of chromoplast development. *Int Rev Cytol* 1995; **163**: 175–247.
73 Jeffery J, Holzenburg A, King S. Physical barriers to carotenoid bioaccessibility. Ultrastructure survey of chromoplast and cell wall morphology in nine carotenoid-containing fruits and vegetables. *J Sci Food Agric* 2012; **92**: 2594–2602.
74 Harris WM, Spurr AR. Chromoplasts of tomato fruits. II. The red tomato. *Am J Bot* 1969; **56**: 380–389.
75 Bangalore DV, McGlynn WG, Scott DD. Effects of fruit maturity on watermelon ultrastructure and intracellular lycopene distribution. *J Food Sci* 2008; **73**: 222–228.
76 Frey-Wyssling A, Schwegler F. Ultrastructure of the chromoplasts in the carrot root. *J Ultrastruct Res* 1965; **13**: 543–559.
77 Paolillo DJ, Garvin DF, Parthasarathy MV. The chromoplasts of Or mutants of cauliflower (*Brassica oleracea* L. var. botrytis). *Protoplasma* 2004; **224**: 245–253.
78 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.
79 Lois LM, Rodríguez-Concepción M, Gallego F, Campos N, Boronat A. Carotenoid biosynthesis during tomato fruit development: regulatory role of 1-deoxy-D-xylulose 5-phosphate synthase. *Plant J* 2000; **22**: 503–513.

80 Hugueney P, Bouvier F, Badillo A, Quennemet J, d'Harlingue A *et al*. Developmental and stress regulation of gene expression for plastid and cytosolic isoprenoid pathways in pepper fruits. *Plant Physiol* 1996; **111**: 619–626.
81 Kato M, Ikoma Y, Matsumoto H, Sugiura M, Hyodo H. Accumulation of carotenoids and expression of carotenoid biosynthetic genes during maturation in citrus fruit. 2004; **134**: 824–837.
82 Clotault J, Peltier D, Berruyer R, Thomas M, Briard M *et al*. Expression of carotenoid biosynthesis genes during carrot root development. *J Exp Bot* 2008; **59**: 3563–3573.
83 Obrero Á, González-Verdejo CI, Die JV, Gómez P, Del Río-Celestino M *et al*. Carotenogenic gene expression and carotenoid accumulation in three varieties of *Cucurbita pepo* during fruit development. *J Agric Food Chem* 2013; **61**: 6393–6403.
84 Tuan PA, Kim JK, Park N Il, Lee SY, Park SU. Carotenoid content and expression of phytoene synthase and phytoene desaturase genes in bitter melon (*Momordica charantia*). *Food Chem* 2011; **126**: 1686–1692.
85 Ampomah-Dwamena C, McGhie T, Wibisono R, Montefiori M, Hellens RP *et al*. The kiwifruit lycopene beta-cyclase plays a significant role in carotenoid accumulation in fruit. *J Exp Bot* 2009; **60**: 3765–3779.
86 Blas AL, Ming R, Liu Z, Veatch OJ, Paull RE *et al*. Cloning of the papaya chromoplast-specific lycopene beta-cyclase, CpCYC-b, controlling fruit flesh color reveals conserved microsynteny and a recombination hot spot. *Plant Physiol* 2010; **152**: 2013–2022.
87 Galpaz N, Burger Y, Lavee T, Tzuri G, Sherman A *et al*. Genetic and chemical characterization of an EMS induced mutation in *Cucumis melo CRTISO* gene. *Arch Biochem Biophys* 2013; **539**: 117–125.
88 Zhang J, Yuan H, Fei Z, Pogson BJ, Zhang L *et al*. Molecular characterization and transcriptome analysis of orange head Chinese cabbage (*Brassica rapa* L. ssp. pekinensis). *Planta* 2015; **241**: 1381–1394.
89 Su T, Yu S, Zhang JWF, Yu Y, Zhang D *et al*. Loss of function of the carotenoid isomerase gene BrCRTISO confers orange color to the inner leaves of chinese cabbage (*Brassica rapa* L. ssp. pekinensis). *Plant Mol Biol Report* 2014; **33**: 648–659.
90 Arango J, Jourdan M, Geoffriau E, Beyer P, Welsch R. Carotene hydroxylase activity determines the levels of both α-carotene and total carotenoids in orange carrots. *Plant Cell* 2014; **26**: 2223–2233.
91 Yamamizo C, Kishimoto S, Ohmiya A. Carotenoid composition and carotenogenic gene expression during Ipomoea petal development. *J Exp Bot* 2010; **61**: 709–719.
92 LaFountain AM, Frank HA, Yuan YW. Carotenoid composition of the flowers of Mimulus lewisii and related species: implications regarding the prevalence and origin of two unique, allenic pigments. *Arch Biochem Biophys* 2015; **573**: 32–39.
93 Castillo R, Fernández J-A, Gómez-Gómez L. Implications of carotenoid biosynthetic genes in apocarotenoid formation during the stigma development of Crocus sativus and its closer relatives. *Plant Physiol* 2005; **139**: 674–689.
94 Zhu C, Yang Q, Ni X, Bai C, Sheng Y *et al*. Cloning and functional analysis of the promoters that upregulate carotenogenic gene expression during flower development in *Gentiana lutea Physiol Plant* 2014; **150**: 493–504.
95 Yang Q, Yuan D, Shi L, Capell T, Bai C *et al*. Functional characterization of the Gentiana lutea zeaxanthin epoxidase (GlZEP) promoter in transgenic tomato plants. *Transgenic Res* 2012; **21**: 1043–1056.
96 Dalal M, Chinnusamy V, Bansal KC. Isolation and functional characterization of lycopene beta-cyclase (*CYC-B*) promoter from *Solanum habrochaites*. *BMC Plant Biol* 2010; **10**: 61–76.
97 Corona V, Aracri B, Kosturkova G, Bartley GE, Pitto L *et al*. Regulation of a carotenoid biosynthesis gene promoter during plant development. *Plant J* 1996; **9**: 505–512.
98 Imai A, Takahashi S, Nakayama K, Satoh H. The promoter of the carotenoid cleavage dioxygenase 4a-5 gene of *Chrysanthemum morifolium (CmCCD4a-5*) drives petal-specific transcription of a conjugated gene in the developing flower. *J Plant Physiol* 2013; **170**: 1295–1299.
99 Del Villar-Martínez AA, García-Saucedo PA, Carabez-Trejo A, Cruz-Hernández A, Paredes-López O. Carotenogenic gene expression and ultrastructural changes during development in marigold. *J Plant Physiol* 2005; **162**: 1046–1056.
100 Welsch R, Arango J, Bär C, Salazar B, Al-Babili S *et al*. Provitamin A accumulation in cassava (*Manihot esculenta*) roots driven by a single nucleotide polymorphism in a phytoene synthase gene. *Plant Cell* 2010; **22**: 3348–3356.
101 Fu X, Feng C, Wang C, Yin X, Lu P *et al*. Involvement of multiple phytoene synthase genes in tissue-and cultivar-specific accumulation of carotenoids in loquat. *J Exp Bot* 2014; **65**: 4679–4689.
102 Kishimoto S, Ohmiya A. Carotenoid isomerase is key determinant of petal color of Calendula officinalis. *J Biol Chem* 2012; **287**: 276–285.
103 Schledz M, Al-Babili S, Lintig JV, Haubruck H, Rabbani S *et al*. Phytoene synthase from *Narcissus pseudonarcissus*: functional expression, galactolipid requirement, topological distribution in chromoplasts and induction during flowering. *Plant J* 1996; **10**: 781–792.
104 Al-Babili S, von Lintig J, Haubruck H, Beyer P. A novel, soluble form of phytoene desaturase from *Narcissus pseudonarcissus* chromoplasts is Hsp70-complexed and competent for flavinylation, membrane association and enzymatic activation. *Plant J* 1996; **9**: 601–612.
105 Bonk M, Tadros M, Vandekerckhove J, Al-Babili S, Beyer P. Purification and characterization of chaperonin 60 and heat-shock protein 70 from chromoplasts of *Narcissus pseudonarcissus*. *Plant Physiol* 1996; **111**: 931–939.
106 Von Lintig J, Welsch R, Bonk M, Giuliano G, Batschauer A *et al*. Light-dependent regulation of carotenoid biosynthesis occurs at the level of phytoene synthase expression and is mediated by phytochrome in *Sinapis alba* and *Arabidopsis thaliana* seedlings. *Plant J* 1997; **12**: 625–634.
107 Toledo-Ortiz G, Huq E, Rodríguez-Concepción M. Direct regulation of phytoene synthase gene expression and carotenoid biosynthesis by phytochrome-interacting factors. *Proc Natl Acad Sci USA* 2010; **107**: 11626–11631.
108 Welsch R, Beyer P, Hugueney P, Kleinig H, von Lintig J. Regulation and activation of phytoene synthase, a key enzyme in carotenoid biosynthesis, during photomorphogenesis. *Planta* 2000; **211**: 846–854.
109 Zhou X, Welsch R, Yang Y, Álvarez D, Riediger M *et al*. Arabidopsis OR proteins are the major posttranscriptional regulators of phytoene synthase in controlling carotenoid biosynthesis. *Proc Natl Acad Sci USA* 2015; **112**: 3558–3563.
110 Luo Z, Zhang J, Li J, Yang C, Wang T *et al*. A STAY-GREEN protein SlSGR1 regulates lycopene and β-carotene accumulation by interacting directly with SlPSY1 during ripening processes in tomato. *New Phytol* 2013; **198**: 442–452.
111 Pulido P, Toledo-Ortiz G, Phillips MA, Wright LP, Rodríguez-Concepción M. Arabidopsis J-protein J20 delivers the first enzyme of the plastidial isoprenoid pathway to protein quality control. *Plant Cell* 2013; **25**: 4183–4194.
112 Shumskaya M, Bradbury LMT, Monaco RR, Wurtzel ET. Plastid localization of the key carotenoid enzyme phytoene synthase is altered by isozyme, allelic variation, and activity. *Plant Cell* 2012; **24**: 3725–3741.
113 Shumskaya M, Wurtzel ET. The carotenoid biosynthetic pathway: thinking in all dimensions. *Plant Sci* 2013; **208**: 58–63.
114 Beltrán J, Kloss B, Hosler JP, Geng J, Liu A *et al*. Control of carotenoid biosynthesis through a heme-based cis-trans isomerase. *Nat Chem Biol* 2015; **11**: 598–605.
115 Auldridge M, McCarty D, Klee H. Plant carotenoid cleavage oxygenases and their apocarotenoid products. *Curr Opin Plant Biol* 2006; **9**: 315–321.
116 Ohmiya A, Kishimoto S, Aida R, Yoshioka S, Sumitomo K. Carotenoid cleavage dioxygenase (CmCCD4a) contributes to white color formation in chrysanthemum petals. *Plant Physiol* 2006; **142**: 1193–1201.
117 Rubio-Moraga A, Rambla JL, Fernández-de-Carmen A, Trapero-Mozos A, Ahrazem O *et al*. New target carotenoids for CCD4 enzymes are revealed with the characterization of a novel stress-induced carotenoid cleavage dioxygenase gene from Crocus sativus. *Plant Mol Biol* 2014; **86**: 555–569.
118 Ohmiya A, Sumitomo K, Aida R. 'Yellow Jimba': suppression of carotenoid cleavage dioxygenase (*CmCCD4a*) expression turns white chrysanthemum petals yellow. *J Japanese Soc Hortic Sci* 2009; **78**: 450–455.
119 Yoshioka S, Aida R, Yamamizo C, Shibata M, Ohmiya A. The carotenoid cleavage dioxygenase 4 (*CmCCD4a*) gene family encodes a key regulator of petal color mutation in chrysanthemum. *Euphytica* 2012; **184**: 377–387.
120 Liu Y, Zeng S, Sun W, Wu M, Hu W *et al*. Comparative analysis of carotenoid accumulation in two goji (*Lycium barbarum* L. and L. ruthenicum Murr.) fruits. *BMC Plant Biol* 2014; **14**: 1–14.
121 Ilg A, Bruno M, Beyer P, Al-Babili S. Tomato carotenoid cleavage dioxygenases 1A and 1B: relaxed double bond specificity leads to a plenitude of dialdehydes, mono-apocarotenoids and isoprenoid volatiles. *FEBS Open Bio* 2014; **4**: 584–593.
122 García-Limones C, Schnäbele K, Blanco-Portales R, Bellido ML, Caballero JL *et al*. Functional characterization of FaCCD1: a carotenoid cleavage dioxygenase from strawberry involved in lutein degradation during fruit ripening. *J Agric Food Chem* 2008; **56**: 9277–9285.
123 Beisel KG, Jahnke S, Hofmann D, Köppchen S, Schurr U *et al*. Continuous turnover of carotenes and chlorophyll a in mature leaves of Arabidopsis revealed by $^{14}CO_2$ pulse-chase labeling. *Plant Physiol* 2010; **152**: 2188–2199.
124 Vishnevetsky M, Ovadis M, Vainstein A. Carotenoid sequestration in plants: the role of carotenoid-associated proteins. *Trends Plant Sci* 1999; **4**: 232–235.
125 Li L, Van Eck J. Metabolic engineering of carotenoid accumulation by creating a metabolic sink. *Transgenic Res* 2007; **16**: 581–585.
126 Li L, Yang Y, Xu Q, Owsiany K, Welsch R *et al*. The *Or* gene enhances carotenoid accumulation and stability during post-harvest storage of potato tubers. *Mol Plant* 2012; **5**: 339–352.
127 Lopez AB, Van Eck J, Conlin BJ, Paolillo DJ, O'Neill J *et al*. Effect of the cauliflower *Or* transgene on carotenoid accumulation and chromoplast formation in transgenic potato tubers. *J Exp Bot* 2008; **59**: 213–223.
128 Yuan H, Owsiany K, Sheeja T, Zhou X, Rodriguez C *et al*. A single amino acid substitution of the orange protein causes carotenoid accumulation in Arabidopsis. *Plant Physiology* 2015; [Epub ahead of print].

129 Neta-Sharir I, Isaacson T, Lurie S, Weiss D. Dual role for tomato heat shock protein 21: protecting photosystem II from oxidative stress and promoting color changes during fruit maturation. *Plant Cell* 2005; **17**: 1829–1838.
130 Liu Y, Roof S, Ye Z, Barry C, van Tuinen A *et al.* Manipulation of light signal transduction as a means of modifying fruit nutritional quality in tomato. *Proc Natl Acad Sci USA* 2004; **101**: 9897–9902.
131 Kolotilin I, Koltai H, Tadmor Y, Bar-Or C, Reuveni M *et al.* Transcriptional profiling of *high pigment-2dg* tomato mutant links early fruit plastid biogenesis with its overproduction of phytonutrients. *Plant Physiol* 2007; **145**: 389–401.
132 Mustilli AC, Fenzi F, Ciliento R, Alfano F, Bowler C. Phenotype of the tomato *high pigment-2* mutant is caused by a mutation in the tomato homolog of *DEETIOLATED1*. *Plant Cell* 1999; **11**: 145–157.
133 Davuluri GR, van Tuinen A, Fraser PD, Manfredonia A, Newman R *et al.* Fruit-specific RNAi-mediated suppression of *DET1* enhances carotenoid and flavonoid content in tomatoes. *Nat Biotechnol* 2005; **23**: 890–895.
134 Galpaz N, Wang Q, Menda N, Zamir D, Hirschberg J. Abscisic acid deficiency in the tomato mutant *high-pigment 3* leading to increased plastid number and higher fruit lycopene content. *Plant J* 2008; **53**: 717–730.
135 Pan Y, Bradley G, Pyke K, Ball G, Lu C *et al.* Network inference analysis identifies an *APRR2-like* gene linked to pigment accumulation in tomato and pepper fruits. *Plant Physiol* 2013; **161**: 1476–1485.
136 Pozueta-Romero J, Rafia F, Houlné G, Cheniclet C, Carde JP *et al.* A ubiquitous plant housekeeping gene, *PAP*, encodes a major protein component of bell pepper chromoplasts. *Plant Physiol* 1997; **115**: 1185–1194.
137 Vishnevetsky M, Ovadis M, Itzhaki H, Levy M, Libal-Weksler Y *et al.* Molecular cloning of a carotenoid-associated protein from *Cucumis sativus* corollas: homologous genes involved in carotenoid sequestration in chromoplasts. *Plant J* 1996; **10**: 1111–1118.
138 Simkin AJ, Gaffé J, Alcaraz JP, Carde JP, Bramley PM *et al.* Fibrillin influence on plastid ultrastructure and pigment content in tomato fruit. *Phytochemistry* 2007; **68**: 1545–1556.
139 Kilcrease J, Rodriguez-Uribe L, Richins RD, Arcos JMG, Victorino J *et al.* Correlations of carotenoid content and transcript abundances for fibrillin and carotenogenic enzymes in *Capsicum annum* fruit pericarp. *Plant Sci* 2015; **232**: 57–66.
140 Leitner-Dagan Y, Ovadis M, Shklarman E, Elad Y, Rav David D *et al.* Expression and functional analyses of the plastid lipid-associated protein CHRC suggest its role in chromoplastogenesis and stress. *Plant Physiol* 2006; **142**: 233–244.
141 Kilambi HV, Kumar R, Sharma R, Sreelakshmi Y. Chromoplast-specific carotenoid-associated protein appears to be important for enhanced accumulation of carotenoids in hp1 tomato fruits. *Plant Physiol* 2013; **161**: 2085–2101.
142 Zhang MK, Zhang MP, Mazourek M, Tadmor Y, Li L. Regulatory control of carotenoid accumulation in winter squash during storage. *Planta* 2014; **240**: 1063–1074.
143 Ariizumi T, Kishimoto S, Kakami R, Maoka T, Hirakawa H *et al.* Identification of the carotenoid modifying gene *PALE YELLOW PETAL 1* as an essential factor in xanthophyll esterification and yellow flower pigmentation in tomato (*Solanum lycopersicum*). *Plant J* 2014; **79**: 453–465.
144 Vrebalov J, Ruezinsky D, Padmanabhan V, White R, Medrano D *et al.* A MADS-box gene necessary for fruit ripening at the tomato ripening-inhibitor (*rin*) locus. *Science* 2002; **296**: 343–346.
145 Martel C, Vrebalov J, Tafelmeyer P, Giovannoni JJ. The tomato MADS-box transcription factor RIPENING INHIBITOR interacts with promoters involved in numerous ripening processes in a COLORLESS NONRIPENING-dependent manner. *Plant Physiol* 2011; **157**: 1568–1579.
146 Fujisawa M, Nakano T, Shima Y, Ito Y. A large-scale identification of direct targets of the tomato MADS box transcription factor RIPENING INHIBITOR reveals the regulation of fruit ripening. *Plant Cell* 2013; **25**: 371–386.
147 Welsch R, Maass D, Voegel T, Dellapenna D, Beyer P. Transcription factor RAP2.2 and its interacting partner SINAT2: stable elements in the carotenogenesis of Arabidopsis leaves. *Plant Physiol* 2007; **145**: 1073–1085.
148 Li F, Vallabhaneni R, Wurtzel ET. *PSY3*, a new member of the phytoene synthase gene family conserved in the Poaceae and regulator of abiotic stress-induced root carotenogenesis. *Plant Physiol* 2008; **146**: 1333–1345.
149 Welsch R, Wüst F, Bär C, Al-Babili S, Beyer P. A third phytoene synthase is devoted to abiotic stress-induced abscisic acid formation in rice and defines functional diversification of phytoene synthase genes. *Plant Physiol* 2008; **147**: 367–380.
150 Kachanovsky DE, Filler S, Isaacson T, Hirschberg J. Epistasis in tomato color mutations involves regulation of *phytoene synthase 1* expression by cis-carotenoids. *Proc Natl Acad Sci USA* 2012; **109**: 19021–19026.
151 Liu L, Wei J, Zhang M, Zhang L, Li C *et al.* Ethylene independent induction of lycopene biosynthesis in tomato fruits by jasmonates. *J Exp Bot* 2012; **63**: 5751–5762.
152 Weng L, Zhao F, Li R, Xu C, Chen K *et al.* The zinc finger transcription factor SlZFP2 negatively regulates abscisic acid biosynthesis and fruit ripening in tomato. *Plant Physiol* 2015; **167**: 931–949.
153 Jia H-F, Chai Y-M, Li C-L, Lu D, Luo J-Jet al. Abscisic acid plays an important role in the regulation of strawberry fruit ripening. *Plant Physiol* 2011; **157**: 188–199.
154 Seymour GB, Østergaard L, Chapman NH, Knapp S, Martin C. Fruit development and ripening. *Annu Rev Plant Biol* 2013; **64**: 219–241.
155 Pan Z, Zeng Y, An J, Ye J, Xu Q *et al*. An integrative analysis of transcriptome and proteome provides new insights into carotenoid biosynthesis and regulation in sweet orange fruits. *J Proteomics* 2012; **75**: 2670–2684.
156 Grassi S, Piro G, Lee JM, Zheng Y, Fei Z *et al.* Comparative genomics reveals candidate carotenoid pathway regulators of ripening watermelon fruit. *BMC Genomics* 2013; **14**: 781–800.
157 Barsan C, Zouine M, Maza E, Bian W, Egea I *et al.* Proteomic analysis of chloroplast-to-chromoplast transition in tomato reveals metabolic shifts coupled with disrupted thylakoid biogenesis machinery and elevated energy-production components. *Plant Physiol* 2012; **160**: 708–725.
158 Martínez-López LA, Ochoa-Alejo N, Martínez O. Dynamics of the chili pepper transcriptome during fruit development. *BMC Genomics* 2014; **15**: 143–160.
159 Tranbarger TJ, Dussert S, Joët T, Argout X, Summo M *et al.* Regulatory mechanisms underlying oil palm fruit mesocarp maturation, ripening, and functional specialization in lipid and carotenoid metabolism. *Plant Physiol* 2011; **156**: 564–584.
160 Zeng Y, Pan Z, Ding Y, Zhu A, Cao H *et al.* A proteomic analysis of the chromoplasts isolated from sweet orange fruits [*Citrus sinensis* (L.) Osbeck]. *J Exp Bot* 2011; **62**: 5297–5309.
161 Wang YQ, Yang Y, Fei Z, Yuan H, Fish T *et al.* Proteomic analysis of chromoplasts from six crop species reveals insights into chromoplast function and development. *J Exp Bot* 2013; **64**: 949–961.
162 Nashilevitz S, Melamed-Bessudo C, Izkovich Y, Rogachev I, Osorio S *et al.* An orange ripening mutant links plastid NAD(P)H dehydrogenase complex activity to central and specialized metabolism during tomato fruit maturation. *Plant Cell* 2010; **22**: 1977–1997.