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.

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 \(\zeta\)-carotene desaturase (ZDS) to introduce cis double bonds, and isomerizations by \(\zeta\)-carotene isomerase (Z-ISO) and 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.
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 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 \(\beta\)-ring and an \(\alpha\)-ring forms the \(\beta,\alpha\)-branch of carotenoids, including \(\alpha\)-carotene and its derivatives; the formation of two \(\beta\)-rings creates the \(\beta,\beta\)-branch of carotenoids, containing \(\beta\)-carotene and its derivatives. Lycopene \(\epsilon\)-cyclase (LCYE) and lycopene \(\beta\)-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 \(\epsilon\)- or \(\beta\)-rings, respectively, generating orange \(\alpha\)-carotene or \(\beta\)-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 LCYE exist in papaya and citrus species.\(^{24-26}\) Carotenoids that contain only hydrocarbons are grouped as carotenones, which include phytoene and all types of carotenones.

The addition of oxygen by hydroxylases and epoxidases to cyclic carotenones produces xanthophylls (Figure 1). There are two different types of hydroxylases: the CHY (BCH) type, which hydroxylates the \(\beta\)-ring of cyclic carotenones, and the cytochrome P450 type. Of the latter type, hydroxylases CYP97A and CYP97C hydroxylate the \(\beta\)- and \(\alpha\)-rings, respectively. The orange-colored \(\alpha\)-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 \(\beta,\beta\)-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 \(\beta\)-carotene in the \(\beta,\beta\)-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 \(\beta,\beta\)-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.\(^{28}\)

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 denoted 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 (NCED2, 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, \(\alpha\)-ionone, \(\beta\)-ionone, \(\beta\)-cyclotrin, and \(\beta\)-carotin.\(^{32-38}\) CCD7 and CCD8 are involved in the synthesis of strigolactones from \(\beta\)-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 Cucrus 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.45 A deep crimson fruit (βt106 mutant) produces elevated lycopene and is nearly devoid of β-carotene.46 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 carotenoids, such as capsanthin, lutein, and/or β-carotene, depending on the variety.47 In general, considerably higher levels of total carotenoids are observed in red vegetables than in non-red ones.29,48 A three-locus model (c, y1, and y2) has been proposed for pepper fruit color inheritance.49 CCS appears to be responsible for y and P5Y for c2 in red pepper.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 y1, 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. Additionally, broccoli and many dark leafy vegetables such as spinach, mustard green, and kail 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

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**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 | Lutein | 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 | 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 | 52 |
| Cauliflower | Or      | Orange | β-carotene | 52 |
|             | Orange | Lutein, β-carotene | α-carotene, zeaxanthin | 52 |
| Fruit       | Melon   | Cantaloupe | Orange | β-carotene | 54 |
|             | Citrus  | Orange | Viola-xanthin | β-cryptoxanthin, phytoene | 15 |
|             | Mandarin | Orange | β-cryptoxanthin | Phytoene, violaxanthin, β-carotene | 15 |
|             | Grapefruit | Red | β-carotene or lycopene, phytoene | Phytolfluene | 15 |
| Watermelon  | Red     | Lycopene | β, β-carotene, Viola-xanthin | 21, 41 |
|             | Yellow  | Viola-xanthin or Neoxanthin | Lutein | 21 |
|             | Orange  | β-carotene | Phytoene, lycopene, ζ-carotene | 41 |
| Peach       | Red     | 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 | 66 |
|             | Sunkist | Orange | Viola-xanthin, β-carotene | Lutein | 66 |
|             | Osmanthus | Zi Yingui | Butter yellow | β-carotene | 67 |
|             | Jingui  | Golden yellow | β-carotene, lutein | α-carotene | 67 |
|             | Chenghong Dangu | Orange-red | β-carotene, α-carotene | Lutein | 67 |
| Lily        | Connecticut king | Yellow | Antheraxanthin, violaxanthin, lutein | β-carotene | 68 |
|             | Saja and tiger lily | Red | Capsanthin | Antheraxanthin | 28, 68 |
|             | Red     | Astaxanthin, adonirubin | β-carotene, Astaxanthin | 69 |
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inheritance is controlled by a single gene, green-flesh, which was recently revealed to be the melon or gene. Watermelon is predominantly 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, \( \beta \)/-carotene, and phytene, 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 \( \beta \)/-cryptoxanthin, violaxanthin, lutein, and zeaxanthin; grapefruit and pummelo accumulate primarily phytene, phytofluene, \( \xi \)/-carotene, and \( \beta \)/-carotene; and citrus, 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, phytene, or \( \beta \)/-cryptoxanthin.\(^{57}\) Papaya is commonly observed with red or yellow flesh. Its yellow-fleshed fruit accumulates mainly \( \beta \)/-carotene, \( \beta \)/-cryptoxanthin, and \( \xi \)/-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, \( \beta \)/-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 \( CCDF \).\(^{59-62}\)

For example, carotenoid crystals usually form only from the massive accumulation of lycopene and \( \beta \)/-carotene, such as in red tomato, watermelon, 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.\(^{1,13}\) Thus, regulation of these 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 \( CHYB \).\(^{64,78-79}\) 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 \( \delta \)/-carotene or \( \beta \)/-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 \( \beta \)/-carotene is concomitant with the dramatically increased expression of almost all the upstream genes, including those in the MEP pathway (i.e., \( DXS \), \( DXR \), \( GGR \), \( PSY \), \( ZDS \), \( PDS \), and \( LCYB \)), and the reduced expression of the downstream genes \( CHYB \) and \( LCYE \), channeling metabolic flux away from the \( \alpha \)/\( \beta \) branch toward \( \beta \)/-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 \( \beta \)/-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.\(^{5,19,57,81}\) 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. 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. 

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. Similarly, mutations in melon and Chinese cabbage CRTISO lead to the accumulation of pro-lycopene in mature fruit and inner head leaves, respectively. A recent study in orange carrots revealed that a loss of function in the carotene hydroxylase LCYE promoter from a green-branch product is preferred, whereas in yellow chrysanthemum, LCYE is highly expressed and the β, 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 and in the stigmas of different Cucurbitaceae. 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.

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. The ShLCYB promoter from a green-fruited tomato (Solunum tuberosum) exhibits over fivefold activity in flowers and fruits but shows only a basal level of activity in leaves. High PDS promoter activity was also found in the organs and developmental stages where chloroplasts are formed in tomato. Similarly, the GLCyB, GlBCH, and GlZEP promoters from Gentiana lutea are highly active in chromoplast-containing organs but have minimal activity in non-chromoplast-containing tissues. The promoter of CmCCD4a-S was revealed to drive petal-specific transcription in the developing chrysanthemum flower. 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.

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. 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, during the fruit ripening of normal and red orange, in yellow-versus red-fleshed watermelon, and in flowers, such as between white and yellow marigold, between white chrysanthemum and its yellow bud mutant, and in lilies, 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 mode of 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. 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, in LCYB2 for the accumulation of lycopene in red flesh papaya, in CYP97A3 for the high α-carotene content of orange carrot, in CRTISO for the production of pro-lycopene in mature melon fruit, and in CCS for the formation of orange-colored pepper fruit. The insertion of additional amino acids in CRTISO also disrupts its activity and results in the formation of orange head Chinese cabbage. 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.

Carotenoid biosynthesis occurs in the plastidial membrane. 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 chloroplasts: 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. 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. 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.\textsuperscript{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.\textsuperscript{13,35} 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.\textsuperscript{105} A tomato STAY-GREEN protein, SISR1, regulates lycopene accumulation during fruit maturation via direct interaction with PSY1 in the nucleus to negatively control PSY1 activity by suppressing its transcription.\textsuperscript{310}

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.\textsuperscript{111} Suborganelle localization represents another method to control the competence of biosynthetic enzymes in mediating carotenoid biosynthesis, probably by affecting enzyme stability.\textsuperscript{112,113} Recently, it was discovered that Z-ISO depends on a redox-regulated heme cofactor for enzyme activity to control carotenogenesis.\textsuperscript{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.\textsuperscript{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.\textsuperscript{37} Both CCD1 and CCD4 have been identified as gene families in many horticultural crops.\textsuperscript{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, *CnCCD4a* is highly expressed in white petals but nearly undetectable in yellow petals.\textsuperscript{116} The suppression of *CnCCD4a* expression by RNAi and in *CnCCD4a* in white flower cultivars changed the flower color from white to yellow.\textsuperscript{116,118,119} These findings indicate that in chrysanthemum, *CnCCD4a* 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*\textsuperscript{33,117} and *fragrans*,\textsuperscript{34,167} 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.\textsuperscript{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-fleshed varieties.\textsuperscript{60–62} The attractive orange-reddish color in the peal of citrus fruit is due to the accumulation of the carotenoid cleavage product β-carotin from β-cryptoxanthin and zeaxanthin. The transcription of *CCD4b*, one of the five *CCD4* genes in citrus, is directly related to C30 apocarotenoid production during fruit development and affected by ripening regulators, indicating its important role in regulating carotenoid turnover in citrus fruit.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{64} A correlation between increased *FaCCD1* transcription and decreased lutein content is observed during strawberry ripening.\textsuperscript{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 14CO2 pulse-chase labeling study that showed continuous carotenoid turnover at much high rates than expected in *Arabidopsis* leaves.\textsuperscript{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.\textsuperscript{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 carotenoidal biosynthesis and stable storage.\textsuperscript{126} Lipoprotein-sequestering 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. \textsuperscript{127} Or was originally cloned from an orange curd mutant of cauliflower and encodes a DnaJ cysteine-rich domain-containing protein.\textsuperscript{53} Its mutation triggers the differentiation of non-colored plastids into chromoplasts with an increased capacity to accumulate β-carotene in cauliflower and potato.\textsuperscript{127} Recently, the
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Yellow Petal 1) has been observed to modulate carotenoid level and chromoplast development in tomato flower, 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, APRRR2-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. 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. SISGR1 encodes a STAY-GREEN protein that regulates chlorophyll degradation during fruit ripening in tomato. SISGR1 physically interacts with PSY1 in the nucleus to suppress PSY1 expression and inhibit PSY activity, thus negatively regulating lycopene production. 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. 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. Similarly, a member of the APETALA2 (AP2)/ethylene-responsive element-binding protein transcription factor family, AtAP2P2, binds to the promoters of PSY and PDS to regulate carotenoid biosynthesis in plants.

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, 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. 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.

Light signaling is known to regulate carotenoid accumulation. Light regulates PSY to modulate carotenoid biosynthesis during de-ewilation. Functional analysis of two tomato light signaling genes, HYS and COP1LIKE, has revealed that the suppression of HY4 expression leads to decreased carotenoid levels, and the downregulation of COP1LIKE causes increased carotenoid accumulation, indicating HYS and COP1LIKE play positive and negative roles, respectively, in controlling fruit pigment accumulation. Orange head Chinese cabbage results from a defect in CRTISO. 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
photosomization 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.152 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,18 tomato157 chili pepper158 and oil palm.159 In addition, chloroplast 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.151 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 Or, which has a defect in the NDI (NADH dehydrogenase) complex leading to an altered plastid redox status, has reduced carotenoid accumulation in the chloroplasts 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.

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