

## Review Article

# Research Advances on Biosynthesis, Regulation, and Biological Activities of Apocarotenoid Aroma in Horticultural Plants

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Apocarotenoids, which play important roles in the growth and development of horticultural plants, are produced by the action of carotenoid cleavage oxygenase (CCO) family members or nonenzymatic cleavage actions. Apocarotenoids are commonly found in leaves, flowers, and fruits of many horticultural plants and participate in the formation of pigments, flavors, hormones, and signaling compounds. Some of them are recognized as important aroma components of fruit and flower with aromatic odor, such as  $\beta$ -ionone,  $\beta$ -damascenone, and 6-methyl-5-hepten-2-one in tomato fruit, and have low odor thresholds with  $\beta$ -ionone having odor threshold of only 0.007 ppb. In this review, the main apocarotenoid aroma components in horticultural plants were listed, and factors influencing their production were discussed at first. Then, the biosynthetic pathway of apocarotenoid aromas was briefly introduced, and the CCDs gene family was highlighted, and the nonenzymatic production of apocarotenoid aromas was also mentioned. Next, chemical and molecular regulations of apocarotenoid aromas and their biological activities were summarized. Finally, further exploration aspects needed were suggested. We anticipate that this review can afford some crucial information for comprehensive application of apocarotenoid volatile compounds in horticultural plants.

## 1. Introduction

Aromas of horticultural plants are composed of unique volatile compounds. Although different horticultural plants may have many similar aromas, each plant still has its own inherent aromas, which is determined by the proportion of main components and the existence of unique compounds [1]. Aromatic compounds in horticultural plants include monoterpenes, sesquiterpenes, phenolic derivatives, lipid derivatives, and amino acid derivatives, as well as apocarotenoids [1–4]. Apocarotenoids, such as  $\beta$ -ionone, play important roles in the growth, development, and signaling control of horticultural plants, and they were long assumed to be the products of the oxidative cleavage of carotenoids by the action of carotenoid cleavage oxygenase family members (CCOs) [5, 6]. Apocarotenoids are commonly found in leaves, flowers, and fruits of many horticultural plants and participate in the formation of pigments, flavors, hormones, and signaling compounds

[5, 7–12]. Some of them have aromatic odor and low odor thresholds, which are recognized as important aroma components of fruits, flowers, vegetables, and other horticultural products [13–15]. Moreover, several apocarotenoid aroma compounds are extremely powerful and also can act as beneficial substances for human health [16]. Compared with monoterpenes, sesquiterpenes, phenolic derivatives, lipid derivatives, and amino acid derivatives, the identification, biosynthesis, regulation, and biological activities of apocarotenoids were studied later, and it has attracted widespread attention with the deepening of CCO research studies.

## 2. Apocarotenoid Aroma Components and Influential Factors

Carotenoid degradation (or catabolism) involves a multitude of nonenzymatic and enzymatic processes in horticultural plants and products, which can lead to form various

apocarotenoid aromas or their precursors apocarotenoid glycosides (AGs) [17]. Although AGs can function as a valve regulating carotenoid steady-state levels in leaves contributing to carotenoid homeostasis, they can be used as a flavor reserve and as detoxificants and can be hydrolyzed during development or processing to generate apocarotenoid volatile aroma compounds [13, 18]. For example, AGs usually contribute to fruit and wine aroma in grapevine (*Vitis vinifera*) [18, 19]. The production of apocarotenoid aromas or their precursors AGs is influenced by lots of genetic and environmental factors, which result in rich and colorful aroma components in horticultural plants and products.

**2.1. Apocarotenoid Aroma Components and Classification.** Apocarotenoids, such as  $\beta$ -cyclocitral,  $\beta$ -ionone, geranial, geranyl acetone, theaspiron,  $\alpha$ -damascenone,  $\beta$ -damascenone, and 6-methyl-5-hepten-2-one (MHO), all contribute to the aromas of different flowers and fruits of horticultural plants and are highly valued by the flavor and fragrance industry [8, 20, 21]. Their structures reveal an isoprenoid-based origin and are also named norisoprenoids. These apocarotenoid aromas are characterized in different parts of different horticultural plants (Table 1) and contribute to good quality of many horticultural products.

The aroma components of apocarotenoids can be divided into cyclic and linear types (Figure 1). Cyclic apocarotenoids, including  $\beta$ -ionone,  $\beta$ -cyclocitral, theaspiron,  $\alpha$ -damascenone, and  $\beta$ -damascenone, have very low odor thresholds (our ability to sense it), such as  $\beta$ -ionone whose odor threshold is only 0.007 ppb. Therefore, although they are usually not abundant in fruits or flowers, they are extremely important to the quality of fruits and flowers and have a strong impact on people's perception of the aroma of horticultural products. Linear apocarotenoids, such as 6-methyl-5-hepten-2-one (MHO), geranyl acetone, and geranial, are also important aroma components in fruits and flowers, but their odor thresholds are much higher than those of cyclic apocarotenoids, such as MHO whose odor threshold is 2000 ppb [3, 49, 50].

**2.2. Factors Influencing Apocarotenoid Aromas.** Due to the complex nature of apocarotenoid aromas, many factors influence these aroma components including the genetic makeup, developmental stages, cultural practices, postharvest handlings, storage conditions, and so on. To date, we have limited information about how these factors affect apocarotenoid aroma compositions and their contents resulting to different horticultural product quality.

**2.2.1. Genetics.** Apocarotenoid aromas vary with different cultivars or different organs in horticultural plants. In mandarin hybrid fruit, seven carotenoid-derived volatiles were found in 'Temple': nerol, neral, geranial, neryl acetate,  $\alpha$ -ionone, geranyl acetone, and  $\beta$ -ionone. In contrast, only two of these, neryl acetate and geranyl acetone, were found in 'Murcott' [51]. In the juice of four varieties of citrus (Powell Navel orange (*Citrus sinensis* L.), Clemenules mandarin (*C.*

*reticulata* Blanco.), Fortune mandarin (*C. reticulata* Blanco.) and Chandler Pummelo (*C. maxima* Merr.), 'Clemenules' showed the highest levels of  $\beta$ -ionone [28]. In apple fruits,  $\beta$ -damascenone and estragole in 'Zaofengtian,' which were not detected in 'Vista Bella' or 'Liaofu,' could be unique components to 'Zaofengtian' [52]. The volatiles of 6-methyl-5-hepten-2-one and  $\beta$ -ionone specifically existed in 'Sui hong' papaya fruit at harvest and during 15 d storage period at 25°C, but they were not detected in 'Sui huang' papaya fruit [39]. The content of  $\beta$ -ionone, dihydro- $\beta$ -ionone, and total apocarotenoids in 'Luntaixiaobaixing' (LT) and 'Baixing' (BX) were significantly higher than those in 'Hongyuxing' (HY), 'Danxing' (DX), and 'Yechengheiyeyxing' (YC), especially the  $\beta$ -ionone. Similarly, the contents of apocarotenoids in peels were significantly higher than those in the pulps of cultivars tested [22]. Reidel et al. [27] analyzed the volatile compounds of different organs of *Prunus cerasifera* and *P. cerasifera* 'Pissardii' and found that stem, flowers, and ripe fruits of *P. cerasifera* 'Pissardii' and flowers, gynoecium-androecium-chalice, green fruits, and ripe fruits of *P. cerasifera* emit apocarotenoid aromas. In carrot roots, Yahya et al. [26] found that the volatile norisoprenoids farnesyl acetone,  $\alpha$ -ionone, and  $\beta$ -ionone accumulated in 'Nairobi,' 'Rothild,' and 'Purple Haze' cultivars but not in 'Yellowstone' and 'Creme de Lite' in a pattern reflecting their carotenoid content.

**2.2.2. Developmental Stages.** In tomato fruit, biosynthesis of the apocarotenoids  $\beta$ -ionone, geranyl acetone, and 6-methyl-5-hepten-2-one increased 10- to 20-fold as fruits reached a fully ripened stage [53]. Three apocarotenoids, including  $\beta$ -damascenone,  $\beta$ -ionone, and dihydro- $\beta$ -ionone, were the major aroma compounds in apricot fruits, with  $\beta$ -ionone representing 90% of the three total apocarotenoid aroma volatiles. As the most abundant aroma compounds in apricot, the contents of these three apocarotenoids increased dramatically during fruit ripening [23]. The content of  $\beta$ -ionone increased from 155 to 1875  $\mu\text{g}/\text{kg}$  FW in peels and from 30 to 1026  $\mu\text{g}/\text{kg}$  FW in pulps of all cultivars tested [19]. During flower development in *Boronia megastigma* (Nees),  $\beta$ -ionone levels reached a maximum in opening flowers (stage 3) with the levels reducing to less than half of that in the open flowers (stage 4), and this contrasted with the large increases in  $\beta$ -carotene in open flowers [25].

**2.2.3. Culture Practices.** Variation in N supply had a greater impact on the volatile compositions of grape berries than did variation in P or K supply. Reducing N supply to 'Pinot noir' grapevines resulted in lower total (free + bound)  $\beta$ -damascenone and  $\text{C}_6$  compounds in berries in all three consecutive years, and low K supply resulted in lower total  $\beta$ -damascenone in two of three years. Nutrient supply had a relatively small impact on monoterpenes and other volatile compounds in 'Pinot noir' berries [54].

**2.2.4. Postharvest Handling.** Harvested flowers of *Boronia megastigma* incubated at between 12 and 25°C for up to 24 h showed increased levels of extract of up to 25% with

TABLE 1: Apocarotenoid aroma components and related carotenoid cleavage dioxygenases (CCDs) in some horticultural plants.

Species	Organ (or product)	Components	CCDs	References
Apricot ( <i>Prunus armeniaca</i> L.)	Fruit	$\beta$ -Damascenone, $\beta$ -ionone, dihydro- $\beta$ -ionone	PaCCD1	[22, 23]
Blackberry ( <i>Rubus laciniata</i> L.)	Fruit	$\beta$ -Ionone	Not mentioned	[24]
Boronia ( <i>Boronia megastigma</i> Nees)	Flower	$\beta$ -Ionone and various C <sub>27</sub> apocarotenoids (10'-apocaroten-10'-oic acid, 10'-apocaroten-10'-al, methyl-10'-apocaroten-10'-oate, hydroxy-10'-apocaroten-10'-oic acid, 3-palmitoyloxy-10'-apocaroten-10'-oic acid)	Not mentioned	[25]
Carrot ( <i>Daucus carota</i> L.)	Root	Farnesyl acetone, $\alpha$ -ionone, and $\beta$ -ionone	DcCCD1	[26]
Cherry plums ( <i>Prunus cerasifera</i> Ehrhart)	Stem, gynoecium-androecium-chalice flower, fruit	$\alpha$ -Cyclocitral, 4-keto-isophorone, $\beta$ -cyclocitral, geranyl acetone, $\beta$ -ionone, hexa-hydro farnesyl acetone	Not mentioned	[27]
Citrus ( <i>Citrus</i> spp.)	Fruit	6-Methyl-5-hepten-2-one, geranyl acetone, $\beta$ -ionone	Not mentioned	[28, 29]
Date palm ( <i>Phoenix dactylifera</i> L.)	Fruit	$\alpha$ -Ionone, $\beta$ -ionone, geranyl acetone	Not mentioned	[30]
Elm-leaf blackberry ( <i>Rubus ulmifolius</i> Schot)	Fruit, flower	Geranyl acetone, 6-methyl-5-hepten-2-one, $\beta$ -ionone	Not mentioned	[31]
Grape ( <i>Vitis</i> spp.)	Wine	$\alpha$ -Ionone, $\beta$ -ionone, $\beta$ -damascenone	Not mentioned	[32, 33]
Jasmine ( <i>Jasminum sambac</i> (L.) Ait)	Flower scented green tea	$\beta$ -Ionone, $\beta$ -cyclocitral	Not mentioned	[34]
Kiwifruit ( <i>Actinidia</i> spp.)	Fruit	Geranyl acetone, 6-methyl-5-hepten-2-one, $\beta$ -ionone	Not mentioned	[35, 36]
Mango ( <i>Mangifera indica</i> L.)	Fruit	$\beta$ -Ionone	Not mentioned	[37, 38]
Papaya ( <i>Carica papaya</i> L.)	Fruit	6-Methyl-5-hepten-2-one, $\beta$ -ionone	CpCCD1	[39]
Peach ( <i>Prunus persica</i> L.)	Fruit	3-Hydroxy- $\beta$ -damascenone, 3-hydroxy-5,6-epoxy- $\beta$ -ionone, 4-hydroxy-3,5,6-trimethyl-4-(3-oxo-1-butenyl)-2-cyclohexen-1-one, unknown norisoprenoid-1, unknown norisoprenoid-2, unknown norisoprenoid-3	PpCCD4	[40]
Petunia ( <i>Petunia hybrida</i> Vilm)	Flower	$\beta$ -Ionone	PhCCD1	[41]
Raspberry ( <i>Rubus idaeus</i> L.)	Fruit	$\alpha$ -Ionone, $\beta$ -ionone	Putative RiCCD	[42, 43]
Red grape ( <i>Vitis</i> spp.)	Fruit	$\beta$ -Damascenone	Not mentioned	[44]
Strawberry tree ( <i>Arbutus unedo</i> L.)	Fruit	$\alpha$ -Ionone, $\beta$ -ionone	Not mentioned	[45]
Sweet passion fruit ( <i>Passiflora alata</i> Curtis)	Fruit	Dihydro- $\beta$ -ionone, $\beta$ -ionone	Not mentioned	[46]
Tomato ( <i>Lycopersicon esculentum</i> Mill.)	Fruit	$\beta$ -Ionone, geranyl acetone	LeCCD1A, LeCCD1B	[47, 48]

increases in some compounds including  $\beta$ -ionone [55]. Fresh-cut cantaloupe melon fruit treated with ascorbic acid and sodium azide had higher concentrations of  $\beta$ -ionone and geranyl acetone and retained these compounds better with storage time at both 4°C and 22°C [56].

**2.2.5. Storage Conditions.** Although  $\beta$ -ionone had the highest concentration in raspberry fruits of both 'Sevillana' and 'Maravilla' cultivar, opposing trends in the volatile compound compositions for the cultivars during storage at low temperature (0.5°C, 90–95% RH) were observed and

caused important changes in the volatile compound profile of raspberry. Under low temperature storage, significant decreases in C<sub>13</sub> norisoprenoids (including 1,1,6-trimethyl-1,2-dihydronaphthalene,  $\beta$ -damascenone, dihydro- $\beta$ -ionone,  $\alpha$ -ionone, and  $\beta$ -ionone) and increases in terpenes were observed in 'Sevillana.' These changes are most likely responsible for the aromatic differences between the cultivars because of the presence of terpenes in 'Sevillana' and C<sub>13</sub> norisoprenoids in 'Maravilla' [43]. Storage of cantaloupe melon fruit at 4°C caused a considerable synthesis of the apocarotenoid compounds  $\beta$ -ionone and geranyl acetone and decrease in concentration of esters over a period of 24 h [56].

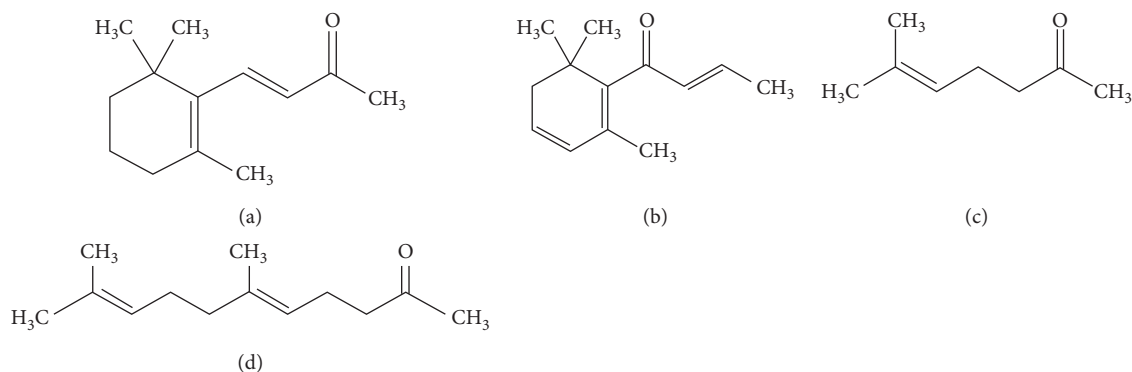


FIGURE 1: Molecular structure of several apocarotenoid aromas in tomato fruit: (a)  $\beta$ -ionone; (b)  $\beta$ -damascenone; (c) 6-methyl-5-hepten-2-one; (d) geranyl acetone.

### 3. Biosynthetic Pathway and Related Genes

As a subclass of isoprenoids, the biosynthesis of carotenoids and apocarotenoids is mainly originated from lycopene which is produced from two universal isoprenoid precursors, isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP). These precursors are produced by the 1-deoxy-D-xylulose-phosphate (DXP) pathway in plastid and the mevalonate (MVA) pathway in cytoplasm (Figure 2) [57].

The key enzymes to form apocarotenoids in horticultural plants are CCOs, which can decompose the carotenoid polyene chain-specific double bonds [5, 6, 16, 61]. CCOs include carotenoid cleavage dioxygenases (CCDs) and nenicis-epoxy-carotenoid dioxygenase (NCEDs). CCOs in *Arabidopsis thaliana* contain nine homologous genes, *AtCCD1*, *AtCCD4*, *AtCCD7*, *AtCCD8*, *AtNCED2*, *AtNCED3*, *AtNCED5*, *AtNCED6*, and *AtNCED9*, which are located on different chromosomes [62]. There are five groups by clustering with CCOs amino acid sequences of different plants, namely, CCD1, CCD4, CCD7, CCD8, and NCEDs [5], while Chen et al. [6] divided 90 CCO genes from 12 species into six groups (i.e., CCD1, CCD4, CCD7, CCD8, NCED, and CCD-like) and found that some CCD8 genes from *Sorghum bicolor*, *Zea mays*, and *Oryza sativa* were not grouped with the CCD8 genes from the other nine species, but clustered in the CCD-like group.

Previous studies have shown that the CCOs related to the formation of apocarotenoid aroma components, which are important for fruit and flower quality, are mainly CCD1 and CCD4 branches [58]. They use different carotenoids as substrates and have different specificities and cleavage sites, thus contributing to the formation of the diversity of apocarotenoid aroma components in horticultural plants (Figure 2) [40, 57–60]. CCD7 and CCD8 branches are mainly involved in the biosynthesis of an important hormone, strigolactone [10, 63, 64], while NCED branch is the key rate-limiting step in ABA biosynthesis [65–67].

*AtCCD1* can catalyze the cleavage of linear and cyclic carotenoids at the 9-10 and the 9'-10' positions, and 1-2 ionone molecules or different oxy-functionalized derivatives were produced based on the different properties of

carotenoid substrates. *In vitro*, *AtCCD1* can use phytoene, zeaxanthin, and neoxanthin as reaction substrates [68, 69]. In addition, studies also have shown that CCD1 can cleave lycopene double bonds at 5, 6 (5', 6') and 7, 8 (7', 8') positions [70, 71]. At present, the homologous genes of CCD1 have been identified in carambola [72], saffron [73], petunia [41], tomato [47], grape [74], nectarine [75], melon [76], citrus [77], strawberry [78], rose [79], osmanthus [80], and other horticultural plants. In tomato fruits, all apocarotenoids could be produced directly from carotenoid precursors through the action of *LeCCD1A* and *LeCCD1B* except for  $\beta$ -damascenone. However, some studies suggest that  $C_{40}$  carotenoids in plastids are firstly decomposed into  $C_{13}$  and  $C_{27}$  apocarotenoids by CCD7 or CCD4, and then the  $C_{27}$  apocarotenoids transported to the cytoplasm were further cleaved under the action of CCD1 to produce aroma components [81–83]. Therefore, the differential expression of CCD1 did not affect the composition and content of carotenoids in fruits of different citrus varieties, but might affect the apocarotenoid aroma compositions of fruits [84].

Compared with CCD1, the research of CCD4 is mainly focused on the relationship between CCD4 and carotenoid contents (i.e., fruit or flower color), and few studies involved apocarotenoid content. Brandi et al. [40] found that *PpCCD4* played an important role in peach flesh color and aroma formation. The carotenoid content of white flesh mutant was lower than that of yellow flesh peach, while the apocarotenoid aroma concentration was higher than that of yellow flesh peach. In citrus fruits, CCD4b1 is responsible for the biosynthesis of  $C_{30}$  apocarotenoids  $\beta$ -citraurin (3-hydroxy- $\beta$ -apo-8'-carotenal) which are key pigments in fruit coloration [85]. *AtCCD4* plays an important role in dark-induced decomposition of carotenoids [86]. RNA interference (RNAi) studies have shown that *CmCCD4a* contributes to the formation of chrysanthemum white petals by decomposing carotenoids [87]. Two *CmCCD4a* RNAi vectors were introduced into white chrysanthemum ('Jimba') by *Agrobacterium tumefaciens*, which could effectively inhibit the expression of *CmCCD4a* in petals and result in yellow petals [88]. Like cultivated chrysanthemum, wild chrysanthemum with white ray petals contains *CmCCD4a* homologous gene, while wild chrysanthemum



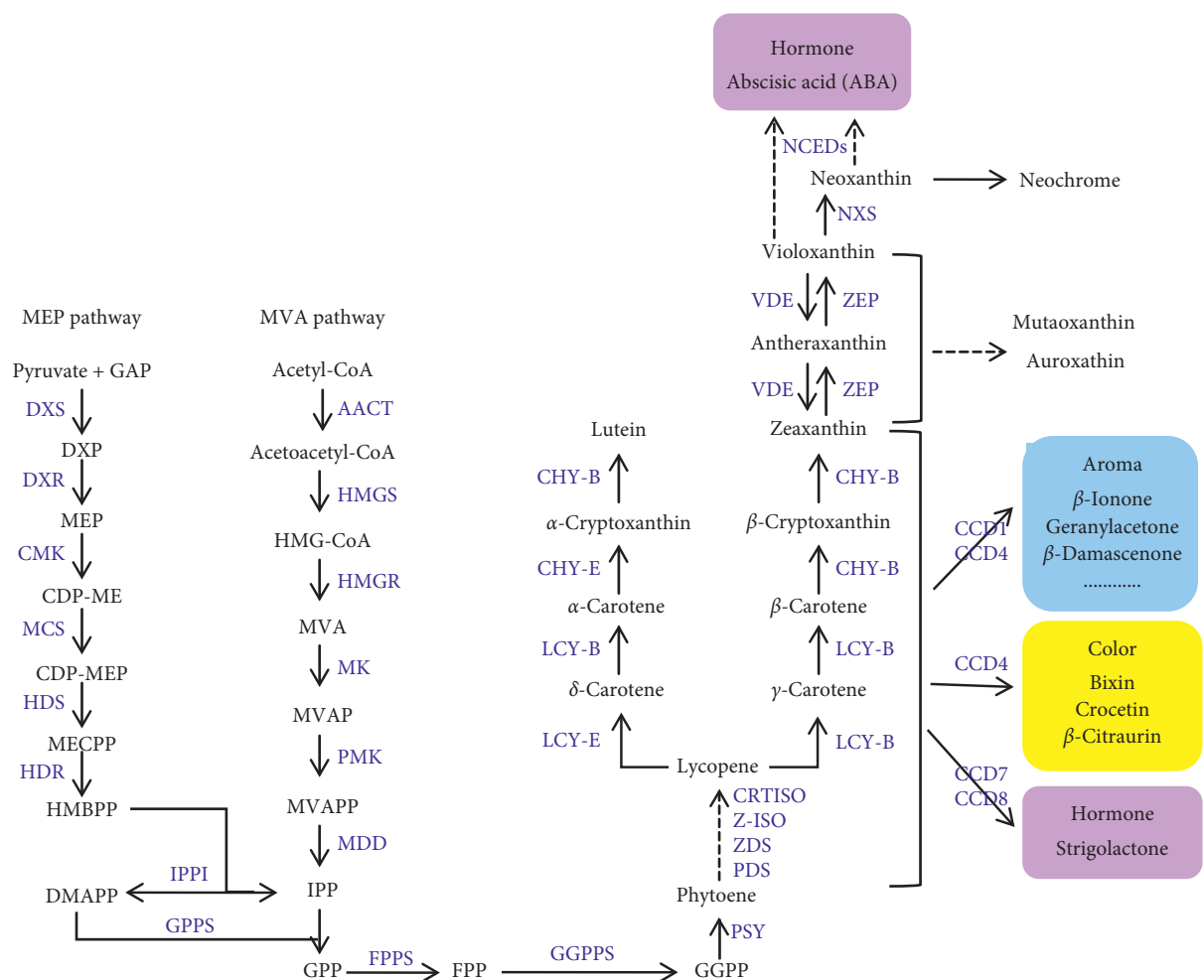


FIGURE 2: Biosynthetic pathway of apocarotenoid aroma in horticultural plants. GAP, glyceraldehyde-3-phosphate; DXP, 1-deoxy-D-xylulose-5-phosphate; DXS, 1-deoxy-D-xylulose-5-phosphate synthase; DXR, 1-deoxy-D-xylulose-5-phosphate reductase; MEP, 2-C-methyl-D-erythritol-4-phosphate; CMK, 2-phospho-4-(cytidine 5'-diphospho)-2-C-methyl-D-erythritol kinase; CDP-ME, 4-(cytidine 5'-diphospho)-2-C-methyl-D-erythritol; MCS, 2-C-methyl-D-erythritol-2,4-cyclodiphosphate synthase; CDP-MEP, 2-phospho-4-(cytidine 5'-diphospho)-2-C-methyl-D-erythritol; HDS, 4-hydroxy-3-methyl-2-E-butenyl-4-disphosphate; MECPP, 2-C-methyl-D-erythritol-2,4-cyclodiphosphate synthase; HDR, 4-hydroxy-3-methyl-2-E-butenyl-4-disphosphate reductase; HMBPP, 4-hydroxy-3-methyl-2-E-butenyl-4-disphosphate; DMAPP, dimethylallyl pyrophosphate; IPP, isopentenyl pyrophosphate; IPPI, isopentenyl pyrophosphate isomerase; GPPS, geranyl pyrophosphate synthase; AACT, aceto acetyl-CoA thiolase; HMGS, 3-hydroxy-3-methyl glutaryl CoA synthase; HMG-CoA, 3-hydroxy-3-methyl glutaryl CoA; HMGR, 3-hydroxy-3-methyl glutaryl CoA reductase; MVA, mevalonate; MK, mevalonate kinase; MVAP, mevalonate pyrophosphate; GPP, geranyl pyrophosphate; FPPS, farnesyl pyrophosphate synthase; FPP, farnesyl pyrophosphate; GGPPS, geranylgeranyl pyrophosphate synthase; GGPP, geranylgeranyl pyrophosphate; PDS, phytoene desaturase; ZDS,  $\zeta$ -carotene desaturase; Z-ISO, CRTISO,  $\zeta$ -carotene isomerase; LCY-B, lycopene- $\beta$ -cyclase; LCY-E, lycopene- $\epsilon$ -cyclase; CHY-B, carotene  $\beta$ -hydroxylase; CHY-E, carotene- $\epsilon$ -hydroxylase; ZEP, zeaxanthin epoxidase; VDE, violaxanthin de-epoxidase; NXS, neoxanthin synthase; NCED, 9-*cis*-epoxycarotenoid dioxygenase; CCD, carotenoid cleavage dioxygenase [36, 57–60].

with yellow ray petals does not contain *CmCCD4a* homologous gene [89].

The expression levels of *MdCCD4*, *CmCCD4a*, *RdCCD4*, *OfCCD4*, and *AtCCD4* were all detected in their respective flowers. The expression levels of *RdCCD4* in rose flowers were 42, 150, and 240 times higher than those in leaves, stems, and roots, respectively. A small amount of carotenoids accumulated in perianth slices of pink and white lily cultivars was not regulated by transcription level of synthetic

related genes, which may be related to the reduction of carotenoid content by *CCD4* cleavage [90].

Besides catabolism by related CCD gene family members (CCDs), the unstable carotenoid can also generate apocarotenoid aromas through nonenzymatic cleavage at different sites by reactive oxygen species produced in oxidizing environments such as high (or low) temperature, strong light, metal catalysts, and water stress. All of the in-chain double bonds within carotenoid molecular can be attacked, leading

to produce different apocarotenoid components, and the number of apocarotenoid species formed nonenzymatically exceeds the number of those that are enzymatically formed [91].

#### 4. Chemical and Molecular Regulation

Apocarotenoid aromas occur in all sorts of horticultural plants and products with different components and concentrations. On account of their beneficial effects, the predominant components can be regulated through chemical treatment, gene overexpression or silencing, and transcription factor expression.

**4.1. Enzyme Inhibitor.** An inhibitor of CCD enzymes, Abamine SG, decreased the levels of both isoprenoid and nonisoprenoid volatiles such as 3-hydroxy- $\beta$ -damascenone and 3-hydroxy-5,6-epoxy- $\beta$ -ionone in 'Redhaven Bianca' peach fruit (white-fleshed bud sport mutant of yellow-fleshed 'Redhaven') [40].

**4.2. Overexpression of CCD Gene.** The additional integration of the carotenoid cleavage dioxygenase gene from the plant *Petunia hybrida* (*PhCCD1*) led to the production of low amounts of  $\beta$ -ionone ( $0.073 \pm 0.01$  mg/g DCW) and changed the color of the strain of *Saccharomyces cerevisiae* from orange to yellow [92]. Carotenoid-accumulating *E. coli*-strains were transformed either with pTHIO-*StCCD4* or the void pTHIO-plasmid which served as negative control; *StCCD4* catalyzed the cleavage of all-*trans*- $\beta$ -carotene at the C9'-C10' double bond, leading to  $\beta$ -ionone and all-*trans*- $\beta$ -apo-10'-carotenal, both *in vivo* and *in vitro* [93]. Overexpression of *OfCCD4* from *Osmanthus fragrans* in *E. coli* increased CCD4 activity and the concentration of  $\beta$ -ionone [94].

**4.3. Silencing of CCD Gene.** In tomato fruit, silencing *LeCCD1A* and *LeCCD1B* could result in a significant decrease of  $\beta$ -ionone content in mature fruit [47]. Successful knockout of *InCCD4* using the CRISPR/Cas9 system in the white-flowered cultivar *Ipomoea nil* cv. AK77 caused the white petals to turn pale yellow, and the total amount of carotenoids in the petals of *ccd4* plants was increased 20-fold relative to nontransgenic plants [95], but the change of apocarotenoid aroma components deserved further study.

**4.4. Transcriptional Regulation.** *OfWRKY3* in *Osmanthus fragrans*, which can bind to the W-box palindrome motif present in the *OfCCD4* promoter, is a positive regulator of the *OfCCD4* gene and might partly account for the biosynthesis of  $\beta$ -ionone in sweet osmanthus [96]. The expression pattern of *OfERF61* closely resembled that of *OfCCD4*, and overexpression of *OfERF61* upregulates the transcript levels of *NbCCD4* in tobacco leaves, suggesting that ERF61 may participate in CCD4 regulation and  $\beta$ -ionone production in sweet osmanthus [97].

#### 5. Biological Activities

As secondary metabolites, apocarotenoid aromas not only affect plant growth, development, and signaling control but also have numerous biological activities, including free radical scavenging, sunburn preventive, chemopreventive, antimicrobial, insect repellent, and attractive effects. Based on *in vitro* and *in vivo* experiments, some apocarotenoid aromas will finally be used to treat human ailments and protect the environment.

**5.1. Free Radical Scavenging Activity.** Both as fragrant acyclic terpenoids, C13 apocarotenoid geranyl acetone was significantly more effective as an ABTS (2,2'-amino-di-2-ethylbenzothiazoline sulphonic acid-6-ammonium salt) and DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenger than monoterpene geraniol. According to the computational results, the presence of allylic H-atom (at the position <sup>1</sup>C) close to the -OH group seemed to be essential for the observed antiradical activity of geraniol, while the scavenging ability of geranyl acetone was associated with the presence of both allylic hydrogen and alkylic hydrogen in the close vicinity of the carbonyl functionality [98].

**5.2. Sunburn Prevention Effect.** To establish sunburn preventive activity, female Skh-1 mice were given oral  $\beta$ -damascenone followed by irradiation with UVR from fluorescent 'sunlamps.' The findings demonstrated that  $\beta$ -damascenone protected against sunburn by activating a sebaceous gland-based pathway that fortified and thickened the cornified envelope plus sebum layer in a way that previously has been observed to occur only in keratinocytes [99].

**5.3. Chemopreventive Effect.**  $\beta$ -Ionone demonstrates potent anticancer activity both *in vitro* and *in vivo* [100].  $\beta$ -Ionone showed promising chemopreventive effects during promotion of hepatocarcinogenesis by acting through distinct mechanism of actions:  $\beta$ -ionone may inhibit cell proliferation and modulate 3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase, and geraniol can induce apoptosis and inhibit RhoA activation [101].  $\beta$ -Ionone also effectively ameliorated the lung carcinogenesis, which is attributed to the antiproliferative and antioxidant potential through free radical scavenging property [102].  $\beta$ -Ionone has potent ability to arrest cell cycle of SGC-7901 cells and decrease proliferation through a MAPK pathway by transcriptional downregulation of cell cycle proteins [103].  $\beta$ -Ionone also can suppress DMBA-initiated mammary cancer in rats [100].

**5.4. Antimicrobial Activity.** Essential oils from lots of horticultural plants contain apocarotenoid volatiles, such as  $\beta$ -damascenone and  $\beta$ -ionone from underground parts (root and rhizome) of *Gentiana asclepiadea* [104], 2-hydroxy- $\beta$ -ionone from *Sisymbrium officinale* [105], 6-methyl-5-hepten-2-one from *Anthemis tenuisecta* [106], and *cis*-

geranyl acetone from sterile stems of *Equisetum arvense* [107]. The disk diffusion method or minimum inhibitory concentration procedure was used for the evaluation of the antimicrobial activity of essential oil, and results demonstrated that all the essential oils exhibit great potential antimicrobial activity against all tested strains [104–107].

**5.5. Insect Repellent.** In plants, the oxidative cleavage of carotenoid substrates produces volatile apocarotenoids, including  $\alpha$ -ionone,  $\beta$ -ionone, and dihydro- $\beta$ -ionone, compounds that are important in herbivore-plant communication.  $\beta$ -Ionone has a strong repellent effect toward both the flea beetle and the spider mite and significant oviposition deterrence to whiteflies. In contrast, dihydro- $\beta$ -ionone had attractant properties, especially to the crucifer flea beetle, while  $\alpha$ -ionone did not show any significant activity [108]. *AtCCD1* overexpression and induced  $\beta$ -ionone emission might find application in the control of pests for *Brassica* crops grown in greenhouse operations. Potentially,  $\beta$ -ionone could also be used on crops grown in open-air ecosystems if this allomone is released in sufficient quantities to discourage herbivore foragers [109].

**5.6. Insect Attractant.**  $\beta$ -Ionone can be used as baits to attract insects. Males of *Euglossa mandibularis* were consistently captured in scent traps baited with  $\beta$ -ionone in areas of Mixed Ombrophilous Forests or transition between this latter physiognomy and Montane Semideciduous Forest at Parque Nacional do Iguaçu, Paraná state, Brazil [110]. Geranyl acetone may be a relatively common pheromone structure for species in the subfamily Spondylidinae. *Asemum caseyi* are attracted to geranyl acetone alone, while *Asemum nitidum* are attracted to blends of fuscumol and geranyl acetone [111]. By using this kind of aroma as pheromone to attract insects, less pesticide will be used and environmentally conscious production will be achieved.

## 6. Perspective

At present, many studies of apocarotenoid aromas were carried out on model plants or microorganisms. An optimized HS-SPME-GC/MS method for volatile apocarotenoid detection and quantification was validated with *Arabidopsis in vitro* and *in planta* [11]. This method can enable us to find more apocarotenoid aromas in horticultural plants and products. The strategy of *Escherichia coli*-based modular pathway optimization and enzyme engineering to overproduce natural  $\alpha$ -ionone and  $\beta$ -ionone was developed, demonstrating the great potential of using microbes or horticultural plants in production of natural flavors and fragrances [57, 86]. Although many studies proved that essential oils containing apocarotenoid aromas from horticultural plants possess antimicrobial activity, little is known about the effect of individual apocarotenoid aromas in different essential oils [104–107].

Apocarotenoid aromas are widely present in different parts (fruits, flowers, leaves, stems, and roots) of horticultural plants and possess different biological activities. The

research on apocarotenoid aromas in horticultural plants is incomplete and unsystematic. The following aspects need further exploration: (1) characterization of more components of apocarotenoid aromas; (2) separation and purification of individual apocarotenoid aroma; (3) biological activities of individual apocarotenoid aroma to plants, animals, and humans; (4) dynamic changes of apocarotenoid aromas during growth; (5) relationship between concentration of apocarotenoid aromas and contents of carotenoids in fruits or flowers; (6) development of new products of apocarotenoid aromas; (7) production of fruits and flowers with elevated levels of apocarotenoid aromas; and (8) production of natural flavors and fragrances by transgenic engineering. With the development of science and technology, more research studies will be carried out to demonstrate the function of apocarotenoid aromas, and these volatile compounds will be exploited not only for plant protection but also for human and animal health.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

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