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Mechanisms suppressing carotenoid accumulation in flowers differ depending on the hybrid groups of lilies (*Lilium* spp.)

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ABSTRACT

White is among the most important flower color hues in floricultural plants. Accumulation of visible pigments, including carotenoids, is restricted in white flowers. Mechanisms that suppressed carotenoid accumulation were evaluated in lily (*Lilium* spp.) tepals. Carotenoid content reduced with the development of flower buds in white and pink (caused by anthocyanins) tepals of lilies. As the enzyme carotenoid cleavage dioxygenase 4 (CCD4) degrades carotenoids and is responsible for white flower development in some plant species, the *CCD4* gene was isolated in lilies and its expression profiles were investigated: one day before anthesis when tepals became white, CCD4 was expressed in Asiatic and Oriental hybrid lilies, but was expressed only at low levels in *L.* × *formolongi* and *L. longiflorum* (a longiflorum group). Next, carotenoid biosynthesis genes were isolated and their expression was evaluated. Expression of most of the biosynthesis genes was seen in Asiatic hybrid lilies; however, expression of phytoene synthase (*PSY*) and β-ring hydroxylase (*HYB*) genes was downregulated in the longiflorum group, and expression of several genes, including *PSY* and ε-ring hydroxylase, was suppressed in Oriental hybrid lilies. Thus, the mechanisms that suppress carotenoid accumulation might be different among the lily groups: Suppression of *PSY* and *HYB* expression was the major mechanism in the longiflorum group lilies, elevated expression of *CCD4* was mainly responsible in Asiatic hybrid lilies, and both biosynthesis gene suppression and upregulation of *CCD4* were involved in Oriental hybrid lilies.

1. Introduction

Flowers often owe their colors to anthocyanins and carotenoids that play a major role in attracting pollinators. Anthocyanins are responsible for purple, blue, and pink pigmentation, while carotenoids are the major pigments that offer yellow and orange colors to petals (Tanaka et al., 2008). Carotenoids also play essential roles in the construction of light-harvesting antenna complexes in photosynthesis and in photoprotection against photooxidative damage to plant cells (Grotewold, 2006; Walter and Strack, 2011), and act as precursors of the plant hormones abscisic acid (Tan et al., 2003) and strigolactones (Zwanenburg et al., 2016).

Carotenoids are 40-carbon isoprenoid compounds made of the five-carbon units isopentenyl diphosphate (IPP) and its double-bond isomer dimethylallyl diphosphate (DMAPP), which are mainly produced by the plastidial 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway (Giuliano, 2014). Most of the genes necessary for carotenoid biosynthesis have been identified in plants (Fig. 1, Cunningham and Gantt, 1998; Ruiz-Sola and Rodríguez-Concepción, 2012). The addition of three IPP molecules to DMAPP generates geranylgeranyl diphosphate (GGPP), which is catalyzed by GGPP synthase (GGPS). Phytoene synthase (PSY) produces colorless carotenoid phytoene by the condensation of GGPP. Subsequent desaturation and isomerization of phytoene eventually results in the production of lycopene, a red carotenoid; and the four enzymes phytoene desaturase (PDS), zeta-carotene isomerise (Z-ISO), zeta-carotene desaturase (ZDS), and carotenoid isomerise (CRTISO), are involved in this step. The cyclization of the ends of the lycopene polyene chain is the first branch point in the pathway, and lycopene β-ring cyclase (LCYB) and/or lycopene ε-ring cyclase (LCYE) produce β-carotene (two β-rings: β,β-carotenoid) or α-carotene (one β-ring and one ε-ring: β,ε-carotenoid). The hydroxylation of β- and ε-rings of carotenes generates xanthophylls such as lutein (β,ε-carotenoid) and zeaxanthin (β,β-carotenoid). Two different types of carotenoid hydroxylases have been found in plants; non-heme di-iron enzymes (β-ring

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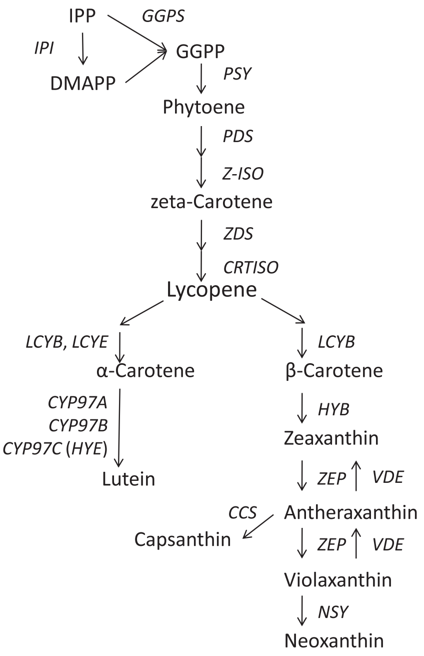

Fig. 1. Carotenoid biosynthesis pathway in higher plants. IPP, isopentenyl diphosphate; DMAPP, dimethylallyl diphosphate; GGPP, Geranylgeranyl diphosphate; *IPI*, IPP isomerase; *GGPS*, GGPP synthase; *PSY*, phytoene synthase; *PDS*, phytoene desaturase; *Z-ISO*, zeta-carotene isomerase; *ZDS*, zeta-carotene desaturase; *CRTISO*, carotenoid isomerase; *LCYB*, lycopene β-cyclase; *LCYE*, lycopene ε-cyclase; *HYB*, β-ring hydroxylase; *HYE*, ε-ring hydroxylase; *CYP97*, cytochrome P450 family 97; *CCS*, capsanthin-capsorubin synthase; *ZEP*, zeaxanthin epoxidase; *VDE*, violaxanthin de-epoxidase; *NSY*, neoxanthin synthase.

hydroxylase, HYB), which are most active in the hydroxylation of the β-ring in β,β-carotenoids, and cytochrome P450 enzymes (cytochrome P450 family 97, CYP97), which catalyze the hydroxylation of β,ε-carotenoids. CYP97A and CYP97B are active on β-rings, whereas CYP97C can efficiently hydroxylate both β- and ε-rings. Zeaxanthin is epoxidated twice to synthesize violaxanthin, and the activities of zeaxanthin epoxidase (ZEP) and violaxanthin de-epoxidase (VDE) balance the ratio of zeaxanthin, antheraxanthin, and violaxanthin (the xanthophyll cycle). Zeaxanthin is subsequently catalyzed by neoxanthin synthase (NSY) to form neoxanthin. These carotenoids are further modified to generate the wide variety of carotenoids, e.g., capsanthin and/or capsorubin accumulated in pepper fruits are synthesized by capsanthin-capsorubin synthase (CCS, Hirschberg, 2001).

The main carotenoids accumulated in leaves are lutein, violaxanthin, neoxanthin, and β-carotene, and there are little differences in them among higher plants. However, carotenoids show the highest variation in composition in fruits, roots, and flowers, depending on plant species (Tanaka et al., 2008). For example, lycopene is the major carotenoid in ripe tomato fruits (Rosati et al., 2000). High levels of α- and β-carotene accumulate in orange carrot taproots, but lutein is the major carotenoid in yellow carrot cultivars (Ma et al., 2017). In flowers, Asteraceae species, including marigold (*Tagetes* sp.) and chrysanthemum (*Chrysanthemum morifolium*), predominantly accumulate lutein and its derivatives (β,ε-carotenoids, Kishimoto et al., 2004; Moehs et al., 2001). The petals of *Adonis* species accumulate large amounts of astaxanthin and other ketocarotenoids, which are often found in bacteria, fungi, and algae, but are very rare in higher plants (Cunningham and Gantt, 2005). Capsanthin and/or capsorubin are major pigments found in orange lily flowers (Deli et al., 1998; Yamagishi et al., 2010a).

In higher plants, carotenoid cleavage dioxygenases (CCDs) enzymatically cleave carotenoids and produce a diverse set of apocarotenoids. The CCD enzyme family consists of CCD1, CCD4, CCD7, CCD8, and nine-cis-epoxycarotenoid dioxygenases (NCEDs). NCEDs are specialized CCDs involved in ABA biosynthesis, and NCED3, NCED5, NCED6, and NCED9 in Arabidopsis contribute to ABA synthesis in developing seeds. A novel apocarotenoid hormone strigolactone is produced by CCD7 and CCD8 in roots and transported to shoots, where it inhibits shoot branching. CCD1 is a cytosolic enzyme involved in flavor and fragrance production in flowers and fruits (Auldridge et al., 2006; Walter and Strack, 2011). CCD4 acts within plastids and is associated with the decrease of carotenoid content in flowers, fruits, and tubers; for example, in chrysanthemum, CmCCD4a is specifically transcribed in flowers and metabolizes yellow carotenoids into colorless derivatives resulting in white petals (Ohmiya et al., 2006). CCD4s contribute to the range of yellow and white color in potato tubers (Campbell et al., 2010) and peach fruit flesh (Brandi et al., 2011; Falchi et al., 2013). CCD4 is also involved in volatile emission in saffron flowers (Rubio et al., 2008) and grape fruits (Lashbrooke et al., 2013), and in the production of β-citraurin, an apocarotenoid pigment, in citrus fruits (Ma et al., 2013).

White is an important color hues in flowers of floricultural plants. Accumulation of visible pigments, including anthocyanins and carotenoids, is restricted in white flowers. Regulation mechanisms of anthocyanin accumulation are well evaluated: The presence or absence of anthocyanin pigments in flowers is predominantly regulated by R2R3-MYB transcription factors, such as AN2 in petunias and ROSEA in snapdragons, and when plants do not express these *R2R3-MYB* genes, their flowers do not accumulate anthocyanins (Yuan et al., 2013). In contrast, mechanisms that change carotenoid levels are not fully understood. Two major mechanisms that reduce carotenoid contents have been reported in flowers of higher plants; carotenoid degradation (Tanaka et al., 2008), and low-level expression of carotenoid biosynthesis genes (Yamamizo et al., 2010). *CmCCD4a* contributes to the white color formation in ray florets of chrysanthemum (Yoshioka et al., 2012), and the silencing of *CmCCD4a* by RNAi alters the petal color from white to yellow (Ohmiya et al., 2006, 2009). Involvement of CCD4 in white flower color has also been reported in *Brassica* species (Zhang et al., 2015). *PSY* is an important gene catalyzing the first step of the carotenoid biosynthesis pathway and controls the metabolic flow of carotenoid biosynthesis (Rodríguez-Villalón et al., 2009). Morning glory (*Ipomoea nil*) flowers accumulate trace amounts of carotenoids because the transcription of carotenoid biosynthesis genes, including *PSY*, is suppressed (Yamamizo et al., 2010). Expression levels of *PSY* and *LCYE*, and those of *PSY*, *LCYB*, and *LCYE*, are low in white flowers of carnation (Ohmiya et al., 2013) and *Eustoma* (Liu et al., 2013), respectively.

Lily is among the most valuable floricultural plants around the world. The genus *Lilium* consists of more than 90 species and is further classified into seven sections (Comber, 1949). Interspecific hybridization is the principal method of lily breeding; species belonging to sections Sinomartagon and Daurolirion have been used to develop Asiatic hybrid lilies (*Lilium* spp.). Oriental hybrid lilies (*Lilium* spp.) are derived from interspecific hybridization among species of the section Archelirion, including *L. auratum* and *L. speciosum*, which are mainly distributed in Japan. *Lilium* × *formolongi* (longiflorum hybrid) is a hybrid of *L. formosanum* and L. longiflorum, belonging to the section Leucolirion (Leslie, 1982).

Anthocyanins and carotenoids are the major pigments accumulated in lily flowers (Yamagishi, 2013). In Asiatic hybrid lilies, many cultivars contain carotenoid pigments (yellow or orange) in their tepals, and the tepals of some cultivars show anthocyanin pigmentation (pink, Nakano et al., 2005). The yellow tepals contain antheraxanthin, (9Z)-violaxanthin, *cis*-lutein, and violaxanthin, and orange tepals accumulate capsanthin (Jeknić et al., 2012; Yamagishi et al., 2010a). Oriental hybrid lily cultivars and the species of the section Archelirion develop white or pink flowers and scarcely accumulate any carotenoids in their tepals, but *L. auratum* develops yellow stripes along the midribs on