Review

Studies on Carotenoids in the Petals of Compositae Plants

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Carotenoids are one of the most important pigments for petal coloration in the yellow to red range; however, knowledge of carotenoids in petals is relatively limited. To better understand flower carotenoids, we analyzed carotenoid composition, the expression of carotenogenic genes, and the relationship between pigment composition and petal color in petals of Compositae plants, including chrysanthemums. We found that petals of yellow-flowered chrysanthemums have a unique carotenoid composition, and that the formation of white petal color in chrysanthemums involves carotenoid degradation catalyzed by carotenoid cleavage dioxygenase (CmCCD4a). We also showed three routes to an orange petal color via carotenoid components in 9 Compositae plants. In addition, we identified (5Z)-carotenoids that contribute orange color formation in calendula petals. In this review, we summarize our studies on carotenoids in the petals of Compositae plants.

Key Words: Calendula officinalis L., carotenoid cleavage dioxygenase (CmCCD4a), Chrysanthemum morifolium Ramat., cis-isomers, petal color.

Introduction

Carotenoids are responsible for petal colors in the yellow to red range. The role of these pigments is thought to be the attraction of insects that aid in pollination. In the green tissues of higher plants, carotenoids have an important function in photosynthesis and protecting tissues against photooxidative damage (Robert et al., 2004; Ruban et al., 2007). Carotenoids also have critical roles in human health as antioxidants and vitamin A precursors.

With a few exceptions, carotenoids possess a terpenoid moiety and are C40 isoprenoids. More than 700 naturally occurring carotenoids have now been identified (Britton et al., 2004). Carotenoid biosynthesis starts from one ‘isoprene unit’, C5-isopentenyl pyrophosphate (IPP) (Fig. 1; see reviews by Cunningham and Gantt, 1998; Fraser and Bramley, 2004; Giuliano et al., 2008; Sandmann et al., 2006). Four IPPs are condensed to C20-geranylgeranyl pyrophosphate (GGPP), and two molecules of GGPP are converted to phytoene, the first C40 carotenoid, by phytoene synthase (PSY). In plants, phytoene is converted via ζ-carotene, the first yellow carotenoid, to lycopene by two structurally and functionally similar enzymes, phytoene desaturase (PDS) and ζ-carotene desaturase (ZDS). This pathway produces poly-cis intermediates that are converted to all-trans configurations through the action of two types of isomerases, ζ-carotene isomerase (Z-ISO), recently identified in maize (Li et al., 2007), and carotenoid isomerase (CRTISO). Subsequently, the ends of the linear carotenoid lycopene can be cyclized by lycopene β-cyclase (LCYB) and/or lycopene ε-cyclase (LCYE), and the molecule is then modified to express a variety of structural features by hydroxylation, epoxidation, or isomerization. Genes encoding enzymes of the carotenoid biosynthetic pathway were first identified in bacteria (Marrs, 1981). Most of those genes have subsequently been identified in various organisms (see reviews by Cunningham and Gantt, 1998; Fraser and Bramley, 2004; Sandmann et al., 2006).

Chrysanthemum (Chrysanthemum morifolium Ramat.) is one of the most important ornamental plants in the world. Their petal color originates mainly from carotenoid and anthocyanin pigments (Kawase and Tsukamoto, 1976). Yellow- and white-flowered cultivars are in great demand for funeral ceremonies in Japan, but the quality of yellow-flowered cultivars for cut-flower production, such as vigor, stem elongation ability and early flowering ability, is generally inferior to that of white-flowered cultivars. For this reason, the production of yellow-flowered cultivars has been falling gradually. Presently, the development of yellow-flowered cultivars with the same quality as white-flowered cultivars is in great demand from both floriculture markets and...
floriculture farmers. The production of orange-flowered cultivars is markedly lower than that of white- and yellow-flowered cultivars. The orange petal color in chrysanthemums results from a mixture of anthocyanins and carotenoids. It therefore lacks brightness, especially under fluorescent lights. In addition, the orange color is unstable because anthocyanin expression is susceptible to plant growth condition, mainly temperature (Dela et al., 2003; Nozaki et al., 2006). These are considered to be negative factors for the distribution of orange-flowered cultivars.

It is important to control petal color in order to improve these drawbacks of yellow- and orange-flowered chrysanthemum cultivars; however, little was known about carotenoids in chrysanthemum petals, which is the responsible for both yellow and orange petal coloration. Consequently, we tried to analyze the carotenoid components, the genes that regulate flower color due to the presence of carotenoids, and the relationship between pigment composition and petal color in the petals of Compositae plants, including chrysanthemum. In this review, we will summarize our recent study with the major focus on carotenoid composition in the petals of Compositae plants.

1. Carotenoid composition and expression of carotenogenic genes in petals and leaves of chrysanthemum

1) Carotenoid composition in petals and leaves

The green tissues of most plants show similar carotenoid profiles, containing both β,ε-carotenoids (α-carotene derivatives), carotenoids having both β- and ε-
ionone rings at each end, and β,β-carotenoids (β-carotene derivatives), carotenoids having β-ionone rings in both sides (Goodwin and Britton, 1988). The essential carotenoids for plant photosynthesis, such as lutein, zeaxanthin, violaxanthin, and antheraxanthin, are invariably found in the green tissues. In contrast, carotenoids in flowers show distinctive compositions that depend on the plant species. For example, petals of tiger lily (Lilium lancifolium Thunb.) contain only β-carotene derivatives (Deli et al., 2000). Compositae plants tend to accumulate mainly lutein and lutein derivatives, α-carotene derivatives, in their petals; for example, African marigolds (Tagetes erecta L.) accumulate a large amount of lutein (~91% of their total carotenoids) (Khachik et al., 1999).

Some carotenoids in chrysanthemum petals have been characterized by TLC and absorption spectroscopy (Karrer and Jucker, 1943; Karrer et al., 1945; Kawase and Tsukamoto, 1976); however, accurate molecular structures had not been determined. Tóth and Szabolcs (1981) characterized eight carotenoids, including five mono-Z-isomers, using synthetic standard preparations, but they were unable to identify several unknown components. We therefore analyzed the carotenoid composition of chrysanthemum petals by FAB-MS and NMR and identified sixteen xanthophylls (Table 1 and Fig. 2; Kishimoto et al., 2004). All of these carotenoids, except for (9Z)-violaxanthin and (9Z,8R)-luteoxanthin, were lutein and its derivatives (approximately 92% of the total carotenoids). Five di-Z geometrical isomers of lutein-5,6-epoxide and (3S,5S,6R,3'R,6'R)-5,6-dihydro-5,6-dihydroxylutein (1) had never before been identified as natural products (Table 1 and Fig. 3). We also isolated various isomers, such as eight geometrical isomers of lutein-5,6-epoxide (i.e., the all-E form (2), two mono-Z forms, and five di-Z forms (3); Fig. 3), three geometrical

### Table 1. Carotenoid composition in petals of chrysanthemum (‘Sunny Orange’).

<table>
<thead>
<tr>
<th>Peak no. (Fig. 2)</th>
<th>Carotenoids</th>
<th>% of total carotenoids</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(3S,5S,6R,3'R,6'R)-5,6-Dihydro-5,6-dihydroxylutein (1)</td>
<td>5.1</td>
</tr>
<tr>
<td>2</td>
<td>(9Z,13Z)-Lutein-5,6-epoxide</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>(13Z,9Z)-Lutein-5,6-epoxide</td>
<td>1.8</td>
</tr>
<tr>
<td>3</td>
<td>(9Z,13Z)-Lutein-5,6-epoxide (3)</td>
<td>2.2</td>
</tr>
<tr>
<td>4</td>
<td>(9Z,13Z)-Lutein-5,6-epoxide</td>
<td>2.0</td>
</tr>
<tr>
<td>5</td>
<td>(all-E)-Lutein-5,6-epoxide (2)</td>
<td>7.7</td>
</tr>
<tr>
<td>6</td>
<td>(9Z,9Z)-Lutein-5,6-epoxide</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>(9Z)-Violaxanthin</td>
<td>2.7</td>
</tr>
<tr>
<td>7</td>
<td>(8S)-Lutein-5,8-epoxide</td>
<td>5.0</td>
</tr>
<tr>
<td>8</td>
<td>(8R)-Lutein-5,8-epoxide</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>(9Z,8R)-Luteoxanthin</td>
<td>1.8</td>
</tr>
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<td>9</td>
<td>(9Z)-Lutein-5,6-epoxide</td>
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<td>(9Z)-Lutein-5,6-epoxide</td>
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</tr>
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<td>11</td>
<td>(all-E)-Lutein</td>
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</tr>
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<td>12</td>
<td>(9Z)-Lutein</td>
<td>11.3</td>
</tr>
<tr>
<td>13</td>
<td>(9Z)-Lutein</td>
<td>6.0</td>
</tr>
</tbody>
</table>

* Nobel carotenoid as natural products.

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Fig. 2. HPLC separation of carotenoids of an extract of chrysanthemum petals (‘Sunny Orange’). Peak numbers as in Table 1. Reprinted from Phytochemistry Vol. 65, Kishimoto et al., Carotenoid composition in petals of chrysanthemum (Dendranthema grandiflorum (Ramat.) Kitamura), p. 2781–2787, Copyright (2004), with permission from Elsevier.

Fig. 3. Structures of (3S,5S,6R,3'R,6'R)-5,6-Dihydro-5,6-dihydroxylutein (1), (all-E)-Lutein-5,6-epoxide (2), and (9Z)-Lutein-5,6-epoxide (3).
isomers of lutein (the all-\(E\) form and two mono-\(Z\) forms), and two epimeric lutein-5,8-epoxides. Recent research has revealed that various cis-form carotenoids occur in petals (Deli et al., 1988; Kull and Pfander, 1995; Melendez-Martinez et al., 2006; Molnár et al., 1986); however, the di-\(Z\) forms of cyclized carotenoids are particularly rare molecular structures and have been reported as natural products only in the petals of rape (\(Brassica napus\) L.) ((9\(Z\),9\(Z\))-lutein; Kull and Pfander, 1997), calendula (\(Calendula officinalis\) L.) ((9\(Z\),9\(Z\))-lutein and (13\(Z\),13\(Z\))-lutein; Bakó et al., 2002), African marigold ((13\(Z\),13\(Z\))-lutein; Khachik et al., 1999), and pansy (\(Viola \times wittrockiana\) Gams) ((9\(Z\),9\(Z\))-violaxanthin, (9\(Z\),13\(Z\))-violaxanthin, (9\(Z\),13\(Z\))-violaxanthin, and (9\(Z\),15\(Z\))-violaxanthin; Molnár et al., 1986). The function of these carotenoids in petals is still unknown.

The carotenoid composition of leaves was completely different from that of petals, showing a typical composition among plant leaves, including carotenoids essential for photosynthesis, such as lutein, violaxanthin, zeaxanthin, and antheraxanthin (Kishimoto and Ohmiya, 2006). β-Carotene derivatives accounted for approximately 43% in leaves. Various cis-forms of lutein and lutein-5,6-epoxide found in petals were not detected in leaves.

2) Comparison of carotenogenic gene expression between petals and leaves

In order to identify the genetic trait which causes the different carotenoid composition between petals and leaves, we analyzed the expressions of 13 genes encoding carotenoid and isoprenoid biosynthetic enzymes by Northern blot and real-time PCR analyses (Kishimoto and Ohmiya, 2006). The apportioning of substrates into the pathways leading to \(\alpha\)-carotene and \(\beta\)-carotene derivatives could be determined simply by the relative amounts or activities of LCYB and LCYE (Cunningham et al., 1996; Cunningham and Gantt, 2001). This hypothesis was supported by our findings that both the ratio of \(\alpha\)-carotene derivatives to total carotenoids and the LCYE gene expression were remarkably high in comparison than those of LCYB. In contrast, LCYE showed extremely lower levels in leaves. The carotenoid biosynthetic pathway after lycopene branches into two pathways: \(\beta\)-carotene derivatives and \(\alpha\)-carotene derivatives (Fig. 1). The apportioning of substrates into the pathways leading to \(\alpha\)-carotene and \(\beta\)-carotene derivatives could be determined simply by the relative amounts or activities of LCYB and LCYE (Cunningham et al., 1996; Cunningham and Gantt, 2001). This hypothesis was supported by our findings that both the ratio of \(\alpha\)-carotene derivatives to total carotenoids and the LCYE gene expression were remarkably high in comparison.
with the ratio of β-carotene derivatives to total carotenoids and the LCYB gene expression in chrysanthemum petals. In contrast, the ratio of β-carotene derivatives to total carotenoids in leaves was higher than that of α-carotene derivatives, reflecting the high expression levels of LCYB.

3) Comparison of carotenogenic gene expression between white and yellow petals

The importance of several enzymes in the regulation of carotenoid accumulation in green tissues and fruits has been reported; these include PSY and PDS in tomato (Solanum lycopersicum L.) (Fraser et al., 1994; Giuliano et al., 1993; Pecker et al., 1992) and GGPS, PSY, and PDS in bell pepper (Capsicum annuum L.) (Hugueney et al., 1996). Moehs et al. (2001) reported that DXS and PSY might be responsible for the color development from pale yellow to deep yellow in African marigold petals. In general, transcriptional activation of carotenoid biosynthetic enzymes is thought to be the major factor in the up-regulation of carotenoid accumulation in many fruits and flowers (see reviews by Fraser and Bramley, 2004; Hirschberg, 2001; Sandmann et al., 2006).

In order to identify the key regulatory step that controls carotenoid biosynthesis in chrysanthemum petals, we first compared the expression of genes encoding carotenoid and isoprenoid biosynthesis enzymes between yellow and white petals (Kishimoto and Ohmiya, 2006). Carotenoid concentration markedly increased during petal development of the yellow-flowered cultivar ‘Yellow Paragon’. On the other hand, carotenoid contents in petals of the white-flowered cultivar ‘Paragon’ decreased gradually and were below the detection limit at the fully developed stage; however, most genes for carotenogenic enzymes showed increasing levels of expression during petal development of both ‘Yellow Paragon’ and ‘Paragon’. Among the three white-flowered cultivars tested, ‘Paragon’ and ‘Fiducia’ had lower DXS expression levels than yellow-flowered cultivars, but those of ‘White Marble’ were similar to those of yellow-flowered cultivars (Fig. 4). There was only a slight difference in the expression levels of all genes tested between ‘White Marble’ (white) and ‘Florida Marble’ (yellow), a variant of ‘White Marble’. Consequently, no genes showed distinct differences in expression levels between petal colors. These results showed that the mechanism of the white color formation of petals cannot be fully explained by transcriptional regulation of the carotenoid biosynthetic pathway. In addition, white petals did not accumulate pathway intermediates, including either yellow carotenoids or colorless carotenoids, such as phytoene and phytofluene (data did not shown). These results suggested that individual steps in the isoprenoid and carotenoid biosynthetic pathways were not disrupted in white petals.

The white color of chrysanthemum petals is dominant over yellow; in addition, yellow-flowered bud sport arise from white-flowered cultivars, but the reverse mutation rarely occurs (Jank, 1957; Machin and Scopes, 1978). Generally, variants that arise from radiation breeding or bud sports contain genomic deletions (Vizir et al., 1994); therefore, yellow-flowered mutants that arise from white-flowered cultivars are assumed to lose a gene that is responsible for carotenoid accumulation. Hattori (1991) suggested that this is the ‘carotenoid biosynthesis inhibitor gene’. Because all carotenoid and isoprenoid biosynthetic genes tested were expressed in white petals during the course of development, we supposed that the gene does not down-regulate carotenoid biosynthesis, but inhibits carotenoid accumulation in a way that is not affected by carotenoid biosynthetic activity.

Next, to find the factor that determines the amount of carotenoids in petals, we performed polymerase chain reaction-select subtraction screening and searched for a clone differentially expressed in white and yellow petals (Ohmiya et al., 2006). We found a clone highly expressed in the petals of white-flowered cultivars but extremely low in yellow-flowered cultivars (Fig. 6). The deduced amino acid sequence of the protein encoded by the clone was highly homologous to the sequence of carotenoid cleavage dioxygenase (designated CmCCD4a). To determine the role of CmCCD4a gene products in the formation of petal color, we introduced the RNAi construct of CmCCD4a into the white-flowered chrysanthemum cultivar, ‘Sei-Marine’. We could obtain transformants whose expression levels were reduced to 2–4% of the wild-type level. The petals of the transformants accumulated carotenoids and appeared yellow.

These results indicated that in white petals of chrysanthemums, carotenoids are synthesized but are subsequently degraded into colorless compounds, which results in the white color. In Southern blot analysis, the
yellow-flowered cultivar ‘Florida Marble’, a bud sport of ‘White Marble’, lacks the band corresponding to CmCCD4a, while the other bands existed in common in both ‘White Marble’ and ‘Florida Marble’. As mentioned above, ‘Florida Marble’ showed almost the same expression levels of all carotenogenic genes tested as those of ‘White Marble’; therefore, it was assumed that yellow-flowered bud sports lost the CmCCD4a gene during somatic mutation, and loss of the gene results in the accumulation of carotenoids. Among the yellow-flowered cultivars tested, only ‘Yellow Paragon’, a bud sport of ‘Paragon’, expressed CmCCD4a in petals (Fig. 6). The petals of ‘Yellow Paragon’ are periclinal chimera, consisting of a yellow L1 layer and a white L2 layer. The L2 layer of the petals of ‘Yellow Paragon’, which may behave genetically in a manner identical to that of its white progenitor ‘Paragon’, expresses CmCCD4a, and the L1 layer lost the CmCCD4a gene; consequently resulting in a lower expression level in petals of ‘Yellow Paragon’ than in white-flowered cultivars.

Another mechanism controlling carotenoid accumulation that is not affected by carotenoid biosynthetic activity was elucidated in cauliflower (Brassica oleracea L. var. botrytis L.) Orange (Or) mutant (Lu et al., 2006). The mutant accumulated a large amount of β-carotene in various tissues normally devoid of carotenoids. The isolated Or gene encodes a plastid-associated protein containing a DnaJ Cys-rich domain, and the presumable function of Or is associated with a cellular process that triggers the differentiation of proplastids and/or non-colored plastids into chromoplasts for carotenoid accumulation. It is becoming clear that the formation of carotenoid storage structures, chromoplasts, provides a metabolic sink to facilitate the accumulation of carotenoids.

The mechanism that controls carotenoid accumulation is mostly unknown. These two different regulatory mechanisms found in chrysanthemum and cauliflower could provide additional approaches for the metabolic engineering of carotenoids in plants.

2. Relationship between pigment composition and petal color in Compositae plants

1) Three routes to orange petal color via carotenoid components in 9 Compositae plants

Orange coloration in petals is generated by a combination of yellow and red pigments in many cases. In Alstroemeria (Alstroemeria spp.) (Tatsuzawa et al., 2004), rose (Rosa spp.) (Yokoi and Saito, 1973), and zinnia (Zinnia elegans Jacq.) (Boyle and Stimart, 1989), orange petals contain both carotenoids and anthocyanins, as yellow and red pigments, respectively. On the other hand, orange is produced only with carotenoids in some species, such as California poppy (Eschscholzia californica Cham.) (Strain, 1938), tiger lily (Deli et al., 2000), and African marigold (Moehs et al., 2001).

Many plants belonging to the Compositae family including chrysanthemum, have both yellow- and orange-flowered cultivars. The orange petal color in this family has a broad range of color tone; for example, that in chrysanthemum tends to be dark, and that in calendula and marigold is very bright. Carotenoids are mainly responsible for the yellow coloration in this family with the exception of some species, such as dahlia (Dahlia spp.), lance coreopsis (Coreopsis lanceolata L.) and yellow cosmos (Cosmos sulphureus Cav.), which contain xanthophylls and coreopsins, types of auroxanthins, as yellow pigments (Harborne et al., 1990; Shimokoriyama and Hattori, 1953). We tried to determine the pigments responsible for the red coloration by qualitative and quantitative comparison of pigments between yellow and orange petals and to clarify the relationship between color tone and pigment composition in the orange petals of 9 species of the Compositae family, African daisy (Osteospermum ecklonis (DC.) Norl.), African marigold, calendula, chrysanthemum, French marigold (Tagetes patula L.), gazania (Gazania spp.), gerbera (Gerbera jamesonii Bol. ex Adl.), sunflower (Helianthus annuus L.), and zinnia.

We have revealed three different ways for orange-flowered cultivars to add redness to the yellow carotenoid base in order to form an orange petal color (Kishimoto et al., 2007). The first is to accumulate more anthocyanins than yellow-flowered cultivars; the orange petal color of chrysanthemum, gerbera and zinnia is mainly formed in this way. These species showed slight differences in carotenoid contents and components between orange- and yellow-flowered cultivars, but orange-flowered cultivars had remarkably higher anthocyanin contents than yellow-flowered cultivars. The second way is to accumulate more carotenoids than yellow-flowered cultivars; orange petals of African marigold, French marigold, and sunflower are formed in this way. These species had only slight differences in anthocyanin contents and carotenoid components between orange- and yellow-flowered cultivars, but remarkably higher carotenoid contents in orange-flowered cultivars than in yellow-flowered cultivars. They had a brilliant color, showing high chroma values. The third way is to accumulate more reddish carotenoids than yellow-flowered cultivars; orange petals of African daisy, calendula, and gazania are mainly formed in this way. Orange-flowered petals of those species had a brilliant color and showed relatively high chroma values.

The orange petal colors of chrysanthemum, gerbera and zinnia appeared dark and dull, and their lightness and color saturation were generally lower than those of the other 6 species; therefore, the accumulation of anthocyanins decreases the lightness and color saturation of petals. In contrast, orange-flowered petals of African marigold, calendula, French marigold, gazania and sunflower, whose petal color was formed only with carotenoids, had a brilliant color. The accumulation of
carotenoids had little effect on lightness and color saturation. These three ways could be combined through inbreeding to produce orange flowers that vary in color tones through variations in lightness, color saturation, and redness.

2) Carotenoid composition in petals of orange- and yellow-flowered calendula cultivars

The petal colors of yellow- and orange-flowered cultivars of calendula originate from carotenoid pigments. As mentioned above, calendula petals have characteristic differences in carotenoid composition between orange- and yellow-flowered cultivars (Fig. 7). Some carotenoids have been characterized by TLC and HPLC (Bakó et al., 2002; Tóth and Szabolcs, 1981), but their precise molecular structures had not been determined and several unknown components remained to be elucidated.

We then made a detailed investigation by FAB-MS and NMR analyses using extracts of petals of orange- and yellow-flowered cultivars (Kishimoto et al., 2005). Nineteen carotenoids were identified, ten of which, (5'Z,9'Z)-rubixanthin, α-carotene, (5'Z)-rubixanthin, δ-carotene, (5Z,9Z,5'Z,9'Z)-lycopene, γ-carotene, (5'Z)-γ-carotene, (5Z,9Z,5Z)-lycopene, (5Z,9Z)-lycopene, and (all-E)-lycopene, are present in orange-flowered but not yellow-flowered cultivars (Figs. 7 and 8, and Table 2). The UV-vis absorption maxima of these ten carotenoids were at longer wavelengths than that of flavoxanthin, the main carotenoid of calendula petals, and it was clear that these carotenoids are responsible

<table>
<thead>
<tr>
<th>Peak no. (Fig. 7)</th>
<th>Carotenoid</th>
<th>% of total carotenoids</th>
<th>λ_max (nm)</th>
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<tr>
<td>1</td>
<td>(8'R)-Luteoxanthin</td>
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<td>398, 422, 448</td>
</tr>
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<td>2</td>
<td>Lutein-5,6-epoxide</td>
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<td>416, 438, 469</td>
</tr>
<tr>
<td>3</td>
<td>Flavoxanthin</td>
<td>28.5</td>
<td>398, 420, 448</td>
</tr>
<tr>
<td>4</td>
<td>(8R,8'R)-Auroxanthin</td>
<td>7.1</td>
<td>380, 401, 425</td>
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<td>5</td>
<td>(9Z)-Lutein-5,6-epoxide</td>
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<td>413, 435, 463</td>
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<tr>
<td>6</td>
<td>Lutein</td>
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<td>444, 473</td>
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<td>Antheraxanthin</td>
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<td>(5Z,9Z)-Rubixanthin*</td>
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<td>18</td>
<td>(5Z,9Z)-Lycopene (4)*</td>
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<td>19</td>
<td>(all-E)-Lycopene*</td>
<td>8.7</td>
<td>446, 473, 505</td>
</tr>
</tbody>
</table>

* Percentage of peak area in the HPLC chromatogram at 450 nm.
* Nobel carotenoid as natural products.
* Reddish carotenoid whose main absorption maxima is detected in the range from 446 to 473 nm.
for the orange color of the petals. Six of these 10 carotenoids had a cis structure at C-5 (C-5'). Among them, (5Z,9Z)-lycopene (4), (5Z,9Z,5'Z)-lycopene, (5Z,9Z,5'Z,9'Z)-lycopene, (5'Z)-γ-carotene, and (5'Z,9'Z)-rubixanthin has never before been identified as a natural compound. Generally, carotenoids having a (5Z)- or (5Z')-configuration are very rare in plants. The best-known carotenoid with such a structure is (5Z')-rubixanthin (5), which is known as gazaniaxanthin, the main carotenoid in the petals of gazania (Bartlett et al., 1969). It has also been isolated from rose petals (Rosa spp.) (Euguster and Märti-Fischer, 1991) and rose hips (Hornero-Méndez and Mínguez-Mosquera, 2000; Märti-Fischer et al., 1983). We assume that lycopene cyclases, such as LCYB and LCYE, cannot convert the (5Z)-end into a ring structure, such as a β-ring or ε-ring, and that (5Z)-carotenoids accumulate accordingly. The reason why only orange-flowered cultivars accumulate (5Z)-carotenoids in petals remains unclear and further research is needed.

3. Future study

The mechanism of white color formation in chrysanthemum petals was clarified in our study, leading to the expectation that we can produce a yellow-flowered cultivar with the same quality as that of white-flowered cultivars by suppressing CmCCD4a expression in petals. It is of particular interest whether this mechanism in chrysanthemum is applicable to other plant species. We also showed that the brilliant-orange color in petals could be formed by the accumulation of large amounts of yellow carotenoids, as in marigold, or reddish carotenoids, as in calendula. Genetic engineering of the carotenoid pathway in petals to produce higher carotenoid levels and/or reddish carotenoids would be the best shortcut to produce bright-orange-flowered chrysanthemums.

Many approaches to alter carotenoid levels and profiles in various plant tissues have been adopted (see reviews by Giuliano et al., 2008; Tanaka and Ohmiya, 2008; Taylor and Ramsay, 2005); however, only a few were successful, such as chrysanthemum (Ohmiya et al., 2006), Lotus corniculatus L. var. japonicus Regel (Suzuki et al., 2007), rape (Shewmaker et al., 1999), and rice (Oryza sativa L.) (Paine et al., 2005; Schaub et al., 2005). One of the main reasons is that various regulatory factors for carotenoid biosynthesis and accumulation, such as key enzymes, transcription factors and storage structures, are still unclear. Future work that sheds light on the mechanisms controlling plant carotenogenesis would clear the way for metabolic engineering of chrysanthemum petals in order to change the color.

Significant progress in studies on plant carotenoids has been made in the past two decades; however, knowledge of flower carotenoids is relatively limited. We hope that our findings help in further understanding of carotenoids in petals.

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