

研究資料

Carotenoid Composition in the Yellow and Pale Green Petals of
Primula Species

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Summary

The carotenoid composition in the yellow petals of *Primula* × *polyantha* and *P. helodoxa*, and in the pale green petals of *P. × polyantha* was analyzed by high-performance liquid chromatography. The major carotenoids detected in the yellow petals were (9*Z*)-violaxanthin, (all-*E*)-violaxanthin, lutein, and antheraxanthin. The carotenoid composition in the pale green petals was completely different from that in the yellow petals; the former accumulated predominantly lutein and β -carotene. Carotenoids in the yellow petals were present in the esterified form, while those in the pale green petals were in the free form.

Key Words: carotenoid, flower color, *Primula*

Introduction

Primula is one of the largest plant genera in the northern hemisphere, containing more than 400 species, which are subdivided into 37 sections (Richards, 1993). In Japan, *Primula sieboldii* has developed into an important ornamental plant, and approximately 300 different cultivars have been produced (Torii, 1985). The petal colors of *P. sieboldii* are limited to white, pink, and red, and the differences are the result of variations in the concentration of anthocyanin in the petals (Yoshioka et al., 2004). On the other hand, there are some yellow-flowered cultivars of *P. × polyantha* and yellow-flowered wild species of *P. helodoxa*. Cao and Liang (2008) reported that the brightness of the yellow color in *P. vulgaris* is largely determined by the carotenoid content. However, little data is available on the carotenoid profiles in the petals of *Primula* species. In the present study, we analyzed the carotenoid composition in the yellow petals of *P. × polyantha* and *P. helodoxa* by using high-performance liquid chromatography (HPLC). Not only yellow petals but also pale green petals contain carotenoids together with chlorophylls. We then compared the carotenoid composition of the yellow petals with that of the pale green petals of *P. × polyantha*.

Materials and Methods

1. Plant materials

Yellow- and pale-green-flowered cultivars of the 'Primrose' series (*P. × polyantha*; Japan Agribio Co. Ltd., Tokyo, Japan) and yellow-flowered wild species of *P. helodoxa* (Fig.1) were purchased from the local market in Tsukuba, Ibaraki, Japan. Petals were harvested and stored at -80°C until use.

2. Extraction of carotenoids

The petals were ground to powder in 2.0-mL microtubes using a Shake Master BMS-12 (Inter Bio Techno, Tokyo, Japan), and the powder was extracted with acetone. The extract was partitioned between diethyl ether and aqueous NaCl phases. The organic layer was washed with water. Half of the organic layer was dried in a vacuum dryer and dissolved in MeOH for HPLC analysis (unsaponified sample). The rest of the organic layer was saponified with the same volume of 5% KOH-MeOH for 2 h at room temperature in the dark. The

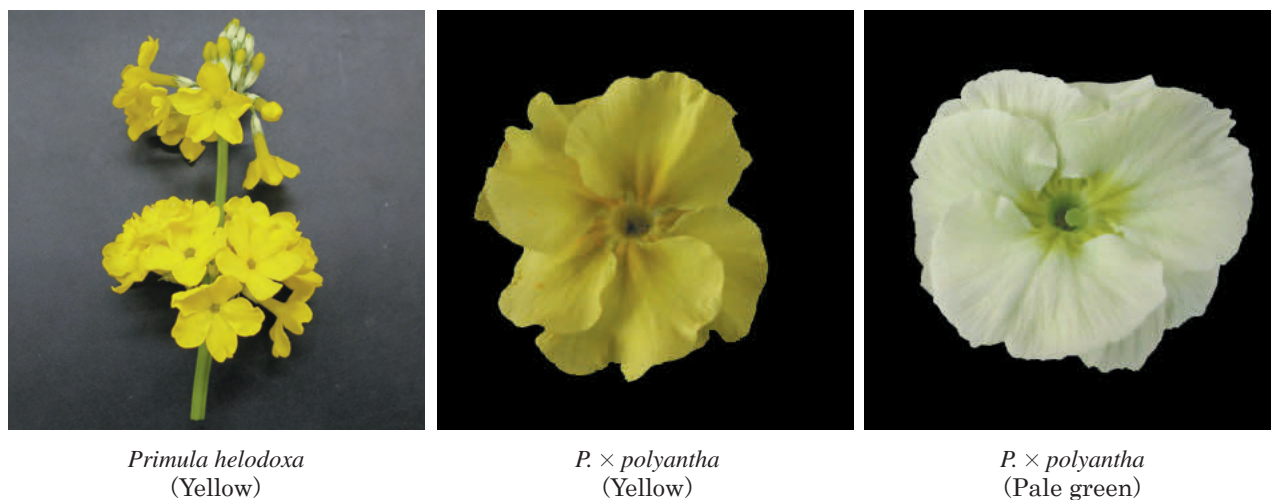


Fig. 1. Photographs of fully opened flowers tested in this study.

saponified matter was then extracted with diethyl ether and washed with water. The organic layer was dried in a vacuum dryer and dissolved in MeOH for HPLC analysis (saponified sample).

3. HPLC analysis of carotenoids

Each sample was analyzed by HPLC (X-LC, JASCO, Tokyo, Japan) equipped with a photodiode array detector. The column was a YMC Carotenoid Column (100×4.6 mm, i.d.; 3µm; YMC, Tokyo, Japan). Solvent A was MeOH:(CH₃)₂COCH₃:H₂O = 95:1:4 (v/v/v); solvent B was MeOH:(CH₃)₂COCH₃:H₂O = 25:71:4 (v/v/v). The gradient was 0/100, 5/100, and 33/0 (min/% solvent A). The flow rate was 1.0 mL·min⁻¹, column temperature was 35°C, and UV/visible monitoring range was 200–750 nm. Peaks were identified by comparing retention times and absorbance spectra with those of previously identified compounds in the petals of *Chrysanthemum morifolium* (Kishimoto et al., 2004), and with those of standard compounds. The total carotenoid contents were estimated from the total peak area of the HPLC chromatograms at a wavelength of 450 nm, and are expressed as lutein equivalents [µg·g⁻¹ fresh weight (FW)] of the tissue. The percentage of each carotenoid component was calculated according to the peak area of the HPLC chromatograms at a wavelength of 450 nm.

Results and Discussion

At an early stage of development, the petals of all the *Primula* plants examined were pale green and contained around 10 µg·g⁻¹ FW carotenoids (data not shown). The mature petals of the yellow-flowered *P. × polyantha* and *P. helodoxa* contained high levels of carotenoids (> 270 µg·g⁻¹ FW, Table 1), while those of the pale-green-flowered *P. × polyantha* contained only small amounts (< 7 µg·g⁻¹ FW, Table 1). The petals of the yellow-flowered *P. × polyantha* and *P. helodoxa* showed similar carotenoid profiles, predominantly accumulating (9Z)-violaxanthin. The levels of (all-E)-violaxanthin, lutein, and antheraxanthin (Fig. 2A, Table 1) were also high. The HPLC chromatograms of the unsaponified and saponified samples were different. Most of the peaks identified in the saponified sample were absent in the unsaponified samples. This indicates that the majority of carotenoids in the yellow petals are present in the esterified form (Fig. 2B). On the other hand, the major carotenoids in the pale green petals of *P. × polyantha* were lutein and β-carotene (Fig. 2A, Table 1). The HPLC chromatograms of the unsaponified and saponified samples were similar, indicating that these carotenoids were present in the free form (Fig. 2B).

Table 1. Carotenoid composition in the petals of *Primula* species

Total carotenoid (µg·g ⁻¹ FW)	Carotenoid composition (%)					
	1** Unknown	2 Violaxanthin	3 (9Z)-Violaxanthin	4 Lutein	5 Antheraxanthin	6 β-Carotene
<i>P. helodoxa</i> (Yellow)						
669.91 ± 53.60*	7.51	12.68	49.20	9.11	10.35	0.46
<i>P. × polyantha</i> (Yellow)						
270.74 ± 28.95	9.62	8.33	47.03	5.94	11.52	0.79
<i>P. × polyantha</i> (Pale green)						
6.71 ± 1.00	4.92	8.05	6.56	40.09	3.13	16.39

* Mean values (±SE) are shown (n = 3).

** Peak numbers correspond to those shown in Fig. 2.

The carotenoid composition in petals differs from species to species, and even between cultivars of the same plant species (Ohmiya, 2011). On the other hand, the leaves of different plant species have similar carotenoid compositions (Goodwin, 1992). The predominant carotenoids in leaves are those essential for photosynthesis, such as lutein and β -carotene. The carotenoid composition of the pale green petals of *P. × polyantha* was similar to that of the leaves, while the carotenoids in the yellow petals of *P. × polyantha* and *P. helodoxa* were different from those in the leaves. Leaf carotenoids are localized in the chloroplasts, while petal carotenoids reside in the chromoplasts. Therefore, we designated the carotenoids in the yellow petals of *Primula* sp. as “chromoplast-type carotenoids” and those in the pale green petals of *Primula* sp. as “chloroplast-type carotenoids”. The results are in agreement with a previous study on *Ipomoea* species (Yamamizo et al., 2010): the petals of the pale-yellow-flowered *I. obscura* accumulated chloroplast-type carotenoids, while those of the yellow-flowered *Ipomoea* sp. accumulated chromoplast-type carotenoids. In leaves, carotenoids and chlorophylls bind to chlorophyll-

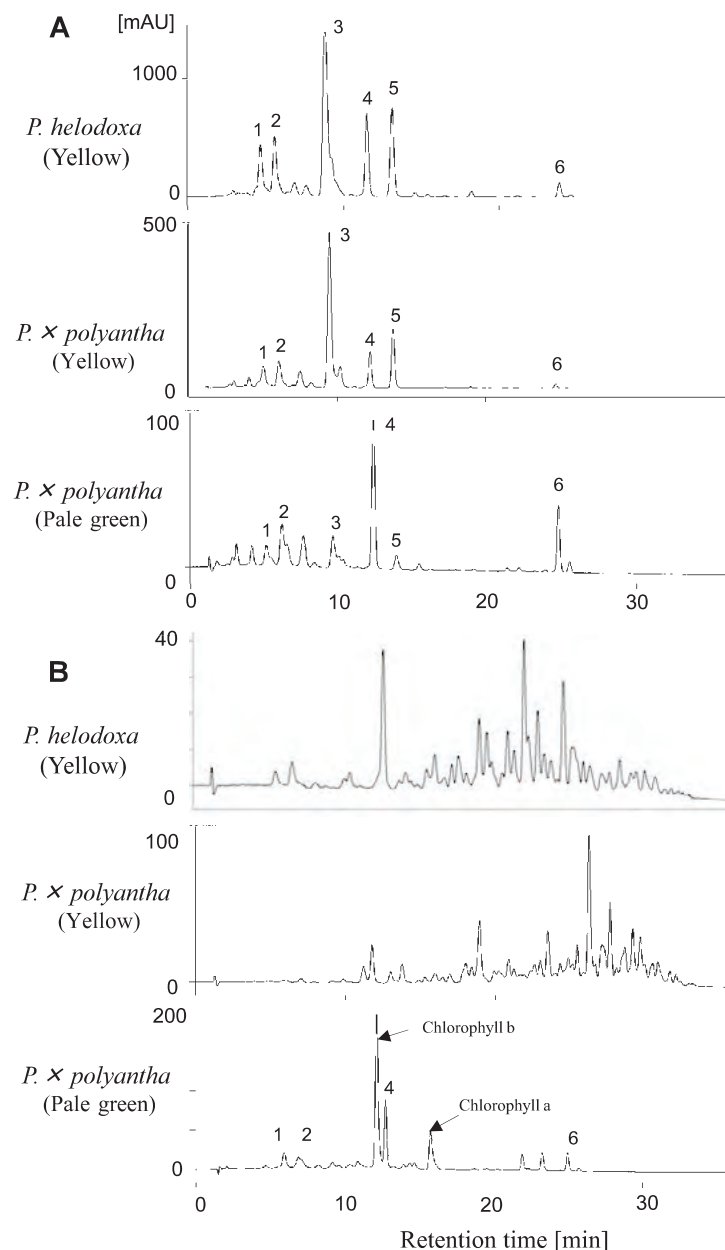


Fig. 2. HPLC chromatograms (absorbance at 450 nm) of saponified (A) and unsaponified (B) carotenoid extracts from *Primula helodoxa* and *P. × polyantha*. 1: unknown; 2: (all-*E*)-violaxanthin; 3: (9*Z*)-violaxanthin; 4: lutein; 5: antheraxanthin; 6: β -carotene.

carotenoid proteins, and are functionally integrated into the thylakoid membrane (Nelson and Yocum, 2006). Immature petals of *I. obscura* have a pale green color, and they contain chlorophylls and chloroplast-type carotenoids (Yamamizo et al., 2010). During flower development, the chlorophylls disappear, and chloroplast-type carotenoids persist, while the petals remain pale yellow. In the case of the pale-green-flowered *P. × polyantha*, we speculate that the carotenoids coexist with the chlorophylls in the chloroplast, because the petals contain chlorophylls (Fig. 2B) even during the later stages of petal development.

Not only did the carotenoid composition differ between the chloroplast- and chromoplast-type carotenoids, but their esterification also differed in the *Primula* plants tested in this study. Vishnevetsky et al. (1999) reported that the carotenoids contained in chromoplasts are generally esterified and are associated with carotenoid-lipoprotein structures. The importance of esterification for carotenoid sequestration into the chromoplasts has been well documented in pepper fruits (Minguez-Mosquera and Hornero-Méndez, 1994a, b; Hornero-Mendez and Minguez-Mosquera, 2000). The transition from chloroplast (in immature green fruits) to chromoplast (in mature red fruits) was correlated with xanthophyll esterification. However, only a small amount of data is presently available on carotenoid esterification in flowers. In this study, we showed that the chromoplast-type carotenoids in yellow petals existed in the esterified form, while chloroplast-type carotenoids in the pale green petals were in the free form. Similar phenomena were also reported in *Ipomoea* plants (Yamamizo et al., 2010). Therefore, we assume that esterification is an important process for the sequestration of carotenoids into the chromoplasts in flower petals.

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サクラソウ属植物の黄色および黄緑色花卉における カロテノイド組成の比較

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和文摘要

サクラソウ属植物のプリムラ・ポリアンサ (*Primula* × *polyantha*) および黄花クリンソウ (*P. helodoxa*) の花卉のカロテノイド組成を HPLC により解析した。プリムラ・ポリアンサの黄花品種と黄花クリンソウの花卉は、(9Z)-violaxanthin, (all-E)-violaxanthin, lutein および antheraxanthin を主成分とする花卉特有のカロテノイド組成を示した。また、カロテノイドの多くはエステル化された状態で蓄積していた。一方、プリムラ・ポリアンサの黄緑花品種の花卉は、葉と同様のカロテノイド組成および存在形態を示した。すなわち、lutein と β -carotene を主成分とし、その多くはエステル化されていない遊離の状態が存在していた。