Analyses of Anthocyanidins and Anthocyanins in Flower Petals of *Lychnis senno* and Its Related Species (Caryophyllaceae)

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Flower petal anthocyanidins were analyzed by high-performance liquid chromatography (HPLC) in *Lychnis senno*, a traditional ornamental plant conserved in Japan, and its related species (Caryophyllaceae). According to the anthocyanidin composition, 8 species analyzed were classified into 4 types. For Type-I species (*L. senno*), flower color was vivid red (JHS 0707), and petals accumulated both 3'-hydroxylated anthocyanidins (cyanidin and peonidin) and pelargonidin. Both Type-II and Type-III species contained only 3'-hydroxylated anthocyanidins in their flower petals. For Type-II species (*Silene. coeli-rosa* 'Cherry Blossom' and *S. yunnanensis*), flower color was light purplish pink (JHS 9203) or deep purplish pink (JHS 9205), and the relative level of cyanidin in petals was much higher than peonidin. On the other hand, for Type-III species (*L. coronaria*), flower color was vivid reddish purple (JHS 9207), and the relative level of peonidin in petals was much higher than cyanidin. For Type-IV species (*L. chalcedonica*, *L. coronata*, *L. miqueliana* and *L. wilfordii* 'Karafuto-enbisennou'), flower color was vivid reddish orange (JHS 0706), strong orange (JHS 1305) or strong reddish orange (JHS 0713), and flower petals exclusively accumulated pelargonidin. When *L. senno* (Type-I) and *L. miqueliana* (Type-IV) were analyzed by HPLC for anthocyanin composition in flower petals, only No. 4 peak was common to these species, indicating that this peak may be a pelargonidin-type anthocyanin. The results obtained in the present study may be helpful in systematic breeding for flower color alteration of *L. senno* and its related species.

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Key words: anthocyanidin, anthocyanin, flower color, HPLC analysis, *Lychnis senno*

Lychnis senno Siebold et Zucc., generally called 'Senno' in Japan, is a species belonging to Caryophyllaceae and native to China. Although this species had widely been cultivated in the Muromachi and the Edo eras in Japan, it is recently on the way of extinction, and we can hardly see it as a garden plant. From 1996 onward, several strains of *L. senno* were found in Chugoku and Kyushu districts, Japan, and they were introduced to the Botanic Gardens of Toyama as research materials (Godo *et al*., 2000, 2004).

Flower color of *L. senno* is characteristic bright crimson (Godo *et al*., 2004), and this attractive trait is desired to be introduced via sexual or somatic hybridization into related species. In order to carry out systematic breeding for flower color alteration, analysis of the pigment composition in flower petals is one of the prerequisites. In the present study, therefore, we analyzed anthocyanidins and anthocyanins by HPLC in flower petals of *L. senno* and its related species.

MATERIALS AND METHODS

Plant materials

Six *Lychnis* species, *L. chalcedonica*, *L. coronaria*, *L. coronata*, *L. miqueliana*, *L. senno* and *L. wilfordii* 'Karafuto-enbisennou', and two *Silene* species, *S. coeli-rosa* 'Cherry Blossom' and *S. yunnanensis* (**Fig. 1**), were used in the present study. Potted plants of these species were cultivated in the greenhouse without heating at the Faculty of Agriculture, Niigata University and the Botanic Gardens of Toyama.

Description of flower color using the JHS color chart

Flower color of each species was checked visually with an aid of the JHS Color Chart (Japan Horticultural Plant Standard Color Chart, Japan Color Research Institute, 1984). Flower petals were harvested immediately after anthesis from potted plants and used for check. Flower color was expressed using ISCC-NBS color name (Inter-Society Color Council, National Bureau of Standards) as well as JHS Color Chart No.

Analyses of anthocyanidins and anthocyanins

Extraction and HPLC analyses of anthocyanidins and anthocyanins were performed according to Mori et al. (2002). Flower petals were harvested immediately after anthesis from potted plants and stored at −80℃ until use. For extraction, ca. 0.2 g fresh weight of petal tissues were incubated for 1 h in 3 ml of an acidic methanolic solution (0.1% HCl in methanol) at room temperature. For *L. senno* and *L. miqueliana*, crude extracts obtained after centrifugation (3,000 cycles min^{-1} for 10 min) were used directly for anthocyanin analysis. For all species, crude extracts obtained after centrifugation were fully hydrolyzed with 2N-HCl for 30 min at 100℃ for liberating anthocyanidins and then

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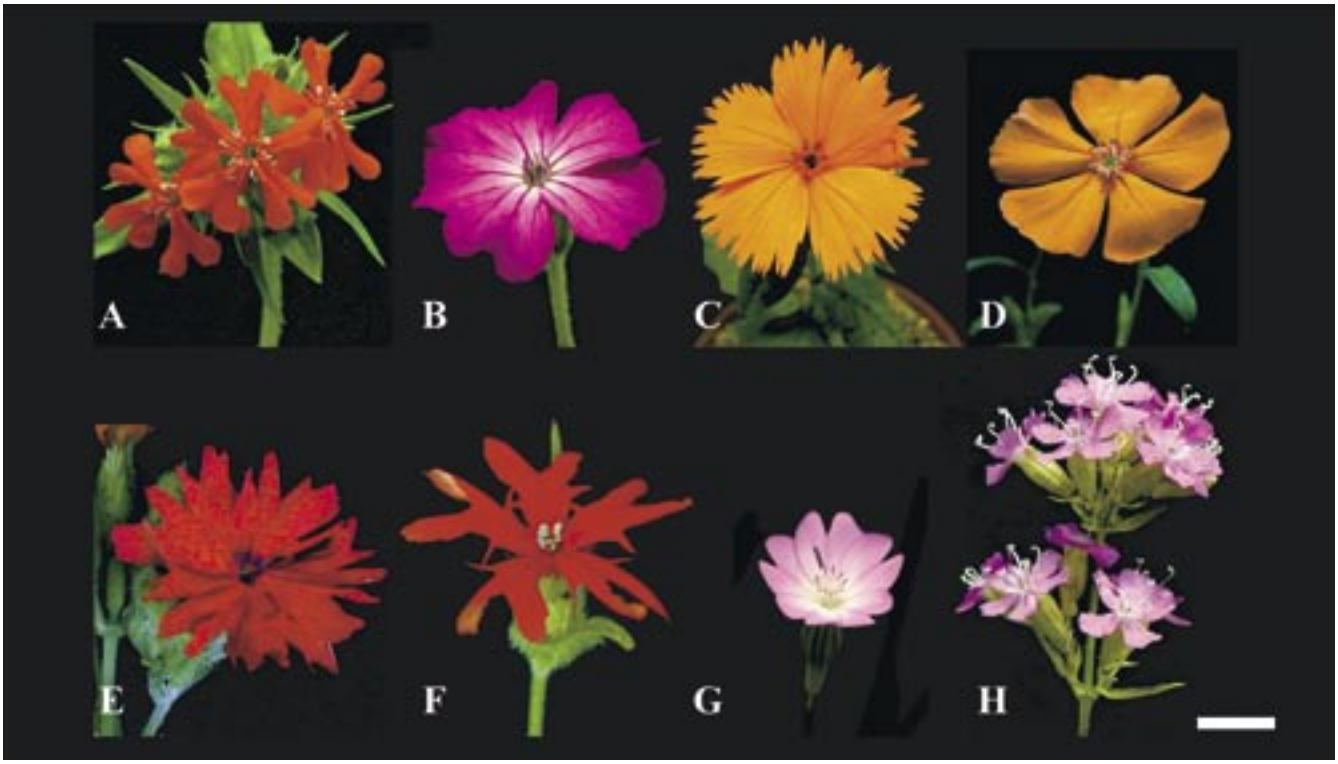

Fig. 1. Flowers of *Lychnis* and *Silene* species analyzed in the present study. (A) *L. chalcedonica*, (B) *L. coronaria*, (C) *L. coronata*, (D) *L. miqueliana*, (E) *L. senno*, (F) *L. wilfordii* 'Karafuto-enbisennou', (G) *S. coeli-rosa* 'Cherry Blossom', (H) *S. yunnanensis*. Bar = 1 cm.

used for anthocyanidin analysis. Crude extracts and their hydrolysates were subjected to HPLC (L-7100 pump with L-7420 spectrophotometric detector; HITACHI, Japan) with linked analytical ODS column (Wakosil-II 5C18 AR, 4.6 mm i.d. 250 mm; Wako Pure Chemical, Japan), by eluting with 7% of solvent B (50% CH_3CN, 40% CH_3COOH and 0.5% CF_3COOH in H_2O) for the first 10 min, followed by elution with a linear gradient from 7 to 35% of solvent B in solvent A (0.5% CF_3COOH in H_2O) for 45 min, at a flow rate of 1.0 ml min^{-1} at 40℃. The elution pattern was monitored at 530 nm. Cyanidin, pelargonidin, delphinidin, petunidin and malvidin extracted from berry pericarps of *Vitis*×*labruscana* 'Kyoho', and peonidin from berries of *Fragaria*×*ananassa* 'Toyonoka', were used as standards for identification of each anthocyanidin.

RESULTS AND DISCUSSION

Table 1 shows flower color checked visually using

Table 1. Flower color and relative anthocyanidin composition in flower petals of *Lychnis senno* and its related species

Species	Flower color [a]		Anthocyanidin composition [b]			Classification according to the anthocyanidin composition
	JHS Color Chart No.	ISCC-NBS color name	Cyanidin	Peonidin	Pelargonidin	
Lychnis chalcedonica	0706	vivid reddish orange	0.3		99.7	Type-IV
L. coronaria	9207	vivid reddish purple	10.4	89.6		Type-III
L. coronata	1305	strong orange			100	Type-IV
L. miqueliana	0713	strong reddish orange			100	Type-IV
L. senno	0707	vivid red	83.4	1.7	14.9	Type-I
L. wilfordii 'Karafuto-enbisennou'	0706	vivid reddish orange			100	Type-IV
Silene coeli-rosa 'Cherry Blossom'	9203	light purplish pink	64.1	35.9		Type-II
S. yunnanensis	9205	deep purplish pink	98.7	1.4		Type-II

[a] Flower color of each species was checked visually with an aid of the JHS Color Chart (Japan Horticultural Plant Standard Color Chart, Japan Color Research Institute, 1984).

[b] Data represent the relative level of each anthocyanidin (area % at 530 nm) analyzed by HPLC.

Table 2. Relative anthocyanin composition in flower petals of *Lychnis miqueliana* and *L. senno*

Species	Peak No.	1	2	3	4	5	6
	Rt (min.)	21.9	23.7	26.5	28.0	29.9	34.0
L. miqueliana				55.6	42.8		1.6
L. senno		3.9	82.2		12.2	1.7	

Data represent the relative level of each anthocyanin (area % at 530 nm) analyzed by HPLC.

the JHS Color Chart and the relative anthocyanidin composition in flower petals analyzed by HPLC. All the 6 *Lychnis* species and 2 *Silene* species analyzed in the present study accumulated some anthocyanidins, 3'-hydroxylated anthocyanidins (cyanidin and peonidin) and/or pelargonidin, in their flower petals. No 3',5'-hydroxylated anthocyanidins (delphinidin, petunidin and malvidin) were detected in all the 8 species. The 8 species were classified into 4 types (Type-I to IV) according to the anthocyanidin composition in flower petals. For Type-I species, both 3'-hydroxylated anthocyanidins (cyanidin and peonidin) and pelargonidin were detected, and *L. senno* solely belonged to this type. Flower color of *L. senno* was expressed as vivid red (JHS 0707) when checked visually using the JHS Color Chart. For Type-II species, only 3'-hydroxylated anthocyanidins (cyanidin and peonidin) were detected, and the relative level of cyanidin was much higher than peonidin. *S. coeli-rosa* 'Cherry Blossom', whose flower color was light purplish pink (JHS 9203), and *S. yunnanensis*, whose flower color was deep purplish pink (JHS 9205), belonged to Type-II. For Type-III species, only 3'-hydroxylated anthocyanidins (cyanidin and peonidin) were detected as in the case of Type-II species, but the relative level of peonidin was much higher than cyanidin. *L. coronaria* was solely classified into Type-III, and flower color of this species was vivid reddish purple (JHS 9207). For Type-IV species, flower petals accumulated exclusively pelargonidin with (*L. chalcedonica*) or without (*L. coronata*, *L. miqueliana* and *L. wilfordii* 'Karafuto-enbisennou') a slight cyanidin. *L. chalcedonica* and *L. wilfordii* 'Karafuto-enbisennou' were classified into Type-IV, and flower color of both species was vivid reddish orange (JHS 0706). *L. coronata*, whose flower color was strong orange (JHS 1305), and *L. miqueliana*, whose flower color was strong reddish orange (JHS 0713), were also belonged to Type-IV. Generally, there is an excellent correlation between flower color and the anthocyanidin type: cyanidin provides most of magenta and crimson flower colors; pelargonidin is responsible for orange, salmon, pink and red colors; and delphinidin is the source of purple, mauve and blue flowers (Forkmann, 1991). A similar relationship between flower color and anthocyanidin composition in flower petals was observed in *Lychnis* and *Silene* species analyzed in the present study.

L. senno (Type-I) and *L. miqueliana* (Type-IV) were further analyzed by HPLC for anthocyanin composition in flower petals (**Table 2**). Among totally 6 peaks observed, only No. 4 peak was detected in both species. Since only pelargonidin was the common anthocyanidin to these species (**Table 1**), No.4 peak may be a pelargonidin derivative. On the other hand, major anthocyanins in flower petals of *L. miqueliana* have been reported to be pelargonidin 3-glucoside and pelargonidin 3-rhamnosylglucoside (Yoshitama and Ishikura, 1991). Therefore, it is possible that No.4 peak is either of these anthocyanins. Further analyses are necessary to identify each anthocyanin in flower petals of *Lychnis* and *Silene* species.

The results obtained in the present study on flower color and pigment composition in flower petals may be helpful in systematic breeding for flower color alteration of *L. senno* and its related species. Interspecific hybridization among these species as well as detailed anthocyanin analyses are now in progress.

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ナデシコ科センノウ (*Lychnis senno*) およびその近縁種の花弁における アントシアニジンおよびアントシアニン分析

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要　約

日本における伝統的な園芸植物であるナデシコ科のセンノウ（*Lychnis senno*）およびその近縁種について、HPLC により花弁のアントシアニジンを分析した。実験に供試した８種は、アントシアニジンの組成により４つのタイプに分類された。タイプ I に分類された種（センノウ）の花弁にはシアニジン、ペオニジンおよびペラルゴニジンが含まれ、花色は vivid red（JHS 0707）であった。タイプ II に分類された種（*Silene coeli-rosa* 'Cherry Blossom' および *S. yunnanensis*）の花弁には主にシアニジンが含まれ、ペオニジンも蓄積されていた。これらの種の花色は light purplish pink（JHS 9203）または deep purplish pink（JHS 9205）であった。一方、タイプ III に分類された種（*L. coronaria*）の花弁には主にペオニジンが含まれ、シアニジンも蓄積されていた。この種の花色は vivid reddish purple（JHS 9207）であった。また、タイプ IV に分類された種（*L. chalcedonica*、*L. coronata*、*L. miqueliana* および *L. wilfordii*‘カラフトエンビセンノウ’）の花弁には主にペラルゴニジンが含まれ、花色は vivid reddish orange（JHS 0706）、strong orange（JHS 1305）または strong reddish orange（JHS 0713）であった。タイプ I に分類されたセンノウとタイプ IV に分類された *L. miqueliana* について、HPLC により花弁のアントシアニンを分析した。その結果、No. 4のピークのみが両種において検出され、このピークがペラルゴニジンを骨格にもつ色素であることが示唆された。本実験において得られた結果は、センノウおよびその近縁種の花色改変に関する育種を計画的に行う際に有用であると考えられる。

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キーワード：アントシアニジン、アントシアニン、花色、HPLC 分析、センノウ

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