

Coloration and Anthocyanin Profile in Tulip Flowers

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Abstract

Correlation of coloration and anthocyanin profile in tulip flowers was investigated. Pelargonidin 3-rutinoside, cyanidin 3-rutinoside, delphinidin 3-rutinoside and their acetyl derivatives were major anthocyanins. Anthocyanins in purple, red, orange and pink perianthes of tulip were analyzed by HPLC. The occurrence of delphinidin 3-rutinoside and its acetyl derivatives is responsible for purple coloration. Pelargonidin 3-rutinoside, cyanidin 3-rutinoside and their 2"-acetyl esters were major anthocyanins in perianthes of the red, orange and pink cultivars with their variant composition ratios. Tissue specific coloration and distribution of anthocyanins among the perianth, perianth-bottom, anther and pollen of the tulip flower were analyzed by using the cultivars 'Ben van Zanten' and 'Ile de France' in detail. Pelargonidin 3-rutinoside, cyanidin 3-rutinoside and their acetyl esters were major anthocyanins in the red perianthes while the cyanidin 3-rutinoside and delphinidin 3-rutinoside were major anthocyanins in the dark purple perianth-bottoms. Delphinidin 3-rutinoside and its acetyl esters were present in the dark purple anthers and pollens. Anthocyanins seem to be tissue-specifically biosynthesized, resulting in formation of a color pattern between inner and outer parts of the tulip flower.

Discipline: Horticulture

Additional key words: perianthes, perianth-bottom, acetyl anthocyanins

Introduction

There is a large variation in flower color of floricultural tulip (*Tulipa gesneriana* L.) cultivars. These color variations seem to be due to anthocyanin composition. Profiling data of anthocyanins in cultivars are useful information for breeding of floricultural plants. We chose purple, red, orange and pink tulip cultivars and analyzed endogenous anthocyanins in perianthes of these cultivars. Pelargonidin 3-rutinoside, cyanidin 3-rutinoside and delphinidin 3-rutinoside had been identified as major anthocyanins in tulip perianthes^{1,3-5}, and recently pelargonidin 3-(2"-acetylrutinoside) and cyanidin 3-(2"-acetylrutinoside) were additionally identified⁸. The relation between coloration of perianthes and concentration ratios of anthocyanins was studied.

Some tulip cultivars have a perianth-bottom whose color is different from its perianth. 'Ben van Zanten' and

'Ile de France' are such typical cultivars and have red perianthes and dark purple perianth-bottoms. Anthers and pollens of these cultivars are also dark purple and we have identified two novel anthocyanins, delphinidin 3-(2"-acetylrutinoside) and delphinidin 3-(3"-acetylrutinoside), in these tissues². We also discuss the distribution of anthocyanins and the tissue specific coloration in the tulip flower.

Materials and methods

1. Plant materials

All tulip plants used in this study were grown in the field of Toyama Agricultural Research Center Vegetable and Ornamental Plants Experiment Station. Seven cultivars of each color possessing purple, red, orange or pink perianthes were used for the study on perianth coloration. 'Ben van Zanten' and 'Ile de France' were among these cultivars and were used for the study on tissue specific

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coloration as well.

2. Identification of anthocyanins in the perianth

Tulip perianthes containing anthocyanins 1–5 (190 g fresh weight) were extracted with 5% HCO₂H (5 L) two times. The extract was applied to an Amberlite XAD-7 column and eluted with EtOH (5% HCO₂H). The eluate was evaporated *in vacuo*, again dissolved in EtOH (5% HCO₂H) and passed through a cellulose column. The eluate was evaporated *in vacuo* and dissolved in 5% HCO₂H and then subjected to an ODS column. Anthocyanin 4 and 2 were eluted in the 10% EtOH (5% HCO₂H) fraction and the 15% EtOH (5% HCO₂H) fraction, respectively. Each fraction was subjected to a Sephadex LH-20 chromatography and eluted with EtOH-HCO₂H-H₂O (10:1:9). The anthocyanins were further purified by a HP 1100 HPLC system (Hewlett-Packard) using a Senshu Pak ODS-4253-D column ($\phi 10 \times 250$ mm) (Senshu Kagaku). Two solvents were used for elution: solvent A; 10% HCO₂H and solvent B; 40% CH₃CN (10% HCO₂H).

The elution was performed on 40% of the solvent B for anthocyanin 2 and 35% of the solvent B for anthocyanin 4 at 40°C at a flow rate of 3 mL/min. Anthocyanins were isolated at 10.3 min (2; 12 mg) and at 10.1 min (4; 10 mg).

3. Spectroscopy

The ¹H-NMR spectra were analyzed in CF₃CO₂D-CD₃OD (1:9) at 400 MHz on a JEOL EX-400 system. The ESI-MS spectra were analyzed on a Thermo Quest TSQ system. The absorption spectra of UV-Vis 240–600 nm were recorded by an on-line HPLC system during analytical HPLC.

4. Analytical HPLC

Perianth-bottoms were excised from perianthes of purple, red, orange or pink cultivars. Anthocyanins of the perianth of each cultivar were extracted with 5% HCO₂H. The extracts were loaded on an ODS-Sep Pak (Waters) and eluted with 50% EtOH (5% HCO₂H).

Table 1. Analytical data of anthocyanin 1–7

Anthocyanins	M ⁺	λ max (nm)	ODS-HPLC Rt (min)
1 Pelargonidin 3-rutinoside	579	505, 430, 280, 265	20.8
2 Pelargonidin 3-(2'''-acetylrutinoside)	621	505, 430, 280, 265	27.0
3 Cyanidin 3-rutinoside	595	520, 280	18.4
4 Cyanidin 3-(2'''-acetylrutinoside)	637	520, 280	24.3
5 Delphinidin 3-rutinoside	611	525, 275	16.1
6 Delphinidin 3-(2'''-acetylrutinoside)	653	525, 275	21.6
7 Delphinidin 3-(3'''-acetylrutinoside)	653	525, 275	17.7

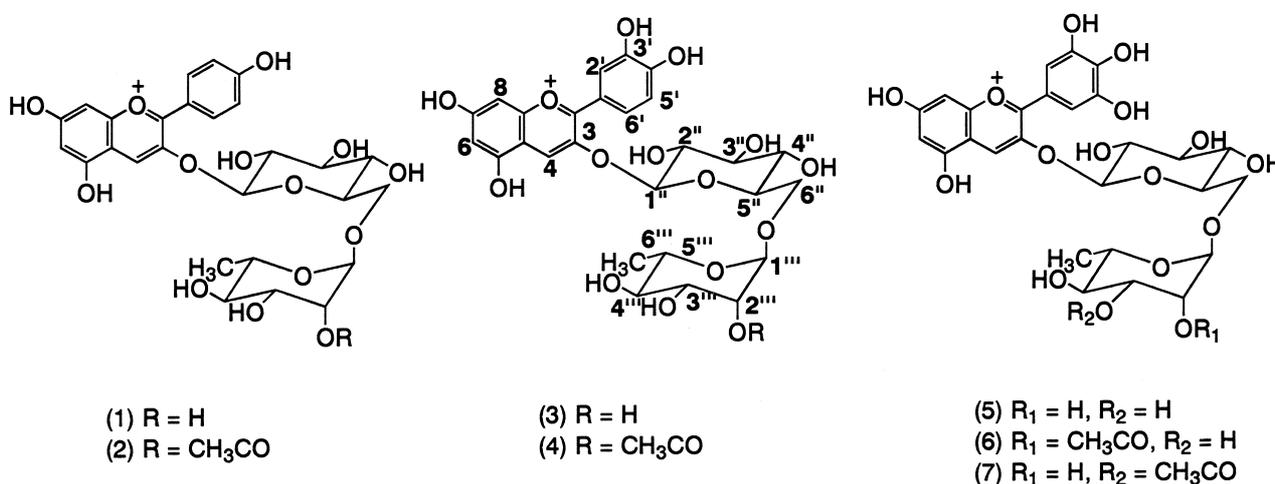


Fig. 1. Structures of anthocyanin 1–7

These samples were analyzed by HPLC on a HP 1100 system. An Inertsil ODS-2 column ($\phi 10 \times 250$ mm) (GL Science) was used. Two solvents were used for elution: solvent A; 1.5 % phosphoric acid and solvent B; 1.5% phosphoric acid, 25% CH_3CN , and 20% HCO_2H . The elution was performed on a linear gradient from 20 to 100% of the solvent B at 40°C at a flow rate of 0.6 mL/min monitoring Vis 520 nm by photodiode array detector. Perianthes, perianth-bottoms, anthers and pollens of 'Ben van Zanten' and 'Ile de France' were divided and again analyzed by using the same methods.

Results

1. Identification of endogenous anthocyanins

We have already identified delphinidin 3-rutinoside (5), delphinidin 3-(2''-acetylrutinoside) (6) and delphinidin 3-(3''-acetylrutinoside) (7), which occur in anthers of tulip². Endogenous anthocyanins of the perianth of tulip cultivars were analyzed by HPLC and five major antho-

cyanins 1–5 were detected (Table 1 and Fig. 1). The retention times and absorption spectra of anthocyanin 1 and 3 coincided with those of pelargonidin 3-rutinoside and cyanidin 3-rutinoside, respectively. Anthocyanins 2 and 4 were extracted from tulip perianthes and purified by using several chromatography methods. The molecular weights were 621 and 637, suggesting that these anthocyanins are the acetyl ester of pelargonidin 3-rutinoside and cyanidin 3-rutinoside.

Most of these ¹H-NMR spectra of anthocyanin 2 and 4 were similar to those of delphinidin 3-(2''-acetylrutinoside) (Table 2). Additional ¹H-NMR spectra were detected and these were assigned to 3' and/or 5' positions of anthocyanidin. These anthocyanin structures were determined to be pelargonidin 3-(2''-acetylrutinoside) (2) and cyanidin 3-(2''-acetylrutinoside) (4).

2. Perianth coloration and anthocyanins

Endogenous anthocyanins of perianthes from purple, red, orange, or pink cultivars were analyzed by

Table 2. ¹H-NMR spectra data for acetyl derivatives of anthocyanins (2, 4 and 6)

H	2	4	6
Anthocyanidin			
4	8.99 (s)	8.92 (s)	8.90 (s)
6	6.67 (d, 2)	6.65 (d, 2)	6.66 (s)
8	6.90 (d, 2)	6.87 (d, 2)	6.83 (s)
2'	8.56 (d, 9)	8.01 (d, 2)	7.76 (s)
3'	7.03 (d, 9)		
5'	7.03 (d, 9)	7.00 (d, 9)	
6'	8.56 (d, 9)	8.21 (dd, 2, 9)	7.76 (s)
Glucose			
1''	5.28 (d, 7)	5.28 (d, 8)	5.32 (d, 8)
2''	3.63 (t, 8)	3.66 (t, 9)	3.72 (t, 9)
3''	3.52 (t, 9)	3.54 (t, 9)	3.56 (t, 9)
4''	3.39 (t, 10)	3.40 (t, 9)	3.42 (t, 9)
5''	3.70 (m)	3.71 (m)	3.73 (m)
6''	3.59 (m)	3.60 (m)	3.61 (m)
	4.04 (dd, 1, 11)	4.04 (dd, 1, 11)	4.05 (dd, 2, 11)
Rhamnose			
1'''	4.63 (d, 2)	4.63 (d, 1)	4.63 (d, 1)
2'''	4.94 (dd, 2, 4)	4.94 (dd, 1, 3)	4.95 (dd, 1, 4)
3'''	3.78 (dd, 4, 10)	3.78 (dd, 3, 10)	3.79 (dd, 4, 10)
4'''	3.29 (t, 10)	3.29 (t, 10)	3.29 (t, 10)
5'''	3.57 (m)	3.58 (m)	3.59 (m)
6'''	1.16 (d, 6)	1.16 (d, 6)	1.16 (d, 6)
Acetyl			
CH ₃	1.97 (s)	1.98 (s)	1.98 (s)

Coupling constants (*J*, Hz) in parentheses.

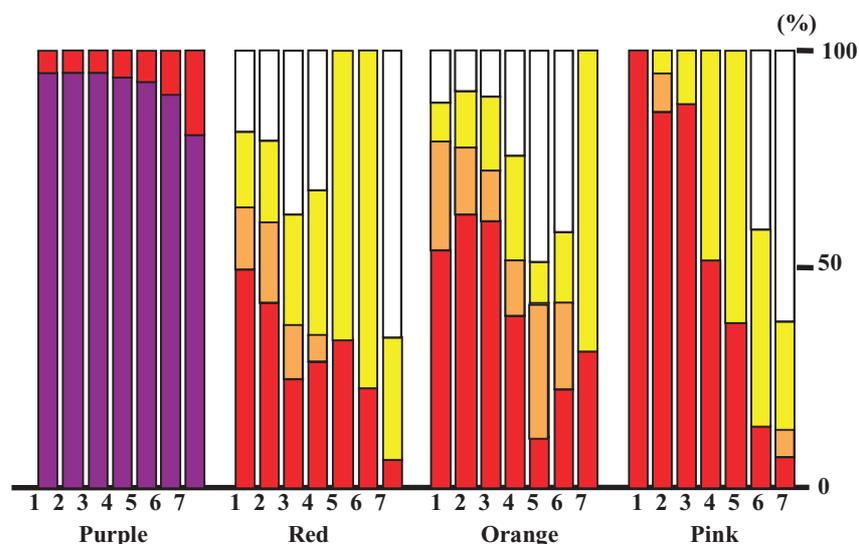


Fig. 2. Composition ratios of anthocyanins in perianthes of the representative cultivars in color variation

■ : 1, pelargonidin 3-rutinoside; □ : 2, pelargonidin 3-(2''-acetyl)rutinoside; ■ : 3, cyanidin 3-rutinoside; ■ : 4, cyanidin 3-(2''-acetyl)rutinoside; ■ : 5, delphinidin 3-rutinoside.

Color and names of cultivars are as follows. Purple: 1, Bleu Aimable; 2, Valentine (purple); 3, Amethyst; 4, Burgundy; 5, Zintsu; 6, Murasakisuisho; 7, Queen of Night. Red: 1, Paul Richter; 2, Ile de France; 3, Ben van Zanten; 4, Red Matador; 5, Utopia; 6, Exotic Bird; 7, Oxford. Orange: 1, Apricot Beauty; 2, Orange Favourite; 3, Bonanza Gold; 4, Hadley; 5, Orange Princess; 6, Alice Lechters; 7, Orange Bouquet. Pink: 1, Pink Diamond; 2, Maywonder; 3, Pink Trophy; 4, Angeliq; 5, True Love; 6, Spring; 7, Lefeber's Memory.

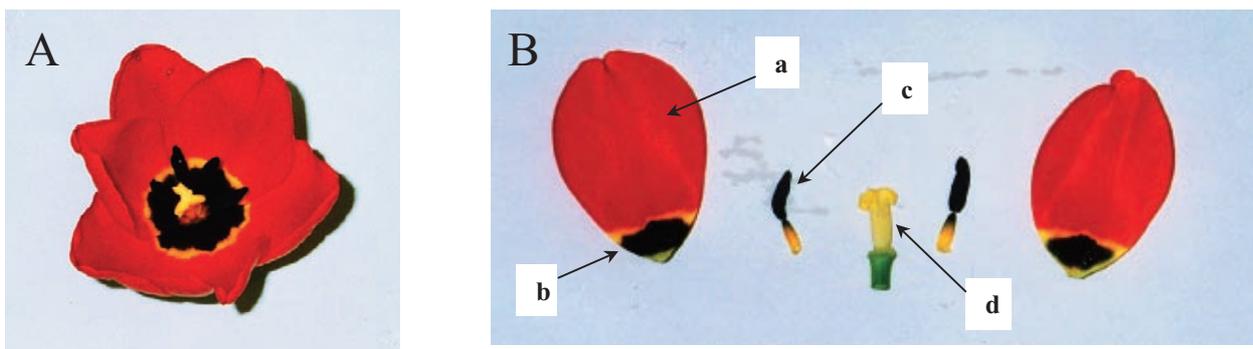


Fig. 3. Pictures of a 'Ben van Zanten' flower

A: the flower complex, B: individual tissues of the flower.
a: perianth, b: perianth-bottom, c: anther and pollen, d: pistil.

HPLC (Fig. 2). Occurrence of delphinidin 3-rutinoside was found in the perianth of purple color cultivars. Furthermore, perianthes containing delphinidin 3-rutinoside showed a purple color. Pelargonidin type anthocyanins and cyanidin type anthocyanins occurred in perianthes of red color cultivars with various compositions. A similar anthocyanin profile was found in orange and pink color cultivars.

3. Tissue specific anthocyanin distribution

'Ben van Zanten' and 'Ile de France' have a white pistil, dark purple anthers, pollens and perianth-bottoms, and red perianthes (Fig. 3). These compose a white cen-

ter, dark purple inner ring and red outer ring in the flower. Endogenous anthocyanins of these cyanic tissues of these cultivars were analyzed by HPLC.

The red perianth of 'Ben van Zanten' contained pelargonidin type anthocyanins and cyanidin type anthocyanins with the ratio of 3:1 (Table 3). The concentration ratio of pelargonidin 3-rutinoside was one-half of that of its acetyl ester while the concentration ratio of cyanidin 3-rutinoside was similar to that of its acetyl ester. The dark purple perianth-bottom contained cyanidin 3-rutinoside and delphinidin 3-rutinoside with an equal concentration ratio. There was little concentration of the acetyl esters in the perianth-bottom. Delphinidin type anthocy-

Table 3. Concentration ratios of anthocyanins 1–7 in tulip flower tissues

Tissue	Anthocyanin Type		
	Pelargonidin (1, 2)	Cyanidin (3, 4)	Delphinidin (5, 6, 7)
‘Ben van Zanten’			
Perianth	74% (24%, 50%)	26% (16%, 10%)	0%
Perianth-bottom	0%	52% (48%, 4%)	48% (44%, 4%, 0%)
Anther	0%	4% (4%, 0%)	96% (35%, 38%, 23%)
Pollen	0%	1% (1%, 0%)	99% (31%, 44%, 24%)
‘Ile de France’			
Perianth	33% (11%, 22%)	67% (40%, 27%)	0%
Perianth-bottom	4% (0%, 4%)	51% (41%, 10%)	45% (45%, 0%, 0%)
Anther	0%	0%	100% (29%, 44%, 27%)
Pollen	0%	0%	100% (23%, 53%, 24%)

anins were present in the dark purple anther and pollen each with an equal concentration ratio.

The red perianth of ‘Ile de France’ contained pelargonidin type anthocyanin and cyanidin type anthocyanin with the ratio of 1:2 (Table 3). Concentration ratios of anthocyanins and each acetyl ester were similar to those of ‘Ben van Zanten’. Anthocyanin profiles of the dark purple tissues of the ‘Ile de France’ flower were also similar to those of ‘Ben van Zanten’.

Discussion

The following 7 anthocyanins were detected in tulip flowers: pelargonidin 3-rutinoside, cyanidin 3-rutinoside, delphinidin 3-rutinoside and their 2nd-acetyl esters, and 3rd-acetyl ester of delphinidin 3-rutinoside (Fig. 1). Pelargonidin 3-(2nd-acetylrutinoside) and cyanidin 3-(2nd-acetylrutinoside) were major anthocyanins in perianthes (Fig. 2). Delphinidin 3-(2nd-acetylrutinoside) and delphinidin 3-(3rd-acetylrutinoside) were major anthocyanins in anthers and pollens (Table 3) in several cultivars. While anthocyanidin 3-rutinosides widely occur in monocotyledoneae and dicotyledoneae plants⁶, plants containing anthocyanidin 3-acetylrutinoside are rare⁷. The occurrence of anthocyanidin 3-acetylrutinoside is peculiar to tulip^{2,8}.

Hydroxylation of anthocyanidin changes the absorption spectrum. Pelargonidin, cyanidin, and delphinidin show orange, red and red purple, respectively. The hydroxylation must contribute to the coloration of tulip flowers. Anthocyanins do not change their absorption spectrum by acetyl derivatization at the 2nd or 3rd position (Table 1). The acetyl derivatization of anthocyanins seems to contribute less to the coloration of tulip (Table 1).

Close correlation between the occurrence of delphinidin 3-rutinoside and purple coloration was found in tulip perianthes (Fig. 2). Delphinidin 3-rutinoside is responsible for purple coloration of tulip perianthes. The pelargonidin type anthocyanins and the cyanidin type anthocyanins were major anthocyanins in perianthes of the red, orange and pink color cultivars with variant composition ratios. It seems that concentrations of the anthocyanins and other yellow pigment carotenoids are concerned with these colorations. We speculate that red color is generated in the case of higher concentrations of anthocyanins than carotenoids, orange color is generated *vice versa*, and pink color is generated in the case of low concentrations of both pigments.

The perianth-bottom, anther and pollen are dark purple tissues in ‘Ben van Zanten’ and ‘Ile de France’. The perianth-bottom color was lighter than the anther and the pollen color (data not shown). The presence of cyanidin type anthocyanins in the perianth-bottom may cause this difference (Table 3). Delphinidin 3-rutinoside and its acetyl esters occurred only in these dark purple tissues of these cultivars. These delphinidin type anthocyanins are responsible for dark purple coloration of these tissues like the perianth.

The structures of ‘Ben van Zanten’ and ‘Ile de France’ make their central parts prominent. This could be regarded as a nectar guide system to attract pollinators and prompt pollination. The difference in anthocyanin composition among tissues suggests that the hydroxylation and the acetyl derivatization of anthocyanin operate in a tissue specific manner. The tissue specific regulation of anthocyanin biosynthesis may contribute to the color pattern formation in the tulip flower.

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