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Anthocyanin Constitutions of Flowers in Newly Established Colored Tuberoses (*Polianthes*)

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The anthocyanin constitutions of the flowers of *Polianthes tuberosa* 'Single', 'Double', *P. howardii* and their nine hybrids with reddish purple, pale purple, pale red, reddish pink and orange obtained by the crossings and backcrossings were analyzed.

Among the parents and hybrids, two white flowered cultivars of *P. tuberosa* had no anthocyanins. Other hybrids and *P. howardii* contained anthocyanins in their petals. The main anthocyanidin in the petals of anthocyanin containing flowers was cyanidin with which some hybrids also contained delphinidin. Reddish purple or pale purple flower hybrids contained delphinidin glycoside with cyanidin glycoside, whereas only cyanidin glycosides were contained in the flowers of orange, pale red and reddish pink.

On the basis of these findings, several aspects for future breeding of tuberose were discussed.

INTRODUCTION

Tuberose (*Polianthes tuberosa*) is one of the most important cut flowers in Taiwan and it occupies a prime position in the floriculture industry (Shen *et al.*, 1987; Shen *et al.*, 1991). There are, however, only two white flowered cultivars 'Single' and 'Double' cultivated in Taiwan. *Polianthes howardii* having reddish-purple flowers, native to Mexico (Howard, 1985), was introduced to Taiwan in 1985 and the breeding for colored tuberose have been started (Shen *et al.*, 1993). And then more than 50 hybrids of colored tuberose were obtained (Shen *et al.*, 1997). The pigment compositions of the petals of the newly established hybrid tuberoses having pink, reddish-purple, purple, orange and yellow flowers which were bred by the crosses and backcrosses of *P. tuberosa* and *P. howardii* have been previously reported (Huang *et al.*, 1998).

In the present study, we analyzed the anthocyanin constitutions of the petals of the hybrids and discussed the relationship between flower colors and anthocyanin constituents. The possibilities of the establishment of the hybrids having blue flowers were also discussed.

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MATERIALS AND METHODS

Plant materials

Petals of *P. tuberosa* 'Single', 'Double', *P. howardii* and their nine anthocyanin-containing hybrids were collected in June 1998 at the farm of National Chiayi University, Chiayi, Taiwan, Republic of China. The petal colors were investigated by visual examination and determined on the basis of Royal Horticultural Society Colour Chart (RHSCC). The petal samples were dried at 40 °C and stored in a desiccator.

Anthocyanin constitutions of the colored tuberose

The dried petals were soaked in 0.1% hydrochloric acid–methanol at 5 °C for 12 h. The extracts were passed through 0.45 μm Millipore filter and analyzed by high performance liquid chromatography (HPLC) (Shimadzu LC-6 A pump, SPD-6 AV spectrophotometric detector) using Cosmosil-$5C_{18}$ column (4.6 mm i.d. × 250 mm, Nakarai Tesque) set at 530 nm. Elution (1.0 ml·min^{-1}) was performed using solvent system comprising solvent A (4% phosphoric acid) and acetonitrile (solvent B) and a linear gradient starting with 90% A, decreasing to 88% A at 20 min.

Isolation of the anthocyanins and determination of their aglycones

To characterize the seven anthocyanins found the extracts were purified successively by preparative paper chromatography using a 40 × 40 cm filter paper (No. 526, Toyo) in two solvent systems BAW and AHW (Table 1). After each development, the pigment band was marked under VIS light, cut out, and then eluted with 5% acetic acid–methanol. The eluate obtained was concentrated under reduced pressure and used for identification.

For the identification of the aglycones of isolated anthocyanins, a small portion of the each purified anthocyanin was hydrolyzed for 60 min at 100 °C with 2 ml of 2 N hydrochloric acid. The hydrolysates obtained were analyzed with some authentic specimens by HPLC, in which chromatograms were developed with an LC-6 A pump equipped with a 4.6 mm I.D. × 250 mm Cosmosil $5C_{18}$ column and detected at 530 nm. A flow rate of 1.0 ml·min^{-1} was maintained and a mixture of acetonitrile–4% phosphoric acid (15:85, v/v) was employed as the eluant. Standard anthocyanidins were prepared from the following sources. Delphinidin (petals of *Commelina communis*), cyanidin (petals of *Rhododendron kaempferi*), petunidin (petals of *Petunia hybridum*), pelargonidin (fruits of *Fragaria* × *ananassa*), peonidin (petals of *Paeonia officinalis*) and malvidin (petals of *Tibouchina urvilleana*).

Three anthocyanins of which the aglycones were clarified were co-chromatographed

Table 1. Solvent systems used for chromatography in this study.

Abbreviation	Composition	Proportion (v/v)	Phase used
AHW	Acetic acid / conc. HCl / Water	15 : 3 : 82	Miscible
BAW	n-Butanol / Acetic acid / Water	4 : 1 : 5	Top
BH	n-Butanol / 2N-HCl	1 : 1	Top
HW	conc. HCl / Water	3 : 97	Miscible

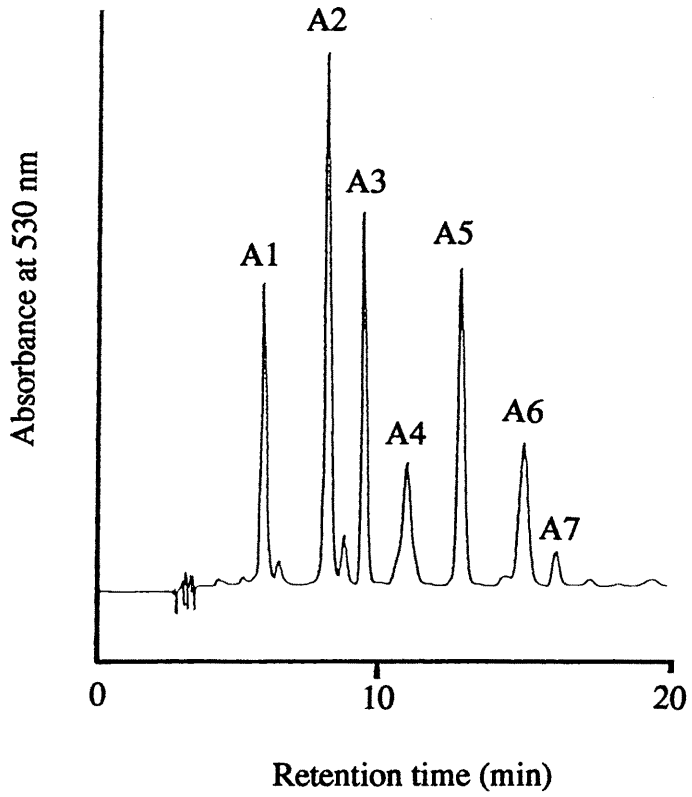

Fig. 1. HPLC tracing of anthocyanins in the petals of reddish–purple tuberose '77A05'.

Table 2. Percentage of appearance of seven anthocyanins in the petals of colored tuberose.

Species and strain	Anthocyanin[z]						
	A1	A2	A3	A4	A5	A6	A7
P. tuberosa 'Single'	–	–	–	–	–	–	–
P. tuberosa 'Double'	–	–	–	–	–	–	–
P. howardii	1.3	7.4	20.3	3.2	50.7	3.9	13.2
77A05	13.4	26.6	16.0	10.7	19.1	11.9	2.3
82O04	0.0	66.3	0.0	0.0	8.6	22.9	2.2
82R16	9.5	45.0	9.6	2.5	23.7	9.8	0.0
84D03	0.0	53.4	0.0	0.0	31.4	15.2	0.0
84D04	0.0	67.3	0.0	0.0	8.1	24.6	0.0
84E14	0.0	24.8	0.0	0.0	55.4	3.7	16.2
84G02	1.5	9.9	15.9	0.0	65.3	2.7	4.8
84J08	2.3	13.4	15.5	1.5	58.1	2.5	6.7
85A05	0.0	29.2	11.0	0.0	49.6	10.2	0.0

[z] A1–A7; correspond to Fig. 1.

Table 3. Aglycones of seven anthocyanins isolated from the petals of colored tuberose.

Sample	Retention time (min)
Aglycone[z]	
A1	6.1
A2	9.8
A3	6.1
A4	6.1
A5	10.1
A6	9.8
A7	9.9
Standard	
Delphinidin	6.3
Cyanidin	10.0
Petunidin	12.7
Pelargonidin	17.5
Peonidin	22.4
Malvidin	27.3

[z] A1–A7; correspond to Fig. 1.

Table 4. Rf values of three major anthocyanins isolated from the colored tuberose.

Sample	Rf values (×100) in				Color
	AHW[z]	BAW[z]	BH[z]	HW[z]	
A3[y]	14	33	9	3	Purple
A5[y]	26	45	20	9	Red
A7[y]	41	45	32	18	Red
Cy3G[x]	27	44	20	9	Red
Cy3G5G[w]	40	34	20	18	Red

[z] Abbreviations of solvent systems shown in Table 1.
[y] correspond to Fig. 1.
[x] Cyanidin 3–glucoside.
[w] Cyanidin 3, 5–diglucoside.

with authentic anthocyanin glycosides; cyanidin 3–glucoside and cyanidin 3, 5–diglucoside.

RESULTS AND DISCUSSION

Maximum seven anthocyanins were detected from the colored tuberose flowers examined in the present study, whereas no anthocyanins in white flowered tuberose 'Single' and 'Double' (Fig. 1 and Table 2). The aglycones of the isolated seven anthocyanins were either delphinidin or cyanidin (Table 3). An anthocyanin (A5 in Fig. 1) was identified as cyanidin 3–glucoside (Table 4). Reddish purple or pale purple flowers contained delphinidin glycoside along with cyanidin glycoside, whereas only cyanidin glycosides were contained in the orange, pale red and reddish pink flowers (Table 5).

Table 5. Petal colors and pigment distribution of tuberose species and their hybrids.

Species and strain	Petal color by visual examination	R. H. S. Colour Chart No.		% of	
		Inside of petal	Outside of petal	Delphinidin glycosides	Cyanidin glycosides
P. tuberosa 'Single'	White	155D	155D	–	–
P. tuberosa 'Double'	White	155D	155D	–	–
P. howardii	Reddish purple	64A	67D	24.8	75.2
77A05	Reddish purple	78D	68C	40.1	59.9
82O04	Orange	23C	43C	0.0	100.0
82R16	Pale purple	63C	63C	21.6	78.4
84D03	Pale red	66D	66D	0.0	100.0
84D04	Reddish pink	52B	52B	0.0	100.0
84E14	Pale red	62D	62D	0.0	100.0
84G02	Reddish purple	64C	63D	17.4	82.6
84J08	Deep reddish purple	59A	59D	19.3	80.7
85A05	Pale purple	80C	80C	11.0	89.0

Thus, it is clear that the co–occurrence of delphinidin glycosides gives much bluer tone, as compared with the forms with pure cyanidin glycosides.

Most of research on the genus *Polianthes* have been carried out on *P. tuberosa*, currently only found in cultivation (Trueblood, 1973). The flowers are only white and the goals for tuberose hybridization are to obtain potential garden hybrids, incorporating more colors, fragrance, and ease of culture (Shen *et al.*, 1987). At present, only white–flowered 'Single' and 'Double' are widely cultivated in the world (Shen *et al.*, 1987). However, as described in the previous paper (Huang *et al.*, 1998), we succeeded to obtain many hybrids of colored tuberose by intra– or interspecific crossings between *P. tuberosa* and *P. howardii*; the latter species has both anthocyanins and carotenoids in its petals.

The anthocyanins are the most important and widespread group of coloring matters in plants. These intensely colored water–soluble pigments are in vacuoles and responsible for nearly pink, scarlet, red, mauve, violet and blue colors in the petals of higher plants (Harborne, 1984). It has been well known that the flower colors based on the anthocyanins are modified by various factors. One of the most important factors is the hydroxylation of anthocyanin. In general, blue flowers have delphinidin glycosides, whereas crimson and magenta flowers have cyanidin glycosides (Harborne, 1976). In this investigation, the hybrid '77A05' showed the highest delphinidin percentage (Table 5), but its flower color is reddish purple. Accordingly, the hybrid containing high delphinidin glycosides without cyanidin glycosides should be bred to obtain blue tuberose.

Co–pigmentation is known as another important factor for blue color or blueing effect in flowers (Asen *et al.*, 1971; De Loose, 1978). In preliminary experiments, quercetin was detected from the white flowered tuberoses and it is known as the co–pigment of anthocyanin. Thus, it might intensify the blue shade to fill simultaneously the high amount of the flavonols sufficient to form full co–pigments.

It is known that anthocyanins 3, 5–diglycoside may afford bluer shade than the corresponding 3–monoglycoside (Hayashi, 1991). The accumulation of 3, 5–diglycosides

of anthocyanidin also should be considered in further breeding.

From these findings, it would be quite reasonable to presume that the systematic and composite planning of future breeding might realize much bluer shades of tuberoses than the hybrids attained until nowadays.

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